



**Targeted delivery of iron, cobalt and magnesium
nanoparticles incorporating anticancer constituents of
Euphorbia species for treatment of breast cancer**

By

Ipeleng Kopano Rosinah Kgosiemang

Dissertation submitted in fulfilment of the requirements of the degree

Doctor of Health Sciences in Biomedical Technology

Department of Health Sciences

Central University of Technology, Free State

24 September 2024

Promoter: Prof Mamello Sekhoacha

Co-Promoter: Prof Samson Sitheni Mashele

DECLARATIONS

I, **IPELENG KOPANO ROSINAH KGOSIEMANG** (SOUTH AFRICAN ID NUMBER: #####) hereby certify that the dissertation submitted by me for the degree DOCTOR OF HEALTH SCIENCES IN BIOMEDICAL TECHNOLOGY is my own independent work and complies with the Code of Academic Integrity, as well as other relevant policies, procedures, rules and regulations of the Central University of Technology (Free State). I hereby declare that this research project has not been previously submitted to any university or faculty for the attainment of any qualification. I further waive copyright of the dissertation in favour of the Central University of Technology (Free State).

IPELENG KOPANO ROSINAH KGOSIEMANG

DATE

I, **MAMELLO PATIENCE SEKHOACHA**, the supervisor of the doctoral dissertation titled: Targeted delivery of iron, cobalt and magnesium nanoparticles incorporating anticancer constituents of Euphorbia species for treatment of breast cancer, hereby certify that the work in this project was done by **IPELENG KOPANO ROSINAH KGOSIEMANG**. I hereby approve the submission of this dissertation and affirm that this has not been submitted previously neither to this or any other institution for admission to a degree or any other qualification.

MAMELLO P SEKHOACHA

____25 September 2024____

DATE

Dedication

This thesis is dedicated to my late

Grandparents

**Makgalathebe Mariam Kgosiemang and Ishmael Tolo
Kunene**

ACKNOWLEDGEMENTS

I am profoundly grateful for the realization of this thesis, a journey that would have remained a mere aspiration without the unwavering support of my supervisor, Prof Mamello SEKHOACHA. Her continuous guidance, patience, motivation, enthusiasm, prayers and profound knowledge in ethnopharmacology research have been instrumental in shaping this endeavour. Prof Sekhoacha's faith and trust in me have been pivotal, propelling me to the academic milestone I now proudly stand upon.

I extend my heartfelt appreciation to Prof Samson Sitheni MASHELE for his steadfast support and attentive ear to our concerns. Special thanks to Dr. ADEGOKE for believing in me during moments of self-doubt, pushing me to overcome challenges and encouraging me to see this journey through. My gratitude also goes to Dr. CHIKA for his steadfast belief and invaluable counsel during challenging times.

I express sincere thanks to the National Research Foundation of South Africa and the Research Innovation Fund of the Central University of Technology in the Free State for generously funding my studies and transforming my dreams into a reality.

To my fellow lab mates: our collaborative efforts, stimulating discussions, prayers and the camaraderie shared over years have enriched this academic pursuit. A special acknowledgment is owed to Justinah MOFOLO – your unwavering support has been my indispensable pillar throughout this journey. Thank you for everything. Relebohile LEFOJANE, from the very beginning until this moment, you've been my dedicated prayer warrior, and for that, I am profoundly grateful.

My deepest appreciation goes to my husband, Kagiso XABA, for his unwavering support, prayers, continuous encouragement and immense patience throughout my years of study, research and thesis writing. I am profoundly grateful to my mother, Lydia KUNENE, for her enduring belief, unwavering support during challenging times, your prayers (JESU) and her empathetic ear. None of this would have been possible without her.

I extend heartfelt thanks to my prayerful sisters, Bankaetse TSHINYANI, Viola MAKHUNAMISHA and Bonolo KUNENE, for their prayers and unwavering moral support. To my uncle Tshepo KUNENE, your support has been invaluable.

A special acknowledgment goes to my grandmother, Makgalathebe KGOSIEMANG, for nurturing and guiding me with love, and also to my grandfather, Tolo KUNENE, for his open home and encouragement for me to pursue my dreams.

Above all, I attribute the success of this endeavor to the Almighty God, whose wisdom, health and strength sustained me through the challenges of this research journey, enabling its completion.

Amidst the challenges of a formidable journey, she emerged resilient, turning obstacles into stepping stones. Her strength echoed in the applause that celebrated not only her triumph but the indomitable spirit of a remarkable woman.

Table of Content

Chapter 1: Introduction

1.1. Background	1-3
1.2. Problem statement	3
1.3. Research aim and objectives	
1.3.1. Aim of the study	3
1.3.2. Objectives of the project	3-4

Chapter 2: Literature review

2.1. Introduction	5-6
2.1.1. Prevalence of cancer	6-9
2.2. Breast cancer	9
2.2.1. Clinical features of breast cancer	9
2.2.2. Breast cancer classification based on gene expression	10
2.3. Therapy and its limitations	10-12
2.4. Medicinal plants	12-13
2.4.1. <i>Euphorbia</i> species used in this study	13
2.5. Nanotechnology and its application	14
2.5.1. Applications	15
2.5.2. Nanotechnology in drug delivery of cancer therapy	16
2.6. Single-Pot eco-friendly synthesis	19-20
2.7. In vivo studies	20-22
2.8. Reference.....	23-45

Chapter 3: Pharmacological significance, medicinal use and toxicity of extracted and isolated compounds from *Euphorbia* species found in Southern Africa: a review

Abstract

3.1. Introduction	48-49
3.2. Methodology	50-51
3.3. Results	51-81

3.4. Discussion	81-146
3.7 Conclusion and future perspective	146-147
3.8. References	148-189

Chapter 4: *In vitro* antiproliferative activity and selective cytotoxicity of sixteen South African *Euphorbia* species against breast cancer cell lines

Abstract

4.1. Introduction	192-193
4.2. Materials and Method	
4.2.1. Plant collection and processing	193-194
4.2.2. Phytochemical analysis	194-195
4.2.3. Antiproliferation and Cytotoxic screening of <i>Euphorbia</i> extracts.....	195
4.2.3.1. Cell culture	195
4.2.3.2. MTT assay.....	195
4.2.3.3. CC ₅₀ determination.....	196
4.2.3.4. Selectivity index.....	196-197
4.3. High-Performance Liquid Chromatography (HPLC)	197
4.2.3. Statistical analysis	197-198
4.4. Results	198-229
4.5. Discussion	230-236
4.6. Conclusion.....	236-237
4.7. Reference.....	238-247

Chapter 5: Green synthesis of magnesium, cobalt, iron oxide and iron dioxide nanoparticles using *Euphorbia tirucalli*: characterization and antiproliferative evaluation against breast cancer cell lines

Abstract

5.1. Introduction	250
5.2. Materials and Methods	
5.2.1. Plant extraction and preparation	252
5.2.2. Phytochemical Screening.....	252
5.2.3. Preparation of MgONPs, CoONPs, and FeONPs.....	252-253
5.2.4. Characterization of synthesized nanoparticles.....	253

5.2.5. Anti-proliferation screening of <i>Euphorbia</i> extracts.....	253-254
5.3. Results	254-272
5.4. Discussion	272-277
5.5. Conclusion.....	278
5.6. References	279-287

Chapter 6: Synergistic Potential of Electroacupuncture and Superparamagnetic Iron Oxide Nanoparticles in Inhibiting proliferation of Triple-Negative Breast Cancer tumor in a Mouse Xenograft Model

Abstract

6.1. Introduction	290-294
6.2. Materials and Methods	
6.2.1. Breast cancer preparation	294
6.2.2. In vivo model	294-295
6.2.3. In vivo TNBC xenograft mouse model.....	295
6.2.4. Treatment grouping.....	296-297
6.2.5. Electroacupuncture intervention.....	297-298
6.2.6. Histological studies	298
6.2.7. Statistical analysis	299
6.3. Results	299-305
6.4. Discussion	305-308
6.5. Conclusion.....	308-309
6.6. References	310-317

Chapter 7: Summary

7.1. Introduction	317
7.2. Results summary	317-319

7.3. Conclusion.....	319-320
7.4. Limitations and recommendation	320-322
❖ Appendices	323-329

List of Abbreviations and Acronyms

MRI: Magnetic resonance imaging

SPION(s): Super Paramagnetic Iron Oxide Nanoparticle(s)

FeONPs: Iron oxide nanoparticles

MgONPs: Magnesium oxide nanoparticles

CoONPs: Cobalt oxide nanoparticles

PBS: Phosphate-buffered saline

DMSO: Dimethyl sulfoxide

TEM: Transmission electron microscopy

DMEM: Dulbecco's Modified Eagle Medium

EDTA: Ethylenediaminetetraacetic Acid

XRD: X-ray diffraction

TG-DSC: Thermogravimetric-Differential Scanning Calorimetry

UV: Ultraviolet

DLS: Dynamic Light Scattering

FTIR: Fourier transform infra-red

MCF-7: Michigan cancer foundation-7

WHO: World health organisation

CANSA: Cancer Association of South Africa

Pb: Lead

Zn: Zinc

Mg: Magnesium

Ag: Silver

Au: Gold

Co: Cobalt

Ni: Nickle

Ti: Titanium

ER: Estrogen receptor

PR: Progesterone receptor

HER2: Human epidermal growth factor receptor 2

ERBB2: Erythroblastic leukemia viral oncogene homolog

DOX: Doxorubicin

FBS: Fetal bovine serum

DMSO₄: Dimethyl sulfoxide

MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5- diphenyltetrazolium bromide

DMEM: Dulbecco's Modified Eagle's Medium

MCF-7, T47D, BT474, MDA-MB-361, SKBR3

HCC202, BT20, MDA-MB-231, MDA-MB-468: Breast cancer cell lines

DNA: Deoxyribonucleic acid

E. trigona: *Euphorbia trigona*

E. clavaroides Boiss: *Euphorbia clavaroides* Boiss

E. caerulea: *Euphorbia caerulea*

E. ammak: *Euphorbia ammak*

E. cooperi: *Euphorbia cooperi*

E. cupularis: *Euphorbia cupularis*

E. sekukunesis: *Euphorbia sekukunesis*

E. polygona: *Euphorbia polygona*

E. ferox: *Euphorbia ferox*

E. ledienii: *Euphorbia ledienii*

E. horrida: *Euphorbia horrida*

E. var. noorsveldensis: *Euphorbia var.noorsveldensis*

E. enopla (Rooidoringnoors): *Euphorbia enopla*

E. bupleurifolia (pine cone plant): *Euphorbia bupleurifolia*

E. Limpopoana: *Euphorbia limpopoana*

E. gorgonis vingerpol: *Euphorbia gorgonis*



E. stellate: Euphorbia stellata

E. Arabica: Euphorbia Arabica

SCID: Severely compromised immunodeficient

UFS: University of the Free State

List of Figures

Figure 1.1 World annual number of deaths

Figure 1.2. Estimated incidence and mortality of all cancers

Figure 1.3. Magnetic drug Targeted drug delivery system

Figure 1.4. Schematic diagram of the preparation of one pot green synthesis

Figure 3.1. *Euphorbia trigona* tree and pot plant

Figure 3.2. *Euphorbia ledienii* plant with stem and flowers

Figure 3.3. *Euphorbia horrida* plant with stem and flowers

Figure 3.4. *Euphorbia enopla* plant with stem and flowers

Figure 3.5. *Euphorbia coerulescences* plant with stem and flowers

Figure 3.6. *Euphorbia cooperi* tree and fruits

Figure 3.7. *Euphorbia tirucalli* plant and flower

Figure 3.8. *Euphorbia ammak* tree and flower

Figure 3.9. *Euphorbia clavarioides* plant with flowers and stem

Figure 3.10 *Euphorbia gorgonis* plant with flowers and stem

Figure 3.11. *Euphorbia bupleurifolia* plant with stem and flowers

Figure 3.12. *Euphorbia polygona* plant with stem and flowers

Figure 3.13. *Euphorbia arabica* plant with stem and flowers

Figure 3.14. *Euphorbia ferox* plant with stem and flowers

Figure 3.15. *Euphorbia stellata* plant with stem and flowers

Figure 3.16. Terpene biosynthesis in the cytosol through the mevalonate path

Figure 4.1. Anti-proliferative effect of doxorubicin against MCF-7, MDA-MB231, and Vero cell lines

Figure 4.2. Anti-proliferative effect of *E. bupleurifolia* extracts against MCF-7 and Vero cell lines

Figure 4.3. Anti-proliferative effect of *E. trigona* extracts against MCF-7 and Vero cell lines

Figure 4.4. Anti-proliferative effect of *E. gorgonis* extracts against MCF-7 and MDA-MB231 cell lines

Figure 4.5. Anti-proliferative effect of *E. polygona* extracts against MDA-MB231 cell line

Figure 4.6. Anti-proliferative effect of *E. ferox* extract against MDA-MB231 cell line

Figure 4.7. Anti-proliferative effect of *E. clavarioides* extracts against MCF-7, MDA-MB231 and Vero cell lines

Figure 4.8. Anti-proliferative effect of *E. ammak* extracts against MCF-7, MDA-MB231 and Vero cell lines

Figure 4.9. Anti-proliferative effect of *E. enopla* extracts against MCF-7, MDA-MB231 and Vero cell lines

Figure 4.10. Anti-proliferative effect of *E. stellata* extracts against MCF-7, MDA-MB231 and Vero cell lines

Figure 4.11. Anti-proliferative effect of *E. ledienii* extracts against MCF-7 and Vero cell lines

Figure 4.12. Anti-proliferative effect of *E. arabica* extracts against MCF-7 and Vero cell lines

Figure 4.13. Anti-proliferative effect of *E. cooperi* extracts against MCF-7 and MDA-MB231 cell lines

Figure 4.14. Anti-proliferative effect of *E. tirucalli* extracts against MCF-7 (A) and MDA-MB231 (B) cell lines

Figure 4.15. HPLC chromatograms of selective extracts of *E. clavarioides* Hex and DCM for MCF-7

Figure 4.16. HPLC chromatograms of selective extract of *E. trigona* DCM for MCF-7

Figure 4.17. HPLC chromatograms of selective extract of *E. gorgonis* Hex for MCF-7 and MDA-MB231

Figure 4.18. HPLC chromatograms of selective extracts of *E. ledienii* Hex and EtoAC for MCF-7 and MDA-MB231

Figure 4.19. HPLC chromatograms of selective extract *E. arabica* DCM for MFC-7 and MDA-MB231

Figure 4.20. HPLC chromatograms of selective extracts of *E. cooperi* Hex and DCM for MCF-7 and MDA-MB231

Figure 4.21. HPLC chromatograms of selective extracts of *E. ammak* Hex and DCM for MCF-7

Figure 4.22. HPLC chromatograms of selective extract *E. ferox* DCM

Figure 4.23. HPLC chromatograms of selective extract of *E. polygona* Hex and DCM for MDA-MB231

Figure 4.24. HPLC chromatograms of selective extract *E. enopla* Hex and DCM for MFC-7 and MDA-MB231

Figure 4.25. HPLC chromatograms of selective extract *E. tirucalli* Hex and DCM for MFC-7 and MDA-MB231.

Figure 5.1. Ultraviolet-visible spectroscopy of FeO from *E. tirucalli*

Figure 5.2. Ultraviolet-visible spectroscopy of MgO and CoO.

Figure 5.3. FTIR spectrum analysis of iron oxide (FeO) NPs

Figure 5.4. FTIR spectrum analysis of MgONPs and CoONPs

Figure 5.5. X-ray diffraction analysis of reduced FeO NPs

Figure 5.6 X-ray diffraction analysis of reduced MgO and CoONPs

Figure 5.7. XPS analysis of FeO NPs

Figure 5.8. XPS analysis of MgONPs

Figure 5.9. XPS analysis of CoONPs

Figure 5.10. SEM imaging of FeO

Figure 5.11. SEM imaging of MgONPs

Figure 5.12. SEM imaging of CoONPs

Figure 5.13. EDS analysis and TEM imaging FeONPs

Figure 5.14. EDS analysis and TEM imaging MgONPs

Figure 5.15. EDS analysis and TEM imaging CoONPs

Figure 5.16. Cell growth inhibitory activity of MgONPs and CoONPs against MCF-7 cell line

Figure 5.15. Cell growth inhibition % of four *Euphorbia* crude extracts, FeO-RT, FeO-500 NPs against MCF-7, SK-BR-3, MDA-MB231 cell lines

Figure 6.1. Mouse under anaesthesia with nose cone during 4T1 Cell Inoculation

Figure 6.2. Electroacupuncture intervention on the xenograft model

Figure 6.3. Anti-proliferative effect of the EA IONPs, *E. tirucalli*, doxorubicin and PBS treatment groups

Figure 6.4. Differential Tumor Size Variation Across Treatment Modalities

Figure 6.5. Body weights of mice

List of Tables

- Table 1.1.** Estimated incidence, mortality and prevalence percentage (%) of cancers
- Table 3.1.** *Euphorbia* spp of Southern Africa and its neighboring countries, their toxicity and traditional use
- Table 3.2.** Comprehensive overview of Isolated Compounds from Individual Plants
- Table 3.3.** Phytochemicals extracted from the selected *Euphorbia* spp
- Table 3.4.** Classification of Crude and Isolated Compounds from 15 *Euphorbia* Species
- Table 3.5.** Bioactive compounds isolated from 15 *Euphorbia* species alongside their biological activities and subclasses
- Table 3.6.** Type of toxicity caused by some *Euphorbia* species
- Table 3.7.** Compounds with high cytotoxic probability against 3 or more cancer cell lines
- Table 3.8.** Physicochemical parameters and solubility tendency
- Table 4.1.** List of 16 selected *Euphorbia* plant species used in this study
- Table 4.2.** CC₅₀ criteria for cytotoxicity
- Table 4.3.** HPLC gradient elution
- Table 4.4.** Percentage yields of organic extract
- Table 4.5.** Percentage yields of inorganic extracts
- Table 4.6.** Qualitative phytochemical analysis of sixteen selected *Euphorbia* plant species
- Table 4.7.** Cytotoxicity of doxorubicin against MCF-7, MDA-MB231, and Vero cell lines
- Table 4.8.** Cytotoxicity of *E. bupleurifolia* against MCF-7, MDA-MB231, and Vero cell lines
- Table 4.9.** Cytotoxicity assessment of extracts from *E. trigona* on MCF-7, MDA-MB231, and Vero cell lines
- Table 4.10.** Cytotoxicity assessment of extracts from *E. gorgonis* on MCF-7, MDA-MB231, and Vero cell lines
- Table 4.11.** Cytotoxicity assessment of extracts from *E. polygona* on MCF-7, MDA-MB231, and Vero cell lines
- Table 4.12.** Cytotoxicity assessment of extracts from *E. horrida* Var on MCF-7, MDA-MB231, and Vero cell lines
- Table 4.13.** Cytotoxicity assessment of extracts from *E. horrida* Indigenous on MCF-7, MDA-MB231, and Vero cell lines

Table 4.14. Cytotoxicity assessment of extracts from *E. ferox* on MCF-7, MDA-MB231, and Vero cell lines

Table 4.15. Cytotoxicity assessment of extracts from *E. clavarioides* on MCF-7, MDA-MB231, and Vero cell lines

Table 4.16. Cytotoxicity assessment of extracts from *E. ammak* on MCF-7, MDA-MB231, and Vero cell lines

Table 4.17. Cytotoxicity assessment of extracts from *E. enopla* on MCF-7, MDA-MB231, and Vero cell lines

Table 4.18. Cytotoxicity assessment of extracts from *E. stellata* on MCF-7, MDA-MB231, and Vero cell lines

Table 4.19. Cytotoxicity assessment of extracts from *E. ledienii* on MCF-7, MDA-MB231, and Vero cell lines

Table 4.20. Cytotoxicity assessment of extracts from *E. coerulescenes* on MCF-7, MDA-MB231, and Vero cell lines

Table 4.21. Cytotoxicity assessment of extracts from *E. arabica* on MCF-7, MDA-MB231, and Vero cell lines

Table 4.22. Cytotoxicity assessment of extracts from *E. cooperi* on MCF-7, MDA-MB231, and Vero cell lines

Table 4.23. Cytotoxicity assessment of extracts from *E. tirucalli* on MCF-7, MDA-MB231, and Vero cell lines.

Table 5.1. Phytochemical profiling of *E. tirucalli*

Table 6.1. Summary of cancer metastasis results in lung and liver samples following different treatments

List of Appendices

1. Ethical approval (UFS)
2. Ethical approval (NWU)

Publications from this Thesis

❖ **Kgosiemang, I. K.**, Lefojane, R., Direko, P., Madlanga, Z., Mashele, S. and Sekhoacha, M. 2020. Green synthesis of magnesium and cobalt oxide nanoparticles using *Euphorbia tirucalli*: Characterization and potential application for breast cancer inhibition. *Inorganic and Nano-Metal Chemistry*, 50(11), 1070-1080.

❖ **Kgosiemang, I. K. R.**, Adegoke, A. M., Mashele, S. S. and Sekhoacha, M. P. 2023. Green synthesis of Iron oxide and Iron dioxide nanoparticles using *Euphorbia tirucalli*: characterization and antiproliferative evaluation against three breast cancer cell lines. *Journal of Experimental Nanoscience*, 18(1), p. 2276.

❖ In vitro Antiproliferative activity and Selective Cytotoxicity of Sixteen South African *Euphorbia* species against Breast cancer cell lines [BMC Complementary Medicine and Therapies, Submission ID:17ac429c-389d- 4961-9a69-c2ee7755181d].

Under review

❖ Pharmacological Significance, Medicinal Use And Toxicity Of Extracted And Isolated Compounds From *Euphorbia* Species Found In Southern Africa: A Review [Plants, MDPI, Manuscript ID: plants-3355477].

Under review

Conference presentation

Presentation of e-poster: Synergistic Potential of Electroacupuncture and Superparamagnetic iron oxide Nanoparticles in inhibiting Proliferation on Triple-negative Breast Cancer Tumor in a Mouse Xenograft Model,



Central University of
Technology, Free State

Ipeleng Kopano Rosinah Kgosiemang, Samson Sitheni Mashele, Mamello Patience

Sekhoacha, **South African society for basic and clinical pharmacology**, held in

South Africa, from 15-17 September 2024.

CHAPTER 1

INTRODUCTION

1.1. Background

Cancer is a devastating disease that affects millions of people worldwide, and a major contributor to global mortality rates (NIH, 2022). Cancer occurs when cells in the body grow uncontrollably and impact neighboring organs, overall health and the well-being of patients. The World Health Organization (WHO) reported nearly 10 million new cases of cancer in 2020, with breast cancer being one of the leading causes of cancer-related deaths among women (WHO, 2022). Unfortunately, the conventional treatment of cancer remains a daunting challenge due to the lack of specificity of some commonly used drugs, such as those used in chemotherapy. The commonly used chemotherapeutic drugs unfortunately target both cancerous and healthy cells, which leads to severe side effects. Hence, there exists a critical necessity for innovative and alternative therapeutic strategies, notably the identification of novel drugs and to improve treatment efficacy while mitigating the adverse effects endured by cancer patients.

One promising approach for developing less toxic cancer treatment involves the investigation of natural compounds derived from medicinal plants. These compounds may offer novel mechanisms of action and reduced side effects, thereby presenting a valuable opportunity for advancing cancer therapeutics. Medicinal plants are increasingly recognized globally for their affordability, accessibility and efficacy in cancer therapy. Certain medicinal plants can even enhance the effectiveness of cancer treatment and reduce its toxicity (Jain *et al.*, 2016). The Euphorbiaceae family, known for its numerous plant species that are

commonly used as traditional medicinal herbs in different parts of the world, contains plant species that exhibit anti-cancer properties (Hartwell, 1969; Mwine & Damme, 2011). In addition to these natural compounds, the impact of nanotechnology, especially in medicine, has been transformative, and its potential continues to be revealed (Landesman *et al.*, 2016). Integrating the bioactive compounds from medicinal plants with advanced nanotechnology techniques offers a compelling strategy to enhance the precise drug delivery and efficacy of cancer treatment, thereby minimizing harm to healthy cells.

Nanoparticles, specifically those with magnetic cores, have displayed immense potential in delivering drugs. The targeted magnetic drug delivery system relies on an external magnetic field that directs the magnetic nanoparticles such as iron-based magnetite to their intended destination. The system boasts remarkable multifunctional loading capability (Mody *et al.*, 2013; Liu *et al.*, 2017). Furthermore, integrating the two fields mentioned above with Traditional Chinese Medicine presents a holistic approach that addresses multiple signaling pathways.

Traditional Chinese Medicine encompasses various modalities including herbal medicine, massage (Tui na), dietary therapy, qigong exercises and acupuncture; all aimed at restoring balance and promoting health (Xu & Yang, 2009). Acupuncture, a widely recognized technique within Traditional Chinese Medicine, involves the insertion of thin needles into specific points on the body to stimulate therapeutic effects across various conditions (WHO, 2002). Extensive studies have shown that electro-acupuncture can enhance drug delivery, reduce drug resistance and minimize side effects, particularly when combined with advancement in nanotechnology, potentially serving as a promising drug delivery system (Yang *et al.*, 2019;

Ma *et al.*, 2019). By combining acupuncture with nanotechnology, cancer treatment can diversify, potentially enhancing therapy precision.

1.2. Problem statement

Cancer treatment is a multifaceted process that involves overcoming major challenges. The first challenge is the harm of chemotherapeutic drugs to normal healthy cells, due to lack of specificity to cancerous cells leading to severe side effects experienced by cancer patients. The second challenge is the non-targeted delivery of drugs to the cancerous sites specifically. The combination of non-targeted drug delivery and lack of specificity is the root cause of these adverse effects. The primary focus of this study is to address these critical issues by developing an affordable and effective cancer treatment strategy that mitigates harm to healthy cells while selectively targeting cancer cells. This will be achieved by integrating medicinal plants, nanotechnology and Traditional Chinese Medicine into cancer therapy. Specifically, the study will explore how these elements can enhance targeted drug delivery and provide a holistic, multifactorial approach to effectively combat the complex nature of cancer.

1.3. Research aim and objectives

1.3.1 Aim of the study

The current study aims to develop a magnetic nanoparticle acupuncture delivery system that can be used to direct the treatment to the tumor location, by using an external magnetic field. The safety and effectiveness of this system will be evaluated in a breast cancer mouse model.

1.3.2 Objectives of the project

Objective 1: To extract selected Euphorbia plant species and screen the extracts for anti-proliferative activity against breast cancer cells.

Objective 2: To synthesize and characterize iron, cobalt and magnesium metal nanoparticles using selected plant species through green synthesis.

Objective 3: To develop a targeted acupunctural magnetic drug delivery Nano system *in vivo* in a breast cancer mouse model, for testing the efficacy and safety of the delivery system and the nanoparticles.

CHAPTER 2

LITERATURE REVIEW

2.1. Introduction

Cancer is the second leading cause of death globally and claims around 10 million lives annually (Ritchie & Roser, 2023). It is predicted that cancer will soon surpass cardiovascular disease as the primary cause of death in high-income countries (Bray *et al.*, 2021). Cancer affects both males and females, while other types of cancer, like prostate and ovarian cancer are gender specific (WHO, 2018).

Cancer is a collection of ailments distinguished by uncontrolled and irregular cell division. It originates from a single mutated cell that multiplies into a cluster of cells known as tumor cells (Hanahan & Weinberg, 2000). These cells have the potential to migrate to other areas of the body via the lymphatic system, disrupting the regular operations of the digestive, nervous and circulatory systems, impacting the body's usual functions. (Hanahan & Weinberg, 2000; CANSA).

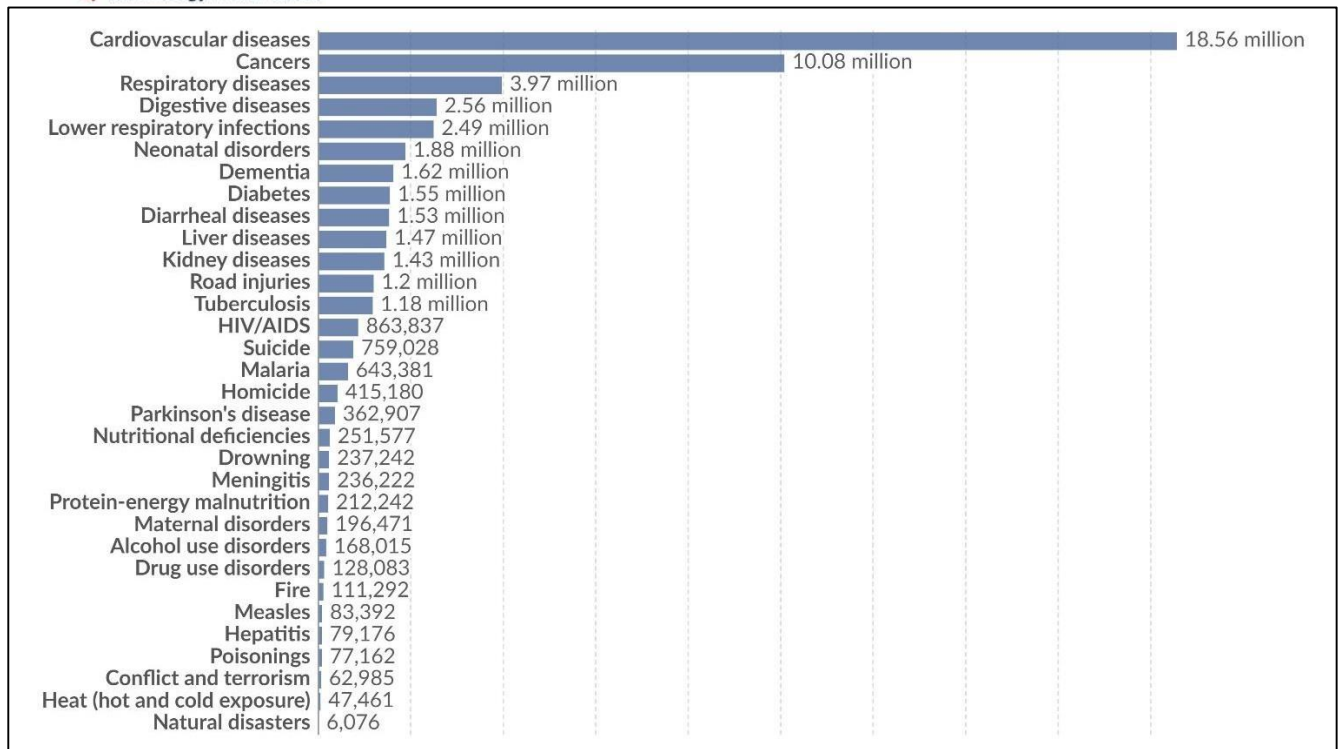


Figure 1.1 World annual number of deaths by cause reported in 2023 (Dattani *et al.*, 2023).

There exist more than 200 variations of cancer, each with their own distinct characteristics. They are classified based on their point of origin (Pardee *et al.*, 2008). The majority, approximately 90%, stem from epithelial tissues that shield and line the surfaces of organs. The remaining 10% consist of sarcomas, leukemias and lymphomas (Wang *et al.*, 2012). Among the most prevalent cancer types are lung, colon, prostate and breast cancers.

2.1.1. Prevalence of cancer

2.1.1.1. Global prevalence

Cancer continues to be a major global health challenge, affecting millions of people each year. According to the World Health Organization (WHO), there were over 18 million new cases of cancer and approximately 9.5 million cancer-related deaths worldwide in 2018

(WHO, 2019a). These alarming numbers reflect a trend of increasing incidence and mortality rates, influenced by factors such as population growth, aging and lifestyle choices.

The highest number of new cases and deaths was recorded in Asia in 2018, with an estimated 8 million and 5 million respectively, followed by Europe, North America, Latin America and the Caribbean, Africa and Oceania, see Figure 1.2 (WHO, 2019a).

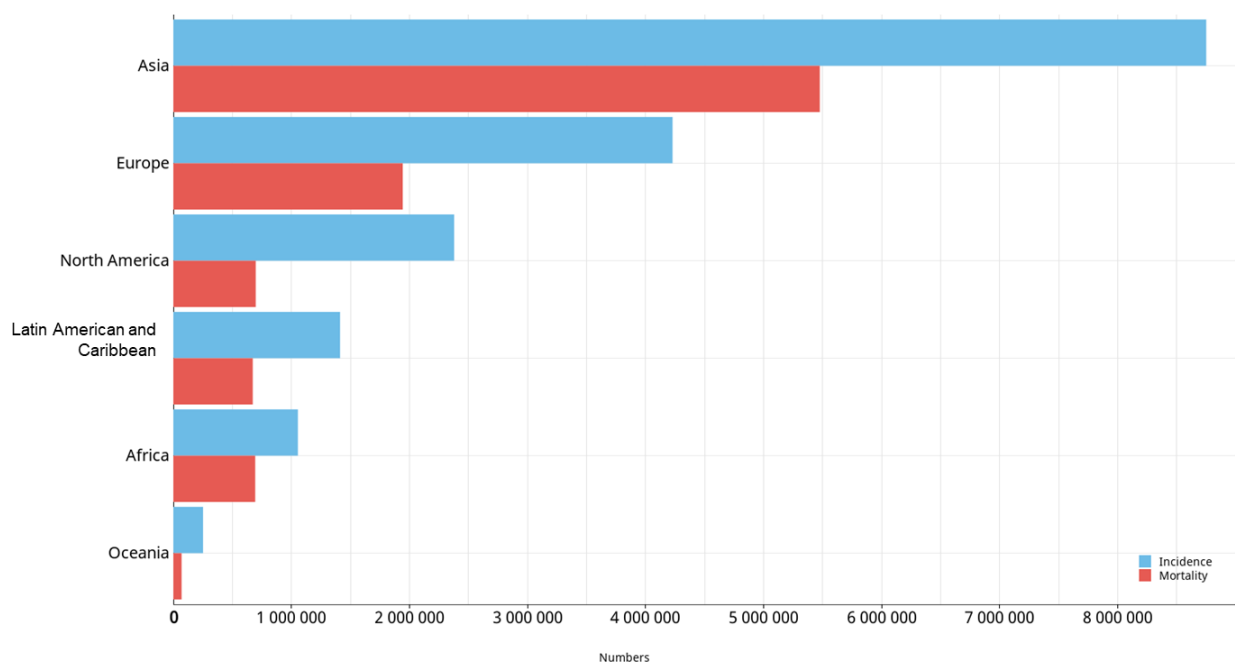


Figure 1.2. Estimated incidence and mortality of all cancers for both sexes and all ages in six continents (WHO, 2019).

2.1.1.2. Prevalence in Africa

Egypt has reported the highest number of cases and deaths from Africa, followed by Nigeria, South Africa, Ethiopia, Algeria, Morocco, Democratic Republic of Congo, Kenya, Tanzania and Uganda.

2.1.1.3. Prevalence in South Africa

In 2018, according to WHO, South Africa had approximately 107 467 new cases reported, with 47 764 cases affecting males and 59 703 cases affecting females. Of those cases, around 28 138 males (14.1% of all deaths) and 29 235 females (10.1% of all deaths) died, resulting in an estimated total of 57 373 deaths (11.7% of all deaths worldwide).

Table 1.1. Estimated incidence, mortality and prevalence percentage (%) of cancers reported in South Africa in 2022, for both sexes and all ages (GLOBOCAN, 2022).

Type of cancer	Incidence rate	%	Mortality rate	%
Breast	14 712	13.2	5 232	8.1
Prostate	12 903	12.1	5 411	8.4
Cervix uteri	10 523	9.5	5 976	9.3
Lung	9 446	8.5	8 672	13.4
Colon	7 338	6.6	4 591	7.1
Kaposi sarcoma	4 119	3.7	706	1.1
Oesophagus	3 312	3.0	3 025	4.7
Non-Hodgkin lymphoma	3 259	2.9	1 867	2.9
Pancreas	2 779	2.5	2 360	3.7
Liver	2 650	2.4	2 401	3.7
Melanoma	2 31	2.0	649	1.1
Bladder	2 148	1.9	1 002	1.6
Leukaemia	2 022	1.8	1 719	2.7
Stomach	1 919	1.7	1 622	2.5
Corpus uteri	1 839	1.7	792	1.2

The data presented in Table 1.1 highlights the leading causes of cancer-related deaths in South Africa. Lung cancer is responsible for 13.4% of all cancer deaths, while cervix uteri, prostate, breast and colon cancers follow closely behind at 9.3%, 8.4%, 8.1%, and 7.1%, respectively. When it comes to incident cases reported, breast cancer is the most widespread at 13.2%, with cervix uteri at 12.1%, lung cancer at 8.5%, colon cancer at 6.6%, and cervix uteri at 9.5%.

2.2. Breast cancer

Breast cancer is a highly invasive form of cancer that primarily affects women and is the leading cause of death among female cancer patients worldwide, according to WHO's 2024 report. In 2024, there were roughly 2.3 million new cases of breast cancer reported, resulting in roughly 670 000 deaths across the globe. In South Africa, GLOBOCAN report indicates that roughly 14 712 cases of breast cancer were reported in 2022, resulting in roughly 5 232 deaths. Inherited susceptibility, being overweight or inactive, alcohol consumption, poor dietary habits, smoking and exposure to chemicals are all contributing factors to the rise in mortality and morbidity, according to the Cancer Association of South Africa.

2.2.1. Clinical features of breast cancer

Breast cancer commonly arises in the milk ducts, glandular tissues or other breast tissues. Among women, it can manifest in different ways such as lumps, swelling and skin changes. Some women may also observe changes in the shape and size of their breasts, nipple discharge that is not breast milk or breast pain.

2.2.2. Breast cancer classification based on gene expression

Breast cancer is not a single disease but rather a collection of diseases with varying molecular and genetic characteristics. This makes a one-size-fits-all approach to treatment ineffective (Done, 2011; Siegel *et al.*, 2019).

Breast cancers are classified into four receptor subtypes: Luminal A, Luminal B, HER2-positive and triple-negative. Luminal A and Luminal B express the estrogen and progesterone receptors. Luminal B is more aggressive and grows faster than Luminal A. Examples of Luminal A breast cancer strains include MCF-7 and T47D, while examples of Luminal B breast cancer strains include BT474 and MDA-MB-361. HER2-positive expresses the human epithelial receptor and is genomically amplified for ERBB2, making it aggressive. Examples of HER2-positive breast cancer strains include SKBR3 and HCC202. Triple-negative doesn't express any of these markers and is the most aggressive of the four classes (Kalimutho *et al.*, 2015; Cardiff & Kenney, 2010). Examples of triple-negative breast cancer strains include BT20, MDA-MB-231 and MDA-MB-468.

The treatment of breast cancer depends on the receptor status of the tumor, particularly estrogen and progesterone. Different treatments are required based on the expression of these receptors and the aggressiveness of the tumor. Hormonal therapy such as tamoxifen and aromatase inhibitors have proven effective in treating Luminal A and B. HER2-positive has been treated using trastuzumab, though drug resistance with this regimen is a concern (Nixon & Verma, 2016). Triple-negative, however, remains a difficult subtype to treat due to lack of receptors.

2.3. Therapy and its limitations

Breast cancer treatment options primarily include surgery, radiotherapy, chemotherapy and hormone therapy, with surgery being most effective if the cancer is detected early (Jin & Ye,

2013; Palmieri *et al.*, 2014).

Surgery treatment involves the physical removal of tumors from the body, as well as some healthy tissue around them and often serves as a curative option for localized cancer (Mayo clinic staff, 2019). Its primary mechanism is excision, promptly eliminating the tumor mass (Chandraprasad *et al.*, 2022). Nonetheless, it is important to note that surgery, though impactful, is an invasive procedure that can potentially lead to complications such as infection, discomfort, blood clots, loss of organ function and bleeding (Mayo clinic staff, 2019). Additionally, it may not be as effective for metastatic cancer that has spread to other parts of the body (Chandraprasad *et al.*, 2022). Moreover, patients with concurrent health issues may encounter heightened surgical risks.

Furthermore, radiation treatment utilizes high-energy particles or waves, like X-rays, to specifically target and disable cancer cells by affecting their DNA, which inhibits their ability to grow and divide (Zeman *et al.*, 2020). Although it can effectively reduce tumor size and eliminate cancer cells in certain areas, it may also cause damage to nearby healthy tissue and organs, resulting in side effects such as fatigue and skin irritation (Schaue & McBride, 2015). Furthermore, it typically involves multiple treatment sessions over an extended period.

Hormone therapy on the other hand, is utilized to combat cancers that depend on hormones for their growth, such as breast and prostate cancers (Desai *et al.*, 2021). This treatment works by either halting the body's hormone production or disrupting hormone activity, effectively impeding the growth of cancer cells (Capper *et al.*, 2016). While it is successful for hormone-sensitive cancers, hormone therapy is ineffective against non-hormone-sensitive

cancers and may lead to side effects such as hot flushes, fatigue and bone loss (Fisher *et al.*, 2013). It may not result in a complete cure and is frequently used alongside other treatments. Similarly, chemotherapy, which operates by interfering with DNA synthesis and cell division to induce the death of unregulated growing cells (Senapati *et al.*, 2018), has its own set of challenges. Despite significant achievements through conventional treatments, chemotherapy's limitations are notable. The limitations include: a large dose that leads to high toxicity to normal cells, limited bio-accessibility of drugs to tumor tissue, rapid biodegradation of drugs, lack of selectivity of cancer medication and untargeted delivery; these are major causes of serious side effects in cancer patients (Conte, 2013; Yuan *et al.*, 2016; CANSA, 2016; Senapati *et al.*, 2018). To avoid these side effects, physicians lowered the chemotherapeutic dose, which resulted in unsuccessful treatment and the development of drug resistance and metastasis. Hence, eradication of cancer is still a major problem due to its heterogeneous nature and the inability of chemotherapeutics to reach the tumor site without damaging the normal healthy tissue. These limitations interfere with cancer treatment and the development of a new regimen. Hence, alternative forms of therapy, such as the use of herbs/ medicinal plants is considered for the treatment of cancer.

2.4. Medicinal plants

Plants are considered as a valuable source of bioactive compounds. Some medicinal plants have shown to possess the ability to enhance the activity or reduce toxicity in cancer treatment (Jain *et al.*, 2016). Medicinal plants are being utilised by a majority of the population as they are affordable, can be easily accessed, have little or no side effects and most of them are effective towards cancer therapy. Therefore, researchers all over the world are paying a lot of attention to them.

Species of the genus *Euphorbia* from the *Euphorbiaceae* family are mostly found in Africa and America. They are in the form of herbs, shrubs, trees; sometimes succulent and cactus- like (Adedapo *et al.*, 2004). Most plant species from the *Euphorbiaceae* family are widely used as traditional medicinal herbs in different parts of the world (Hartwell, 1969). Owing to a wide variety of *Euphorbias* chemical composition, the genus has caught the interest of many researchers for its remarkable therapeutic properties such as antimicrobial, antidiabetic, antiviral and anticancer properties (Betancur-Galvis *et al.*, 2002; Mwine & Damme, 2011). However, the white sticky latex sap found in these plants is highly toxic and can cause skin irritation, but can also be used for medicinal purposes (Villanueva *et al.*, 2015). Some of the plants from this family comprise of various constituents including: steroids, phenolic, cerebrosides, glycerols flavonoids, proteins such as proteases, chitinases, oxidases and lectins for defense against their preys, and terpenes including diterpenes and triterpenes for the treatment of certain cancers (Vogg *et al.*, 1999; Shi *et al.*, 2008; Villanueva *et al.*, 2015).

2.4.1. *Euphorbia* species used in this study

In the current study, the focus is on 15 *Euphorbia* species. These were selected based on their historic use for the treatment of various cancers and other ailments. *Euphorbia trigona* (*E. trigona*), *Euphorbia tirucalli* (*E. tirucalli*), *Euphorbia ammak* (*E. ammak*), *Euphorbia bupleurifolia* (*E. bupleurifolia*), *Euphorbia enopla* (*E. enopla*), *Euphorbia polygona* (*E. polygona*), *Euphorbia cooperi* (*E. cooperi*), *Euphorbia stellata* (*E. stellata*), *Euphorbia ferox* (*E. ferox*), *Euphorbia clavioides* (*E. clavioides*), *Euphorbia gorgonis* (*E. gorgonis*), *Euphorbia coerulescens* (*E. coerulescens*), *Euphorbia horrida* (*E. horrida*), *Euphorbia arabica* (*E. arabica*) and *Euphorbia ledienii* (*E. ledienii*) - some have been traditionally used

in various therapeutic applications. These plants and their potential medicinal properties will be discussed in detail in the next chapter, providing a comprehensive understanding of their role in modern medicine.

2.5. Nanotechnology and its application

Nanotechnology is an exciting and rapidly growing scientific field that enables us to measure, manipulate and create objects that exist at a nanoscale, typically between one and 100 nanometres (nm) (Bayda *et al.*, 2019). Despite their minuscule size, these tiny objects have an enormous surface area volume, making them highly attractive to researchers around the globe. Nanotechnology has broad applications across a range of scientific fields, including engineering, material science, mechanics, optics, electronics, biotechnology, microbiology, environmental remediation and medicine.

Due to this technology, there has been an increasing interest in the biotechnology and medical field to implement the use of nanomaterials for diagnostic purpose in the laboratory by injecting, inhaling and digesting the nanoparticles as a form of treatment (Kulkarni & Kulkarni, 2015). It is important to note that nanoparticles can be designed in different shapes for instance a sphere, wire, rod, tubes, core-shell; this is to assist some functional molecules to attach to the targeted molecule as desired, with the aim of archiving successful drug delivery or molecular recognition. Nanoparticles have unique properties such as being good heat or light absorbers, as well as high magnetic activity. In the medical field, nanotechnology has been applied in medical imaging (Mantle, 2013), cancer, tumor detection and destruction in the early stages (Wang *et al.*, 2008), surgery (Loizidou & Seifalian, 2010), wound healing (Mihai *et al.*, 2019), cardiology (Giannouli *et al.*, 2018), Alzheimer and Parkinson treatment (Kulkarni *et al.*, 2010; Niu *et al.*, 2017), diabetes treatment (Sharifi *et al.*, 2014), dentistry (Kanaparthi & Kanaparthi, 2011), vision (Liu *et al.*, 2012) and hearing prosthesis (Tamura *et al.*, 2005), drug delivery (Kuppusamy *et al.*, 2016) and so forth.

2.5.1. Application of nanoparticles

2.5.1.1. Imaging

Early detection is a vital factor for effective treatment when it comes to battling cancer. However, the inherent limitations of traditional cancer diagnostic techniques have hampered the identification of cancer in the early stage. Therefore, nanotechnology offers promising advancement in cancer diagnosis through its high sensitivity, specificity and multiplexed measurement capabilities. Traditional diagnostic techniques often struggle with early cancer detection due to inherent limitations. Nanotechnology addresses these challenges by enabling the identification of extracellular cancer biomarkers and cancer cells, as well as facilitating *in vivo* imaging. For example, immune superparamagnetic iron oxide nanoparticles (SPIONs) have been utilized in MRI imaging to detect lung cancer metastasis (Wan *et al.*, 2016). Nanoparticles also enhance the capture of cancer biomarkers like proteins, circulating tumor DNA, cells and exosomes, improving assay sensitivity and specificity (Kumar *et al.*, 2017). Additionally, positron emission tomography (PET) imaging, integrated with nanotechnology, provides ultra-high resolution and sensitivity, enabling the detection of low-glucose-uptake tumors that conventional methods may miss (Garrigue *et al.*, 2018). This advancement highlights nanotechnology's potential in revolutionizing early cancer detection and diagnosis.

2.5.1.2. Hyperthermia

Hyperthermia, a conventional tumor treatment method, involves heating tumor cells to temperatures between 40-45 °C to induce thermally mediated metabolic changes like

apoptosis (Jose *et al.*, 2020). This therapy not only addresses tissue hypoxia but also enhances the efficacy of radiation or anti-cancer drugs when used in combination (Colombo *et al.*, 2003; Huilgol *et al.*, 2010). However, its effectiveness is hindered by challenges in efficiently delivering heat to targeted tumors, resulting in non-selective tissue heating and significant side effects. Nanotechnology offers a promising solution by utilizing magnetic nanoparticles for hyperthermia, which absorb energy from external sources and intensify therapeutic effects (Beik *et al.*, 2016). This approach selectively heats tumors by applying an oscillating magnetic field, overcoming the limitations of conventional methods and enhancing the precision of cancer therapy (Sohail *et al.*, 2017; Bañobre-López *et al.*, 2013).

2.5.2. Nanotechnology in drug delivery of cancer therapy

In recent years, nanotechnology has positively contributed towards diagnosis and cancer therapy, focusing mostly on a targeted drug delivery system (Abedin, 2008; Bagherifam, 2013). In a targeted drug delivery system, therapeutic drugs are incorporated into compatible nanoparticles, which are then functionalized with specific targeting agents, such as antibodies for specific antigens, or are designed to respond to external stimuli like magnetic fields or pH changes. This approach ensures targeted delivery to the desired cells, sustains the drug's effect and protects the drug from enzymatic degradation (Seidu *et al.*, 2022; Panyam *et al.*, 2012). The size and shape of the nanoparticle is crucial in targeted drug delivery and should be taken into consideration and optimized to prevent any errors during drug release and pharmacokinetics *in vivo* (Petros & DeSimone, 2010; Ding *et al.*, 2017). These nanoparticles can be synthesized from different materials such as lipids, polymers, inorganic materials and metals (Rivera, 2003; Mornet *et al.*, 2004; Kumari *et al.*, 2010).

From the different nanoparticles used for therapeutic deliver the magnetic nanoparticle has shown great potential in cancer therapy application, as carriers in targeted drug delivery

systems (Santhosh *et al.*, 2013). The magnetic nanocarriers can simply be guided to the cancerous site by using an external magnetic field as shown on Figure 1.3.

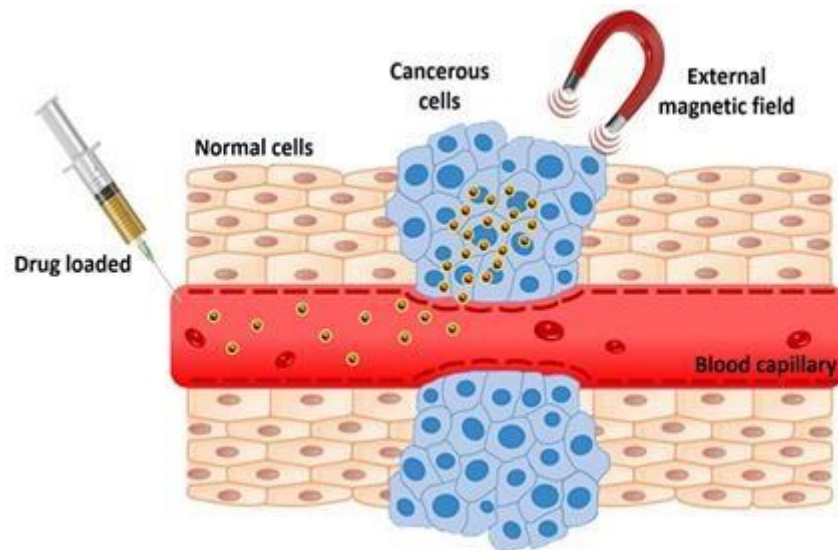


Figure 1.3: Magnetic drug Targeted drug delivery system (Yew *et al.*, 2018).

2.5.2.1. Magnetic delivery system

The magnetic nanoparticles are classified into three different metals: pure metal, metal oxide and magnetic nanocomposite (Guo *et al.*, 2018). The most common magnetic nanoparticles are gold (Au), silver (Ag), iron(Fe), cobalt (Co), nickel (Ni), titanium (Ti), iron oxide and some ferrites which possess a high magnetic field (Chithrani *et al.*, 2006; Jeyaraj *et al.*, 2013). The Fe_2O_3 and Fe_3O_4 magnetic nanoparticles are frequently used during diagnosis and treatment of all kinds of tumors due to their low toxicity (Pankhurst *et al.*, 2003; Li *et al.*, 2016). A lot of researchers have shown interest in elemental metals such as Fe, Ni and Co due to their distinctive characteristics such as high saturation induction, high permeability, low core loss, ability to concentrate and high-density magnetic storage media (Fedlheim & Foss, 2001).

However, these metals are very susceptible to oxidation, making the nanoparticles unstable. Therefore, considerable precaution has to be taken when working with these metals. Lu *et al.* (2008) were able to come up with a solution to resolve the water oxidation problem by producing a water-soluble magnetic Co nanoparticle. Building on this advancement, the current study focuses on Co, Fe and Mg nanoparticles, with particular emphasis on the properties and applications of Co and Fe nanoparticles; understanding their significance in targeted drug delivery systems.

2.5.2.1.1 Cobalt nanoparticles

Among these elemental metals, nanoparticles made from cobalt possess unique characteristics, such as high surface area, high saturation magnetization, large anisotropy field and unique catalytic properties (Dumestre *et al.*, 2002). These properties make it eligible to store a large amount of information. Cobalt nanoparticles are commonly used in cancer therapy through a process called hyperthermia (use of heat to kill cancer cells) (Pankhurst *et al.*, 2009; Thanh & Green, 2010; Alexiou *et al.*, 2010). Studies by Ansari *et al.* (2017) also confirmed that Co nanoparticles revealed a mild anti-proliferative character against cancer cells and have also shown that they are not harmful to normal cells.

2.5.2.1.2 Fe nanoparticles

The Fe nanoparticle, on the other hand, possesses high magnetic properties, high surface area, high electrical and thermal conductivity and can maintain superparamagnetism (Fahmy *et al.*, 2018). The superparamagnetic property is one of the unique properties the Fe nanoparticle possesses and is crucial for its application in targeted drug delivery and magnetic resonance imaging (Pankhurst *et al.*, 2003). The Fe nanoparticles have received a lot of attention due to their biocompatibility and low toxicity (Hasan, 2015). Fe nanoparticles are also used in different applications such as spintronics, biology and medical science

(Fahmy *et al.*, 2018). It has also been found to be useful for labeling and magnetic separation of biological materials, MRI contrast enhancement, directed drug delivery and hyperthermia treatment (Pankhurst *et al.*, 2003).

Magnesium is one of the most abundant intracellular cation found in living cells and it takes part in most cellular activities. The blood plasma contains about 1% of the total amount of magnesium found in the body (Liao *et al.*, 1998). Magnesium is well known for its role as a storehouse for hydrogen through its nanocomposite structure (Jeon *et al.*, 2011). Studies by Heublein *et al.* (2003) also showed that magnesium is a promising metal in a cardiovascular implant due to its biocompatibility and biodegradable property (Heublein *et al.*, 2003). Wang and her group introduced magnesium nanoparticles in thermal cancer therapy, demonstrating that targeted tissues injected with magnesium nanoparticles reached higher temperatures than tissues without magnesium nanoparticles, leading to the effective destruction of cancer cells through hyperthermia (Wang *et al.*, 2012).

2.6. Single-Pot Eco-Friendly Synthesis

The use of green chemistry for synthesis of nanoparticles has shown to be advantageous over chemical and physical methods. The chemical and physical methods can result in bioaccumulation of toxic materials and can add hazardous materials to the environment. Therefore, it is not advisable to utilize them in health fields due to their toxicity to living cells. The green approach is less expensive, produces nanoparticles at a large scale, no addition of any chemical agent for reducing and capping, no use of high pressure, temperature and energy, eco-friendly and free of chemical contamination (Chandran *et al.*, 2006; Huang *et al.*, 2007; Iravani, 2011; Mittal *et al.*, 2013). It has previously been shown that bacteria, algae, fungi, as well as plants extracts can be used as an alternative to the

physical and chemical method to reduce precursor metal salts to metal nanoparticles. However, the use of dried plants and plant extracts has received a lot of attention compared to other biological systems. Plants are known to contain secondary metabolites such as phenolics, alkaloids, steroids, flavonoids, tannins, amino acids, proteins and saponins which are known to be responsible for the reduction and stabilization of metal precursors to metal nanoparticles. Figure 1.4 below shows a quick process of green synthesis.

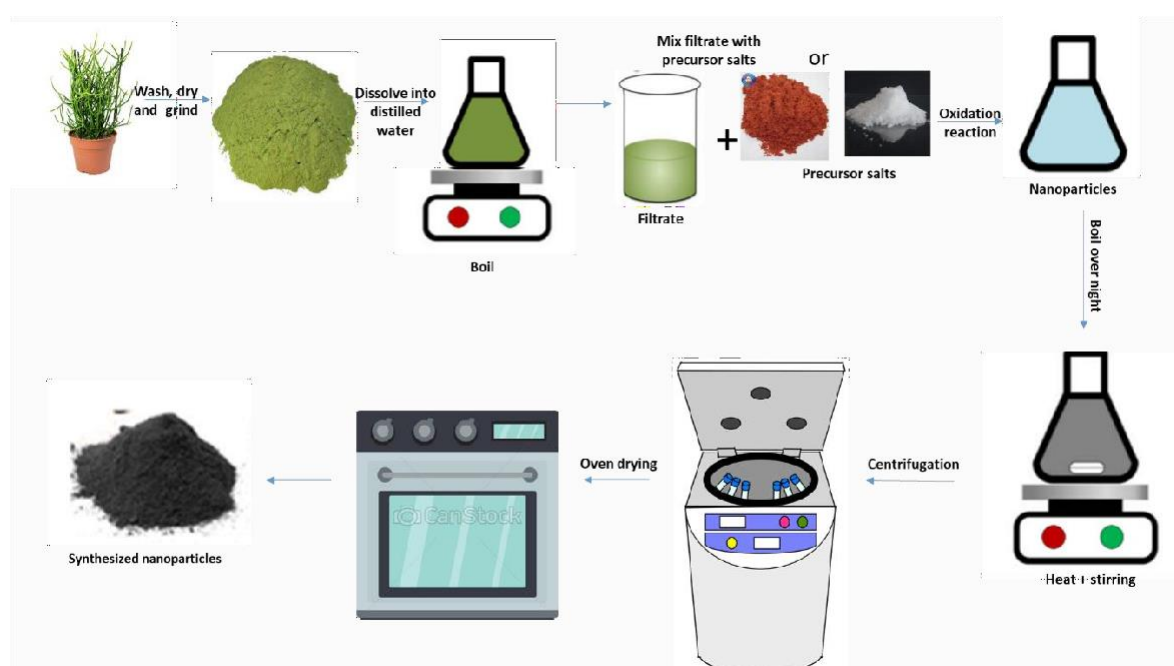


Figure 1.4: Schematic diagram illustrating the one-pot green synthesis process.

2.7. *In vivo* studies

Extensive research has been done on *in vitro* screening of cancerous cells and has been successful (El-Abd *et al.*, 2014). However, most drugs fail to make it to the *in vivo* phase due to a lack of appropriate facilities and the technology required for such experiments. Animals such as rat, mice, hamsters, rabbits, zebrafish, cats, fish, dogs and livestock are used in *in vivo* studies (Mulas & Reymundo, 2000; Yee *et al.*, 2015; eMICE a and b). In cancer research

mice and rats are the most commonly used animal models. The reason being that they mimic the human immune system better than other known models. The application of animal models in cancer research plays a crucial role in mechanical studies and pre-clinical research. There have been different traditional preclinical animal models that have been currently explored for cancer diagnosis and treatment.

Cancer animal models are divided into 4 categories: (a) animals in which cancers occur spontaneously without any alteration of the animal's genes or initiation of cancer by prior treatments; (b) animals whose genes are altered so that they develop spontaneous tumors of the same types and with similar properties to that of humans; (c) animals that develop spontaneous tumors if they are exposed to other factors, such as chemicals or radiation; and (d) animals whose natural, unaltered genetic makeup permits researchers to identify the genes that generate susceptibility to cancer development (eMICE b). The importance of these models differs according to the species, available research reagents and tools to support the studies, similarities to human tumors and the compatibility of the cells used with the selected animal strain (eMICE b).

Animal models, such as genetically engineered tumor models, primary human tumor grafts, ectopic xenografts and orthotopic xenografts are mostly used for *in vivo* studies. The xenograft and genetically engineered tumor models have been used for anticancer drug discovery and testing and have also been utilised for investigating tumor initiation, growth and metastasis (Yee *et al.*, 2015). In the xenograft cancer model, cancerous cells from humans or animals are grafted ectopically, meaning beneath the skin, or orthotopically, which is directly in the organ of the tumor origin e.g. mammary fat pad (El-Abd *et al.*, 2014). Animal models used in breast cancer research, e.g. cell-derived xenograft and patient derived xenograft mice have limitations when it comes to engraftment of primary tumors (Johnstone

et al., 2015). However, the syngeneic BALB/c albino mouse model has shown potential to initiate a primary tumor when engrafted with 4T1 mouse tumor cells, which closely mimics the human breast cancer cell (Park *et al.*, 2018).

BALB/c nude mice are considered an established model among syngeneic and are used in cancer therapy (Johnstone *et al.*, 2015). This strain is more reliable compared to C57BL/6, C3H and DBA/2 models, as it has resulted in successful engraftment of mouse tumor cells (Silva *et al.*, 2015). BALB/c nude model is not only suitable for engraftment but for drug delivery as well (Malik *et al.*, 2017). The utilization of BALB/c nude mice for implanting 4T1 breast cancer cells presents numerous benefits. These mice lack a functioning thymus, resulting in a weakened T-cell mediated immune response, which facilitates the successful acceptance and growth of human and murine tumors (Panaampon *et al.*, 2021; Rossa *et al.*, 2019). The deficiency in their immune system leads to a high rate of tumor acceptance when introducing 4T1 breast cancer cells, making it a dependable model for investigating tumor growth and spread (Ottewell *et al.*, 2021). Moreover, 4T1 cells are recognized for their highly metastatic nature. When implanted in BALB/c nude mice, they replicate the aggressive characteristics of human breast cancer, making them an excellent model for studying metastasis and evaluating anti-metastatic therapies (Piranlioglu *et al.*, 2019). The use of 4T1 cells in BALB/c nude mice has been firmly established in the field of cancer research. It comes with a wide range of existing literature and standardized protocols, which make it easier to conduct comparative studies and improve reproducibility (Rajaratnam *et al.*, 2022). Moreover, these mice facilitate straightforward monitoring of tumor progression and response to treatments due to their lack of fur, which makes the tumors more visible and palpable. The absence of fur and pigmentation in nude mice also enhances the precision and effectiveness of various imaging techniques, such as bioluminescence or fluorescence imaging, commonly utilized to track tumor growth and metastasis.

2.8. References

- Abdel-Sattar, E., Abou-Hussein, D., & Petereit, F. (2015). Chemical constituents from the leaves of *Euphorbia ammak* growing in Saudi Arabia. *Pharmacognosy Research*, 7(1), 14.
- Abdel-Sattar, E., Maes, L., & Salama, M. M. (2010). In vitro activities of plant extracts from Saudi Arabia against malaria, leishmaniasis, sleeping sickness and Chagas disease. *Phytotherapy Research*, 24(9), 1322–1328.
- Abedin, F. (2011). Magnetic and albumin targeted drug delivery for breast cancer treatment (Doctoral dissertation, Wichita State University, College of Engineering, Department of Mechanical Engineering).
- Adedapo, A. A., Abatan, M. O., & Olorunsogo, O. O. (2004). Toxic effects of some plants in the genus *Euphorbia* on haematological and biochemical parameters of rats. *Veterinarski Arhiv*, 74(1), 53–62.
- Afolayan, A. J., Grierson, D. S., & Mbeng, W. O. (2014). Ethnobotanical survey of medicinal plants used in the management of skin disorders among the Xhosa communities of the Amathole District, Eastern Cape, South Africa. *Journal of Ethnopharmacology*, 153(1), 220–232.
- Alexiou, C., Tietze, R., Schreiber, E., & Lyer, S. (2010). Nanomedicine: Magnetic nanoparticles for drug delivery and hyperthermia–new chances for cancer therapy. *Bundesgesundheitsblatt-Gesundheitsforschung-Gesundheitsschutz*, 53, 839-845.
- Al-Hajj, M. M. A., Al-Shamahy, H. A., Alkhatib, B. Y., & Moharram, B. A. (2018). In vitro anti-leishmanial activity against cutaneous leishmania parasites and preliminary phytochemical analysis of four Yemeni medicinal plants. *Universal Journal of Pharmaceutical Research*, 3, 48-54.

Al-Harbi, N. A. (2017). Diversity of medicinal plants used in the treatment of skin diseases in Tabuk region, Saudi Arabia. *Journal of Medicinal Plants Research*, 11(35), 549-555.

Al-Shanwani, M. (1996). *Plants used in Saudi folk medicine*. Riyadh: King Abdul Aziz City for Science and Technology.

Ansari, I. A., & Akhtar, M. S. (2019). Current insights on the role of terpenoids as anticancer agents: A perspective on cancer prevention and treatment. In *Natural Bio-active Compounds: Volume 2: Chemistry, Pharmacology and Health Care Practices* (53-80).

Ansari, S. M., Bhor, R. D., Pai, K. R., Sen, D., Mazumder, S., Ghosh, K., Kolekar, Y. D., & Ramana, C. V. (2017). Cobalt nanoparticles for biomedical applications: Facile synthesis, physiochemical characterization, cytotoxicity behavior and biocompatibility. *Applied Surface Science*, 414, 171–187.

Assefa, A., & Bahiru, A. (2018). Ethnoveterinary botanical survey of medicinal plants in Abergelle, Sekota and Lalibela districts of Amhara region, Northern Ethiopia. *Journal of Ethnopharmacology*, 213, 340-349.

Bagherifam, S. (2013). pH responsive nano carriers for anti-cancer drug delivery (Doctoral dissertation, Middle East Technical University).

Bañobre-López, M., Teijeiro, A., & Rivas, J. (2013). Magnetic nanoparticle-based hyperthermia for cancer treatment. *Reports of Practical Oncology & Radiotherapy*, 18(6), 397-400.

Beik, J., Abed, Z., Ghoreishi, F. S., Hosseini-Nami, S., Mehrzadi, S., Shakeri-Zadeh, A., & Kamrava, S. K. (2016). Nanotechnology in hyperthermia cancer therapy: From fundamental principles to advanced applications. *Journal of Controlled Release*, 235, 205-221.

Betancur-Galvis, L. A., Morales, G. E., Forero, J. E., & Roldan, J. (2002). Cytotoxic and antiviral activities of Colombian medicinal plant extracts of the Euphorbia genus. *Memórias do Instituto Oswaldo Cruz*, 97(4), 541-546.

Bray, F., Laversanne, M., Cao, B., Varghese, C., Mikkelsen, B., Weiderpass, E., & Soerjomataram, I. (2021). Comparing cancer and cardiovascular disease trends in 20 middle- or high-income countries 2000–19: A pointer to national trajectories towards achieving sustainable development goal target 3.4. *Cancer Treatment Reviews*, 100, 102290.

Bouquet, A. J. (1969). Natural products as alternative remedy. In *Royal Botanic Gardens Kew* (166-179).

Campbell, J. P., Merkel, A. R., Masood-Campbell, S. K., Elefteriou, F., & Sterling, J. A. (2012). Models of bone metastasis. *Journal of Visualized Experiments*, (67).

CANSA. (2016). Chemotherapy fact sheet [Internet]. [Cited 2018 May 27]. Available from: <http://www.cansa.org.za/files/2016/07/Fact-Sheet-Chemotherapy-July-2016.pdf>

CANSA. (2018). About cancer [Internet]. [Cited 2018 May 27]. Available from: <http://www.cansa.org.za/about-cancer/>

CANSA. (2019). Woman's health [Internet]. [Cited 2019 December 09]. Available from: <https://www.cansa.org.za/womens-health/>

Capper, C. P., Rae, J. M., & Auchus, R. J. (2016). The metabolism, analysis, and targeting of steroid hormones in breast and prostate cancer. *Hormones and Cancer*, 7, 149-164.

10), 3277-3286.

Carter, S. (1994). A preliminary classification of Euphorbia subgenus Euphorbia. *Annals of the Missouri Botanical Garden*, 368-379.

Chandraprasad, M. S., Dey, A., & Swamy, M. K. (2022). Introduction to cancer and treatment approaches. In *Paclitaxel* (1-27). Academic Press.

Chithrani, B. D., Ghazani, A. A., & Chan, W. C. (2006). Determining the size and shape dependence of gold nanoparticle uptake into mammalian cells. *Nano Letters*, 6(4), 662-668.

Cock, I. E., Ndlovu, N., & Van Vuuren, S. F. (2020). The use of South African botanical species for the control of blood sugar. *Journal of Ethnopharmacology*, 113234.

Colombo, R., Da Pozzo, L. F., Salonia, A., Rigatti, P., Leib, Z., Baniel, J., Caldarera, E., & Pavone-Macaluso, M. (2003). Multicentric study comparing intravesical chemotherapy alone and with local microwave hyperthermia for prophylaxis of recurrence of superficial transitional cell carcinoma. *Journal of Clinical Oncology*, 21(23), 4270-4276.

Conte, C. (2013). Core-shell sustained release nanosystems for the intravenous delivery of anticancer drugs (Ph.D. thesis, University of Naples Federico II, Italy). core.ac.uk.

Curtis, C., Shah, S. P., Chin, S. F., Turashvili, G., Rueda, O. M., Dunning, M. J., Speed, D., Lynch, A. G., Samarajiwa, S., Yuan, Y., & Gräf, S. (2012). The genomic and transcriptomic architecture of 2,000 breast tumors reveals novel subgroups. *Nature*, 486(7403), 346.

Dagenais, G. R., Leong, D. P., Rangarajan, S., Lanas, F., Lopez-Jaramillo, P., Gupta, R., Diaz, R., Avezum, A., Oliveira, G. B., Wielgosz, A., & Parambath, S. R. (2019). Variations in common diseases, hospital admissions, and deaths in middle-aged adults in 21 countries from five continents (PURE): A prospective cohort study. *The Lancet*.

Mulas, J., & Reymundo, C. (2000). Animal models of human breast carcinoma: Canine and

feline neoplasms. *Clinical & Translational Oncology*, 2(6).

Desai, K., McManus, J. M., & Sharifi, N. (2021). Hormonal therapy for prostate cancer. *Endocrine Reviews*, 42(3), 354-373.

de Souza, L. S., Puziol, L. C., Tosta, C. L., Bittencourt, M. L., Santa Ardisson, J., Kitagawa, R. R., Filgueiras, P. R., & Kuster, R. M. (2019). Analytical methods to access the chemical composition of an *Euphorbia tirucalli* anticancer latex from traditional Brazilian medicine. *Journal of Ethnopharmacology*, 237, 255-265.

Ding, Q., Liu, D., Guo, D., Yang, F., Pang, X., Che, R., Zhou, N., Xie, J., Sun, J., Huang, Z., & Gu, N. (2017). Shape-controlled fabrication of magnetite silver hybrid nanoparticles with high performance magnetic hyperthermia. *Biomaterials*, 124, 35-46.

Done, S. (Ed.). (2011). *Breast cancer: Recent advances in biology, imaging and therapeutics* (190-196). Toronto, Canada.

Dumestre, F., Chaudret, B., Amiens, C., Fromen, M. C., Casanove, M. J., Renaud, P., & Zurcher, P. (2002). Shape control of thermodynamically stable cobalt nanorods through organometallic chemistry. *Angewandte Chemie International Edition*, 41(22), 4286-4289.

El-Abd, E. A., Sultan, A. S., Shalaby, E. A., & Matalkah, F. (2014). Animal models of breast cancer. In *Omics Approaches in Breast Cancer: Towards Next-Generation Diagnosis, Prognosis and Therapy* (pp. 297-314).

El-Sherei, M. M., Islam, W. T., El-Dine, R. S., El-Toumy, S. A., & Ahmed, S. R. (2015). Phytochemical investigation of the cytotoxic latex of *Euphorbia cooperi* NE Br. *Australian Journal of Basic & Applied Sciences*, 9(11), 488-493.

eMICE. (2018a). Other laboratory animal cancer models [Internet]. Available from: <https://emice.nci.nih.gov/aam/other-laboratory-animal-cancer-models>

eMICE. (2018b). What are animal models of cancer [Internet]. Available from: <https://emice.nci.nih.gov/aam>

Fahmy, H. M., Mohamed, F. M., Marzouq, M. H., Mustafa, A. B. E. D., Alsoudi, A. M., Ali, O. A., Mohamed, M. A., & Mahmoud, F. A. (2018). Review of green methods of iron nanoparticles synthesis and applications. *BioNanoScience*, 8(2), 491-503.

Fedlheim, D. L., & Foss, C. A. (2001). *Metal nanoparticles: Synthesis, characterization, and applications*. CRC Press.

Fisher, W. I., Johnson, A. K., Elkins, G. R., Otte, J. L., Burns, D. S., Yu, M., & Carpenter, J. S. (2013). Risk factors, pathophysiology, and treatment of hot flashes in cancer. *CA: A Cancer Journal for Clinicians*, 63(3), 167-192.

Garrigue, P., Tang, J., Ding, L., Bouhrel, A., Tintaru, A., Laurini, E., Huang, Y., Lyu, Z., Zhang, M., Fernandez, S., & Balasse, L. (2018). Self-assembling supramolecular dendrimer nanosystem for PET imaging of tumors. *Proceedings of the National Academy of Sciences*, 115(45), 11454-11459.

Gelband, H., & Sloan, F. A. (Eds.). (2007). *Cancer control opportunities in low-and middle-income countries*. National Academies Press.

Giannouli, M., Karagkiozaki, V., Pappa, F., Moutsios, I., Gravalidis, C., & Logothetidis, S. (2018). Fabrication of quercetin-loaded PLGA nanoparticles via electrohydrodynamic atomization for cardiovascular disease. *Materials Today: Proceedings*, 5(8), 15998-16005.

Globocan. (2022). Statistics at a glance, 2022 Top 5 most frequent cancers Number of new cases 111 321 Number of deaths 64 547 Number of prevalent cases (5-year) 282 418. Available at: <https://gco.iarc.who.int/media/globocan/factsheets/populations/710-south-africa-fact-sheet.pdf>

Guillarmod, A. J. (1971). *Flora of Lesotho (Basutoland)*.

Gundidza, E. (1992). A skin irritant phorbol ester from *Euphorbia cooperi* NE Br. *Central African Journal of Medicine*, 38(12), 444-447.

Gupta, N., Vishnoi, G., Wal, A., & Wal, P. (2013). Medicinal value of *Euphorbia tirucalli*. *Systematic Reviews in Pharmacy*, 4(1), 40.

Hanahan, D., & Weinberg, R. A. (2000). The hallmarks of cancer. *Cell*, 100(1), 57-70.

Hargreaves, B. J. (1991). The spurges of Botswana. *Botswana Notes and Records*, 23, 115-158.

Harrison, R. J., Dunin-Borkowski, R. E., & Putnis, A. (2002). Direct imaging of nanoscale magnetic interactions in minerals. *Proceedings of the National Academy of Sciences*, 99(26), 16556–16561.

Hartwell, J. L. (1969). Plants used against cancer. A survey. *Lloydia*, 32, 79-107.

Hasan, S. (2015). A review on nanoparticles: Their synthesis and types. *Research Journal of Recent Sciences*, 2277, 2502.

Hedberg, I., & Staugard, F. (1989). *Traditional medicinal plants: Traditional medicine in Botswana*. Ipeleng, Gaborone.

Heublein, B., Rohde, R., Kaese, V., Niemeyer, M., Hartung, W., & Haverich, A. (2003).

Biocorrosion of magnesium alloys: A new principle in cardiovascular implant technology? *Heart*, 89(6), 651-656.

Hirenallur-Shanthappa, D. K., Ramírez, J. A., & Iritani, B. M. (2017). Immunodeficient mice: The backbone of patient-derived tumor xenograft models. In *Patient Derived Tumor Xenograft Models* (57-73). Academic Press.

Huang, M., Lu, J. J., Huang, M. Q., Bao, J. L., Chen, X. P., & Wang, Y. T. (2012). Terpenoids: Natural products for cancer therapy. *Expert Opinion on Investigational Drugs*, 21(12), 1801-1818.

Huilgol, N. G., Gupta, S., & Sridhar, C. R. (2010). Hyperthermia with radiation in the treatment of locally advanced head and neck cancer: A report of randomized trial. *Journal of Cancer Research and Therapeutics*, 6(4), 492.

Hyde, M. A., Wursten, B. T., Ballings, P., & Palgrave, C. M. (2015). Flora of Zimbabwe: Species information. *Grewia flavescens*.

Iorns, E., Drews-Elger, K., Ward, T. M., Dean, S., Clarke, J., Berry, D., El Ashry, D., & Lippman, M. (2012). A new mouse model for the study of human breast cancer metastasis. *PloS One*, 7(10), e47995.

Ishikawa, F., Yasukawa, M., Lyons, B., Yoshida, S., Miyamoto, T., Yoshimoto, G., Watanabe, T., Akashi, K., Shultz, L. D., & Harada, M. (2005). Development of functional human blood and immune systems in NOD/SCID/IL2 receptor γ chainnull mice. *Blood*, 106(5), 1565-1573.

Iwalewa, E. O., McGaw, L. J., Naidoo, V., & Eloff, J. N. (2007). Inflammation: The foundation of diseases and disorders. A review of phytomedicines of South African origin used to treat pain and inflammatory conditions. *African Journal of Biotechnology*, 6(25).

Jain, S., Dwivedi, J., Jain, P. K., Satpathy, S., & Patra, A. (2016). Medicinal plants for treatment of cancer: A brief review. *Pharmacognosy Journal*, 8(2).

Jaradat, N., Al-Lahham, S., Al-masri, M., Bsharat, A., Alsaleh, R., & Sabobeh, R. (2019). Antioxidant, anti-enzymatic, antimicrobial and cytotoxic properties of *Euphorbia tirucalli* L. *bioRxiv*, 2019-12.

Jeon, K. J., Moon, H. R., Ruminski, A. M., Jiang, B., Kisielowski, C., Bardhan, R., & Urban, J. J. (2011). Air-stable magnesium nanocomposites provide rapid and high-capacity hydrogen storage without using heavy-metal catalysts. *Nature Materials*, 10(4), 286.

Jeyaraj, M., Sathishkumar, G., Sivanandhan, G., MubarakAli, D., Rajesh, M., Arun, R., Kapildev, G., Manickavasagam, M., Thajuddin, N., Premkumar, K., & Ganapathi, A. (2013). Biogenic silver nanoparticles for cancer treatment: An experimental report. *Colloids and Surfaces B: Biointerfaces*, 106, 86-92.

Jin, S., & Ye, K. (2013). Targeted drug delivery for breast cancer treatment. *Recent Patents on Anti-Cancer Drug Discovery*, 8(2), 143-153.

Johnson, T. (2019). *CRC Ethnobotany Desk Reference*. CRC Press.

Jose, J., Kumar, R., Harilal, S., Mathew, G. E., Prabhu, A., Uddin, M. S., Aleya, L., Kim, H., & Mathew, B. (2020). Magnetic nanoparticles for hyperthermia in cancer treatment: An emerging tool. *Environmental Science and Pollution Research*, 27(16), 19214-19225.

Jurberg, P., Cabral Neto, J. B., & Schall, V. T. (1985). Molluscicide activity of the "Avelós" plant (*Euphorbia tirucalli*, L.) on *Biomphalaria glabrata*, the mollusc vector of schistosomiasis. *Memórias do Instituto Oswaldo Cruz*, 80(4), 423-427.

- Kalimutho, M., Parsons, K., Mittal, D., López, J. A., Srihari, S., & Khanna, K. K. (2015). Targeted therapies for triple-negative breast cancer: Combating a stubborn disease. *Trends in Pharmacological Sciences*, 36(12), 822-846.
- Kanaparthi, R., & Kanaparthi, A. (2011). The changing face of dentistry: Nanotechnology. *International Journal of Nanomedicine*, 6, 2799.
- Khattabi, A. M., Talib, W. H., & Alqdeimat, D. A. (2017). A targeted drug delivery system of anti-cancer agents based on folic acid-cyclodextrin-long polymer functionalized silica nanoparticles. *Journal of Drug Delivery Science and Technology*, 41, 367–374.
- Kiyohara, H., Ichino, C., Kawamura, Y., Nagai, T., Sato, N., Yamada, H., Salama, M.M. & Abdel-Sattar, E. (2012). In vitro anti-influenza virus activity of a cardiotonic glycoside from *Adenium obesum* (Forssk.). *Phytomedicine*, 19(2), 111-114.
- Komoreng, L., Thekiso, O., Lehasa, S., Tiwani, T., Mzizi, N., Mokoena, N., Khambule, N., Ndebele, S. & Mdletshe, N. (2017). An ethnobotanical survey of traditional medicinal plants used against lymphatic filariasis in South Africa. *South African Journal of Botany*, 111, 12-16.
- Kose, L. S., Moteetee, A. & Van Vuuren, S. (2015). Ethnobotanical survey of medicinal plants used in the Maseru district of Lesotho. *Journal of Ethnopharmacology*, 170, 184- 200.
- Krause, S., Brock, A. & Ingber, D. E. (2013). Intraductal injection for localized drug delivery to the mouse mammary gland. *Journal of visualized experiments: JoVE*, (80).
- Kulkarni, P. V., Roney, C. A., Antich, P. P., Bonte, F. J., Raghu, A. V. & Aminabhavi, T. M. (2010). Quinoline-n-butylcyanoacrylate-based nanoparticles for brain targeting for the diagnosis of Alzheimer's disease. *Wiley Interdisciplinary Reviews: Nanomedicine and Nanobiotechnology*, 2(1), 35-47.

- Kulkarni, S. K. & Kulkarni, S. K. (2015). *Nanotechnology: principles and practices*. Springer.
- Kumar, B., Kumar, R., Skvortsova, I. & Kumar, V. (2017). Mechanisms of tubulin binding ligands to target cancer cells: updates on their therapeutic potential and clinical trials. *Current Cancer Drug Targets*, 17(4), 357-375.
- Kumari, A., Yadav, S. K. & Yadav, S. C. (2010). Biodegradable polymeric nanoparticles based drug delivery systems. *Colloids and Surfaces B: Biointerfaces*, 75(1), 1-18.
- Kuppusamy, P., Yusoff, M. M., Maniam, G. P. & Govindan, N. (2016). Biosynthesis of metallic nanoparticles using plant derivatives and their new avenues in pharmacological applications—An updated report. *Saudi Pharmaceutical Journal*, 24(4), 473-484.
- Landesman-Milo, D., Qassem, S. & Peer, D. (2016). Targeting Cancer Using Nanocarriers. *Nanomedicine* (pp. 131-155). Springer, New York, NY.
- Li, X., Wei, J., Aifantis, K.E., Fan, Y., Feng, Q., Cui, F. Z. & Watari, F. (2016). Current investigations into magnetic nanoparticles for biomedical applications. *Journal of Biomedical Materials Research Part A*, 104(5), 1285-1296.
- Liao, F., Folsom, A. R. & Brancati, F. L. (1998). Is low magnesium concentration a risk factor for coronary heart disease? The Atherosclerosis Risk in Communities (ARIC) Study. *American heart journal*, 136(3), 480-490.
- Lim, J. & Majetich, S. A. (2013). Composite magnetic–plasmonic nanoparticles for biomedicine: manipulation and imaging. *Nano Today*, 8(1), 98-113.
- Lin, M. W., Lin, A. S., Wu, D. C., Wang, S. S., Chang, F. R., Wu, Y. C. & Huang, Y. B. (2012). Euphol from *Euphorbia tirucalli* selectively inhibits human gastric cancer cell growth

through the induction of ERK1/2-mediated apoptosis. *Food and chemical toxicology*, 50(12), 4333-4339.

Lirio, L. G., Hermano, M. L. & Fontanilla, M.Q. (1998). Note antibacterial activity of medicinal plants from the Philippines. *Pharmaceutical biology*, 36(5), 357-359.

Liu, S., Jones, L. & Gu, F. X. (2012). Nanomaterials for ocular drug delivery. *Macromolecular bioscience*, 12(5), 608-620.

Liu, Y., Li, M., Yang, F. & Gu, N. (2017). Magnetic drug delivery systems. *Sci. China Mater*, 60, 471-486.

Loizidou, M. & Seifalian, A. M. (2010). Nanotechnology and its applications in surgery. *British journal of surgery*, 97(4), 463-465.

Luseba, D. & Van der Merwe, D. (2006). Ethnoveterinary medicine practices among Tsonga speaking people of South Africa. *Onderstepoort Journal of Veterinary Research*, 73(2), 115-122.

Maliehe, E. B. (1997). *Medicinal plants and herbs of Lesotho*. Mafeteng Development Project.

Mantle, M. D. (2013). NMR and MRI studies of drug delivery systems. *Current opinion in colloid & interface science*, 18(3), 214-227.

Marathe, K., Nashikkar, N., Bundale, S. & Upadhyay, A. (2019). Analysis of quorum quenching potential of *Euphorbia trigona* Mill. *Int J Pharm Sci Res*, 10(3), 1372-1386.

Mayo clinic staff (2019). *What to know about surgery for cancer*. [online] Mayo Clinic. Available at: <https://www.mayoclinic.org/diseases-conditions/cancer/in-depth/cancer-surgery/art-20044171>.

- Mihai, M .M., Dima, M. B., Dima, B. & Holban, A. M. (2019). Nanomaterials for wound healing and infection control. *Materials*, 12(13), p. 2176.
- Mody, V. V., Cox, A., Shah, S., Singh, A., Bevins, W. & Parihar, H. (2014). Magnetic nanoparticle drug delivery systems for targeting tumor. *Applied Nanoscience*, 4(4), 385- 392.
- Moffett, R. (2010). *Sesotho plant and animal names and plants used by the Basotho*. 242-257. UJ Press.
- Mohsenipour, Z. & Hassanshahian, M. (2016). Antibacterial activity of Euphorbia hebecarpa alcoholic extracts against six human pathogenic bacteria in planktonic and biofilm forms. *Jundishapur journal of microbiology*, 9(6).
- Mornet, S., Vasseur, S., Grasset, F. & Duguet, E. (2004). Magnetic nanoparticle design for medical diagnosis and therapy. *Journal of Materials Chemistry*, 14(14), 2161-2175.
- Morsi, S. R. A. (2015). Phytochemical and Biological Study of Certain Euphorbia Species Cultivated in Egypt. *CU Theses*.
- Morton, C. L. & Houghton, P. J. (2007). Establishment of human tumor xenografts in immunodeficient mice. *Nature protocols*, 2(2), p. 247.
- Moteetee, A. & Van Wyk, B. E. (2011). The medical ethnobotany of Lesotho: a review. *Bothalia*, 41(1), 209-228.
- Moteetee, A., Moffett, R. O. & Seleteng-Kose, L. (2019). A review of the ethnobotany of the Basotho of Lesotho and the Free State Province of South Africa (South Sotho). *South African Journal of Botany*, 122, 21-56.
- Munro, B., Vuong, Q. V., Chalmers, A. C., Goldsmith, C. D., Bowyer, M. C. & Scarlett, C. J.

(2015). Phytochemical, antioxidant and anti-cancer properties of *Euphorbia tirucalli* methanolic and aqueous extracts. *Antioxidants*, 4(4), 647-661.

Mwine, J. T. & Van Damme, P. (2011). Why do Euphorbiaceae tick as medicinal plants? A review of Euphorbiaceae family and its medicinal features. *Journal of Medicinal Plants Research*, 5(5), 652-662.

Mwine, T. J. & Van Damme, P. (2011). Why do Euphorbiaceae tick as medicinal plants?: a review of Euphorbiaceae family and its medicinal features. *Journal of medicinal plants research*, 5(5), 652-662.

Nashikkar, N., Begde, D., Bundale, S., Mashitha, P., Rudra, J. & Upadhyay, A. (2012). Evaluation of the immunomodulatory properties of *Euphorbia trigona*—An in vitro study. *Int J Institutional Pharm Life Sci*, 2(1), 88-105.

Niu, S., Zhang, L. K., Zhang, L., Zhuang, S., Zhan, X., Chen, W. Y., Du, S., Yin, L., You, R., Li, C. H. & Guan, Y. Q. (2017). Inhibition by multifunctional magnetic nanoparticles loaded with alpha-synuclein RNAi plasmid in a Parkinson's disease model. *Theranostics*, 7(2), 344.

Nixon, N. & Verma, S. (2016). A value-based approach to treatment of HER2-positive breast cancer: Examining the evidence. *American Society of Clinical Oncology Educational Book*, 36, e56-e63.

Palmieri, C., Patten, D. K., Januszewski, A., Zucchini, G. & Howell, S. J. (2014). Breast cancer: current and future endocrine therapies. *Mol. Cell. Endocrinol*, 382, 695-723.

Pan, A., Zhang, H., Li, Y., Lin, T. Y., Wang, F., Lee, J., Cheng, M., Dall'Era, M., Li, T., deVere White, R. & Pan, C. X. (2016). Disulfide-crosslinked nanomicelles confer cancer- specific drug

delivery and improve efficacy of paclitaxel in bladder cancer. *Nanotechnology*, 27(42), 425103.

Panaampon, J., Sasamoto, K., Kariya, R. & Okada, S. (2021). Establishment of nude mice lacking NK cells and their application for human tumor xenografts. *Asian Pacific Journal of Cancer Prevention: APJCP*, 22(4), 1069.

Pankhurst, Q. A., Connolly, J., Jones, S. K. & Dobson, J. (2003). Applications of magnetic nanoparticles in biomedicine. *Journal of physics D: Applied physics*, 36(13), R167.

Pankhurst, Q. A., Thanh, N. T. K., Jones, S. K. & Dobson, J. (2009). Progress in applications of magnetic nanoparticles in biomedicine. *Journal of Physics D: Applied Physics*, 42(22), 224001.

Panyam, J. & Labhasetwar, V. (2012). Biodegradable nanoparticles for drug and gene delivery to cells and tissue. *Advanced Drug Delivery Reviews*, 64, 61–71.

Pardee, A. B., Stein, G. S. & Bronstein, E.A. (2008). What goes wrong in cancer. *The Biology and Treatment of Cancer: Understanding Cancer*, 1-19.

Parekh, J. & Chanda, S. (2007). In vitro antimicrobial activity and phytochemical analysis of some Indian medicinal plants. *Turkish Journal of Biology*, 31(1), 53-58.

Petros, R. A. & DeSimone, J. M. (2010). Strategies in the design of nanoparticles for therapeutic applications. *Nature reviews Drug discovery*, 9(8), p. 615.

Phillips, E. P. (1917). A contribution to the flora of the Leribe Plateau and environs: with a discussion on the relationships of the floras of Basutoland, the Kalahari, and the South- eastern regions. *Annals of the South African Museum. Annale van die Suid-Afrikaanse Museum*, 16(1), 1–379.

Piranlioglu, R., Lee, E., Ouzounova, M., Bollag, R. J., Vinyard, A. H., Arbab, A. S., Marasco, D., Guzel, M., Cowell, J. K., Thangaraju, M. & Chadli, A. (2019). Primary tumor- induced immunity eradicates disseminated tumor cells in syngeneic mouse model. *Nature communications*, 10(1), 1430.

Puchalapalli, M., Zeng, X., Mu, L., Anderson, A., Glickman, L. H., Zhang, M., Sayyad, M. R., Wangenstein, S. M., Clevenger, C. V. & Koblinski, J. E. (2016). NSG mice provide a better spontaneous model of breast cancer metastasis than athymic (nude) mice. *PloS one*, 11(9), e0163521.

Rajaratnam, H., Rasudin, N. S., Safuan, S., Abdullah, N. A., Mokhtar, N. F., & Fuad, W. E. M. (2022). Passage number of 4T1 cells influences the development of tumor and the progression of metastasis in 4T1 orthotopic mice. *The Malaysian Journal of Medical Sciences: MJMS*, 29(3), 30.

Rezende, J. R., Rodrigues, S. B., Jabor, I. A. S. & Pamphile, J. A. (2004). Antimutagenic effect of *Euphorbia tirucalli* latex on the methionine system in *Aspergillus nidulans*. *Acta Scientiarum. Biological Sciences* , 26 (4), 481-484.

Ritchie, H. & Roser, M. (2023). How many people in the world die from cancer?. *Our World in Data*.

Rivera, E. (2003). Current status of liposomal anthracycline therapy in metastatic breast cancer. *Clinical breast cancer*, 4, S76-S83.

Robinson, I., Ung, D., Tan, B., Long, J., Cooper, A. I., Fernig, D. G., & Thanh, N. T. K. (2008). Size and shape control for water-soluble magnetic cobalt nanoparticles using polymer ligands. *Journal of Materials Chemistry*, 18(21), 2453-2458.

Rossa Jr, C. & D'Silva, N. J. (2019). Immune-relevant aspects of murine models of head and neck cancer. *Oncogene*, 38(21), 3973-3988.

Dattani, S., Spooner, F., Ritchie, H. & Roser, M. (2023). Causes of Death. *Our World in Data*.

SANBI. Red List of South African Plants. Internet]. [Cited 2018 July 07]. Available from: <http://redlist.sanbi.org/genus.php?genus=574>.

Orange Grove Farm. FLORA. Internet]. [Cited 2018 July 07]. Available from: <https://orangegrovekaroo.wordpress.com/category/flora/>

Ottewell, P. D. & Lawson, M. A. (2021). Advances in murine models of breast cancer bone disease. *Journal of Cancer Metastasis and Treatment*, 7.

Santhosh, P. B. & Ulrih, N. P. (2013). Multifunctional superparamagnetic iron oxide nanoparticles: promising tools in cancer theranostics. *Cancer letters*, 336(1), 8-17.

Schmelzer, G. H. & Gurib-Fakim A. (2008). Medicinal plants. *Plant Resour. Trop Afr.* 412-5.

Schmelzer, G. H., Schmelzer, G. H. & Gurib-Fakim, A. (eds.) (2008). *Medicinal plants* (1), 282.

Schmidt, E., Lotter, M. & McClelland, W. (2002). *Trees and shrubs of Mpumalanga and Kruger national park*. Jacana media, 280.

Schaue, D. & McBride, W. H. (2015). Opportunities and challenges of radiotherapy for treating cancer. *Nature reviews Clinical oncology*, 12(9), 527-540.

Seidu, T. A., Kutoka, P. T., Asante, D. O., Farooq, M. A., Alolga, R. N. & Bo, W. (2022). Functionalization of nanoparticulate drug delivery systems and its influence in cancer therapy.

Senapati, S., Mahanta, A. K., Kumar, S. & Maiti, P. (2018). Controlled drug delivery vehicles for cancer treatment and their performance. *Signal transduction and targeted therapy*, 3(1), 7.

Shale, T. L., Stirk, W. A. & van Staden, J. (1999). Screening of medicinal plants used in Lesotho for anti-bacterial and anti-inflammatory activity. *Journal of Ethnopharmacology*, 67(3), 347-354.

Sharifi, E., Salimi, A., Shams, E., Noorbakhsh, A. & Amini, M. K. (2014). Shape-dependent electron transfer kinetics and catalytic activity of NiO nanoparticles immobilized onto DNA modified electrode: fabrication of highly sensitive enzymeless glucose sensor. *Biosensors and Bioelectronics*, 56, 313-319.

Shi, Q. W., Su, X. H., and Kiyota, H. (2008). Chemical and pharmacological research of the plants in genus Euphorbia. *Chemical reviews*, 108(10), 4295-4327.

Siddique, N. A., da Silva, J. A. T. & Bari, M. A. (2010). Preservation of indigenous knowledge regarding important and endangered medicinal plants in Rajshahi district of Bangladesh. *Journal of Plant Sciences*, 5(2), 201-215.

Siegel, R. L., Miller, K. D. & Jemal, A. (2019). Cancer statistics, 2019. *CA: a cancer journal for clinicians*, 69(1), 7-34.

Sohail, A., Ahmad, Z., Bég, O. A., Arshad, S. & Sherin, L. (2017). A review on hyperthermia via nanoparticle-mediated therapy. *Bulletin du cancer*, 104(5), 452-461.

Tada, M. & Seki, H. (1989). Toxic diterpenes from Euphorbia trigona (Saiunkaku: an indoor foliage plant in Japan). *Agricultural and biological chemistry*, 53(2), 425-430.

Tamura, T., Kita, T., Nakagawa, T., Endo, T., Kim, T. S., Ishihara, T., Mizushima, Y., Higaki, M. & Ito, J. (2005). Drug delivery to the cochlea using PLGA nanoparticles. *The Laryngoscope*, 115(11), 2000-2005.

Tanaka, S., Saito, Y., Kunisawa, J., Kurashima, Y., Wake, T., Suzuki, N., Shultz, L. D., Kiyono, H. & Ishikawa, F. (2012). Development of mature and functional human myeloid subsets in hematopoietic stem cell-engrafted NOD/SCID/IL2 γ KO mice. *The Journal of Immunology*, p. 1103660.

Thanh, N. T. & Green, L. A. (2010). Functionalisation of nanoparticles for biomedical applications. *Nano Today*, 5(3), 213-230.

The Encyclopedia of succulants. *Euphorbia clavarioides* [Internet]. [Cited 2018 July 07]. Available from: http://www.llifile.com/Encyclopedia/SUCCULENTS/Family/Euphorbiaceae/32951/Euphorbia_clavarioides_var._truncata

Guo, T., Lin, M., Huang, J., Zhou, C., Tian, W., Yu, H. & Feng, X. (2018). The recent advances of magnetic nanoparticles in medicine. *Journal of Nanomaterials*, 2018(1), 7805147.

Tiwani, T. (2017). *Phytochemical screening, cytotoxicity, antimicrobial and anthelmintic activity of medical plants used in the treatment of lymphatic filariasis in the Eastern Cape, South Africa* (Doctoral dissertation, University of the Free State).

Tiwari, S., Singh, P. & Singh, A. (2003). Toxicity of *Euphorbia tirucalli* Plant Against Freshwater Target and Non-target Organisms. *Pakistan Journal of Biological Sciences*, 6(16), 1423–1429.

- Valadares, M. C., Carrucha, S. G., Accorsi, W. & Queiroz, M. L. (2006). *Euphorbia tirucalli* L. modulates myelopoiesis and enhances the resistance of tumor-bearing mice. *International immunopharmacology*, 6(2), 294-299.
- Van Damme, P. (1989). *Studie van Euphorbia tirucalli L.: morfologie, fysiologie, teeltvoorwaarden* (Doctoral dissertation, Ghent University).
- Van Wyk, B. E., Oudtshoorn, B. V. & Gericke, N. (1997). *Medicinal Plants of South Africa*. Briza. 304.
- Villanueva, J., Quirós, L. M. & Castañón, S. (2015). Purification and partial characterization of a ribosome-inactivating protein from the latex of *Euphorbia trigona* Miller with cytotoxic activity toward human cancer cell lines. *Phytomedicine*, 22(7-8), 689-695.
- Vogg, G., Mattes, E., Rothenburger, J., Hertkorn, N., Achatz, S. & Sandermann Jr, H. (1999). Tumor promoting diterpenes from *Euphorbia leuconeura* L. *Phytochemistry*, 51(2), 289- 295.
- Wan, X., Song, Y., Song, N., Li, J., Yang, L., Li, Y. & Tan, H. (2016). The preliminary study of immune superparamagnetic iron oxide nanoparticles for the detection of lung cancer in magnetic resonance imaging. *Carbohydrate Research*, 419, 33-40.
- Wang, C. and Sun, S. (2007). Chemical synthesis of monodisperse magnetic nanoparticles. *Handbook of Magnetism and Advanced Magnetic Materials*.
- Wang, C. C., Jamal, L. & Janes, K. A. (2012). Normal morphogenesis of epithelial tissues and progression of epithelial tumors. *Wiley Interdisciplinary Reviews: Systems Biology and Medicine*, 4(1), 51-78.

Wang, Q., Xie, L., He, Z., Di, D. & Liu, J. (2012). Biodegradable magnesium nanoparticle-enhanced laser hyperthermia therapy. *International journal of nanomedicine*, (7), 4715.

Wang, X., Yang, L., Chen, Z. & Shin, D. M. (2008). Application of nanotechnology in cancer therapy and imaging. *CA: a cancer journal for clinicians*, 58(2), 97-110.

Watt, J. M. & Breyer-Brandwijk, M. G. (1962). The Medicinal and Poisonous Plants of Southern and Eastern Africa being an Account of their Medicinal and other Uses, Chemical Composition, Pharmacological Effects and Toxicology in Man and Animal. (Edn 2).

WHO (2018a.) World cancer day [Internet]. [Cited 2018May 27]. Available form: <http://www.who.int/cancer/en/>

WHO (2018b). Cancer fact sheet [Internet]. [Cited 2018May 27]. Available form: <http://www.who.int/en/news-room/fact-sheets/detail/cancer>

WHO (2019). International agency for cancer. Cancer Fact sheet [internet].[Cited 2019 December 06]. Available form: <http://gco.iarc.fr/today/data/factsheets/cancers/39-All-cancers-fact-sheet.pdf>.

WHO (2024). International agency for cancer. Cancer Fact sheet [internet].[Cited 2024 April 19]. Available form: <https://www.who.int/news-room/fact-sheets/detail/breast-cancer>

WHO (2019c). International agency for cancer. Cancer Fact sheet [internet].[Cited 2019 December 09]. Available form: <http://gco.iarc.fr/today/data/factsheets/cancers/20-Breast-fact-sheet.pdf>

WHO (2018a). Breast cancer [Internet]. [Cited 2018May 27]. Available from: <http://www.who.int/cancer/prevention/diagnosis-screening/breast-cancer/en/>.

Wright, L. E., Ottewell, P. D., Rucci, N., Peyruchaud, O., Pagnotti, G. M., Chiechi, A., Buijs, J. T. & Sterling, J. A. (2016). Murine models of breast cancer bone metastasis. *BoneKEY reports*, 5.

Xin, Y., Yin, M., Zhao, L., Meng, F., & Luo, L. (2017). Recent progress on nanoparticle-based drug delivery systems for cancer therapy. *Cancer Biology & Medicine*, 14(3), 228.

Xu, J., & Yang, Y. (2009). Traditional Chinese medicine in the Chinese health care system. *Health Policy*, 90(2-3), 133-139.

Yang, W., Chen, X., Li, Y., Guo, S., Wang, Z. & Yu, X. (2020). Advances in pharmacological activities of terpenoids. *Natural Product Communications*, 15(3), 1934578X20903555.

Yee, N. S., Ignatenko, N., Finnberg, N., Lee, N. & Stairs, D. (2015). Animal models of cancer biology. *Cancer growth and metastasis*, 8, CGM-S37907.

Yew, Y. P., Shameli, K., Miyake, M., Khairudin, N. B. B. A., Mohamad, S. E. B., Naiki, T. & Lee, K. X. (2020). Green biosynthesis of superparamagnetic magnetite Fe₃O₄ nanoparticles and biomedical applications in targeted anticancer drug delivery system: A review. *Arabian Journal of Chemistry*, 13(1), 2287-2308.

Yuan, Y., Cai, T., Xia, X., Zhang, R., Chiba, P., & Cai, Y. (2016). Nanoparticle delivery of anticancer drugs overcomes multidrug resistance in breast cancer. *Drug Delivery*, 23(9), 3350-3357.

Zeman, E. M., Schreiber, E. C. & Tepper, J. E. (2020). Basics of radiation therapy.

In *Abeloff's clinical oncology*, Elsevier, 431-460.

Zhang, B., Strauss, A. C., Chu, S., Li, M., Ho, Y., Shiang, K. D., Snyder, D. S., Huettner, C. S., Shultz, L., Holyoake, T. & Bhatia, R. (2010). Effective targeting of quiescent chronic myelogenous leukemia stem cells by histone deacetylase inhibitors in combination with imatinib mesylate. *Cancer cell*, 17(5), 427-442.

Zhang, H. W., Hu, J. J., Fu, R. Q., Liu, X., Zhang, Y. H., Li, J., ... & Gao, N. (2018). Flavonoids inhibit cell proliferation and induce apoptosis and autophagy through downregulation of PI3K γ mediated PI3K/AKT/mTOR/p70S6K/ULK signaling pathway in human breast cancer cells. *Scientific reports*, 8(1), 11255.

CHAPTER 3

PHARMACOLOGICAL SIGNIFICANCE, MEDICINAL USE AND TOXICITY OF EXTRACTED AND ISOLATED COMPOUNDS FROM *EUPHORBIA* SPECIES FOUND IN SOUTHERN AFRICA: A REVIEW

Abstract

This study aims to document plants from the Euphorbiaceae found in Southern Africa in order to understand their traditional medicinal uses, pharmacological properties, toxicity and their active secondary metabolites. Literature was gathered from multiple sources such as scientific journals, books, dissertations and conference papers between 1962-2023. The review was conducted on 15 *Euphorbia* species found in Southern Africa. Recent studies have revealed that certain compounds found in *Euphorbia* plants possess valuable biological and pharmacological properties. Despite this, the white sticky latex sap present in these plants can be highly toxic, though it can also be utilized for medicinal purposes. The phytochemical constituents of these plants have been shown to exhibit a variety of beneficial properties, including antibacterial, antioxidant, antiproliferative, anticancer, anti-inflammatory, antiviral, antifungal and anti-HIV effects. These plants owe their efficacy to a range of phytochemicals, such as euphol, cycloartenol, tirucallol and triterpenoids, each with its own unique biological and pharmacological characteristics. Additionally, several plants contain various proteins, including lectin, Chitobiosidase, Endochitinase, N-acetyl-b-glucosamidase and lysozyme. The data presented in this review sheds light on various species of *Euphorbia* found in Southern Africa. Although some Euphorbiaceae species have been screened for active compounds that have medicinal properties, the majority of documented plants have not been

thoroughly screened, which is a matter of concern. Given their historic use, it is important to conduct further research to scrutinize the medicinal significance of the *Euphorbia* species. This should involve in-depth examinations of isolated compounds, their pharmacokinetics, pharmacodynamics and pharmacotherapeutics, both *in vitro* and *in vivo*. This study will serve as a fundamental resource for researchers to explore the qualities and benefits of the unexplored *Euphorbia* species, and also design meaningful future studies.

3.1. Introduction

The Euphorbiaceae is a highly varied group of flowering plants, having over 300 genera and 8 000 species (Bijekar & Gayatri, 2014). Within this family, the *Euphorbia* genus stands out as one of the largest, with more than 2 000 species (Aleksandrov *et al.*, 2019). These species can take the form of herbs, shrubs and trees, sometimes resembling succulents or cacti. They are mainly found in tropical and subtropical regions of Africa and America, as noted by Adedapo *et al.* in 2004. Many species in this family produce a toxic white sap, but this sap can also have medicinal properties (Villanueva *et al.*, 2015). The genus contains economically significant species, making it a vital genus with high research potential.

South Africa has a rich collection of *Euphorbia* species, with at least 188 indigenous to the country (SANBI). The *Euphorbia* genus has attracted the attention of many researchers due to its diverse chemical compositions, which include euphol, triterpenoids, diterpene ester and tirucallol. These compositions have been found to have remarkable therapeutic properties such as antimicrobial, antidiabetic, antiviral and anticancer properties, as noted by Betancur- Galvis *et al.* (2002) and Mwine and Damme (2011). However, there is a lack of scientific documentation on the anticancer properties of many *Euphorbia* species in Southern Africa. This is due to a lack of research on isolated compounds, as well as a lack of testing for discovery of new compounds. Furthermore, there is limited research on unexplored *Euphorbia* species that may have potential anticancer properties. Additionally, the majority of published articles on *Euphorbia* focus on already explored species, with little to no information on the unexplored ones (Fürstenberger & Hecker, 1985; Cataluna *et al.*, 1999). It is worth noting that there is currently only one comprehensive review available on the

Euphorbia species found in Southern Africa. This review highlights the limited scientific documentation available on these plants and emphasizes the need for further research to unlock their full potential. The review, however, did not focus on the compounds found in the selected plants and anticancer properties (Mavundza *et al.*, 2022). Therefore, the current study focuses on gathering more data on the use of isolated compounds as anticancer lead agents in traditional medicine to advance research in the field of discovery and development of anticancer agents.

For this reason, the purpose of this review is to provide a comprehensive overview of the pharmacological activities of the isolated compounds from various *Euphorbia* species found in Southern Africa. This review also aims to highlight the diversity of the Euphorbiaceae family and its potential for further research. The study focuses on several plants, including *Euphorbia trigona* (*E. trigona*), *Euphorbia tirucalli* (*E. tirucalli*), *Euphorbia ammak* (*E. ammak*), *Euphorbia bupleurifolia* (*E. bupleurifolia*), *Euphorbia enopla* (*E. enopla*), *Euphorbia polygona* (*E. polygona*), *Euphorbia cooperi* (*E. cooperi*), *Euphorbia stellata* (*E. stellata*), *Euphorbia ferox* (*E. ferox*), *Euphorbia clavarioides* (*E. clavarioides*), *Euphorbia gorgonis* (*E. gorgonis*), *Euphorbia coerulescens* (*E. coerulescens*), *Euphorbia horrida* (*E. horrida*), *Euphorbia arabica* (*E. arabica*), and *Euphorbia ledienii* (*E. ledienii*). These plants were selected for their pharmacological activities and toxicological evaluation on breast cancer cells as part of the authors' recent research project. This review serves as a valuable resource for other researchers to explore the remaining *Euphorbia* species for their potentially active compounds and anticancer therapeutic properties.

3.2. Methodology

To conduct this review, original articles from journals indexed in PubMed Central, Springer Link, Scopus, Science Direct, Hindawi, and Google scholars on *Euphorbia* species, their medicinal uses, toxicity and pharmacological properties, were analysed. For this review, only texts published in English between 1962 and 2023 were considered. The years of publication were selected due to the limited data available on *Euphorbia* plants. Throughout the search process, 881 reports were found across various databases. After reviewing the titles and abstracts, the search was refined and narrowed down to 293 articles, which were fully examined and are now being discussed in this study.

The research criteria for inclusion were based on the guidelines set by Lu *et al.* (2020) with a few modifications. The criteria included: (1) studies on the isolated compounds of *Euphorbia* species conducted *in vitro* and *in vivo*; (2) evaluation of their biological activities; (3) studies published in peer-reviewed journals written in English; and (4) studies that provided full-text papers. There were no limitations on the study's location. The study excluded (1) human studies, (2) a combination of isolated compounds and (3) non-original articles.

The retrieved data was carefully examined to identify possible medical applications, biological activities, isolated compounds, toxicity and pharmacological properties. Additional confirmation of the correct plant names was verified from the plantlist.org website. After analyzing all the data collected, conclusive results were obtained.

The cytotoxic tendency of selected compounds on various cancer cell lines and human tumor types was computed using the Cell Line Cytotoxicity Predictor (CLCPred) webserver (<https://www.way2drug.com/Cell-line/>) a free online service for *in silico* evaluation of human cell line cytotoxicity tendency of bioactive compounds (refer Lagunin *et al.*, 2018). This

prediction was based on Prediction of Activity Spectra for Substances (PASS) technology (<https://www.way2drug.com/PASSonline>) where the training set was generated based on cytotoxicity data derived from ChEMBLdb (version 23) (<https://www.ebi.ac.uk/chembl/db/>). Compounds with robust anticancer potential were selected and subjected to further analyses to extract physicochemical and pharmacokinetics parameters using the SwissADME (<http://www.swissadme.ch/index.php>) (Daina *et al.*, 2017) and pKCSM (<http://biosig.unimelb.edu.au/pkcsml/>) (Pires *et al.*, 2015) webserver as demonstrated earlier (Adetunji *et al.*, 2023; Ogunyemi *et al.*, 2022).

3.3. Results

The current study reports on 15 *Euphorbia* species, their traditional and pharmacological properties, and isolated compounds. *Euphorbia* species have been used in folk medicine for various ailments, which is why scientists have taken an interest in investigating this genus and fully documenting the secondary metabolites responsible for these properties, see Table 3.1.

Out of the 15 plant species examined, 8 have been traditionally used as medicine for treating 7 distinct diseases. The most commonly used plants are for treating cancer (7), followed by warts and wounds (5), as well as other ailments, see Table 3.1.

Table 3.1. *Euphorbia* spp of Southern Africa and its neighboring countries, their toxicity and traditional use.

Botanical name Synonym in bold	Vernacular name (E)= English; (S)= Sotho	Location	Growing condition	Traditional use	Bioactive property	Toxicity	Reference
	(Z)= Zulu; (X)= Xhosa; (A)=Afrikans						
	(N)= Ndebele						
	(T)= Tsonga						
	(Ts)= Tswana						
	(V)= Venda						

<i>Euphorbia trigona</i> <i>Euphorbia hermentiana</i> Lem	African milk tree (E)	West Africa, tropical Asia, India	Dry tropical forests and semi arid environment. Direct sunlight	Respiratory infections, urinary tract infections, gonorrhoea, tumors and warts, intestinal parasites, rheumatoid arthritis, hepatitis, inflammation, piles, constipation, epileptic attack.	Anticancer	Skin irritation	Tada & Seki, 1989 Nashikkar <i>et al.</i> , 2012 Marathe <i>et al.</i> , 2019 Siddique <i>et al.</i> , 2010 Bouquet, 1969 Lynn and Clevette-Radford, 1986
---	-----------------------	-----------------------------------	---	---	------------	-----------------	--

<i>Euphorbia cooperi</i> <i>E. cooperi</i> var. <i>cooperi</i> <i>E. cooperi</i> var. <i>calidicola</i>	Candelabra <i>Euphorbia</i> (E), Umhlonhlo (N), Tshikondengala (V), Mokhoto (T) Mohlohlo (S)	South Africa (KwaZulu-Natal, Swaziland, North-West Province, Mpumalanga and Limpopo) Mozambique, Zimbabwe, Botswana	Well-drained soil. Direct sunlight.	Sore stomach, bloatedness, paralysis, wound healing	Breast cancer inhibitor	Skin irritation, blindness, throat burning sensation	Luseba & Van der Merwe, 2006 Gildenhuys, 2006 El-Sherei <i>et al.</i> , 2015 Hedberg & Staugård, 1989 Breyer-Brandwijk, 1962 Morsi, 2015 Gundidza & Hecker, 1992 Engi <i>et al.</i> , 2007 El-Sherei <i>et al.</i> , 2015
--	--	---	-------------------------------------	---	-------------------------	--	--

							Morsi, 2015
<i>Euphorbia ammak</i>	African candelabra /Desert candelabra (E)	Saudi Arabia, Yemen peninsula	No data reported	No data reported	Anticancer Antileishma nial activity, (H1N1) influenza virus, MDCK cells, antiparasitic activity	Skin and eye irritation. Normal melanocyte (HFB4).	Carter, 1994 Almehdar <i>et al.</i> , 2012 Al-Hajj <i>et al.</i> , 2018 Kiyohara <i>et al.</i> , 2012 Abdel-Satter <i>et al.</i> , 2010
<i>Euphorbia tirucalli</i>	Rubber-hedge <i>Euphorbia</i> (E),	Eastern tropical	Rock garden,	Snakebites, sexual impotence, warts,	Antibacteria l,	Skin irritation, blindness, fish	Watt & Breyer- Brandwijk, 1962

<i>Euphorbia laro</i> Drake	umSululu (Z), umHlonthlo (X), motsetse/ setlharesetola (Ts), mahumbana (T), muṭungu (V) kraalmelkbos (A)	Africa, South Africa, Indian Ocean Island	well- drained soils, mild to warm climates.	wounds, skin complaints, swollen glands, oedema, haemorrhoids, rheumatism, epilepsy toothache, earache and tumor.	molluscicid e, antiherpetic, antimutagen ic antibacterial , myelomodul ating activity, antitumor effect on different cell lines. Tumor promotor	poisoning, gastro- intestinal haemorrhage, fatality, Burkitt's lymphoma, Epstein-Barr virus	Baslas & Gupta, 1982 Kajikawa <i>et al.</i> , 2005 Shivkumar, 2015 Hargreaves, 1991 Van Damme, 1989 Gupta <i>et al.</i> , 2013 Voigt, 2007 Macneil <i>et al.</i> , 2003 Van den Bosch <i>et al.</i> , 1993 Jurberg <i>et al.</i> , 1985 Lirio <i>et al.</i> , 1998 Betancur-Galvis <i>et al.</i> , 2002 Tiwari <i>et al.</i> , 2003 Rezende <i>et al.</i> , 2004 Parekh & Chanda, 2007 Altamimi <i>et al.</i> , 2019 Valadares <i>et al.</i> , 2006 de Souza <i>et al.</i> , 2019
--------------------------------	---	---	--	---	--	---	---

							Mwine & Damme, 2011
<i>Euphorbia clavarioides</i> <i>Euphorbia basutica</i>	lions Spoor (E), melkpol (A), iSantilele/ isihlekehleke (Z), sehlehle/sehloko (S)	South Africa (Eastern Cape, KwaZulu-Natal, Northern Cape, Western Cape), Lesotho	Direct sunlight, mineral soil, grassland gardens	Skin rash in children, acne, sores, bruises, burns, eczema, ulcer, cracked heels and wounds, cancerous sores, warts, swollen feet, leprosy remedy, herpes, HIV related infections, high blood pressure, diabetes	No data reported	Skin irritation, bird lime	Moteetee <i>et al.</i> , 2019 Shale <i>et al.</i> , 1999 Maliehe, 1997 Kose <i>et al.</i> , 2015 Cock <i>et al.</i> , 2021
<i>Euphorbia gorgonis</i>	Nkalimasane (Z) melkbol (A)	South Africa (Eastern Cape)	Well-drained soil, Direct sunlight	Wounds, swelling, skin problems.	Antibacterial, antimicrobial activity, cancer cell line (HuTu), hepatoma cells (H411E)	Skin irritation	Johnson, 2019 Komoreng <i>et al.</i> , 2017 Tiwani, 2017
<i>Euphorbia bupleurifolia</i> <i>Tithymalus</i>	Pine cone plant (E), melkbol (A),	South Africa (Eastern Cape Province, Transkei and	Warm and moist condition s,moderat	Cancerous sores, for painful cracked feet, eczema, pimples, rashes and	No data reported	Skin irritation	Afolayan <i>et al.</i> , 2014 Van Wyk, 1997

<i>bupleurifolius</i>	intsele (X), insema (Z)	Natal)	e sun light.	wounds in topical formulation, clean teeth, swellings of the lower limbs, cancer, retained placenta.			Iwalewa <i>et al.</i> , 2019 Assefa & Bahiru, 2018
<i>Euphorbia enopla</i> <i>Euphorbia enopla</i> var. <i>enopla</i>	Milk- barrel/Pincushi on <i>Euphorbia</i> (E)	South Africa (Eastern Cape in the Noorsveld, in arid to semi- arid Karoo)	Well- drained soil, direct sunlight	No data reported	No data reported	Skin irritation, toxic to Vero cell line	Mampa <i>et al.</i> , 2020
<i>Euphorbia coerulescens</i> <i>Euphorbia virosa</i>	Sweetnoors (A)	South Africa (Cape province)	Sunny to half- shady, rocky and gritty- sandy soils	No data reported	No data reported	Inflammation in mice, skin irritation, blindness, throat irritation if swollen	Evans, 1978
<i>Euphorbia polygona</i>	African Milk Barrel (E)	South Africa (Eastern Cape)	Well- drained soil, direct sunlight and high temperat	No data reported	No data reported	Eye irritation, skin irritation, paralysis	Mampa <i>et al.</i> , 2020

			ure environm ent.				
<i>Euphorbia horrida Var</i> <i>Euphorbia horrida</i> Boiss.	African Milk Barrel (E)	South Africa (Wittepoort/ Karoo)	Well- drained soil, direct sunlight and dry condition s.	No data reported	No data reported	Inhibition of Vero cell line	Mampa <i>et al.</i> , 2020
<i>Euphorbia arabica</i> <i>Euphorbia neopolycnemoi des</i>	Klein Bont <i>Euphorbia</i> (E), umhlonhlo (Z)	Botswana, southern Mozambique, Zimbabwe, Limpopo, Mpumalanga and KwaZulu- Natal, South Africa	Stony grassland area.	Warts, stomachache, skin infections.	Antibacteria l	Inhibition of Vero cell line	Al-Harbi, 2017 El-Shanwani, 1996
<i>Euphorbia ledienii</i> <i>Euphorbia ledienii</i> A. Berger var. <i>ledienii</i>	Crested <i>Euphorbia</i> (E)	South Africa (Western Cape)	Well- drained soil, sunny condition s	No data reported	No data reported	Skin irritation	Abdel-Fattah, 1987



<i>Euphorbia ferox</i> <i>Euphorbia capitosa</i>	Milkweed (E)	South Africa (Western Cape)	Drained sandy soil, rocky outcrops and in sunny environment	No data reported	No data reported	Poisonous latex	
<i>Euphorbia stellata</i> <i>Euphorbiasquarrosa</i> <i>Euphorbia radiata</i> <i>Euphorbia scolopendrea</i> <i>Euphorbia uncinata</i>	Spurge (E)	South Africa (Eastern Cape)	Well-drained soil and sunny environment	No data reported	No data reported	Skin and eye irritation.	

An investigation into the chemical composition of various *Euphorbia* species unveiled distinctive profiles of isolated compounds. *E. tirucalli* exhibited the highest diversity with a total of 30 isolated compounds, spanning triterpenoids (Lupane, Oleanane, Tirucallane, Phorbol-type), phenolic compounds (Gallic Acid Derivatives, Ellagic Acid Derivatives, Flavonoids), phytosterols (Sterols), glycosides (Triterpene Glycosides, Other Glycosides), and diterpene esters (Mavundza *et al.*, 2022; Kgosiemang *et al.*, 2020; Shivkumar, 2015). Following closely, *E. cooperi* presented 18 compounds, including triterpenoids (Lupane, Oleanane, Phorbol-type), phytosterols (Sterols), and glycosides (Triterpene Glycosides) (Hlengwa, 2018; Sherei *et al.*, 2015). *E. trigona* showcased 16 compounds, including triterpenoids (Lupane, Oleanane, Phorbol-type), phenolic compounds (Gallic Acid Derivatives, Ellagic Acid Derivatives, Flavonoids), phytosterols (Sterols), and glycosides (Other Glycosides) (Boshara, 2014; Nashikkar *et al.*, 2012; Anjaneyulu & Rao, 1985). *E. coerulescens* and *E. ledienii* exhibited 9 and 8 compounds, respectively, encompassing triterpenoids, phenolic compounds and phytosterols (Sytwala *et al.*, 2015; Evans, 1978; Nielsen *et al.*, 1979); see Table 3.2 and 3.3. Notably, certain plants demonstrated fewer or no isolated compounds.

Table 3.2. Comprehensive overview of Isolated Compounds from Individual Plants

Plant	Compounds	Reference
<i>Euphorbia trigona</i>	Euphol, Cycloartenol, Cycloartanol, Lupeol, α amyrin, β –amyrin, Betulinic acid, Taraxerol, β -sitosterol, Taraxerol acetate, Friedelin, Friedelan 3 α - and 3 $\beta\alpha$ –ols, 24-ethylene cycloartanol, Epi-friedelinyl acetate, 3 β , friedelinol, Rhoiptlenone	Anjaneyulu & Rao, 1985 Nielsen <i>et al.</i> , 1977
<i>Euphorbia ledienii</i>	Isobutyric, 2-Methylbutyric acid, 12-Deoxyphorbol-13-isobutyrate-20-acetate, 12-Deoxyphorbol-13-(2-methylbutyrate)-20-acetate, Phorbol, 12-Deoxy phorbol, 12-Deoxy-16-hydroxy phorbol, Ingol-7,8,12-acetate, ditiglate	Popplewell <i>et al.</i> , 2010 Redei <i>et al.</i> , 2010 Evans & Kinghorn, 1977
<i>Euphorbia tirucalli</i>	Triterpenes, Euphol, Diterpene esters, Euphorbiane, 12-Deoxyphorbol esters, Ingenol, β -Sitosterol, Euphorbol, hexacosonate, 12-Deoxy-4 β -hydroxyphorbol-13-phenyl acetate-20-acetate, 12, 20-Dideoxyphorbol-13-isobutyrate, Glut-5-en-3- β -ol, Tirucallicine, Tri-methyl ellagic acid, Terpenic alcohol, Isoleuphorol, Taraxasterol, Tirucallol, Ketone euphorone, Resin, Ellagic acid, Taraxerol, 3,3'-Di-O-methylellagic acid, Euphorbin A, Euphorbin B, Tirucallin A, Tirucallin B, Euphorbol, Cycloartenol, 24-Methylenecycloartenol, Ingenol triacetate, β -amyrin, Rhoiptlenone, 3 β -friedelinol, Epi-friedelinyl acetate, 24-ethylene cycloartanol, Friedelan 3 α - and 3 $\beta\alpha$ –ols, Friedelin, Taraxerol acetate, Betulinic acid, β –amyrin, α amyrin, Lupeol, Cycloartanol, β -amyrone, glutinone, taraxerone, Glut-5-en-3- β -ol and cycloart-23-ene-3- β , 25-diol, Euphorcinol, Euphorginol, 12, 13,20-tri- O-acetylphorbol, 3,5,20-tri-O-acetylingenol 20-acetate	Mavundza <i>et al.</i> , 2022 Martins <i>et al.</i> , 2020 Lin <i>et al.</i> , 2012 Yu <i>et al.</i> , 2012 Dutra <i>et al.</i> , 2012 Kajikawa <i>et al.</i> , 2005 Cataluna & Rates, 1999 Yoshida <i>et al.</i> , 1991 Khan & Malik, 1990 Khan <i>et al.</i> , 1988a Khan <i>et al.</i> , 1988b Khan <i>et al.</i> , 1989 Rasool <i>et al.</i> , 1989 Khan <i>et al.</i> , 1987 Lynn & Clevette-Radford, 1985 Baslas & Gupta, 1984 Baslas & Gupta, 1982 Yamamoto <i>et al.</i> , 1981

		Biesboer & Mahlberg, 1979 Nielsen <i>et al.</i> , 1979 Kinghorn, 1979 Fürstenberger & Hecker, 1977 Gupta & Mahadevan, 1967
<i>Euphorbia enopla</i>	Euphol, Tirucallol	Ponsinet & Ourisson, 1968
<i>Euphorbia coerulescens</i>	Angelate acetate isobutyrate, Fatty acids, Acetate laurate, α -Methyl butyrate, Heptanoate, Laurate, Euphol, Tirucallol, Euphorbol	Nielsen <i>et al.</i> , 1979 Evans, 1978
<i>Euphorbia ammak</i>	α -glutanol, Stigmasterol, Euphol, Euphorbol	Abdel-Sattar <i>et al.</i> , 2015 Ponsinet & Ourisson, 1968
<i>Euphorbia cooperi</i>	16-Hydroxy-12-desoxyphorbol, Euphol, Obtusifoliol, 12-Deoxyphorbol-13-isobutyrate-16-angelate-20-acetate, Euphorbilactone, Norsesquiterpenoid, Arachiside A, Glutanol, 16-Angeloyloxy-13 α -isobutanoyloxy-4 β ,9 α ,20- trihydroxytiglia-1,5-diene-3,7-dione, 20-Acetoxy-16-angeloyloxy13 α -isobutanoyloxy-4 β ,9 α ,20-tetrahydroxytiglia-1,5-diene-3-one, Gallic acid, Bervifolin, carboxylic acid, Kampferol-3-O- β -D-rutinoside, 1-O-Galloyl-3,6-hexahydroxydiphenyl- β -D glucopyranoside, 3, 3' Dimethoxy ellagic acid 3, 4, 4' Trimethoxyellagic acid, Ellagic acid, Kaempferol, 7-galloyl catechin, kaempferol 3-O- β -(6''- O-galloyl)-glucopyranoside, triesters-16-hydroxy-12-desoxy-phorbol	Mavundza <i>et al.</i> , 2022 Hlengwa, 2018 El-Toumy <i>et al.</i> , 2018 El-Sherei <i>et al.</i> , 2015 Gschwendt & Hecker, 1970
<i>Euphorbia horrida</i>	17-Hydroxyingenol-17-benzoate-20-angelate, Diterpene esters	El-Hawary <i>et al.</i> , 2020

Table 3.3. Phytochemicals extracted from the selected *Euphorbia* s This table outlines the diverse array of crude phytochemicals extracted from carefully chosen *Euphorbia* species, shedding light on the rich chemical composition inherent in these selected plants.

Plant	Phytochemical/s	Reference
<i>Euphorbia trigona</i>	Saponins, alkaloids, flavonoids, glycosides, sterols and triterpenoids, tannins	Boshara, 2014 Nashikkar <i>et al.</i> , 2012 Nielsen <i>et al.</i> , 1977
<i>Euphorbia cooperi</i>	Triterpenoid	Hlengwa, 2018
<i>Euphorbia ammak</i>	alkaloids, saponins, glycosides	Al-Hajj <i>et al.</i> , 2018
<i>Euphorbia tirucalli</i>	Triterpenoid, phenols, flavonoids, tannins, alkaloids, saponins, glycosides, and steroids.	Kgosiemang <i>et al.</i> , 2020 Shivkumar, 2015
<i>Euphorbia clavarioides</i>	Alkaloids, flavonoids, saponins, tannins, terpenoids, phytosterols, glycosides, triterpenoids, anthraquinone	Mampa <i>et al.</i> , 2020 Kose, 2017
<i>Euphorbia gorgonis</i>	Phytosterols, glycosides, triterpenoids, flavonoids, alkaloids, saponins	Mampa <i>et al.</i> , 2020 Tiwani, 2017
<i>Euphorbia bupleurifolia</i>	Phytosterols, tannins, glycosides, triterpenoids, saponins, flavonoids and alkaloids	Mampa <i>et al.</i> , 2020 Van Wyk, 1997
<i>Euphorbia enopla</i>	Phytosterols, glycosides, triterpenoids, flavonoids, alkaloids, tanins, anthraquinone	Mampa <i>et al.</i> , 2020
<i>Euphorbia polygona</i>	Phytosterols, tannins, glycoside, triterpenoids, flavonoids, alkaloids	Mampa <i>et al.</i> , 2020
<i>Euphorbia horrida</i> Var	phytosterols, pentose, tannins, glycosides, triterpenoids, anthraquinones, saponins, flavonoids	Mampa <i>et al.</i> , 2020
<i>Euphorbia arabica</i>	Phytosterols, tannins, glycosides, triterpenoids, anthraquinones, flavonoids	Mampa <i>et al.</i> , 2020
<i>Euphorbia ledienii</i>	No data reported	
<i>Euphorbia ferox</i>	No data reported	
<i>Euphorbia stellata</i>	No data reported	
<i>Euphorbia coerulescens</i>	No data reported	

The breakdown of these compounds into subclasses revealed diverse chemical categories, including Euphane-type Triterpenoids (Euphol, Euphorbol), Cycloartane-type Triterpenoids (Cycloartenol, Cycloartanol, 24-ethylene cycloartanol), Lupane-type Triterpenoids (Lupeol),

Oleanane-type Triterpenoids (α -amyrin, β -amyrin), Pentacyclic Triterpenes (Betulinic acid, Glut-5-en-3- β -ol), Taraxarane-type Triterpenoids (Taraxerol, Taraxerol acetate), Phorbol-type Diterpenoids (12-Deoxyphorbol-13-isobutyrate-20-acetate, Phorbol, 12-Deoxy phorbol, 12-Deoxy-16-hydroxy phorbol, 12-Deoxyphorbol esters, 12, 20-Dideoxyphorbol-13-isobutyrate), Tirucallane-type Triterpenoids (Tirucallicine, Tirucallol, Tirucallin A, Tirucallin B), Steroidal Triterpenoids (Terpenic alcohol), Ingenane-type Triterpenoids (Ingol-7,8,12-acetate, ditiglate, Ingenol, Ingenol triacetate, Angelate acetate isobutyrate), Flavonoids (Trimethyl ellagic acid, 17-Hydroxyingenol-17-benzoate-20-angelate, Kampferol-3-O- β -D-rutinoside), Sterols (β -sitosterol, Stigmasterol, Taraxasterol), Ellagic Acid Derivatives (Ellagic acid, 3,3'-Di-O-methylellagic acid), Medium-chain Fatty Acids (Laurate) and no specific subclass records (Rhoiptlenone, 2-Methylbutyric acid, Euphorbin A, Euphorbin B, Euphorbol hexacosonate, 12-Deoxy-4 β -hydroxyphorbol-13-phenyl acetate-20-acetate, 20-Acetoxy-16-angeloyloxy-13 α -isobutanoyloxy-4 β ,9 α ,20-tetrahydroxytiglic-1,5-diene-3-one, Resin, Arachiside A, 3, 4, 4' Trimethoxyellagic acid, 12-Deoxyphorbol-13-(2-methylbutyrate)-20-acetate, 2-Methylbutyric acid) (see Table 3.4).

E. tirucalli as well as *E. ammak* are among the *Euphorbia* species that received substantial research attention. These findings are in agreement with studies conducted by Mavundza *et al.* (2022), which stated that *E. tirucalli* was among the most studied species.

Table 3.4. Classification of Crude and Isolated Compounds from 15 *Euphorbia* Specie. This table delineates the categorization of both crude extracts and individually isolated compounds obtained from 15 distinct *Euphorbia* species. The compounds are organized based on their respective classes, providing a comprehensive overview of the chemical constituents present in each species.

Compounds	Class	subclass
Euphol (Tetracyclic Triterpene), Cycloartanol (Sterol Lipid), Lupeol, α -amyrin, β -amyrin, Betulinic acid, Taraxerol, Taraxerol acetate, Friedelin, Friedelan-3- β -ol, 3 β -Friedelinol	Terpenoids	Triterpenoids
Cycloartenol, β -sitosterol, 24-Methylenecycloartanol, Tirucallol (Tetracyclic Triterpene), Obtusifoliol, Glutininol, Triterpene euphol (Steroidal Alcohol), 24-Methylene cycloartenol, Ingenol triacetate (Diterpene), Terpenic alcohol (Terpene), Taraxasterol (Phytosterol), Stigmasterol		Phytosterol
12-Deoxyphorbol-13-isobutyrate-20-acetate (Phorbol Ester), Phorbol (Diterpenoid), 12-Deoxyphorbol ester (Diterpenoid), 12-Deoxy-16-hydroxy-phorbol (Component of DHPB), 16-Hydroxy-12-desoxyphorbol (Diterpenoid), 12-Deoxyphorbol-13-isobutyrate-16-angelate-20-acetate (Diterpene), 12-Deoxy phorbol esters (Phorbol Ester), Ingenol (Diterpenoid), Glut-5-en-3- β -ol (Triterpenoid)		Other Terpenoids
Gallic acid (Phenolic Acid), Bervifolin carboxylic acid (Tannin), 3, 3'-Dimethoxy ellagic acid (Tannin), Ellagic acid (Tannin), Kaempferol (Flavonoid), Kampferol-3-O- β -D-rutinoside (Flavonoid), 1-O-Galloyl-3,6-hexahydroxydiphenyl- β -D-glucopyranoside/corilagin (Tannin), Tri-methyl ellagic acid (Tannin), 3'-Di-O-methylellagic acid (Phenol)	Phenolic	
Laurate	Fatty acids	
Diterpene esters	Miscellaneous	
Flavonoids		Polyphenolic compounds
Alkaloids		Organic compounds
Saponins		glycosides
Tannins		Polyphenols
Glycosides		Acetal Derivatives of Monosaccharides

Anthraquinones		Phenolics
----------------	--	-----------

The investigation into the chemical composition of various *Euphorbia* species is presented in Table 3.4, and has revealed a diverse array of isolated compounds, each showcasing unique pharmacological and biological activities. Euphol, classified within the Euphane subclass, demonstrates a diverse profile, including anticancer, cytotoxicity, anti-nociceptive, anti-inflammatory and HIV-1 reverse transcriptase inhibitor activities (Silva *et al.*, 2019 Lin *et al.*, 2012 Yasukawa *et al.*, 2000). The Cycloartenol and Cycloartanol compounds, falling under the Cycloartane subclass, exhibit a wide range of effects such as anti-inflammatory, antitumor, antioxidant, antibiosis, anti-Alzheimer's disease, apoptotic, analgesic and antifungal activities (Heliawati *et al.*, 2023; Salome-Abarca *et al.*, 2020; Sawale *et al.*, 2019; Niu *et al.*, 2018; Zhang *et al.*, 2017; Zare *et al.*, 2015; Zare *et al.*, 2014; Barla *et al.*, 2006). Lupeol, belonging to the Lupane subclass, displays diverse properties like anticancer, anti-inflammatory, antimicrobial, antiprotozoal, antiproliferative, antiangiogenic and cholesterol-lowering effects (Zhang *et al.*, 2015; Gupta *et al.*, 2013; Kumari & Kakkar, 2012; Borgati *et al.*, 2012; Siddique & Saleem, 2011; Gallo & Sarachine, 2009; Sudhahar *et al.*, 2007; You *et al.*, 2003). Oleanane-type triterpenoids, represented by α -amyrin and β -amyrin, demonstrate activities such as cytotoxicity, antifungal, anti-inflammatory, nitric oxide inhibition, reactive oxygen species activation and anticancer effects (Wen *et al.*, 2018; Lin *et al.*, 2011; Jabeen *et al.*, 2011; Shih *et al.*, 2010; Singh *et al.*, 2009; Singh *et al.*, 2009; Abdel-Monem *et al.*, 2008 Johann *et al.*, 2007; Vazquez *et al.*, 1999). Pentacyclic triterpenes, including Betulinic acid, exhibit antitumor, antidiabetic, anti-inflammatory, HIV-1 reverse transcriptase inhibition, antiviral and hepatoprotective activities (Silva *et al.*, 2016; Foo *et al.*, 2015; Damle *et al.*, 2013; Esposito *et al.*, 2013; Mertens-Talcott *et al.*, 2013; Kumar *et al.*, 2013; Alakurtti *et al.*, 2006; Aiken & Chen, 2005; Bernard *et al.*, 2001; Selzer *et al.*, 2000). The Taraxarane subclass, represented

by compounds like Taraxerol and Taraxerol acetate, showcases properties like anticancer, anti-inflammatory, apoptotic, antioxidative, antimicrobial, antifungal and antidiabetic effects (ur Rahman *et al.*, 2016; Rehman *et al.*, 2013; Tan *et al.*, 2011; Biswas *et al.*, 2009; Singh *et al.*, 2002; Takasaki *et al.*, 1999). Sterols, represented by β -sitosterol, display a spectrum of activities, including anti-inflammatory, anticancer, antiproliferative, analgesic and antimicrobial effects (Cheng *et al.*, 2015; Nirmal *et al.*, 2012; Loizou *et al.*, 2010; El-Fiky *et al.*, 2008; Vazquez *et al.*, 1999). Tirucallane-type triterpenoids, such as Tirucalicine and Tirucallol, currently have no specific records of biological activity (Fernandez-Arche *et al.*, 2010; Akihisa *et al.*, 2001). Terpenic alcohol, a steroidal triterpenoid, demonstrates antibacterial and irritant effects (Mali and Panchal, 2017b; Inoue *et al.*, 2004). Ellagic Acid Derivatives, including Tri-methyl ellagic acid, exhibit anticancer properties (Wardana *et al.*, 2022; Mali & Panchal, 2017b; Zhang *et al.*, 2008). Ingenane-type Triterpenoids, represented by Ingenol and its derivatives, showcase cytotoxicity and HIV-1 reverse transcriptase inhibition (Silva *et al.*, 2019; Abreu *et al.*, 2014). Various subclasses such as Taraxarane, Euphane-type, Sterol, Tirucallane and Phorbol-type exhibit diverse pharmacological activities, including anti-inflammatory, antifungal, antibacterial, hepatoprotective, antioxidant, antiproliferative and anti-HIV effects, see Table 3.5.

Table 3.5. Bioactive compounds isolated from 15 *Euphorbia* species alongside their biological activities and subclasses. This table provides an in-depth exploration of bioactive compounds isolated from 15 distinct *Euphorbia* species, highlighting their diverse biological activities.

Compounds	Subclasses	Pharmacological/ biological activity	Reference
Euphol	Euphane	Anticancer Cytotoxicity Anti-nociceptive Antiinflammatory HIV-1 reverse transcriptase inhibitor	Silva <i>et al.</i> , 2019 Lin <i>et al.</i> , 2012 Yasukawa <i>et al.</i> , 2000
Cycloartenol	Cycloartane	Antiinflammatory Antitumor Antioxidant Antibiosis Anti-alzheimer's disease Apoptotic Analgesic Bactericidal	Zare <i>et al.</i> , 2015 Zare <i>et al.</i> , 2014 Zhang <i>et al.</i> , 2017 Niu <i>et al.</i> , 2018 Sawale <i>et al.</i> , 2019
Cycloartanol	Cycloartane	Antifungal Vasodepressor Antitumor	Salome-Abarca <i>et al.</i> , 2020 Barla <i>et al.</i> , 2006 Heliawati <i>et al.</i> , 2023
Lupeol	Lupane	Anticancer Antiinflammatory Antimicrobial Antiprotozoal Antiproliferative	Gallo & Sarachine, 2009 Siddique & Saleem, 2011 You <i>et al.</i> , 2003 Zhang <i>et al.</i> , 2015 Kumari & Kakkar, 2012



		Antiangiogenic Anti-invasive Cholesterol lowering	Borgati <i>et al.</i> , 2012 Gupta <i>et al.</i> , 2013 Sudhahar <i>et al.</i> , 2007
α amyrin	Oleanane	Cytotoxicity Antifungal Antiinflammatory	Abdel-Monem <i>et al.</i> , 2008 Johann <i>et al.</i> , 2007 Singh <i>et al.</i> , 2009 Jabeen <i>et al.</i> , 2011
β -amyrin	Oleanane	Antiinflammatory Nitric oxide inhibitor Reactive oxygen species activator Anticancer	National Center for Biotechnology Information, 2023 Singh <i>et al.</i> , 2009 Vazquez <i>et al.</i> , 1999 Shih <i>et al.</i> , 2010 Wen <i>et al.</i> , 2018 Lin <i>et al.</i> , 2011
Betulinic acid	Pentacyclic triterpenes	Antitumor Antidiabetic Antiinflammatory HIV-1 reverse transcriptase inhibitor Antiviral Hepatoprotective activity	Damle <i>et al.</i> , 2013 Esposito <i>et al.</i> , 2013 Aiken & Chen, 2005 Foo <i>et al.</i> , 2015 Selzer <i>et al.</i> , 2000 Mertens-Talcott <i>et al.</i> , 2013 Kumar <i>et al.</i> , 2013 Silva <i>et al.</i> , 2016 Bernard <i>et al.</i> , 2001 Alakurtti <i>et al.</i> , 2006



Taraxerol	Taraxarane	Anticancer Antiinflammatory Apoptotic Anti-oxidative activity Antimicrobial Antifungal Antidiabetic	Takasaki <i>et al.</i> , 1999 Biswas <i>et al.</i> , 2009 Tan <i>et al.</i> , 2011 Singh <i>et al.</i> , 2002
β -sitosterol	Sterols	Antiinflammatory Anticancer Antiproliferation Analgesic Antimicrobial	Vazquez <i>et al.</i> , 1999 Loizou <i>et al.</i> , 2010 Cheng <i>et al.</i> , 2015 Nirmal <i>et al.</i> , 2012 El-Fiky <i>et al.</i> , 2008
Taraxerol acetate	Taraxarane	Antiinflammatory Cyclooxygenases inhibitor	ur Rahman <i>et al.</i> , 2016 Rehman <i>et al.</i> , 2013
Friedelin	Friedelane	Cytotoxicity Antibacterial	Chen <i>et al.</i> , 2015 Abdul-Hammed <i>et al.</i> , 2023
Friedelan 3 α - and 3 $\beta\alpha$ -ols	Friedelane	Anticancer	Abdul-Hammed <i>et al.</i> , 2023
24-ethylene cycloartanol	Cycloartane	Antiinflammatory Antifungal	Vazquez <i>et al.</i> , 1999 Krstić <i>et al.</i> , 2016
Epi-friedelinyl acetate	Friedelane		Anju & Rameshkumar, 2022
3 β -friedelinol	Friedelane	Antibacterial Cytotoxicity	Ogunnusi <i>et al.</i> , 2010 Anju & Rameshkumar, 2022 Sousa <i>et al.</i> , 2014 Yang <i>et al.</i> , 2017
Rhoiptlenone	No data reported	No data reported	Anju & Rameshkumar, 2022
Isobutyric	Carboxylic acid	irritant	Sosath <i>et al.</i> , 1988



			Redei <i>et al.</i> , 2010
2-Methylbutyric acid	No data reported	No data reported	Redei <i>et al.</i> , 2010
12-Deoxyphorbol-13-isobutyrate-20-acetate	Phorbol	Antifungal	Ourhizif <i>et al.</i> , 2022
12-Deoxyphorbol-13-(2-methylbutyrate)-20-acetate	Phorbol	No data reported	Popplewell <i>et al.</i> , 2010
Phorbol	Phorbol	Tumor promoter Apoptosis	Fürstenberger & Hecker, 1985 Brodie & Blumberg, 2003
12-Deoxy phorbol	Phorbol	Antitumor Apoptotic	Zayed <i>et al.</i> , 1984 Shen <i>et al.</i> , 2015 Alotaibi <i>et al.</i> , 2018 Tsai <i>et al.</i> , 2020
12-Deoxy-16-hydroxy phorbol	Phorbol	Irritant Tumor promoter	Hirota <i>et al.</i> , 1988
Ingol-7,8,12-acetate,ditiglate	Ingenane	No data reported	Sosath <i>et al.</i> , 1988
Triterpenes euphol	Glycosides	Anticancer Antiinflammatory	de Souza <i>et al.</i> , 2019 Dutra <i>et al.</i> , 2011 Biesboer & Mahlberg, 1979
Diterpene esters	Terpenoids	Anticancer Cytotoxicity Tumor promoter Irritant Pro-inflammatory	Wu <i>et al.</i> , 1991 Fatope <i>et al.</i> , 1996 Fürstenberger & Hecker, 1985 Khan & Malik, 1990 Rizk <i>et al.</i> , 1985



12-Deoxyphorbol esters	Phorbol	Irritant Pro-inflammatory Tumor promoter	Kinghorn & Kinghorn, 1979 Driedger & Blumberg, 1980 Priya & Rao, 2011 Adolf & Hecker, 1984
Ingenol	Ingenane	Cytotoxicity HIV- reverse transcriptase Inhibitor	Abreu <i>et al.</i> , 2014 Silva <i>et al.</i> , 2019
Euphorbol hexacosonate	No data reported	No data reported	Mali & Panchal, 2017b
12-Deoxy-4 β -hydroxyphorbol-13-phenyl acetate-20-acetate	No data reported	No data reported	Mali & Panchal, 2017b
12, 20-Dideoxyphorbol-13-isobutyrate	Phorbol	No data reported	Mali & Panchal, 2017b
Glut-5-en-3- β -ol	Pentacyclic triterpene	Antibacterial	Abdel-Monem & Abdelrahman, 2016
Tirucalidine	Tirucallane	No data reported	Khan, 2010 Khan <i>et al.</i> , 1988 Mali & Panchal, 2017b Wal <i>et al.</i> , 2013
Tri-methyl ellagic acid	Ellagic Acid Derivatives	Anticancer	Mali & Panchal, 2017b Wardana <i>et al.</i> , 2022 Zhang <i>et al.</i> , 2008
Terpenic alcohol	Steroidal Triterpenoids	Antibacterial Irritant	Inoue <i>et al.</i> , 2004 Mali & Panchal, 2017b
Isoeuphorol	Ingenane-type Triterpenoids	No data reported	Mali & Panchal, 2017b
Taraxasterol	Sterol	Inhibition of tumor promotion Antiproliferation	Ovesnaâ <i>et al.</i> , 2004



Tirucallol	Tirucallane	Antiinflammatory HIV-Inhibition reverse transcriptase	Fernandez-Arche <i>et al.</i> , 2010 Akihisa <i>et al.</i> , 2001
Ketone euphorone	Euphane-type Triterpenoids	No data reported	Upadhyay <i>et al.</i> , 2010
Resin	No data reported	Digestive enzyme Antioxidant Antispasmodic Hypotensive Hepatoprotective Antiviral Antifungal Anticancer Anxiolytics Anthelmintic	Upadhyay, 2017
Ellagic acid	Ellagic Acid Derivatives	Hepatoprotective activity Antiproliferative activity Antioxidant	Shaimaa, 2015
3,3'-Di-O- methylellagic acid	Ellagic Acid Derivatives	Antioxidant Moderate antibacterial activity Antimicrobial activity Anticancer	Herawati & Firdaus, 2013 Vigbedor <i>et al.</i> , 2022 Aljubiri <i>et al.</i> , 2021 Guo <i>et al.</i> , 2011
Euphorbin A	No data reported	No data reported	Yoshida <i>et al.</i> , 1988
Euphorbin B	No data reported	No data reported	Yoshida <i>et al.</i> , 1988
Tirucallin A	Tirucallane	No data reported	Yoshida <i>et al.</i> , 1991
Tirucallin B	Tirucallane	No data reported	Yoshida <i>et al.</i> , 1991



Euphorbol	Euphane-type Triterpenoids	Antibacterial Antiinflammatory	Abdel-Sattar <i>et al.</i> , 2015 Mazoir <i>et al.</i> , 2011
24-Methylenecycloart enol	Cycloartane	Anti-oxidant Antiinflammatory	National Center for Biotechnology Information, 2023 Sotiroudis <i>et al.</i> , 2010 Ekpo and Pretorius, 2007 Martinez-Vazquez M <i>et al.</i> , 1999
Ingenol triacetate	Ingenane-type Triterpenoids	Antimicrobial Antitumor	Sultana <i>et al.</i> , 2022 Tilabi and Upadhyay, 1983 National Center for Biotechnology Information, 2023
Angelate acetate isobutyrate	Ingenane-type Triterpenoids	No data reported	Evans, 1978
Fatty acids	No data reported	No data reported	Evans, 1978
Acetate laurate	No data reported	No data reported	Evans, 1978
α -Methyl butyrate	No data reported	No data reported	Evans, 1978
Heptanoate	No data reported	No data reported	Evans, 1978
Laurate	Medium-chain fatty acids	Antibacterial	Matsue <i>et al.</i> , 2019 Evans, 1978
α -glutanol	Pentacyclic triterpenes	Ant-proliferation Cytotoxicity	Chen & Li, 2021 Abdel-Sattar <i>et al.</i> , 2015 Ding <i>et al.</i> , 2010
Stigmasterol	Phytosterol	Cytotoxicity Antioxidant Hypoglycemic Thyroid inhibitor	Abdel-Sattar <i>et al.</i> , 2015 Panda <i>et al.</i> , 2009
16-Hydroxy-12-	Phorbol	Antitumor	Dinala <i>et al.</i> , 2022



desoxyphorbol		Tumor promoter	Gschwendt & Hecker, 1970
Obtusifoliol	Lanostane	Cytotoxicity	Hlengwa, 2018 El-sherei <i>et al.</i> , 2015 Ahmed, 2015 Aghaei <i>et al.</i> , 2016
12-Deoxyphorbol-13-isobutyrate-16-angelate-20-acetate	Phorbol-type diterpenoid	Cytotoxicity	Hlengwa, 2018 El-sherei <i>et al.</i> , 2015 Ahmed, 2015
Euphorbilactone	Ingenane-type Triterpenoids	No data reported	Hlengwa, 2018
Norsesquiterpenoid	No data reported	No data reported	Hlengwa, 2018
Arachiside A	Ingenane-type Triterpenoids	No data reported	Hlengwa, 2018
Glutinol	Pentacyclic triterpenes	Antiproliferation	Chen & Li, 2021
16-Angeloyloxy-13 α -isobutanoyloxy-4 β ,9 α ,20-trihydroxytiglia-1,5-diene-3,7-dione	No data reported	No data reported	Hlengwa, 2018
20-Acetoxy-16-angeloyloxy13 α -isobutanoyloxy-4 β ,9 α ,20-tetrahydroxytiglia-1,5-diene-3-one	No data reported	No data reported	Hlengwa, 2018
Gallic acid	Gallic Acid Derivatives	Hepatoprotective activity Antioxidant	Ahmed <i>et al.</i> , 2019
Bervifolin carboxylic acid	Gallic Acid Derivatives	Hepatoprotective activity	Ahmed <i>et al.</i> , 2019



		Antioxidant	
Kampferol-3-O- β -D-rutinoside	Flavonoids	Hepatoprotective activity Antioxidant	Ahmed <i>et al.</i> , 2019
1-O-Galloyl-3,6-hexahydroxydiphenyl- β -D-glucopyranoside	Gallic Derivatives Acid	Antitumor Antiinflammation Antioxidant Hepatoprotective activity Antimicrobial Antihypertensive Antidiabetic Anti-HIV Antifungal	Lipinska <i>et al.</i> , 2014 Tong <i>et al.</i> , 2018 Zheng <i>et al.</i> , 2016 Ming <i>et al.</i> , 2013 Bai <i>et al.</i> , 2019 Kolodziej <i>et al.</i> , 2005 Tang <i>et al.</i> , 2019 Jin <i>et al.</i> , 2013 Miyasaki <i>et al.</i> , 2013 Cheng <i>et al.</i> , 1995 Ahmed, 2015
3, 3' Dimethoxy ellagic acid	Ellagic Derivatives Acid	Hepatoprotective activity Antioxidant	Ahmed <i>et al.</i> , 2019
3, 4, 4' Trimethoxy ellagic acid	Ellagic Derivatives Acid	Hepatoprotective activity Antioxidant	Ahmed <i>et al.</i> , 2019
Kaempferol	Flavonoids	Hepatoprotective activity Antiproliferative activity Antioxidant	Ahmed, 2015
17-Hydroxyingenol-17-benzoate-20-angelate	Flavonoids	No data reported	El-Hawary <i>et al.</i> , 2020

E. trigona has the most isolated anticancer compounds (14), followed by *E. tirucalli* (13), *E. cooperi* (8), and the others have fewer or none. The *Euphorbia* species found in Southern Africa are rich sources of various types of bioactive compounds, including triterpenoids, phorbol esters, alkaloids, flavonoids, phytosterols, glycosides and saponins (Mavundza *et al.*, 2022; Mampa *et al.*, 2020; Tiwani *et al.*, 2017). Furthermore, minor classes that were isolated from *Euphorbia* species from Southern Africa are: anthraquinone, polyphenols and tannins (Mavundza *et al.*, 2022; Mampa *et al.*, 2020; Tiwani *et al.*, 2017).

It has further been reported that most spurge contain an acidic and burning vesicant juice, as well as cyanoglycosides, which can be toxic (Al-Sultan & Hussein, 2006). Reports have shown that ingestion of a large quantity of the latex may cause gastro-intestinal haemorrhage and even result in death (Voigt, 2007). Although incidents of poisoning in children and animals are rare, it is important to handle these plants with great caution (Rizk, 1987). The latex from these plants can also cause blisters on the skin and temporary blindness (Watt & Breyer-Brandwijk, 1962). Furthermore, they have been used as fish poison and bird-lime; see Table 3.6.

Table 3.6. Type of toxicity caused by some *Euphorbia* species

Type of toxicity	<i>Euphorbia</i> species	References
Fish poisoning	<i>Euphorbia scheffleri</i> Pax, <i>Euphorbia tirucalli</i> L., and <i>Euphorbia inaequilatera</i> Sond	Rizk, 1987
Human poisoning	<i>Euphorbia ledienii</i> A. Berger, <i>Euphorbia heterophylla</i> L., <i>Euphorbia cooperi</i> N.E.Br. ex A. Berger, <i>Euphorbia candelabrum</i> Kotschy, <i>Euphorbia virosa</i> Willd., <i>Euphorbia poissonii</i> Pax, <i>Euphorbia unispina</i> N.E.Br. and <i>Euphorbia venenifica</i> Tremaux ex Kotschy;	Rizk, 1987

Domestic animals poisoning	<i>Euphorbia caput-medusae</i> L., <i>Euphorbia silenifolia</i> (Haworth) Sweet, <i>Euphorbia ingens</i> E. Mey. Ex Boiss; as well as irritating ones: <i>E. tirucalli</i> , <i>Euphorbia poissonii</i> , <i>Euphorbia unispina</i> and <i>E. venenifica</i> . I	Rizk, 1987
Carcinogen/ promotor of cell division	<i>E. tirucalli</i> , <i>Euphorbia leuconeura</i> , <i>J. curcas</i>	Hirota & Suttajit, 1988 Van Damme, 2001 Vogg <i>et al.</i> , 1999
Conjunctivitis	<i>E. tirucalli</i> and <i>Euphorbia royleana</i>	Shlamovitz <i>et al.</i> , 2009 Van Damme, 1989

Taxonomic classification of all *Euphorbia* plants discussed in this review:

Domain: Eukaryote; Kingdom: Plantae; Order: Malpighiales; Family: *Euphorbiaceae*; Genus: *Euphorbia* (<https://www.mindat.org/taxon-4691.html>)

3.4. Discussion

3.4.1. Ethnopharmacological use, phytochemistry and toxicity

3.4.1.1 *Euphorbia trigona*

E. trigona, a plant native to Central Africa, tropical Africa and India has been used in traditional Indian Ayurvedic medicine to treat respiratory and urinary tract infections and gonorrhea; Figure 3.1 (Marathe *et al.*, 2019). Studies by Nashikkar *et al.* (2012) have also shown that *E. trigona* is effective in treating various ailments, such as tumors, warts, intestinal parasites, rheumatoid arthritis, hepatitis and inflammation. A combination of the roots and ginger can be consumed in the morning to alleviate piles (Siddique *et al.*, 2010).

Additionally, some people take latex drops in palm wine for severe constipation or during an epileptic attack (Bouquet, 1969).



Figure 3.1. *Euphorbia trigona* tree and pot plant (source: <http://tropical.theferns.info/image.php?id=Euphorbia+trigona>, <https://laidbackgardener.blog/2018/12/09/when-a-red-euphorbia-turns-green/>)

Phytochemistry

The plant *E. trigona* was analyzed for its phytochemical composition and was found to contain saponins, alkaloids, flavonoids, glycosides, sterols and triterpenoids (Boshara, 2014). Additional studies by Nashikkar *et al.* (2012) also identified the presence of sterols, alkaloids, flavonoids and saponins, as well as tannins that were not detected by Boshara (2014). The absence of tannins may be due to environmental factors. Nielsen *et al.* (1977) found that the latex of *E. trigona* contains a high level of sterols, with the main components being euphol

and cycloartenol. Anjaneyulu and Rao (1985) isolated several triterpenoids from the latex, including euphol, cycloartanol, cycloartenol, lupeol, α -amyrin and β -amyrin. They also identified five diterpenes ester skin-irritants. *E. trigona* is also a good source of lectin, which has been shown to have a potency in human erythrocyte agglutination (Lynn & Clevette-Radford, 1986).

Toxicity

A study, conducted by EL-Hawary *et al.* in 2020, tested the cytotoxic effects of a methanolic extract of *E. trigona* against HEPG2, MCF-7 and CACO2 cell lines. The findings revealed a pronounced cytotoxic effect on MCF-7 and Caco-2 cell lines, with IC50 values of 16.1 and 15.6 $\mu\text{g/mL}$, respectively. In 2022, Anju and Rameshkumar evaluated a methanol extract's cytotoxic effect on HeLa and H9C2 cell lines but found no notable cytotoxic effect on either cell line. Another study evaluated the cytotoxicity effect of Hex, DCM, MeOH and EtoAc extract against Vero cell lines (Mampa *et al.*, 2020). The results indicated that none of the four extratcs from *E. trigona* exhibited cytotoxicity, as they did not hinder 50% of the cell growth at concentrations of 10 $\mu\text{g/mL}$ and below.

3.4.1.2 *Euphorbia ledienii*

E. ledienii is a plant species that is indigenous to the Western Cape, South Africa (see Figure 3.2). The plant does not have any traditional or pharmacological data.



Figure 3.2. *Euphorbia ledienii* plant with stem and flowers (source: <https://www.agaveville.org/viewtopic.php?f=54anddt=3571>, http://www.llifile.com/Encyclopedia/SUCCULENTS/Family/Euphorbiaceae/14292/Euphorbia_ledienii)

Phytochemistry

Despite a lack of traditional or pharmacological data, several compounds have been identified from the plant, including 12-Deoxyphorbol-13-isobutyrate-20-acetate and 12-Deoxyphorbol-13-(2-methylbutyrate)-20-acetate (Poppewell *et al.*, 2010). Evans and Kinghorn (1977) discovered three variations of taglines which are phorbol, 12-Deoxyphorbol, and 12-Deoxy-16-hydroxy phorbol. *E. ledienii* has been reported to contain Ingol-7,8,12-acetate and ditiglate (Sosath *et al.*, 1988). Redei *et al.* (2010) identified Isobutyric and 2-methylbutyric acid. Other researchers discovered several hydrolytic proteins in *E. ledienii*, including N- acetyl-b-glucosamidase, chitobiosidase, endochitinase, and lysozyme activity (Sytwala *et al.*, 2015). According to Domsalla *et al.* (2010), the *E. ledienii* plant has been found to have high proteolytic activity.

Toxicity

It is crucial to highlight that the plant is extremely toxic to humans, leading to potential skin irritation (Abdel-Fattah, 1987). It is important to note that none of the isolated compounds have undergone toxicity assessments to date.

3.4.1.3 *Euphorbia horrida*

E. horrida is a plant species that can be found in Wittepoort/Karoo, South Africa, Figure 3.3.

There is currently no known traditional or pharmacological information about this plant.

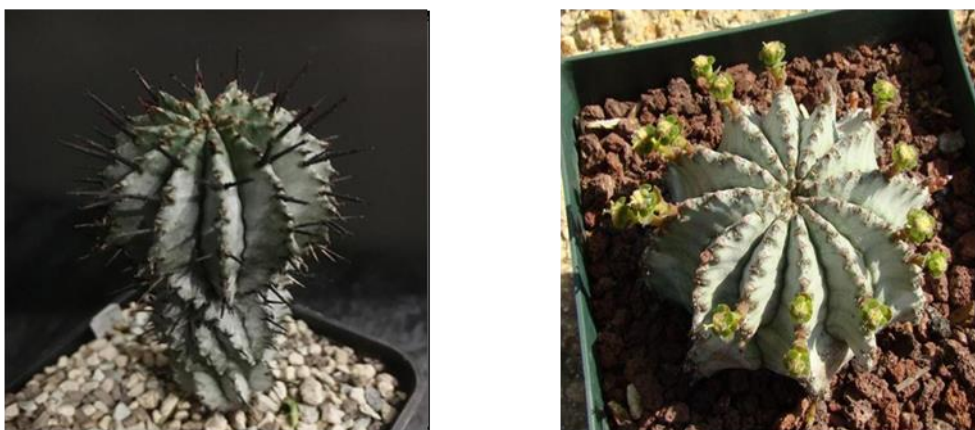


Figure 3.3. *Euphorbia horrida* plant with stem and flowers (source: <http://pza.sanbi.org/euphorbia-horrida>)

Phytochemistry

In a study by El-Hawary *et al.* (2020), 17-Hydroxyingenol-17-benzoate-20-angelate from the plant was isolated. The plant also contains diterpene esters that can cause skin irritation. In addition, Mampa *et al.* (2020) found various other classes of compounds in the plant, including phytosterols, pentose, tannins, glycosides, triterpenoids, anthraquinones, saponins, flavonoids and alkaloids.

Toxicity

The cytotoxic effect of dichloromethane (DCM) extract derived from *E. horrida* var. was evaluated on a Vero cell line. The results demonstrated notable cytotoxicity, as evidenced by its achievement of an IC₅₀ value at a concentration of 10 µg/mL, highlighting its potential cytotoxic effects (Mampa *et al.*, 2020). A study conducted by El-Hawary *et al.* (2020) evaluated the cytotoxicity effect of methanol extract of *E. horrida* against HeLa and H9C2 cell lines and no significant effect was observed.

3.4.1.4 *Euphorbia enopla*

E. enopla, a plant that is native to the Eastern Cape and semi-arid Karoo, South Africa, has no documented traditional or pharmacological data (see Figure 3.4).



Figure 3.4. *Euphorbia enopla* plant with stem and flowers (source: https://www.cactus-art.biz/schede/EUPHORBIA/Euphorbia_enopla/Euphorbia_enopla/Euphorbia_enopla.htm)

Phytochemistry

The plant contains euphol and tirucallol, which were isolated by Ponsinet and Ourisson. (1968). Furthermore, Mampa *et al.* (2020) extracted phytosterols, glycosides, triterpenoids, flavonoids, alkaloids, tanins and anthraquinone. Sytwala *et al.* (2015) isolated N-acetyl-b- glucosamidase, chitobiosidase, endochitinase and lysozyme hydrolytic proteins.

Toxicity

A study conducted by Mampa *et al.* (2020) assessed the toxicity of the hexane extract of *E. enopla* on the Vero cell line. The results indicated that the extract had a substantial inhibitory effect on cell growth, particularly at a concentration of 10 µg/mL. Notably, the highly non-polar hexane fraction exhibited the most potent effects (Mampa *et al.*, 2020).

3.4.1.5 *Euphorbia coerulescens*

E. coerulescens which is native to the Cape province, South Africa, has no documented traditional or pharmacological data (see Figure 3.5). However, it has a number of compounds isolated from it.



Figure 3.5. *Euphorbia coerulescences* plant with stem and flowers (source: [https://www.ebay.com.au/itm/euphorbia-coerulescens-POT-10 CM /233323039902?nma=true&si=DenzQanzHzMK8eHcDW6MHzA0s9s%253D&orig_cvip=true&nordt=true&rt=nc&_trksid=p2047675.l2557](https://www.ebay.com.au/itm/euphorbia-coerulescens-POT-10-CM-/233323039902?nma=true&si=DenzQanzHzMK8eHcDW6MHzA0s9s%253D&orig_cvip=true&nordt=true&rt=nc&_trksid=p2047675.l2557))

Phytochemistry

Studies by Evans (1978) resulted in the isolation of angelate acetate isobutyrate, acetate α -methyl butyrate, acetate laurate, α -Methyl butyrate, heptanoate and laurate. In other studies, euphol, tirucallol and euphorbol were also isolated (Nielsen *et al.*, 1979). In addition, Sytwala *et al.* (2015) isolated several hydrolytic proteins, including N-acetyl-b-glucosamidase, chitobiosidase, endochitinase and lysozyme activity. Lynn and Clevette-Radford (1986) also isolated homogeneous lectins.

Toxicity

During an irritancy test conducted by Evans in 1978, it was observed that the latex of *E. coerulescences* induced ear inflammation in mice. Furthermore, this latex has the potential to cause skin irritation and, if ingested, may result in a burning sensation in the throat. Direct contact with the eyes can lead to severe consequences, including the risk of blindness. It is worth noting that none of the extracted compounds have been subjected to toxicity evaluations at this time.

3.4.1.6 *Euphorbia cooperi*

E. cooperi is a plant that grows naturally in KwaZulu Natal and Limpopo province, South Africa (see Figure 3.6). Research has shown that it may have potential in treating various health conditions. It has been reported that the liquid from the soaked roots and stem has been used as an enema for sore stomachs and bloatedness (Hedberg & Staugård, 1989). In South Africa, the Venda tribe uses this plant to cure paralysis and apply it to infected wounds (Hedberg &

Staugård, 1989). Farmers have historically used this plant to treat various bacterial infections in their livestock (Luseba & Van Der Merwe, 2006; Arnold & Gulumian, 1984). Furthermore, the latex is utilized for poisoning fish in South Africa's Limpopo province (Hargreaves, 1991; Watt & Breyer-Brandwijk, 1962).



Figure 3.6. *Euphorbia cooperi* tree and fruits (source: <http://pza.sanbi.org/euphorbia-cooperi>)

Phytochemistry

Phytochemical analysis of *E. cooperi* revealed that the latex of *E. cooperi* comprises of numerous diesters and triesters (Gschwendt & Hecker, 1970). The chloroform fraction of the plant's latex was found to contain three compounds that had never been isolated before. These were euphol, obtusifoliol and 12-Deoxyphorbol-13-isobutyrate-16-angelate-20-acetate, which belong to the triterpene, steroid and diterpenoid families, respectively (El-Sherei *et al.*, 2015). A study by El-Toumy *et al.* (2018) isolated 7-galloyl catechin, kaempferol 3-*O*- β -(6'-*O*-galloyl)-glucopyranoside and triesters-16-hydroxy-12-desoxy-phorbol from the flower of *E. cooperi*.

A study of the aerial part of *E. cooperi* has uncovered some interesting findings. Hlengwa

(2018) has discovered a unique norsesquiterpenoid called euphorbilactone, as well as its glycoside, arachiside A. The researcher further identified a triterpenoid, glutinol, a known phorbol ester (16-Angeloyloxy-13 α -isobutanoyloxy-4 β ,9 α ,20-trihydroxytiglic-1,5-diene-3,7-dione) and a new phorbol esters (20-Acetoxy-16-angeloyloxy-13 α -isobutanoyloxy-4 β ,9 α ,20-tetrahydroxytiglic-1,5-diene-3-one). Upon a thorough review of existing literature, it has come to light that certain compounds such as Euphol, Obtusifoliol, 12-Deoxyphorbol-13-isobutyrate-16-angelate-20-acetate, Euphorbilactone, Norsesquiterpenoid, Arachiside A, Glutinol, 16-Angeloyloxy-13 α -isobutanoyloxy-4 β ,9 α ,20- trihydroxytiglic-1,5-diene-3,7-dione, 20-Acetoxy-16-angeloyloxy-13 α -isobutanoyloxy-4 β ,9 α ,20-tetrahydroxytiglic-1,5-diene-3-one, Bervifolin, carboxylic acid, Kampferol-3-O- β -D-rutinoside, 1-O-Galloyl-3,6-hexahydroxydiphenyl- β -D glucopyranoside, 3, 3' Dimethoxy ellagic acid and 3, 4, 4' Trimethoxyellagic acid have not been previously discussed.

Toxicity

A study conducted by El-Sherei *et al.* in 2015 evaluated the cytotoxic effect of the chloroform extract of *E. Cooperi*. Their findings revealed that the chloroform extract demonstrated significant cytotoxic effects against MCF-7, HepG2 and HeLa cell lines. The IC₅₀ values for these cell lines were 4.23, 10.80 and 26.6, respectively. The results align with Mavundza *et al.*'s findings (2022), as no further research has been conducted to date.

3.4.1.7 *Euphorbia tirucalli*

E. tirucalli is a plant that is indigenous to Eastern tropical Africa, South Africa and the Indian Ocean Island (see Figure 3.7). It has been proven to be effective in treating various illnesses. Hargreaves (1991) stated that *E. tirucalli* is used to induce vomiting for treating snakebites. Other authors have reported the use of its latex for various purposes such as treating sexual

impotence, skin disorders, swollen glands, edema, haemorrhoids, rheumatoid arthritis, epilepsy, tooth and ear pain, and tumor (Waczuk *et al.*, 2015; Van Damme, 1989; Gupta *et al.*, 2013). In addition, the latex has pharmacological properties that include antibacterial, molluscicidal, antiherpetic and antimutagenic effects (Gupta *et al.*, 2013; Wal *et al.*, 2013; Jurberg *et al.*, 1985; Lirio *et al.*, 1998; Betancur-Galvis *et al.*, 2002; Tiwari *et al.*, 2003; Rezende *et al.*, 2004; Parekh & Chanda, 2007; Altamimi *et al.*, 2019). Studies have demonstrated that extracts from *E. tirucalli* have myelomodulating activity and suppress the formation of colonies (Valadares *et al.*, 2006). Additionally, the latex of the plant contains compounds that have antitumor effects on various cell lines (Viviane *et al.*, 2019; de Souza *et al.*, 2019). In fact, *E. tirucalli* has been patented as a possible medicinal treatment for prostate cancer (Mwine & Damme, 2011).



Figure 3.7. *Euphorbia tirucalli* plant and flower (source: <https://za.pinterest.com/pin/298785756510953587/>; <https://za.pinterest.com/pin/474426141998696380/>)

Phytochemistry

Extensive studies have analyzed the chemical makeup of *E. tirucalli* and diterpenes have been

identified as the primary isolated compound in all parts of the plant (Mavundza *et al.*, 2022).

The latex of *E. tirucalli* contains several phytoconstituents, including triterpenes euphol, diterpene esters of phorbol, 12-Deoxyphorbol esters and ingenol, β -sitosterol, euphorbol hexacosonate, 12-Deoxy-4 β -hydroxyphorbol-13-phenyl acetate-20-acetate, 12, 20-Dideoxyphorbol-13-isobutyrate, glut-5-en-3- β - and euphol, 12-O-2Z-4E-octadienoyl-4-deoxyphorbol-13-acetate, cycloart-23-ene-3- β -, 25-diol, Euphorcinol, 4-Deoxyphorbol diester, cycloeuphordenol, cyclotirucanenol, diterpene ester, serine proteases, euphol, steroids, tirucallicine, tri-methyl ellagic acid, terpenic alcohol, isoeuphorol, taraxasterol, tirucallol (fresh latex), ketone euphorone and resin (Martins *et al.*, 2020; Biesboer & Mahlberg, 1979; Nielsen *et al.*, 1979; Kinghorn, 1979; Fürstenberger & Hecker, 1977; Yamamoto *et al.*, 1981; Baslas & Gupta, 1984; Lynn & Clevette-Radford, 1985; Khan *et al.*, 1988a; Khan *et al.*, 1988b; Khan & Malik, 1990; Khan *et al.*, 1987, Khan *et al.*, 1989; Cataluna & Rates, 1997; Lin *et al.*, 2012; Dutra *et al.*, 2012). Researchers have identified several compounds in the stem of *E. tirucalli*, including ellagic acid, taraxerol, 3,3'-Di-O-methylellagic acid, β - sitosterol, euphorbin A (a type of polyphenol), euphorbin F (dimers), tirucallin A (a type of tannin) and tirucallin B. (Gupta & Mahadevan, 1967; Khan & Malik, 1990; Yoshida *et al.*, 1991). Euphorbiane which is a triterpenoid was isolated from the stem (Yu *et al.*, 2013). Rasool *et al.* (1989) isolated euphorginol from the stem bark of *E. tirucalli*. The bark, on the other hand, was found to contain phorbol, β -sitosterol, cycloartenol, 24-Methylene cycloartenol and ingenol triacetate (Baslas & Gupta, 1982). Other studies have revealed that β -amyrin is present in the leaves of this plant, as reported by Kajikawa *et al.* (2005). Additionally, Shivkumar (2015) found various compounds such as phenols, flavonoids, tannins, alkaloids, saponins, glycosides, triterpenes and steroids. However, Kgosiemang *et al.* (2020) isolated only tannins, glycosides, triterpenoids and saponins from the same plant. Upon review, it was noted that several compounds, such as diterpene esters, 12- Deoxyphorbol esters, ingenol, hexacosonate,

12-Deoxy-4 β -hydroxyphorbol-13-phenyl acetate-20-acetate, 12, 20-Dideoxyphorbol-13 isobutyrate, tirucallicine, tri-methyl ellagic acid, terpenic alcohol, isoeuphorol, taraxasterol, tirucallol, ketone euphorone, resin, ellagic acid, 3,3'-Di-O-methylellagic acid, euphorbin A, euphorbin B, tirucallin A, tirucallin B, cycloartenol, 24-Methylenecycloartenol, ingenol triacetate, rhoiptlenone, 3 β -friedelinol, epi-friedelinyl acetate, 24-ethylene cycloartanol, friedelan 3 α - and 3 $\beta\alpha$ -ols, taraxerol acetate, betulinic acid, α amyrrin, lupeol, Cycloartanol and β -amyrrone were not discussed in other reviews compared to the current review (Mavundza *et al.*, 2022).

Toxicity

Oliveira *et al.* (2018) assessed the antitumor effect of euphol from *E. tirucalli* against a wide range of human cancer cell lines. The study demonstrated that euphol exhibits cytotoxic properties against various cancer cell lines, exhibiting IC₅₀ values ranging from 1.41 to 38.89 μ M. The highest impact was observed in esophageal squamous cell lines (11.08 μ M) and pancreatic carcinoma cells (6.84 μ M), with notable effects also observed in prostate, melanoma and colon cancer cells. Letícia *et al.* (2017) evaluated the antiproliferative efficacy of *E. tirucalli* extracts against leukaemia (HL-60), lymphoma (Daudi) and melanoma (B16F10) cell lines using methyl thiazol tetrazolium assay (MTT) at concentrations of 62, 125, 250 and 500 μ g/mL. There was a notable regional variation in the cytotoxicity of the extracts, displaying a dose-dependent pattern. The extracts exhibited comparable effectiveness against the leukaemia cell line HL-60, resulting in a reduction of cell viability to approximately 60–70%. In a separate study, other researchers assessed anti-proliferation effects of highly diluted latex and *E. tirucalli* homeopathic remedies on melanoma cells *In vitro*. The researchers created solutions of 0.5% and 5% concentration in 70°GL EtOH, which were then prepared for use. The findings

indicated that the 0.5% latex solution at 30cH reduced melanoma cell growth by 19.7%, while the 0.5% *E. tirucalli* solution at 30cH showed a 32.1% reduction (Macedo *et al.*, 2021). In another study, Waczuk *et al.* (2015) conducted an evaluation of the cytotoxic effect of an aqueous extract obtained from *E. tirucalli* on human leukocytes. The results revealed that exposure to high concentrations of the extract induced a notable reduction in cell viability. In a study by Abdel-Aty *et al.* (2019), the cytotoxicity of phenol content from *E. tirucalli* was evaluated against various cancer cell lines (HepG2, MCF7, A549, HL-60, HCT116) and human normal melanocyte, HFB4. The results showed that low concentrations of the phenolic content exhibited significant potent cytotoxicity against HL-60, with an IC₅₀ value of 22.76 ± 2.85 µg/ml. Moreover, the extract demonstrated moderate cytotoxicity against MCF-7 and A549 cells, with IC₅₀ values of

31.65 ± 3.67 and 35.36 ± 3.82 µg/ml, respectively. Upon thorough evaluation of the anti-proliferative potential of the methanolic extract on MiaPaCa-2 cancer cell line, it was unequivocally observed that the extract exhibited a remarkable ability to significantly impede the growth of MiaPaCa-2 cancer cells (Munro *et al.*, 2015). The presented toxicity is in line with what was presented by Mavundza *et al.* (2020).

3.4.1.8 *Euphorbia ammak*

E. ammak is a plant that grows in Saudi Arabia and the Yemen peninsula (see Figure 3.8) (Carter, 1994).



Figure 3.8. *Euphorbia ammak* tree and flower (source: <http://luirig.altervista.org/cpm/albums/bot-units07/euphorbia-ammak16454.jpg>)

Phytochemistry

Abdel-Sattar *et al.* (2015) screened the leaves of *E. ammak* and found three compounds: euphol, α -glutinous and stigmasterol. Furthermore, Ponsinet and Ourisson (1968) isolated euphol and euphorbol. Alkaloids, saponins and glycosides were detected by Al-Hajj and colleagues in 2018. According to Al-Hajj and co-workers, it has been found to have good anti-leishmanial activity against *Cutaneous leishmaniasis*.

Toxicity

Studies have also shown that the methanolic extract of *E. ammak* could potentially reduce the H1N1 influenza virus and exhibits considerable cytotoxic activity against MDCK cells (Kiyohara *et al.*, 2012). Additionally, Abdel-Satter *et al.* (2010) found that the methanolic extract has anti-parasitic properties and that the euphol compound, which was also isolated, demonstrated significant cytotoxic effect against various human cancer cell lines *in vitro*. According to research conducted by Mampa *et al.* (2020), the effect of four distinct *E. ammak* extracts on cell proliferation was evaluated. The extracts were obtained using various solvents

such as Hexane, DCM, MeOH and EtOAc. The findings of the study indicated that the DCM extract exhibited the most significant inhibition of cell growth at concentrations as low as 1 µg/mL. Almehdar *et al.* (2012) evaluated the cytotoxic effect of *E. ammak* latex on MCF-7 breast cancer cells. The study found that the latex showed significant toxicity against MCF-7 cells, with an IC₅₀ value of 14.3.

3.4.1.9 *Euphorbia clavarioides*

E. clavarioides is a plant that originates from South Africa and Lesotho (see Figure 3.9). It has been traditionally used to treat various skin conditions, such as skin rashes in children, acne, sores, bruises, burns, eczema, ulcers, cracked heels and wounds (Guillarmod, 1971; Shale *et al.*, 1999; Moteetee *et al.*, 2019). According to a study by Mbhele (2021), *E. clavarioides* is effective in wound healing, confirming its traditional use. Additionally, in Lesotho, *E. clavarioides* is used for bathing swollen feet and, when mixed with *Berkheya onopordifolia*, it is used as a remedy for leprosy (Mbhele, 2021). Other reports suggest that it can be used to treat herpes, HIV-related infections, high blood pressure, and diabetes (Maliehe, 1997; Kose *et al.*, 2015; Cock *et al.*, 2021).



Figure 3.9. *Euphorbia clavarioides* plant with flowers and stem (source: <https://www.inatura>)

list.org/observations/38217609; [http://www.llifile.com/Encyclopedia/SUCCULENTS/Family/Euphorbiaceae/32951/Euphorbia clavarioides var. truncata](http://www.llifile.com/Encyclopedia/SUCCULENTS/Family/Euphorbiaceae/32951/Euphorbia_clavarioides_var._truncata))

Phytochemistry

In 2019, Selete-Kose *et al.* conducted a phytochemical analysis of *E. clavarioides* and found that it contains alkaloids, flavonoids, saponins, terpenoids and tannins. In 2020, Mampa *et al.* conducted a study on the same plant and isolated phytosterols, glycosides, triterpenoids, anthraquinone, flavonoids and alkaloids. Despite these findings, there is still a lack of research on this plant.

Toxicity

In a study conducted by Mampa *et al.* in 2020, the effect of Hex, DCM, MeOH and EtOAc derived from *E. clavarioides* extracts on cell proliferation was evaluated against the Vero cell line. The Hex and DCM extracts exhibited the highest cell growth inhibition of Vero cells.

3.4.1.10 *Euphorbia gorgonis*

E. gorgonis is a plant species that can only be found in the Eastern Cape of South Africa (see Figure 3.10). Studies have shown that it has medicinal properties that can be used to treat cancer, wounds, swelling and skin problems (Johnson, 2019; Komoreng *et al.*, 2017). The plant has also been found to have high antibacterial and antimicrobial activity (Tiwani, 2017).



Figure 3.10. *Euphorbia gorgonis* plant with flowers and stem (source: <http://www.bihrmann.com/caudiciforms/SUBS/eup-gor-sub.asp>)

Phytochemistry

Studies have identified the presence of phytosterols, glycosides, triterpenoids, flavonoids and alkaloids (Mampa *et al.*, 2020). While Tiwani (2017) reported the presence of tannins, saponins, alkaloids and flavonoids.

Toxicity

When tested on human intestinal cancer cells, the aqueous extracts of *E. gorgonis* extract showed cell reduction, while the acetone and ethanol extracts showed cytotoxic and reduced cell viability patterns, respectively. In another study, the acetone extract was found to reduce cell viability in rat hepatoma cells, while the aqueous extract showed a high percentage of cell viability (Tiwani, 2017). Mampa *et al.* (2020), evaluated the cytotoxicity effect of Hex, DCM, MeOH and EtOAc extracts on a Vero cell line. It was observed that none of the extracts displayed toxicity against the cell line.

3.4.1.11 *Euphorbia bupleurifolia*

E. bupleurifolia is a plant native to South Africa and is commonly found in the Eastern Cape Province and Natal (see Figure 3.11). The milky latex of this plant has been historically used to treat various ailments including cancerous sores, painful cracked feet, eczema, pimples, rashes and wounds (Afolayan *et al.*, 2014). Additionally, the twigs of *E. bupleurifolia* have been utilized as a teeth-cleaning agent (Afolayan *et al.*, 2014). It has also been used to treat swelling in the lower limbs and cancer (Van Wyk *et al.*, 1997; Iwalewa *et al.*, 2019). Some have even used it to help with retained placenta (Assefa & Bahiru, 2018).



Figure 3.11. *Euphorbia bupleurifolia* plant with stem and flowers (source: <https://davesgarde.com/guides/pf/showimage/238493/#b>; <https://planetdesert.com/products/euphorbia-bupleurifolia-large>)

Phytochemistry

Previous studies have revealed that *E. bupleurifolia* contains various secondary metabolites such as phytosterols, tannins, glycosides, triterpenoids, saponins, flavonoids and alkaloids (Mampa *et al.*, 2020). Van Wyk *et al.* (1997) specifically reported the presence of triterpenes

Toxicity

An investigation conducted by Mampa *et al.* in 2020 examined the potential cytotoxicity of *E. bupleurifolia* extracts on the Vero cell line. The findings indicated that both the Hex and DCM extracts had an antiproliferative effect on the Vero cell line. It is worth noting that the Hex and DCM extracts showed significant efficacy at concentrations of 1 and 10 µg/mL respectively.

3.4.1.12 *Euphorbia polygona*

E. polygona is a plant species that originates in the Eastern Cape, South Africa (see Figure 3.12). There is no recorded information on its traditional and pharmacological use.



Figure 3.12. *Euphorbia polygona* plant with stem and flowers (source:

http://www.biodiversityexplorer.info/plants/euphorbiaceae/euphorbia_polygona.htm)

Phytochemistry

A study conducted by Mampa *et al.* in 2020 discovered the presence of various compounds in the plant, including phytosterols, tannins, glycosides, triterpenoids, flavonoids and alkaloids.

The study conducted by Mampa *et al.* (2020) evaluated the antiproliferative effects of the Hex, DCM, MeOH and EtOAc extracts of *E. polygona* against the Vero cell line. The results of the study indicated that the Hex and DCM extracts exhibited the highest antiproliferative effect compared to the MeOH and EtOAc extracts.

3.4.1.13 *Euphorbia arabica*

E. arabica can be found in various regions, including Botswana, southern Mozambique, Zimbabwe and South Africa (see Figure 3.12). This plant has been utilized in the past as an antibacterial agent, according to El-Shanwani in 1996. It has also been used to treat ailments such as warts and stomachaches, while the juice has been used to treat skin infections (El-Shanwani, 1996; Al-Harbi, 2017).



Figure 3.13. *Euphorbia arabica* plant with stem and flowers (source: http://www.biodiversityexplorer.info/plants/euphorbiaceae/images/1362101_658w.jpg, http://www.biodiversityexplorer.info/plants/euphorbiaceae/images/136210-2_658w.jpg)

A recent study by Mampa *et al.* in 2020 has revealed the presence of phytosterols, tannins, glycosides, triterpenoids, anthraquinones and flavonoids.

Toxicity

When the cytotoxic effect of Hex, DCM, MeOH and EtOAc extracts of *E. arabica* were evaluated against a Vero cell line. The findings of the study indicate that the hexane extract of *E. arabica* inhibited cell growth of the Vero cell line, achieving IC₅₀ at all concentrations tested. It is worth noting that the DCM extract showed IC₅₀ at a concentration of 10 µg/mL.

3.4.1.14 *Euphorbia ferox*

E. ferox is a plant species that is indigenous to the Western Cape region of South Africa (see Figure 3.14). Currently, there is no available information on the traditional and pharmacological activities and isolated compounds of *E. ferox*.



Figure 3.14. *Euphorbia ferox* plant with stem and flowers (source:communities/southern-africa/view/observation/586073, <http://gadi.agric.za/Grootfontein%20plants/Euphorbia%20ferox.html>).

3.4.1.15 *Euphorbia stellata*

E. stellata is a plant species that can be found in the Eastern Cape region of South Africa (see Figure 3.15). There is currently no recorded information on the traditional and pharmacological uses or the isolated compounds of *E. stellata*.



Figure 3.15. *Euphorbia stellata* plant with stem and flowers (source:

https://mk0newsiteviodqfcuwv.kinstacdn.com/wp-content/uploads/2020/01/20200317-DSC_2747.jpg, <https://worldofsucculents.com/wp-content/uploads/2016/12/Euphorbia-stellata4.jpg>)

3.4.2. Pharmacological activities

The use of medicinal plants has gained attention due to their effectiveness in treating various ailments, and some of these claims have been supported by scientific evidence. Natural products are significant because of their diverse biological activities and drug-like properties, making them useful in the development of new lead compounds, natural drugs, pharmacological tools and herbal remedies (Shi *et al.*, 2010). The therapeutic properties of

these plants are attributed to their unique bioactive chemicals.

Many species of *Euphorbia* are known for their medicinal properties, with over 5% of them used to treat various ailments such as warts, wounds, skin complications, tumor, respiratory disorder, sexually transmitted diseases, urinary tract infections and intestinal parasites throughout the world (Hedberg & Staugård, 1989; Nashikkar *et al.*, 2012; El-Sherei *et al.*, 2015; Morsi, 2015). This is likely due to the presence of unique secondary metabolites and isolated compounds (Shi *et al.*, 2008). However, it is worth noting that the latex found in most *Euphorbia* species is toxic and can cause serious skin irritation and blindness. Despite the toxicity, latex also contains crucial biological active compounds such as terpenes, diterpenoids and triterpenes (Hua *et al.*, 2017).

Several species of *Euphorbia* have demonstrated medicinal properties, as their extracts have been patented as prescription drugs. For example, *E. lathyris* extract (US 5707631) is used to treat arthritis, high cholesterol, Alzheimer's and blood pressure. Extracts from *E. peplus*, *E. hirta* and *E. drummondii* (US 6844013) have shown selective cytotoxicity against various cancer cell lines and their compounds are used to treat malignant melanomas and squamous cell carcinomas. *E. aaron-rossii*, *E. tirucalli*, *E. tomentella* and *E. tomentosa* (US 2003/0171334 A1) are used in treating prostate cancer. Additionally, *E. tirucalli* latex (US 2009/0142421 A1) has been found to be potent in treating diseases related to cell proliferation or angiogenesis (Goutam *et al.*, 2017). Extract from *E. obesa* (US 6923993) has been shown to stimulate apoptosis and inhibit the growth of cancer cells. *E. hirta* (US 2007/0248694 A1) is used to reduce inflammation and *E. antiquorum* (US 2003/0165579 A1) has been found to inhibit tumor growth (Mwine & Damme, 2011).

This study has analyzed 15 different species of *Euphorbia* and found a diverse range of secondary metabolites, which are important in biomedical sciences. The analysis identified

several phytochemicals, including diterpenoids, triterpenes, sesquiterpenoids, phloracetophenones, cerebroside, glycerols, flavonoids, and steroids, as well as isolated compounds (listed in Table 3.5).

3.4.2.1. Flavonoids, a type of polyphenolic compound, were found in 9 species of *Euphorbia*, including *E. horrida*, *E. trigona*, *E. clavarioides*, *E. enopla*, *E. gorgonis*, *E. bupleurifolia*, *E. polygona*, *E. arabica* and *E. tirucalli*. Additionally, these have been observed in other species of the *Euphorbiaceae* family, including *E. microsciadia*, *E. heterophylla*, *E. hirta*, *E. neriifolia*, *E. paralysis*, *E. lunulata* and *E. larica*, indicating their prevalence within the *Euphorbiaceae* family. Studies have shown that flavonoids have medicinal properties that make them useful for treating different health conditions. These properties include antiinflammatory, enzyme inhibition, antimicrobial, estrogenic activity, antiallergic and antioxidant activity (Cowan, 1999; Tapas *et al.*, 2008; Abdel-Hameed *et al.*, 2013). According to Mai *et al.* (2017), *E. helioscopia* contains a significant amount of flavonoids and has shown to have a cytotoxic effect on triple-negative breast cancer cells. In addition, according to research by Yang *et al.* (2011) found that there was a decrease in the differentiation of 3T3-L1 preadipocytes, a reduction in triglyceride accumulation in mature adipocytes and a decrease in nitric oxide production in RAW 264.7 cells. According to a report by Galleggiante *et al.* (2017), flavonoids can prevent the degradation of cAMP by phosphodiesterases and extend cAMP signaling by the enzyme. This leads to the development of antiinflammatory properties. According to El-Hawary *et al.* (2020), the methanol extracts from *E. trigona* were found to cause cell death in MCF-7 and Caco-2 cells with IC₅₀ values of 16.1 and 15.6 µg/mL, respectively. Previous research has shown that the butanol extract derived from *E. tirucalli* demonstrated effective cytotoxicity against MCF-7 cells and MDA-MB231, with IC₅₀ values of 15 and 30 µg/mL (Choene *et al.*, 2016). Hence, researchers have been drawn towards using this secondary metabolite for pharmaceutical purposes.

3.4.2.2. Alkaloids are a type of organic compound that occur naturally and have at least one nitrogen atom (Seigler & Seigler, 1998). It was found in 9 plant species, including *E. horrida*, *E. trigona*, *E. clavarioides*, *E. gorgonis*, *E. enopla*, *E. bupleurifolia*, *E. ammak*, *E. polygona* and *E. tirucalli*. The fact that alkaloids were found in nearly all of the chosen plants should not come as a surprise since previous studies have documented the presence of alkaloids in species from the *Euphorbiaceae* family. (Kapingu *et al.*, 2005; Boudiar *et al.*, 2010; Ndam *et al.*, 2016; Al-Saraireh *et al.*, 2021). Prescription medicines derived from plants with alkaloids have been used for many years. Their potent effects are attributed to the presence of alkaloids. Morphine, currently used as an analgesic, was the first alkaloid from *Opium poppy* (Demirkapu & Yananli, 2020). Furthermore, other alkaloids such as vinblastine, quinine, morphine, atropine, nicotine, caffeine, ephedrine and strychnine are also used in medicine production. Alkaloids have several physiological effects on humans, such as antibacterial, antimitotic, antiinflammatory, local anesthetic, hypnotic, antitumor activity and several others (Molyneux *et al.*, 1996).

3.4.2.3. Saponins, which are glycosides, were present in 7 plants, namely; *E. horrida*, *E. trigona*, *E. clavarioides*, *E. gorgonis*, *E. bupleurifolia*, *E. polygona* and *E. ammak*. According to a report by Bigoniya and Rana (2009), saponins have been found in *E. neriifolia*, *E. paralias* and *E. terracina* (Jannet *et al.*, 2017). Saponins possess pharmacological and medicinal properties such as cell membrane permeability, hemolytic activity, antiviral, antifungal, antiinflammatory and antiallergic (Glauert *et al.*, 1962; El Izzi *et al.*, 1992; Francis *et al.*, 2002). In addition, they have shown potential for cancer treatment by reducing cell invasiveness, induce cell cycle arrest and apoptosis, and also for suppressing angiogenesis (Fuchs *et al.*, 2009). Along with other antitumor medicines, saponins have been added to enhance their cytotoxicity in tumor treatment (Gaidi *et al.*, 2002; Jia *et al.*, 2004). A study conducted by Xiao *et al.* (1999) discovered that triterpene saponin found in the root bark of *Aralia dasyphylla* Miq.

had the ability to inhibit cancer cells in two types of cell lines: KB and HeLa-S3. Another study discovered that eight steroidal saponins found in *Allium porrum* L. were able to inhibit WEHI 164 and J774 cells (Fattorusso et al., 2000). In 2001, Tran *et al.* conducted a study on *Dracaena angustifolia* Roxb. roots and rhizomes, testing spirostanol and furostanol-type saponins for their antiproliferative activity against murine colon 26-L5 carcinoma, human HT-1080 fibrosarcoma and B-16 BL6 melanoma cells. The study found that three of the tested compounds were highly effective in inhibiting the growth of HT-1080 fibrosarcoma cells. According to a study by Yokosuka *et al.* (2002), two types of saponins (ruscogenin glycoside and 26-glycosyloxyfurostanol saponin) demonstrated cytostatic activity against HL-60 human leukaemia cells. Researchers have discovered two new triterpenoid saponins, namely glycoside A and B, in the aerial parts of *Glinus oppositifolius* L. These saponins were found to be effective against *Plasmodium falciparum*, a type of protozoan (Traore et al., 2000). In a study conducted by Iorizzi *et al.* (2002), three new furostanol saponins and seven previously known saponins were extracted from the seeds of *Capsicum annuum* L. var. acuminatum Fingerh. However, the analysis showed that these saponins had little to no effect on the growth of Gram-positive and Gram-negative bacteria.

3.4.2.4. Tannins, which are polyphenols, were found in 9 plants. These plants are *E. horrida*, *E. trigona*, *E. claviroides*, *E. gorgonis*, *E. bupleurifolia*, *E. enopla*, *E. polygona*, *E. arabica* and *E. tirucalli*. These findings are in agreement with a study by Aleksandrov *et al.* (2019), which also observed high levels of tannins in *E. hirta*. According to Fraga-Corral *et al.* (2021), applying tannin topically can help remove irritants from the skin, reduce inflammation and be beneficial in treating burns and wounds due to its antihemorrhagic and antiseptic properties. Tannins have been used in various studies for treating different diseases due to their high antioxidant content, ability to scavenge free radicals and their antimicrobial, antiviral properties, and they have also been found to be effective in cancer chemotherapy (Chung *et*

al., 1998; Amarowicz *et al.*, 2004; Ho *et al.*, 2001; Buzzini *et al.*, 2008; Koleckar *et al.*, 2008).

Tannins were also reported to inhibit various coronavirus strains (Chinsembu, 2020).

3.4.2.5. Glycosides, which are acetal derivatives of monosaccharides, were present in 10 *Euphorbia* spp including *E. horrida*, *E. trigona*, *E. enopla*, *E. clavarioides*, *E. gorgonis*, *E. bupleurifolia*, *E. polygona*, *E. arabica*, *E. tirucalli* and *E. ammak*. Studies by Mshvildadze *et al.* (2007) discovered that glycosides from the bark of *Betula papyrifera* and they had a significant cytotoxic effect against lung carcinoma, colorectal adenocarcinoma and normal skin fibroblast. Liu *et al.* (2013) further reported the cytotoxic activity of glycosides from *Antiaris toxicaria* against human NIH460 lung cancer cells.

3.4.2.6. Anthraquinones, which are phenolics, were present in 4 *Euphorbia* s namely; *E. horrida*, *E. enopla*, *E. clavarioides* and *E. arabica*. Anthraquinones are potent compounds found in various plant-based medicines that possess numerous health benefits, including acting as laxatives, diuretics, estrogenic agents and immunomodulators. Additionally, they are also utilized in cancer treatment. Moreover, they exhibit antibacterial, antiparasitic, insecticidal, fungicidal and antiviral properties as well (Yadav *et al.*, 2019).

According to studies by Hanson (2003) and Berdy (2005), several plants including *E. bupleurifolia*, *E. gorgonis*, *E. horrida*, *E. polygona* and *E. coerulescens* contain alkaloids, saponins and terpenoids. These compounds are believed to have pharmacological effects such as anticancer and antibacterial properties (Kemboi *et al.*, 2020). In addition, the report found that a single plant can treat multiple illnesses and many recorded plants have similar secondary metabolites. According to the data, seven different *Euphorbia* plants were used to treat cancer, these include *E. trigona*, *E. tirucalli*, *E. clavarioides*, *E. gorgonis*, *E. bupleurifolia* and *E. cooperi*. Five plants were used for wound healing and warts, which included *E. trigona*, *E. tirucalli*, *E. clavarioides*, *E. arabica* and *E. bupleurifolia*. Three plants, *E. trigona*, *E.*

bupleurifolia and *E. gorgonis*, were used for inflammation, while *E. arabica* and *E. cooperi* were used for stomach aches, skin infections and cracked heels. The seven plants with cancer-fighting properties all contained common secondary metabolites, including phytosterols, tannins, glycosides, triterpenoids, saponins, upholds, flavonoids and alkaloids. It has been reported that the latex from *Euphorbia* species can be highly toxic if ingested and can cause severe skin irritation (Watt & Breyer-Brandwijk, 1962; Voigt, 2007)

3.4.1.7. Terpenoids

3.4.1.7.1. Triterpenoids

Triterpenoids which are triterpenes, are present in most *Euphorbia* s In this study, it was discovered in 10 plants, including *E. horrida*, *E. enopla*, *E. trigona*, *E. clavarioides*, *E. gorgonis*, *E. bupleurifolia*, *E. polygona*, *E. cooperi*, *E. arabica* and *E. tirucalli*. It has been found that these secondary metabolites have medicinal properties, including being anticarcinogenic, antimalarial, antiulcer, antiinflammatory, cytotoxic, antiHIV, antiangiogenic, hepaticidal, antimicrobial and antiviral (Langenheim, 1994; Dudareva *et al.*, 2004; Si *et al.*, 2018). According to Munro *et al.* (2015), the tetracyclic triterpene euphol found in *E. tirucalli* latex has been proven to have anticancer properties. According to Yasukawa *et al.* (2000), *E. kansui* contains lanostane-type triterpenes that can inhibit inflammation caused by TPA. The main triterpene in this plant, euphol, was found to effectively prevent tumor promotion induced by TPA.

Euphol, a tetracyclic triterpene, is commonly found in the *Euphorbiaceae* family, including *E. trigona*, *E. enopla*, *E. tirucalli*, *E. coerulescens* and *E. ammak*. Popplewell *et al.* (2010) discovered that this compound showed moderate activity against the HepG2 cells. Ahmed *et al.* (2019) also reported remarkable cytotoxic effect of euphol from *E. bothae* against the MCF-

7 cells. Silva *et al.* (2019) evaluated the antitumor effect of euphol on a number of human cancer cell lines and discovered that euphol from *E. tirucalli* was cytotoxic to several cancer cell lines, including esophageal squamous and pancreatic carcinoma cells. Meanwhile, Abdel-Sattar *et al.* (2015) found that euphol from *E. ammak* exhibited notable cytotoxic effect against HeLa cells. In other studies, euphol from *E. umbellata* was found to exhibit noteworthy cytotoxic effect against HL-60, K-562 and B16F10 cells, while Yasukawa *et al.* (2000) discovered that topical application of euphol from *E. kansui* greatly reduced the cancer-promoting impact of TPA in mouse skin, lowering it by 90%. Akihisa *et al.* (2001) reported that euphol was a successful inhibitor of HIV-1 reverse transcriptase. Additionally, euphol has shown potential in reducing inflammation and pain by blocking PGE2 and protein C kinase epsilon mediators (Dutra *et al.*, 2011). However, data has yet to be documented on the euphol of *E. trigona*, *E. enopla*, *E. tirucalli*, *E. coerulescens* and *E. ammak*.

Cycloartanol is a type of sterol lipid that has been found in various plants including *E. trigona*, *E. glareosa* Pall. Ex M. Bieb., *E. amygdaloides* L. and *E. palustris* L. (Salome- Abarca *et al.*, 2020). According to a recent report by Salome-Abarca *et al.* (2020), cycloartenol extracted from these plants was noted to have antifungal properties against *B. cinerea*. Barla *et al.* (2006) also discovered cycloartanol in *E. helioscopia*, but observed no vasodepressor activity. Meanwhile, Heliawati *et al.* (2023) conducted a study on the cytotoxicity of cycloartanol extracted from the bark of *Cory-pha utan* Lamk on leukemia cells. The research indicated that cycloartanol was able to restrict the growth of these cells. Nevertheless, there is currently no information available on the effects of cycloartanol from *E. trigona*.

Lupeol, a triterpenoid, is found in many plants including *E. trigona*, *Tamarindus indica*, *Celastrus paniculatus*, *Zanthoxylum riedelianum*, *Allanblackia monticola*, *Himatanthus*

sucuuba, *Leptadenia hastata*, *Crataeva nurvala*, *Bombax ceiba*, *Sebastiania adenophora*, *Aegle marmelos* and *Embllica officinalis* (Siddique & Saleem, 2011; Saleem, 2009; Erazo *et al.*, 2008; Imam & Iqbal Azhar, 2007; de Miranda *et al.*, 2000). You *et al.* (2003) studied the effects of Lupeol from *Bombax ceiba* on various cells such as HUVEC, SK-MEL-2, A549, and B16-F10 melanoma cells. Their findings showed that lupeol effectively inhibited the formation of HUVEC tubes by over 80% at a concentration of 50 µg/mL. However, there was no notable cytotoxic effect observed in the three cancer cell lines. Nguemfo *et al.* (2009) conducted a study on the antiinflammatory properties of lupeol found in *Allanblackia monticola* Staner. The findings demonstrated that within just 30 minutes, lupeol effectively reduced paw edema by approximately 57.14%. Zhang *et al.* (2015) conducted a study on lupeol's ability to inhibit the growth of liver cancer cells called HCCLM3. It was found that lupeol effectively stopped liver cancer cell growth by suppressing the secretion of Brain-Derived Neurotrophic Factor, phosphatidyl inositol 3-kinase and Wnt pathways. Borgati. (2017) conducted a study on the effectiveness of lupeol from *Parahancornia fasciculata* against chloroquine-resistant W2 clones in treating malaria. The study found that the compound had limited activity. Other studies have explored the antidiabetic properties of lupeol found in *Solanum xanthocarpum* (Gupta *et al.*, 2012). The findings showed that lupeol can effectively hinder the advancement of diabetes by decreasing glucose levels, nitric oxide, further increases serum insulin and antioxidant levels. Sudhakar *et al.* (2007) conducted a study on the impact of lupeol from the *Crataeva nurvala* plant on cholesterol levels. They discovered that this compound can reduce oxidative stress and inflammatory cytokines, which in turn leads to a decrease in nitric oxide production and ultimately results in lower cholesterol levels. However, data has yet to be documented on the lupeol of *E. trigona*.

α-amyrin, a type of triterpenoid, can be found in several plants including *E. trigona*, *E. tirucalli*, *E. aphylla* Brouss, *E. schimperi* C. Presl and *E. hirta* (Ragasa & Cornelio, 2013;

Abdel-Monem *et al.*, 2008; Vazquez *et al.*, 1999). Abdel-Monem *et al.* (2008) also discovered that α -amyrin obtained from *E. schimperi* C. Presl displayed moderate cytotoxicity, resulting in a 40-50% reduction in u251 and MCF-7 cell lines at a concentration of 10 $\mu\text{g/mL}$. Other studies reported insignificant cytotoxicity against HCT 116 cell line (Ragasa & Cornelio, 2013). Jabeen *et al.* (2011) conducted an analysis of the antifungal properties of α -amyrin from *Melia azedarach* L. *in vitro*. The results indicated that the compound was effective against *Ascochyta rabiei*, with a MIC of 0.0156 mg mL^{-1} . However, there is currently no available data on the effects of α -amyrin from *E. trigona* and *E. tirucalli*.

β -amyrin, a triterpenoid, was found in *E. trigona* and *E. hirta* and has demonstrated anticancer properties against Hep-G2 cancer (Wen *et al.*, 2018). The same compound also exhibited weak cytotoxic effect against NTUB1, A549 and HL-60 (Lin *et al.*, 2011). According to Ragasa and Cornelio (2013) insignificant cytotoxicity against HCT 116 cell line was observed. According to Vazquez *et al.* (1999), a study showed that the antiinflammatory activity in mice ears was effective. Another study by Shih *et al.* (2010) found that β -amyrin from *E. hirta* could treat arthritis inflammation by blocking the function of the nitric oxide pathways. According to Lin *et al.* (2011), the use of β -amyrin together with cisplatin caused reactive oxygen species to trigger cell cycle arrest and apoptosis in NTUB1 cells. However, there is currently no documented information on the β -amyrin found in *E. trigona*.

Betulinic acid, a triterpenoid, has been discovered in various plants such as *E. trigona*, *Tetracarpidium conophorum* seeds, *Uapaca paludosa*, *Manniophyton fulvum* (Euphorbiaceae) and *Agathosma betulina* (Oriakhi *et al.*, 2021; Mbeunkeu *et al.*, 2018; Banzouzi *et al.*, 2015; Sekhoacha *et al.*, 2009). A study conducted by Zhang *et al.* (2015) showed that betulinic acid has the potential to inhibit the growth of more than 20 different cancer cells. According to studies conducted by Damle *et al.* (2013), betulinic acid was found to have cytotoxic effects

on MCF-7 cells with an IC_{50} value of 13.5 mg/mL. Additionally, the compound was found to reduce the level of specificity of protein transcription factors, which are known to be overproduced in cancer cells compared to normal cells (Mertens-Talcott *et al.*, 2013). A study conducted by Mbeunkeu *et al.* (2018) found that betulinic acid from *Manniophyton fulyum* had a substantial impact on HeLa cells, resulting in a cell viability rate of only 4%. A study conducted by Foo *et al.* (2015) discovered that the DCM fraction of *Dillenia suffruticosa* can cause cell cycle arrest and apoptosis of MCF-7 cells through the p35/p21 pathway. The compound was found to inhibit the growth and ability of all human melanoma cell lines to form colonies (Selzer *et al.*, 2000). According to Oriakhi *et al.* (2021), there was a 54% inhibition of HepG2 cells as well as hepatoprotective activity, whereby a binding energy of -11.2 kcal/mol to the Hepatitis B virus DNA was observed. Other studies reported antidiabetic activity, where the pancreatic α -amylase is inhibited (Tamamura *et al.*, 2018; Kumar *et al.*, 2013). According to Bernard *et al.* (2001), betulinic acid was found to have an antiinflammatory effect by inhibiting phospholipase A2. Additionally, Mukherjee *et al.* (1997) reported that betulinic acid reduced rat paw edema caused by carrageenan and serotonin. According to additional research, *Hypericum hircinum* L. contains betulinic acid which can prevent HIV-1 from replicating by stopping reverse transcriptase-associated DNA polymerase. This is achieved by either hindering the fusion of HIV or disrupting a certain stage of its maturation process (Esposito *et al.*, 2013; Aiken & Chen, 2005). However, data has yet to be documented on the betulinic acid of *E. trigona*.

Taraxerol, a triterpenoid, has previously been isolated from *E. trigona*, *E. neriifolia* Linn, *Artemisia roxburghiana*, *Taraxacum japonicum* and Fruits of *Dregea volubilis* (ur Rahman *et al.*, 2016; Biswas *et al.*, 2009; Bigoniya & Rana, 2008; Takasaki *et al.*, 1999). Taraxerol isolated from *Vepris punctate* showed limited activity against the A2780 cells (Chaturvedula *et al.*, 2004). Studies by Cao *et al.* (2008) also showed minimal inhibition of cell growth of

A2780 cells at the highest tested concentration. Taraxerol from *Conyza canadensis* was found to have a maximum antiproliferative effect against A431. However, taraxerol displayed no activity against HeLa, MCF-7 and MRC-5 (Csupor-Löffler *et al.*, 2011). In addition, it was found that taraxerol effectively hindered the growth of AGS cells by causing G(2)/M arrest and stimulating cell apoptosis (Tan *et al.*, 2011). According to Takasaki *et al.* (1999), taraxerol has been found to be highly effective in preventing tumors in mice during two-stage carcinogenesis tests. In addition, Singh *et al.* (2002) reported that taraxerol has antiinflammatory properties, reducing paw edema by 48.61%. Furthermore, it was also observed that taraxerol exhibited moderate antimicrobial activity against certain gram negative and gram-positive bacteria (Singh *et al.*, 2002). According to a study conducted by Min *et al.* (2004), taraxerol found in *Styrax japonica* showed low effectiveness in scavenging free radicals based on the DPPH assay. Sangeetha *et al.* (2009) investigated the use of taraxerol from *Mangifera indica* as an antidiabetic agent. According to the findings, the compound has dual activity as a glucose activator and stimulator of glycogen, making it a potential treatment for type 2 diabetes. However, there is currently no documented information available on the taraxerol of *E. trigona*.

Taraxerol acetate, a triterpenoid, was previously isolated from *E. trigona*, *E. pubescens* and *Artemisia roxburghiana* (Carréu, 2020). Studies have shown that taraxerol acetate from *A. roxburghiana* has the ability to significantly reduce edema in mice caused by carrageen (ur Rahman *et al.*, 2016). In addition, Rehman *et al.* (2013) discovered that taraxerol acetate can inhibit Cyclooxygenases enzymes 1 and 2. However, there is currently no documented data on the taraxerol acetate found in *E. trigona*.

Friedelin, which is a type of triterpenoid, has been found in plants such as *E. trigona*, *E. tortilis* Rottler, *Mangifera indica* and *Lentinus edodes* (Anju *et al.*, 2018; Chen *et al.*, 2015; Anjaneyulu *et al.*, 1999). Previous studies have shown that when investigated for its

antibacterial properties against certain gram positive and gram-negative bacteria, friedelin had weak activity with a minimum inhibitory concentration (MIC) value of over 250 µg/mL (Chen *et al.*, 2015). However, it was discovered that friedelin from *Mangifera indica* had anti-colorectal cancer activity (Abdul-Hammed *et al.*, 2023). Although studies have yet to be conducted on friedelin from *E. trigona*, it is an area of interest for future research.

Friedelan-3-β-ol, a triterpenoid, was also isolated from *E. trigona* and *Mangifera indica*. The compound exhibited significant inhibition of thymidylate synthase, thereby displaying its anti-colorectal potential (Abdul-Hammed *et al.*, 2023). However, data has yet to be documented on the friedelan-3-β-ol of *E. trigona*.

3β-Friedelinol, a triterpenoid, has been found in *E. trigona*, *E. vajravelui*, *E. kamerunica* and *Maytenus robusta* (Sousa *et al.*, 2014; Ogunnusi *et al.*, 2010). Sousa *et al.* (2014) discovered that 3β-friedelinol from *Maytenus robusta* exhibited cytotoxicity against 4T1 cells. Meanwhile, Ogunnusi *et al.* (2010) investigated the antibacterial activity of 3β-Friedelinol from *E. kamerunica* against certain bacterias and found that the compound had inhibitory activity. However, there is currently no available data on the 3β-Friedelinol of *E. trigona*. The latex of *E. trigona* has ingenol esters that are highly irritating to the skin. Studies have shown that it can cause dermatitis in open patch tests and blisters in closed patch tests (Tada & Seki, 1989). Two compounds isolated from *E. trigona*, Epi-Friedelinyl acetate and Rhoiptlenone, have no known biological effects.

3.4.1.8. Phytosterols

Phytosterols which are sterols, were present in 9 plants, including *E. horrida*, *E. trigona*, *E. clavarioides*, *E. gorgonis*, *E. enopla*, *E. bupleurifolia*, *E. cooperi*, *E. polygona* and *E. arabica*. Research suggests that phytosterols possess bioactive properties that offer multiple

health benefits, such as reducing inflammation, preventing oxidative stress, fighting against cancer and lowering cholesterol levels (Vilahur *et al.*, 2019; Vezza *et al.*, 2020). A mixture of phytosterols inhibited tumor development in various cancer forms, including cholangiocarcinoma and breast cancer, at physiological doses (Kazłowska *et al.*, 2013; Kangsamaksin *et al.*, 2017).

Cycloartenol is a phytosterol compound found in certain plants such as *E. trigona*, *E. nicaeensis*, *E. broteri*, *E. macrosteigia*, and *E. boetica* (NHI). It serves as a precursor to various sterol compounds and has several pharmacological benefits such as antiinflammatory, antitumor, antioxidant, antibacterial and antiAlzheimer's properties (Zare *et al.*, 2015; Zhang *et al.*, 2017). Niu *et al.* (2018) conducted a study on the potential anticancer properties of cycloartenol in relation to glioma U87 cells. The findings indicated that cycloartenol was able to hinder the growth and ability of the glioma U87 cells to form colonies. This was due to the induction of Sub-G1 cell cycle arrest and apoptosis, both of which contributed to the antiproliferative effects observed. According to Zare *et al.* (2014), cycloartenol has displayed anticancer, analgesic and bactericidal properties. Meanwhile, Sawale *et al.* (2019) discovered that cycloartenol from *E. neriifolia*, at concentrations of 10, 20, 40, 60, 80 and 100µg /mL, exhibited antioxidant activity ranging from 34.56% to 72.87%. However, data has yet to be documented on the Cycloartenol of *E. trigona*.

β-sitosterol, a sterol, was previously isolated from *E. trigona*, *E. abyssinica*, *Pinellia ternate* and *Nyctanthes arbortristis* (Cheng *et al.*, 2015; Nirmal *et al.*, 2012; El-Fiky *et al.*, 2008). β-sitosterol was investigated for its effect on the growth of Caski and HeLa cells; investigating its potential antiproliferation and anticancer effects. The results revealed that the compound was found to reduce the expression of antigen responsible for cell proliferation in both

cervical carcinoma cells (Cheng *et al.*, 2015). In their 2015 study, Cheng *et al.* discovered that elevated levels of P53 and decreased levels of HPVE6 viral oncogenes resulted in anticancer activity in Caski and HeLa cells. Previous research has indicated that β -sitosterol derived from *Nyctanthes arbortristis* leaves had a considerable antiinflammatory impact on the paw edema of rats (Nirmal *et al.*, 2012). β -sitosterol derived from *E. abyssinica* exhibits notable antimicrobial properties against *Candida albicans* (El-Fiky *et al.*, 2008). According to reports, β -sitosterol found in *E. hirta* was able to prevent inflammation caused by TPA (Vazquez *et al.*, 1999). However, there is currently no documented data on the β -sitosterol of *E. trigona*.

24-Methylenecycloartanol, a type of sterol, was previously isolated from *E. hirta*, *E. heteradena*, *E. trigona*, *E. broteri*, *E. palustris*, *Moroccan propolis* and *E. aleppica* (De *et al.*, 1987; Öksüz *et al.*, 1996; Krstić *et al.*, 1996; Öksüz *et al.*, 2002). According to Krstić *et al.* (2016), it was found that 24-Methylenecycloartanol from *E. palustris* has a stronger antifungal effect against *Fusarium sporotrichioides* and *Alternaria alternata*. Previous research has shown that 24-Methylenecycloartanol found in *E. hirta* can reduce inflammation caused by TPA (Vazquez *et al.*, 1999). However, there is currently no available data on the effects of 24-Methylenecycloartanol of *E. trigona*.

Tirucallol, a tetracyclic triterpene, was found in *E. enopla* and *E. lacteal* latex. This compound has antiinflammatory effects. Other studies have shown that mice models experienced a decrease in ear edema and that nitrate production in stimulated macrophages was inhibited (Miranda *et al.*, 2022; Fernandez-Arche *et al.*, 2010). Furthermore, it was discovered that it inhibits HIV-1 reverse transcriptase (Akihisa *et al.*, 2001). However, there is currently no available data on the tirucallol of *E. enopla*.

Obtusifoliol is a type of sterol that is present in various plants, including *E. cooperi*, *E. bothae*, *E. chamaesyce* and *E. sogdiana* (Popplewell *et al.*, 2010; Tanaka *et al.*, 1999). A study by Aghaei *et al.* (2016) found that obtusifoliol from *E. Sogdiana* has reduced the cytotoxic effect against MCF-7 and MDA-231, with an IC₅₀ value of 29.33 ± 1.52 and 41.81 ± 2.42 μ M; respectively. Similarly, Ahmed (2015) reported weak cytotoxicity of obtusifoliol from *E. cooperi* against MCF-7.

Glutinol, a triterpenoid, was isolated from *E. cooperi*, *E. ammak*, *E. chamaesyce* and *Scoparia dulcis* (Abdel-Sattar *et al.*, 2015; NCBI). This compound exhibited a notable cytotoxic effect against HeLa cells (Abdel-Sattar *et al.*, 2015). A study conducted by Ding *et al.* (2010) investigated the potential toxicity of glutinol from *Acer mandshuricum* on different types of cells; including leukaemia, ovary, lung and human colon. The findings showed that the cell lines were significantly inhibited, with GI₅₀ values ranging from 11.6 to 16.0. Another research by Chen and Li (2021) also reported the antiproliferation of ovarian cells. However, there is currently no available data on the effects of glutinous from *E. cooperi* and *E. ammak*.

Triterpene euphol, a steroidal alcohol, is found in plants such as *E. tirucalli*, *E. umbellata* and *Synadenium grantii* (Latansio de Oliveira *et al.*, 2022; de Oliveira *et al.*, 2013). Studies have shown that triterpene euphol, the primary compound present in *E. umbellata* and *E. tirucalli*, shows potential as a supplementary cancer treatment (Latansio de Oliveira *et al.*, 2022; de Souza *et al.*, 2019). According to Lin *et al.* (2000), triterpene euphol exhibited moderate cytotoxic activity against gastric adenocarcinoma cells. Additionally, Silva *et al.* (2019) reported that triterpene euphol had a cytotoxic effect on glioblastoma cells. de Oliveira *et al.* (2013) conducted a study on the antitumoral effect of triterpene euphol from

Synadenium grantii. The study found that the compound did not exhibit any antitumoral effect on B16F10 melanoma cells. A study conducted by Dutra *et al.* (2011) focused on the potential of triterpene euphol found in *E. tirucalli* in preventing and treating inflammation in the colons of mice. Their findings showed that this compound was highly effective in reducing pro-inflammatory mediators *in vitro*.

24-Methylene cycloartenol, a sterol, was previously extracted from the *E. tirucalli* and *E. neriifolia*. The plant was tested for its pharmacological activities, rather than the compound itself. Additionally, 24-Methylene which was isolated from *E. hirta*, has been shown to have significant antiinflammatory effects when used to treat ear inflammation caused by acetate anti-inflammatory (Martinez-Vazquez *et al.*, 1999). There is currently no information available regarding the anticancer properties of 24-Methylene cycloartenol from *E. tirucalli*.

Ingenol triacetate, a diterpene, has shown to have an antimicrobial effect against different pathogens that cause infectious diseases. (Sultana *et al.*, 2022). Ingenol triacetate was also found to be a non-tumor promotor (Sultana *et al.*, 2022). However, a separate study by Tilabi and Upadhyay (1983) discovered that when ingenol triacetate was applied topically to female NMRI mice, it resulted in a high occurrence of lung adenoma. There is currently no information available regarding the anticancer properties of Ingenol triacetate from *E. tirucalli*.

Terpenic alcohol which is a terpene, from *E. tirucalli*, has demonstrated antibacterial properties against *S. aureus*. This is due to its ability to damage cell membrane (Mali & Panchal, 2017; Duong *et al.*, 2019). However, this study was not directly done on terpenic alcohol (Inoue *et al.*, 2004). More studies need to be performed on this compound, especially that which is isolated from *E. tirucalli*.

Taraxasterol, which is a phytosterol was extracted from *E. tirucalli*, *Carthamus tinctorius*, *Chrysanthemum morifolium* and *Helianthus annuus* (Ovesnaâ, *et al.*, 2004; Akihisa *et al.*, 1996). Taraxasterol was effective in reducing ear inflammation caused by TPA in mice and also in preventing tumor growth on mouse skin. When taraxasterol was tested on the skin of mice, it demonstrated strong antitumor activity during two-stage carcinogenesis tests. Futhermore, the research showed that taraxasterol has the ability to suppress spontaneous mammary tumors in C3H/OuJ mice. The experiment was conducted with taraxasterol, which was not extracted from *Euphorbia* species (Ovesnaâ *et al.*, 2004). Further pharmacological testing is required on *Euphorbia* species, especially on *E. tirucalli*, as suggested by Ovesnaâ *et al.* (2004).

Stigmasterol which falls under the sterol class, was extracted from *E. ammak* and *Butea monosperma* (Panda *et al.*, 2009). In a separate study by Abdel-Sattar *et al.* (2015), the cytotoxicity activity of stigmasterol from *E. ammak* was examined. The study showed that the compound was effective against HeLa cells. In 2009, Panda and colleagues conducted a study on the effects of stigmasterol from *Butea monosperma*. Their findings showed that stigmasterol had properties that could inhibit the thyroid and lower blood glucose levels. The study further showed that stigmasterol has the potential to reduce liver damage caused by oxidative stress, as it decreased the level of harmful lipid peroxidation and increased the activity of protective enzymes. Studies have shown that this compund exhibits cytotoxicity against MCF-7 breast cancer cells (El-Sherei *et al.*, 2015; Morsi, 2015). Although some *Euphorbia* diterpenoids (phorbol ester) are toxic, recent research indicates that certain compounds in this plant have noteworthy bioactivity (Gundidza & Hecker, 1992; Engi *et al.*, 2007).

3.4.1.9. Other terpenoids

12-Deoxyphorbol-13-isobutyrate-20-acetate is a type of phorbol ester that can be found in various plants such as *E. ledienii*, *E. coerulescens*, *E. tirucalli*, *E. triangularis*, *E. resinifera* and *E. bothae*. Studies by Ourhzif *et al.* (2022) reported cytotoxicity of 12-Deoxyphorbol-13-isobutyrate-20-acetate from *E. resinifera* latex which inhibited the growth of *A. carbonarius*. However, there is currently no available data on the 12-Deoxyphorbol-13-isobutyrate-20-acetate found in *E. ledienii*, *E. coerulescens* and *E. tirucalli*.

Phorbol, a diterpenoid, was extracted from *E. ledienii* and *Croton tiglium*, a plant belonging to the *Euphorbiaceae* family. It was also discovered in *E. tirucalli* by Fürstenberger and Hecker in 1985. While some research suggests that phorbol can irritate and promote tumor growth, other studies have found that it may induce apoptosis in tumor cells by activating protein kinase C (Brodie & Blumberg, 2003). However, there is currently no available information on the phorbol found in *E. ledienii* and *E. tirucalli*.

12-Deoxyphorbol ester, a diterpenoid, was isolated from *E. ledienii* and *E. grandicornis*. According to a study by Zayed *et al.* (1984), this compound was found to activate protein kinase C instead of promoting tumor growth. Previous research has also shown that this activation can have antiproliferative effects on certain types of cancer cells. According to research conducted by Shen *et al.* (2015), 12-Deoxyphorbol ester was found to prevent the growth of myeloid leukemia cells. Additionally, other studies showed that 12-Deoxyphorbol ester increased cell death in breast cancer cells compared to normal breast epithelial cells (Alotaibi *et al.*, 2018). According to a study by Tsai *et al.* (2020), 12-Deoxyphorbol ester has the capacity to stop the growth and cause cell death in human lung cancer cells by activating

the PKC- δ /PKD/ERK Signaling Pathway. However, there is currently no information available on the effects of this compound from *E. ledienii*.

12-Deoxy-16-hydroxy-phorbol, is a component of DHPB which has been found in the latex of several plants including *E. ledienii*, *E. poisonii* Pax, *E. cooperi*, *Jatropha curcas* and *Jatropha gossypifolia* (Schmidt & Evans, 1977; Hecker, 1977). It was found to stimulate ornithine decarboxylase in mouse skin, restrict the binding of [3H]-12-O-tetradecanoylphorbol-13-acetate to phorbol ester receptors and activate protein kinase C *in vitro* (Hirota *et al.*, 1988). However, data has yet to be documented on the 12-Deoxy-16-hydroxy-phorbol of *E. ledienii* and *E. cooperi*.

16-Hydroxy-12-desoxyphorbol, a diterpenoid, was isolated from *E. cooperi*, *E. bothae*, *E. triangularis*, *E. ingens* and *Croton rhamnifolius*, and *E. rowlandii* (Dinala *et al.*, 2022; Sakata *et al.*, 1971). Dinala *et al.* (2022) documented that this compound showed activity against HCC70 and MCF-7 with EC₅₀ values of 0.592 and 1.003 μ M; respectively. Contrary to this, Gschwendt and Hecker (1970) reported the compound as a tumor promoter. However, data has yet to be documented on the 16-Hydroxy-12-desoxyphorbol of *E. cooperi*.

12-Deoxyphorbol-13-isobutyrate-16-angelate-20-acetate, which belongs to the diterpene class, was discovered in *E. cooperi* and *E. bothae* (Poppewell *et al.*, 2010). Ahmed (2015) reported that 12-Deoxyphorbol-13-isobutyrate-16-angelate-20-acetate from *E. cooperi* demonstrated noteworthy cytotoxicity against MCF-7 cells.

12-Deoxy phorbol esters, which fall under the phorbol ester class, were previously isolated from *E. tirucalli*, *E. triangular*, *E. resinifera* and *Excoecaria bicolor* (Karalai *et al.*, 1995; Zayed *et al.*, 1984; Gschwendt & Becker, 1970). It was reported that 12-Deoxy phorbol esters are carcinogenic and a tumor promotor (Priya & Rao, 2011; Adolf & Hecker, 1984).

Studies by Driedger and Blumberg (1980) reported inflammation in mice ears after exposure to 12-Deoxy phorbol esters.

Ingenol, a diterpenoid, was discovered in *E. tirucalli* and *E. sikkimensis* by Yang *et al.* (2013). In 2019, Silva and colleagues conducted research on the impact of ingenol, derived from *E. tirucalli*, on multiple human cancer cell lines. The findings showed that the compound had varying degrees of effectiveness, ranging from weak to potent, against the different cell lines. In other studies, ingenol effectively prevented the replication of HIV-1 subtype B and C in both MT-4 cells and human PBMCs (Abreu *et al.*, 2014).

Glut-5-en-3- β -ol, a triterpenoid, was discovered in *E. tirucalli*, *E. pseudocactus* Berger (Abdel-Monem & Abdelrahman, 2016). Abdel-Monem and Abdelrahman (2016) conducted a study on Glut-5-en-3- β -ol's ability to fight against various microorganisms. However, the results showed that Glut-5-en-3- β -ol did not exhibit any activity against any of the tested bacteria.

3.4.1.10. phenolic compounds

Gallic acid, a phenolic acid from *E. cooperi*, is effective in protecting the liver and preventing liver damage caused by paracetamol. It also has antioxidant properties that can improve the levels of serum alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase and bilirubin antioxidant (Ahmed *et al.*, 2019).

Bervifolin carboxylic acid, a tannin from *E. cooperi*, has a strong hepatoprotective effect. It also acts as an antioxidant, reducing the levels of serum alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase and bilirubin in rats with paracetamol-induced hepatotoxicity (Ahmed *et al.*, 2019).

3, 3'-Dimethoxy ellagic acid a tannin, from *E. cooperi* displayed significant hepatoprotective effect as well as antioxidant effect. In particular, it was able to lower levels of serum alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase and bilirubin in rats with paracetamol-induced hepatotoxicity. In addition, the compound showed moderate activity against HepG2 cells (Ahmed, 2019).

Ellagic acid, a tannin from *E. cooperi*, has been shown to have many benefits for liver health in rats. In the same study, it has been found to protect the liver, prevent the growth of cells and act as an antioxidant (Ahmed *et al.*, 2019; Ahmed, 2015). It also reduces the levels of certain enzymes and bilirubin in the blood, which are commonly elevated in cases of liver damage caused by paracetamol antioxidant (Ahmed, 2015; Ahmed *et al.*, 2019).

Kaempferol and Kampferol-3-O- β -D-rutinoside, which are a class of flavonoids from *E. cooperi*. They were found to be effective in protecting the liver, preventing excessive cell growth and reducing the levels of certain enzymes and bilirubin in the blood, all of which are associated with liver damage caused by paracetamol antioxidant (Ahmed, 2015; Ahmed *et al.*, 2019).

1-O-Galloyl-3,6-hexahydroxydiphenyl- β -D-glucopyranoside/corilagin a tannin, was extracted from various plants including *E. cooperi*, *E. prostrata*, *Phyllanthus amarus* L., *Phyllanthus niruri* L and *Euphoria longana* Lam (*Euphorbiaceace*) (Yang *et al.*, 2022; Zheng *et al.*, 2016; Kolodziej *et al.*, 2005; Cheng *et al.*, 1995; Chen *et al.*, 1992). In 2013, Ming and colleagues conducted a study on the potential antitumor effects of corilagin from *Phyllanthus niruri* L. on three types of cells - Chang-liver, SMMC7721 and Bel7402. The findings showed that the compound had a moderate level of effectiveness against SMMC7721 and Bel7402 cells. However, it only exhibited weak activity against Chang-liver cells. In a study

by Bai *et al.* (2019), corilagin extracted from *Dimocarpus longan* Lour was found to have a cytotoxic effect on A549 cells. Another research by Tong *et al.* (2018) discovered that corilagin from *Phyllanthus urinaria* induced apoptosis through reactive oxygen species and autophagy. During autophagy mediation, the Akt/mTOR/p70S6K pathway was suppressed and there was an increase in the formation of autophagic vacuoles and conversion of LC3-I to LC3-II. According to a report by Ahmed (2015), corilagin showed notable toxicity against MCF-7 cells. Kolodziej *et al.* (2005) studied the effects of corilagin from the *Phyllanthus amarus* L. plant on various genes related to inflammation including iNOS. Their findings showed that corilagin increased the mRNA levels of iNOS and cytokines in parasitized cells. In 2013, Jin and colleagues conducted a study on animals to explore the effects of corilagin on liver protection and inflammation reduction. The findings indicated that corilagin was able to inhibit the NF- κ B pathway, leading to an increase in the level of superoxide dismutase, a key defense mechanism against superoxide radical toxicity and nitric oxide, which plays an important role in liver metabolism. Previous research has shown that corilagin, derived from *E. longana* Lam, can decrease blood pressure by reducing the release of plasma noradrenaline and inducing direct vasorelaxation (Cheng *et al.*, 1995). Yang *et al.* (2013) studied the impact of corilagin from *Terminalia bellerica* Roxb on HepG2 cellss; specifically its potential to treat diabetes. Their findings showed that corilagin increased PPAR γ signaling, which led to an increase in adipogenesis. According to studies by Latté and Kolodziej (2000), from the *Zeitschrift für Naturforschung*, corilagin has a strong antifungal effect against *Candida glabrata*. Notka *et al.* (2003) reported HIV-1 replication inhibition from corilagan of *Phyllanthus amarus*.

Tri-methyl ellagic acid is a tannin which was previously isolated from *E. tirucalli* and *E. sorori*. In 2008, Zhang *et al.* conducted a study to assess the antibacterial effect of tri-methyl

ellagic acid on various types of bacteria. The results indicated that the compound had a moderate effect on *B. subtilis* and *S. aureus*, but did not show any inhibitory activity against *E. coli*. There is currently no information available regarding the anticancer properties of trimethyl ellagic acid from *E. tirucalli*.

3'-Di-O-methylellagic acid, which is a phenol, has been isolated from *E. tirucalli*, *E. lunulata* and *E. schimperiana* (Aljubiri *et al.*, 2023). Researchers have tested the cytotoxicity of this compound from *E. schimperiana* extract on four human cancer cell lines. The results showed that 3,3'-Di-O-methylellagic acid had promising cytotoxicity against PC3 cells (Aljubiri *et al.*, 2021; Lan *et al.*, 2020), with an IC₅₀ of 5.5 µg/mL. This depicts the likely anticancer activity of the compound (Aljubiri *et al.*, 2021). Research has found that 3'-di-O- methylellagic acid, which was extracted from *E. schimperiana*, has exhibited promising antibacterial properties. This is reported by the same author. Aljubiri *et al.* (2021) confirmed the antiinflammatory activity of 3'-Di-O-methylellagic acid from the *E. lunulate* isolate. Guo *et al.* (2011) discovered that *E. hylonoma* contains 3'-Di-O-methylellagic acid, which has a strong antioxidant effect. A great efficacy of a potential antimicrobial agent by the compound was observed from the isolate of *E. thymifolia*.

3.4.1.11. Fatty Acids

Laurate, a fatty acid, has been found in *E. coerulescens* and has limited antimicrobial activity against beneficial lactic acid bacteria but strong antimicrobial activity against harmful *Bacteroides* and *Clostridium* bacteria (Wu *et al.*, 2021; Matsue *et al.*, 2019). However, there is currently no available data on the effect of laurate of *E. coerulescens*.

3.4.1.12. Miscellaneous

Diterpene esters, a terpene, were extracted from the latex of *E. horrida*, *E. periplus*, *E. tirucalli* and *E. cauducifolia* (Baloch & Baloch, 2010; Fürstenberger *et al.*, 1985). It is a skin irritant and tumor promoter (Baloch & Baloch, 2010; Fürstenberger *et al.*, 1985). Contrary, studies by Kedei *et al.* (2004) reported that diterpene ester is an anticancer agent as it activates the protein kinase C, which has previously been proven to induce an anticancer effect on cells. According to research conducted by Ogbourne *et al.* (2004), applying high doses of diterpene ester topically cured skin cancer in mice through protein kinase C. Another study by Hampson *et al.* (2005) found that applying a much lower dose of diterpene ester resulted in cell death through a protein kinase C mechanism in leukemia cells. Nothias- Scaglia *et al.* (2015) reported that diterpene ester could also inhibit HIV replication at the nanomolar level. However, there is currently no available data on the effects of diterpene esters from *E. horrida* and *E. tirucalli*.

Out of the 15 *Euphorbia* plants that were studied, some of the compounds were left unexplored. These include Ingol-7,8,12-acetate and ditiglate from *E. ledienii*, as well as 12- Deoxyphorbol-13-(2-methyl butyrate)-20-acetate. One compound from *E. horrida* was identified as 17-Hydroxyingenol-17-benzoate-20-angelate. Additionally, five compounds were identified from *E. coerulescens*: Angelate acetate isobutyrate, Fatty acids, Acetate laurate, α -Methyl butyrate and Heptanoate. *E. cooperi* had five compounds identified: Euphorbilactone, Norsesquiterpenoid, Arachiside A, 16-Angeloyloxy-13 α -isobutanoyloxy-4 β ,9 α ,20-trihydroxytiglic-1,5-diene-3,7-dione and 20-Acetoxy-16-angeloyloxy-13 α -isobutanoyloxy-4 β ,9 α ,20-tetrahydroxytiglic-1,5-diene-3-one. Lastly, *E. tirucalli* had ten compounds identified: Euphorbol hexacosonate, 12-Deoxy-4 β -hydroxyphorbol-13-phenyl acetate-20-acetate, 12, 20-Dideoxyphorbol-13-

isobutyrate, tirucallicine, isoeuphorol, ketone euphorone, euphorbin A, euphorbin B, tirucallin A and tirucallin B.

3.4.2. In *silico* evaluation of selected phytochemicals

Euphorbia species are well reported for various biological activities and pharmacological potential indicating their potential as anticancer agents. Some extracts and isolates of the plants are well reported to show cytotoxic effects, as reviewed in this study. However, the phytochemical constituents that may account for the cytotoxic effects and other biological activities are largely unexplored. *In silico* techniques, which are valuable for resource maximization and search space minimization, may help to provide insightful information on the biological activities, pharmacokinetics, pharmacodynamics and pharmacotherapeutics of phytochemicals reported from *Euphorbia* species; and thereby serve as a fundamental resource for researchers to design more targeted experiments and manage future studies into the medicinal benefits of the unexplored and underutilized *Euphorbia* species.

3.4.2.1. Cell Line Cytotoxicity tendency of selected compounds

Promising *Euphorbia*-derived compounds as shown in Table 3.7 were evaluated *in silico* for cytotoxicity and anticancer tendency using the Cell Line Cytotoxicity Predictor (CLCPred) webserver (Lagunin *et al.*, 2018). Among 74 compounds subjected to prediction, 40 compounds were prioritized based on the availability of information and high cytotoxic activity. The compounds predicted to have high cytotoxic activity exhibited high anticancer potential activity when the probability cut-off was set as both $P(a)_{ctive} > P(i)_{nactive}$ and $P_a > 0.50$. These compounds exhibit sufficient cytotoxic tendencies against multiple cancer cell lines and tumor types in human. Among these are the several *Euphorbia*- derived top compounds (EDCs) viz:

Euphol, cycloartenol, lupeol, α -amyrin, betulinic acid, 24-Methylene-cycloartanol, 12 Deoxyphorbol 13 tiglate 20 acetate, Ingol 7,8,12 acetate, diterpene glycoside, Tirucallol, Isobutyl angelate, Kaempferol-3-O-rutinoside exhibit high cytotoxic probability against 3 or more cell lines (Table 3.7). Considering the huge potential of plant-based natural products in drug discovery programs and the contribution of terpenoids in the production of anti-cancer compounds, terpenoids from *Euphorbia* species can be exploited for more reliable economical and environmentally safe bioactive molecules. Collectively, the terpenoids feature cytotoxicity tendencies against the various cell lines of stomach carcinoma, kidney carcinoma, skin melanoma, liver hepatoblastoma, lung carcinoma, brain glioma, colon adenocarcinoma and thyroid carcinoma (see Table 3.7).

Table 3.7: Compounds with high cytotoxic probability against 3 or more cancer cell lines

Compounds	Pa	Pi	Cancer cell line	Cell line full name	Tissue	Tumor type
Euphol (C1)	0.591	0.002	MKN- 7	Gastric carcinoma	Stomach	Carcinoma
	0.555	0.014	UO-31	Renal carcinoma	Kidney	Carcinoma
	0.554	0.014	SK-MEL-2	Melanoma	Skin	Melanoma
Cycloartenol (C2)	0.783	0.001	MKN-7	Gastric carcinoma	Stomach	Carcinoma
	0.577	0.015	HepG2	Hepatoblastoma	Liver	Hepatoblastoma
	0.523	0.029	DMS-114	Lung carcinoma	Lung	Carcinoma
	0.501	0.017	U-251	Glioma	Brain	Glioma
Lupeol (C3)	0.785	0.003	8505C	Thyroid-gland-undifferentiated	Thyroid	Carcinoma

				carcinoma		
	0.62	0.001	FaDu	Hypopharyngeal squamous carcinoma	Upper aerodigestive tract	Carcinoma
	0.563	0.013	SK-MEL- 2	Melanoma	Skin	Melanoma
	0.539	0.004	DLD-1	Colon adenocarcinoma	Colon	Adenocarcinoma
	0.526	0.005	SW480	Colon adenocarcinoma	Colon	Adenocarcinoma
Alpha-amyrin (C4)	0.52	0.005	PANC-1	Pancreatic carcinoma	Pancreas	Carcinoma
	0.632	0.004	8505C	Thyroid-gland-	Thyroid	Carcinoma

				undifferentiated (anaplastic) carcinoma		
	0.523	0.003	MKN-7	Gastric carcinoma	Stomach	Carcinoma
Betulinic acid (C5)	0.695	0.003	8505C	Thyroid-gland- undifferentiated (anaplastic) carcinoma	Thyroid	Carcinoma
	0.667	0.001	FaDu	Hypopharyngeal- squamous carcinoma	Upper aerodigestive tract	Carcinoma
	0.562	0.013	SK-MEL-2	Melanoma	Skin	Melanoma
24-Methylene-	0.555	0.003	MKN-7	Gastric carcinoma	Stomach	Carcinoma

cycloartanol (C6)	0.539	0.023	HL-60	Promyeloblast leukemia	Haematopoietic and lymphoid tissue	Leukemia
	0.52	0.031	DMS-114	Lung carcinoma	Lung	Carcinoma
	0.505	0.026	HL-60	Promyeloblast leukemia	Haematopoietic and lymphoid tissue	Leukemia
12- Deoxyphorbol- 13-tiglate-20- acetate (C7)	0.638	0.033	A549	Lung carcinoma	Lung	Carcinoma
	0.568	0.024	NCI-H838	Non-small cell lung cancer. 3 stage	Lung	Carcinoma
	0.556	0.021	HL-60	Promyeloblast leukemia	Haematopoietic and lymphoid tissue	Leukemia
Ingol-7,8,12- acetate,ditiglate	0.663	0.012	NCI-H838	Non-small cell lung cancer. 3 stage	Lung	Carcinoma

(C8)	0.592	0.005	SK-MEL-1	Metastatic melanoma	Skin	Melanoma
	0.513	0.033	DMS-114	Lung carcinoma	Lung	Carcinoma
Diterpene glycoside (C9)	0.682	0.008	SK-MEL-2	Melanoma	Skin	Melanoma
	0.614	0.015	HL-60	Promyeloblast leukemia	Haematopoietic and lymphoid tissue	Leukemia
	0.534	0.034	NCI-H838	Non-small cell lung cancer. 3 stage	Lung	Carcinoma
Tirucallol (C10)	0.591	0.002	MKN-7	Gastric carcinoma	Stomach	Carcinoma
	0.555	0.014	UO-31	Renal carcinoma	Kidney	Carcinoma
	0.554	0.014	SK-MEL-2	Melanoma	Skin	Melanoma
Isobutyl angelate	0.616	0.012	HepG2	Hepatoblastoma	Liver	Hepatoblastoma

(C11)	0.545	0.03	NCI-H838	Non-small cell lung cancer. 3 stage	Lung	Carcinoma
	0.544	0.052	A549	Lung carcinoma	Lung	Carcinoma
Kaempferol-3-O-rutinoside (C12)	0.546	0.004	Caco-2	Colon adenocarcinoma	Colon	Adenocarcinoma
	0.548	0.016	SK-MEL-1	Metastatic melanoma	Skin	Melanoma
	0.55	0.022	HL-60	Promyeloblast leukemia	Haematopoietic and lymphoid tissue	Leukemia
	0.542	0.031	NCI-H838	Non-small cell lung cancer. 3 stage	Lung	Carcinoma

*Pa - Probability to be Active, Pi - Probability to be Inactive

3.4.2.2. Physicochemical and drug-like properties

The physicochemical parameters and drug-likeness of the EDCs were computed based on their chemical structures using the SwissADME platform as presented in Table 3.8 (physicochemical properties; drug-like properties). Table 3.7 and Table 3.8 show that most of the compounds show good and desirable physicochemical properties. All the EDCs except C8, C9 and C12 have molecular weight less than 500. Other desirable physicochemical properties exhibited by several compounds are lipophilicity (LogP), solubility, Polar Surface Area (PSA), Hydrogen Bond Donors and Acceptors. Moderate molecular weight is preferred for drug-likeness. Extremely large or small molecules might have issues with absorption, distribution, metabolism and excretion (ADME) properties. Balanced lipophilicity is desirable. Too hydrophilic or too hydrophobic molecules might face issues with absorption or distribution in the body. Good solubility in physiological fluids is essential for a drug candidate to be effective. Poorly soluble compounds might have bioavailability issues. Smaller polar surface area often correlates with better oral absorption. Fewer hydrogen bond donors and acceptors often indicate better drug-like properties.

Drug-likeness refers to the set of characteristics that make a molecule more likely to become an effective and safe drug candidate. Understanding physicochemical properties helps in predicting a molecule's behavior in biological systems and its potential as a drug. Many of these properties are essential for drug development and violation of any of these properties may result in delay or failure of the drug candidate to reach market operation. All the compounds except C9 and C12 exhibit desirable Lipinski and Veber features. They also feature Synthetic accessibility. The Lipinski's Rule-of-Five (RO5) is a guideline used to assess drug-likeness based on physicochemical properties. It suggests that for a compound to

have good oral bioavailability, it should have: Molecular weight <500 Daltons, LogP <5, Hydrogen bond donors <5, Hydrogen bond acceptors <10 (Gyebi *et al.*, 2022; Lipinski, 2004). While adherence to the RO5 is not a strict requirement for a compound to become a drug, it serves as a useful guideline in early-stage drug discovery to prioritize compounds likely to have favorable properties.

3.4.2.3. ADMET properties of top compounds

The fate and behavior of a drug or chemical compound within the human body are significantly influenced by Absorption, Distribution, Metabolism, Excretion and Toxicity (ADMET) factors. Understanding these properties is crucial in drug development to ensure both efficacy and safety. Computational tools play a pivotal role in predicting these properties and in this study we utilized the pKCSM webserver (Pires *et al.*, 2015) to predict the ADMET properties of the top compounds, as detailed in Table 3.8.

Table 3.8: Physicochemical parameters and solubility tendency

Descriptors	Euphol	Cycloartenol	Lupeol	α -amyrin	Betulinic acid	24-Methylene-cycloartanol
Physiochemical characteristic						
Molecular weight (g/mol)	426.72	426.72	426.7 2	426.72	456.70	440.74
Num.rotatable bonds	4	4	1	0	2	5
H-bond acceptors	1	1	1	1	1	1
H-bond donors	1	1	1	1	2	1
Molar Refractivity	137.04	135.14	135.1 4	135.14	136.91	139.95
TPS	20.23	20.23	20.23	20.23	57.53	20.23
Solubility (Log Po/w)	5.09	5.17	4.68	4.77	3.79	5.20

Solubility class	Poorly soluble	Poorly soluble	Poorly soluble	Poorly soluble	Poorly soluble	Poorly soluble
Drug-likeness						
Lipinski	Yes	Yes	Yes	Yes	Yes	Yes
Bioavailability	0.55	0.55	0.55	0.55	0.85	0.55
PAINS	0 alert	0 alert	0 alert	0 alert	0 alert	0 alert
Brenk	1 alert	1 alert	1 alert	1 alert	1 alert	1 alert
Synthetic accessibility	Yes	Yes	Yes	Yes	Yes	Yes
Absorption						
Caco2 permeability(log Papp in 10-6 cm/s)	1.203	1.194	1.226	1.227-J	1.175	1.221
Human intestinal absorption (% Absorbed)	93.119	95.248	95.782	94.062	99.763	95.309

P-glycoprotein SUbstrate	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
P-glycoprotein Inhibitor	Pos.	Pos.	Pos.	Pos.	Neg.	Neg.
P-glycoprotein II inhibitor	Pos.	Pos.	Pos.	Pos.	Neg.	Pos.
Distribution						
VDss (human) (log L/kg)	0.661	-0.075	0	0.266	-1.18	-0.072
BBB permeability (Log BB)	0.683	0.794	0.726	0.674,	-0.322	0.845
CNS permeability (Log PS)	-2.254	-1.714	-1.714	-1.773	-1.343	-1.462
Metabolism						
CYP2D6 substrate	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
CYP3A4 substrate	Pos.	Pos.	Pos.	Pos.	Pos.	Pos.
CYP1A2 inhibition	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.

CYP2C19 inhibitor	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
CYP2C9 inhibitor	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
CYP2D6 inhibitor	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
CYP3A4 inhibitor	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Excretion						
Total Clearance (l og ml/min/kg)	0.403	0.262	0.153	0.119	0.116	0.255
Renal OCT2 substrate	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Toxicity						
Max. tolerated dose (human) (log mg/kg/day)	-0.568	-0.46	0.502	-0.571	0.144	0.303
hERG Iinhibitor	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Oral Rat Acute –	1.906	2.627	2.563	2.4	2.256	2.542



Central University of
Technology, Free State

Toxicity (LOSO)(mol/ka)						
Oral Rat Chronic T oxicity (LOAEL)((log mg/ ka bw/day)	0.788	0.806	0.8	0.856	2.206	0.802
Hepatotoxicity	Neg.	Neg.	Neg.	Neg.	Pos.	Neg.

[Neg. – Negative; Pos. – Positive]

The predictions regarding the intestinal absorption of Euphorbia-derived terpenoids in humans, based on computed molecular descriptors, indicate that all the compounds exhibit good human intestinal absorption (%Absorbed). Additionally, most of these compounds are predicted as P-glycoprotein inhibitors (PGIs). PGIs interfere with glycoprotein activity, preventing the pumping of drug compounds out of cells. This inhibition can lead to the maintenance of high intracellular drug concentration, potentially improving therapeutic efficacy. Notably, several FDA-approved drugs and natural compounds are recognized as PGIs (Amin, 2013).

Human volume of distribution (VD_{ss}) is a pivotal parameter for determining the human dose. While some Euphorbia-derived compounds feature low VD_{ss}, the majority exhibit a low plasma binding tendency. A drug with strong plasma protein binding may struggle to dissociate from plasma proteins and bind to target receptors, even if present in the bloodstream. Thus, a candidate with very high plasma levels due to very low VD_{ss} may appear promising, but it is essential to examine the magnitude of the pharmacological effect to avoid potential misinterpretations (White, 2017).

The computed interactions of the compounds with liver phase I drug metabolism, using various cytochrome P450 descriptors, reveal that most of the top compounds do not exhibit inhibitory tendencies against various cytochrome P450s. This suggests that these compounds may not adversely affect phase I drug metabolism in the liver.

Organic cation transporter 2 (OCT2) mediates the initial step in the renal secretion of organic cations. While several compounds exhibit a high clearance rate tendency, all are predicted to be non-substrates of OCT2.

Computed molecular descriptors assessing the toxicity tendency of the compounds indicate that the majority may not be toxic, as indicated by AMES toxicity and maximum dose (human) predictions. hERG I, which blocks the hERG channel, may cause cardiotoxicity and oral rat acute toxicity. However, none of the six phytocompounds in this study exhibit the potential to be hERG channel blockers, suggesting that they may not cause hERG channel- related cardiotoxicity (Gyebi *et al.*, 2022; Kratz *et al.*, 2017).

3.4.3. Biosynthesis of terpenoid class

The *Euphorbia* species contains a diverse array of compounds, including terpenoids, steroids, fatty acids and phenolic compounds. Each compound has its own specific biosynthetic pathway. However, the current study will only focus on the biosynthesis pathway of terpenoid, as it is the main class of compounds isolated from *Euphorbia* species. A schematic diagram of the biosynthesis is presented in Figure 3.16.

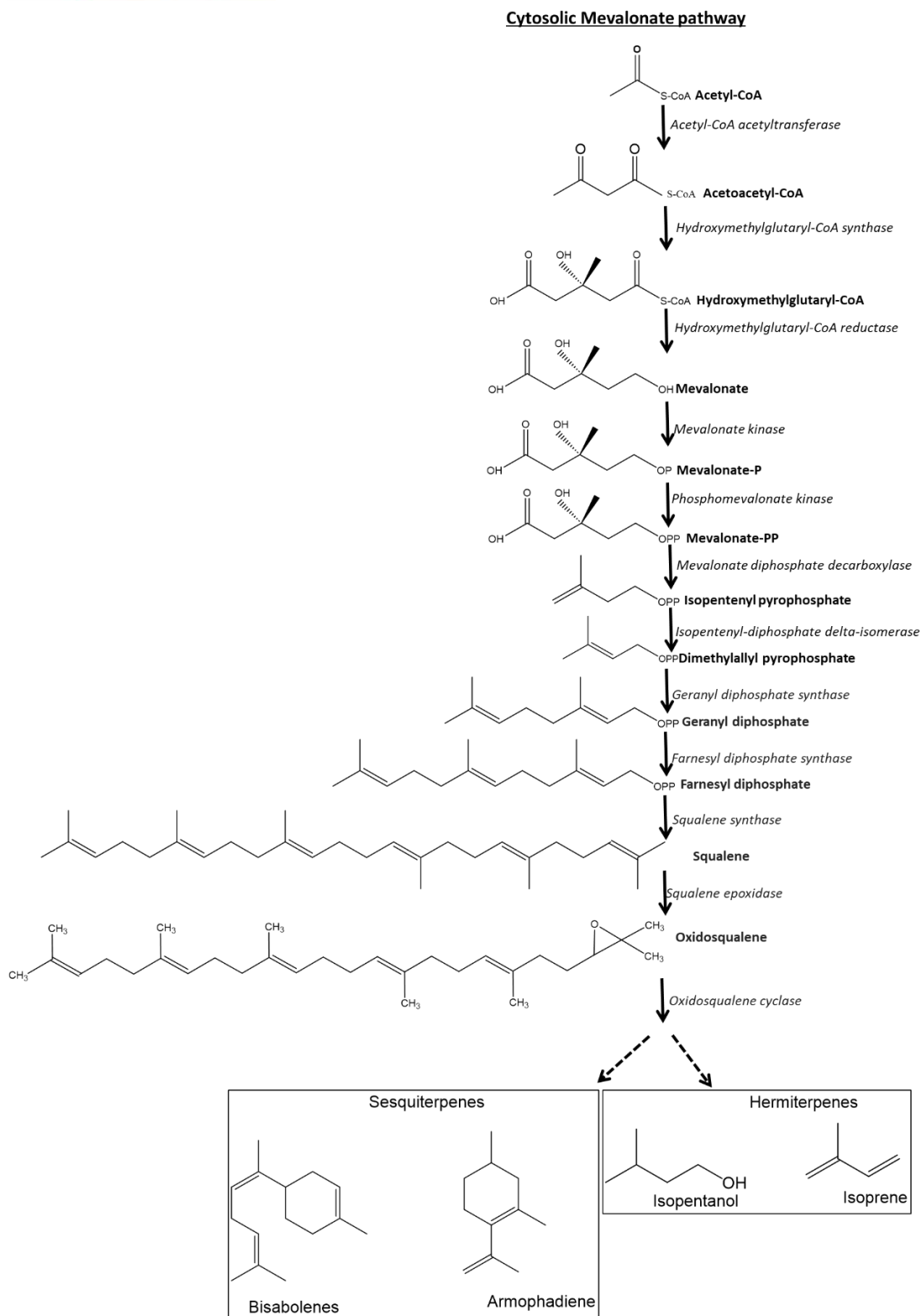


Figure 3.16. Terpene biosynthesis in the cytosol through the mevalonate pathway. Starting with acetyl-CoA, this process produces terpene precursors (isopentenyl pyrophosphate and

dimethylallyl pyrophosphate), serving as building blocks for various terpenes with diverse biological functions. The diagram was adopted from Qiao *et al.* (2018).

3.5. Conclusion and future perspective

This review focuses on the medicinal properties of 15 species of *Euphorbia*, particularly their potential as alternative treatments for cancer. These plants contain unique compounds such as triterpenoids, tannins, diterpene esters and sterols. Several studies have reported that the *Euphorbiaceae* family contains phytochemicals that lead to the isolation of various classes of triterpenoids. Some of these triterpenoids include euphol (found in *E. trigona*, *E. tirucalli*, *E. enopla*, *E. coerulescens*, *E. ammak*, *E. cooperi*), cycloartenol, lupeol, amyryns, taraxerols, friedelin and tirucallol (found in *E. trigona*). Tannins classes include ellagic acid, 1-O-Galloyl-3,6-hexahydroxydiphenyl- β -D-, tri methyl ellagic acid and unknown structures Tirucallin A and Tirucallin B. Sterols classes such as β -sitosterol, Stigmasterol and Obtusifoliol are also present. Diterpenoid classes such as diterpene esters, 12-Deoxyphorbol, phorbol, ingenol, ingenol triacetate, 16-Hydroxy-12-desoxyphorbol, 12-Deoxyphorbol-13-isobutyrate-16-angelate-20-acetate are also found. These compounds have shown cytotoxic effects through various mechanisms of action, including cell proliferation and differentiation, and also apoptosis. Out of all the *Euphorbia* species studied, *E. trigona* had the most anti-cancer isolated compounds with a total of 14. *E. tirucalli* followed closely behind with 13, while *E. cooperi* had 8 and the others had fewer or none at all. While some studies have reported promising results, others have found no significant inhibitory activity against certain cell lines. The insilico results further revealed that several *Euphorbia*-derived top compounds show good cytotoxic potential against multiple cancer cell lines indicating anticancer tendency against stomach carcinoma, kidney carcinoma, skin melanoma, liver

hepatoblastoma, lung carcinoma, brain glioma, colon adenocarcinoma and thyroid carcinoma, etc. Most of these compounds are drug-like as indicated by Lipinski screening and Veber parameters which were derived from good physicochemical properties. Most of the EDCs were predicted to possess good pharmacokinetic tendencies as indicated by the ADMET properties.

Therefore, building upon the diverse compounds identified, such as triterpenoids, tannins, diterpene esters and sterols, requires further in-depth pharmacological studies to unravel the precise mechanisms of action and potential synergies among these compounds. Subsequent clinical trials would be pivotal in assessing the safety and efficacy of these compounds in human subjects. Additionally, exploring combination therapies with existing cancer treatments, investigating the bioavailability of isolated compounds and identifying new, yet-to-be-evaluated compounds like Euphorbin A, Euphorbin B and Tirucallin A are crucial areas of focus. Development of optimized formulations, understanding the factors contributing to variability in study results and fostering collaboration between diverse research fields are imperative for advancing the potential of *Euphorbia* species in cancer therapeutics. Furthermore, public awareness initiatives and the integration of ethnobotanical knowledge can contribute to a holistic understanding of the historical and contemporary significance of these plants in medicinal contexts. This comprehensive and multidisciplinary approach underscores the need for ongoing research to harness the full therapeutic potential of *Euphorbia* species for cancer treatment. Overall, this report aims to provide scientific credibility to the traditional use of *Euphorbia* species for medicinal purposes.

3. 6. References

- Aljubiri, S. M., Elsalam, E. A., Abd El Hady, F. K., Radwan, M. O., Almansour, A. I., & Shaker, K. H. (2023). In vitro acetylcholinesterase, tyrosinase inhibitory potentials of secondary metabolites from *Euphorbia schimperiana* and *Euphorbia balsamifera*. *Zeitschrift für Naturforschung C*, 78(5-6), 209-216.
- Abdel-Aty, A. M., Hamed, M. B., Salama, W. H., Ali, M. M., Fahmy, A. S., & Mohamed, S. A. (2019). Ficus carica, Ficus sycomorus and Euphorbia tirucalli latex extracts: Phytochemical screening, antioxidant and cytotoxic properties. *Biocatalysis and Agricultural Biotechnology*, 20, 101199.
- Abdel-Fattah, M. R. (1987). The chemical constituents and economic plants of the Euphorbiaceae. *Botanical Journal of the Linnean Society*, 94, 293–326.
- Abdel-Hameed, E. S. S., Bazaid, S. A. & Salman, M. S. (2013). Characterization of the phytochemical constituents of Taif rose and its antioxidant and anticancer activities. *BioMed research international*, 2013, 13 pages.
- Abdel-Monem, A. R. & Abdelrahman, E. H. (2016). New abietane diterpenes from *Euphorbia pseudocactus berger* (Euphorbiaceae) and their antimicrobial activity. *Pharmacognosy magazine*, 12(Suppl 3), S346. doi: 10.4103/0973-1296.185768
- Abdel-Monem, A. R., Abdel-Sattar, E., Harraz, F. M. & Petereit, F. (2008). Chemical Investigation of *Euphorbia schimperi* C. Presl. *Records of natural products*, 2(2).
- Abdel-Sattar, E., Abou-Hussein, D. & Petereit, F. (2015). Chemical constituents from the leaves of *Euphorbia ammak* growing in Saudi Arabia. *Pharmacognosy research*, 7(1), 14. doi:



Abdel-Sattar, E., Maes, L., & Salama, M. M. (2010). In vitro activities of plant extracts from Saudi Arabia against malaria, leishmaniasis, sleeping sickness, and Chagas disease. *Phytotherapy Research*, 24, 1322–1328.

Abdul-Hammed, M., Bello, I. A., Olajide, M., Adedotun, I. O., Afolabi, T. I., Ibironke, A. A., *et al.* (2023). Exploration of bioactive compounds from *Mangifera indica* (Mango) as probable inhibitors of thymidylate synthase and nuclear factor kappa-B (NF-Kb) in colorectal cancer management. *Physical sciences reviews*, (0).

Abreu, C. M., Price, S. L., Shirk, E. N., Cunha, R. D., Pianowski, L. F., Clements, J. E., Tanuri, A. & Gama, L. (2014). Dual role of novel ingenol derivatives from *Euphorbia tirucalli* in HIV replication: inhibition of de novo infection and activation of viral LTR. *PloS one*, 9(5), p. e97257.

Adedapo, A. A., Saba, A. B., Dina, O. A. & Oladejo, G. M. A. (2004). Effects of dexamethasone on the infectivity of *Trypanosoma vivax* Y486 and the haematological changes in Nigerian domestic chickens (*Gallus gallus domesticus*). *Veterinarski arhiv*, 74(5), 371-381.

Adetunji, J. A., Ogunyemi, O. M., Gyebi, G. A., Adewumi, A. E., & Olaiya, C. O. (2023). Atomistic simulations suggest dietary flavonoids from *Beta vulgaris* (beet) as promising inhibitors of human angiotensin-converting enzyme and 2-alpha-adrenergic receptors in hypertension. *Bioinformatics Advances*, 3(1).

Adolf, W., & Hecker, E. (1984). On the active principles of the spurge family, X. Skin irritants, cocarcinogens, and cryptic cocarcinogens from the latex of the manchineel tree. *Journal of Natural Products*, 47(3), 482-496.

Afolayan, A. J., Grierson, D. S. & Mbeng, W. O. (2014). Ethnobotanical survey of medicinal

plants used in the management of skin disorders among the Xhosa communities of the Amathole District, Eastern Cape, South Africa. *Journal of ethnopharmacology*, 153(1), 220-232.

Aghaei, M., Yazdiniapour, Z., Ghanadian, M., Zolfaghari, B., Lanzotti, V. & Mirsafaei, V. (2016). Obtusifoliol related steroids from *Euphorbia sogdiana* with cell growth inhibitory activity and apoptotic effects on breast cancer cells (MCF-7 and MDA-MB231). *Steroids*, 115, 90-97.

Ahmed, S. R., Elsherei, M. M., Salah El Dine, R. & Eltomy, S. (2019). Phytoconstituents, Hepatoprotective, and Antioxidant Activities of *Euphorbia cooperi* NE Br. *Egyptian journal of chemistry*, 62(special issue (Part 2) innovation in chemistry), 831-840.

Ahmed, S. R. (2015). Phytochemical and Biological Study of Certain *Euphorbia* Species Cultivated in Egypt. [Doctoral Theses].

Aiken, C. & Chen, C. H. (2005). Betulinic acid derivatives as HIV-1 antivirals. *Trends in molecular medicine*, 11(1), 31-36.

Akihisa, T., Ogihara, J., Kato, J., Yasukawa, K., Ukiya, M., Yamanouchi, S. & Oishi, K. (2001). Inhibitory effects of triterpenoids and sterols on human immunodeficiency virus-1 reverse transcriptase. *Lipids*, 36, 507-512.

Akihisa, T., Yasukawa, K., Oinuma, H., Kasahara, Y., Yamanouchi, S., Takido, M. & Tamura, T. (1996). Triterpene alcohols from the flowers of compositae and their anti- inflammatory effects. *Phytochemistry*, 43(6), 1255-1260.

Alakurtti, S., Mäkelä, T., Koskimies, S. & Yli-Kauhaluoma, J. (2006). Pharmacological properties of the ubiquitous natural product betulin. *European journal of pharmaceutical sciences*, 29(1), 1-13.

Aleksandrov, M., Maksimova, V. & Koleva Gudeva, L. (2019). Review of the anticancer and cytotoxic activity of some species from genus *Euphorbia*. *Agriculturae conspectus scientificus*, 84(1). 1-5.

Al-Hajj, M. M. A., Al-Shamahy, H. A., Alkhatib, B. Y. & Moharram, B. A. (2018). *In vitro* anti-leishmanial activity against cutaneous Leishmania parasites and preliminary phytochemical analysis of four Yemeni medicinal plants. *Universal journal of pharmaceutical research*, 3, 48-54.

Al-Harbi, N. A. (2017). Diversity of medicinal plants used in the treatment of skin diseases in Tabuk region, Saudi Arabia. *Journal of medicinal plants research*, 11(35), 549-555.

Aljubiri, S. M., Mahmoud, K., Mahgoub, S. A., Almansour, A. I. & Shaker, K. H. (2021). Bioactive compounds from *Euphorbia schimperiana* with cytotoxic and antibacterial activities. *South African journal of botany*, 141, 357-366.

Almehdar, H., Abdallah, H. M., Osman, A. M. M. & Abdel-Sattar, E. A. (2012). *In vitro* cytotoxic screening of selected Saudi medicinal plants. *Journal of natural medicines*, 66, 406-412.

Alotaibi, D., Amara, S., Johnson, T. L. & Tiriveedhi, V. (2018). Potential anticancer effect of prostratin through SIK3 inhibition. *Oncology letters*, 15(3), 3252-3258.

Al-Sultan, S. I. & Hussein, Y. A. (2006). Acute toxicity of *Euphorbia heliscopia* in rats. *Pakistan journal of nutrition*, 5(2), 135-140.

Altamimi, M., Jaradat, N., Alham, S., Al-Masri, M., Bsharat, A., Alsaleh, R. & Sabobeh, R. (2019). Antioxidant, anti-enzymatic, antimicrobial and cytotoxic properties of *Euphorbia tirucalli* L. bioRxiv, 2019-12.

Amarowicz, R., Troszynska, A., Barylko-Pikielna, N. I. N. A. & Shahidi, F. (2004).

Polyphenolics extracts from legume seeds: correlations between total antioxidant activity, total phenolics content, tannins content and astringency. *Journal of food lipids*, 11(4), 278- 286.

Anjaneyulu, V., Rao, G. S. & Connolly, J. D. (1985). Occurrence of 24-epimers of cycloart-25-ene-3 β , 24-diols in the stems of *Euphorbia trigona*. *Phytochemistry*, 24(7), 1610-1612.

Anjaneyulu, V., Satyanarayana, P., Viswanadham, K. N., Jyothi, V. G., Rao, K. N. & Radhika, P. (1999). Triterpenoids from *Mangifera indica*. *Phytochemistry*, 50(7), 1229-1236.

Anju, V. & Rameshkumar, K. B. (2022). Phytochemical investigation of *Euphorbia trigona*. *Journal of the Indian chemical society*, 99(1), 100253.

Anju, V., Singh, A., Shilpa, G., Kumar, B., Priya, S., Sabulal, B. & Rameshkumar, K. B. (2018). Terpenes and biological activities of *Euphorbia tortilis*. *Letters in organic chemistry*, 15(3), 221-225.

Arnold, H. J. & Gulumian, M. (1984). Pharmacopoeia of traditional medicine in Venda. *Journal of ethnopharmacology*, 12(1), 35-74.

Assefa, A. & Bahiru, A. (2018). Ethnoveterinary botanical survey of medicinal plants in Abergelle, Sekota and Lalibela districts of Amhara region, Northern Ethiopia. *Journal of ethnopharmacology*, 213, 340-349.

Bai, X., Pan, R., Li, M., Li, X. & Zhang, H. (2019). HPLC profile of longan (cv. Shixia) pericarp-sourced phenolics and their antioxidant and cytotoxic effects. *Molecules*, 24(3), 619.

Baloch, I. B. & Baloch, M. K. (2010). Irritant and co-carcinogenic diterpene esters from the latex of *Euphorbia cauducifolia* L. *Journal of Asian natural products research*, 12(7), 600-613.

Banzouzi, J. T., Soh, P. N., Ramos, S., Toto, P., Cavé, A., Hemez, J. & Benoit-Vical, F. (2015). Samvisterin, a new natural antiplasmodial betulin derivative from *Uapaca paludosa*

(*Euphorbiaceae*). *Journal of ethnopharmacology*, 173, 100-104.

Barla, A., Bırman, H., Kültür, Ş. & Öksüz, S. (2006). Secondary metabolites from *Euphorbia helioscopia* and their vasodepressor activity. *Turkish journal of chemistry*, 30(3), 325-332.

Baslas, R. K. & Gupta, N. C. (1982). Chemical constituents of the bark of *Euphorbia tirucalli*. *Indian journal of pharmaceutical sciences*.

Baslas, R. K. & Gupta, N. C. (1984). Constituents with potential effective agents from the latex of some *Euphorbia* species. *Herba hungarica (Hungary)*.

Berdy, J. (2005). Bioactive microbial metabolites. *The Journal of antibiotics*, 58(1), 1-26.

Bernard, P., Scior, T., Didier, B., Hibert, M. & Berthon, J. Y. (2001). Ethnopharmacology and bioinformatic combination for leads discovery: application to phospholipase A2 inhibitors. *Phytochemistry*, 58(6), 865-874.

Betancur-Galvis, L. A., Morales, G. E., Forero, J. E. & Roldan, J. (2002). Cytotoxic and antiviral activities of Colombian medicinal plant extracts of the *Euphorbia* genus. *Memórias do instituto oswaldo Cruz*, 97, 541-546.

Biesboer, D. D. & Mahlberg, P. G. (1979). The effect of medium modification and selected precursors on sterol production by short-term callus cultures of *Euphorbia tirucalli*. *Journal of natural products*, 42(6), 648- 657.

Bigoniya, P. & Rana, A. (2008). A comprehensive phyto-pharmacological review of *Euphorbia neriifolia* Linn. *Pharmacognosy reviews*, 2(4), 57.

Bigoniya, P. & Rana, A. C. (2009). Radioprotective and in-vitro cytotoxic sapogenin from *Euphorbia neriifolia* (*Euphorbiaceae*) leaf. *Tropical journal of pharmaceutical research*, 86.

Bijekar, S. R. & Gayatri, M. C. (2014). Ethanomedicinal properties of *Euphorbiaceae* family-

a comprehensive review. *International journal of phytomedicine*, 6(2), 144.

Biswas, M., Biswas, K., Ghosh, A. & Haldar, P. (2009). A pentacyclic triterpenoid possessing antiinflammatory activity from the fruits of *Dregea volubilis*. *Pharmacognosy magazine*, 5(19), 64.

Borgati, T. F., Pereira, G. R., Brandão, G. C. & Oliveira, A. B. D. (2012). Synthesis of triazol derivatives of lupeol with potential antimalarial activity. *Orbital: The electronic journal of Chemistry*, 4(1), 21-22.

Boshara, O. A. A. (2014). *Phytochemical Screening for Leaves, Cortex and Pith of the Cactus Euphorbia trigona L* [Doctoral dissertation]. University of Gezira.

Boudiar, T., Hichem, L., Khalfallah, A., Kabouche, A., Kabouche, Z., Brouard, I. &

Bruneau, C. (2010). A new alkaloid and flavonoids from the aerial parts of *Euphorbia guyoniana*. *Natural product communications*, 5(1), 1934578X1000500109.

Bouquet, A. J. (1969). *Natural products as an alternative remedy* 24th ed. Royal botanic Garden Kew, 166 -179.

Breyer-Brandwijk, M. G. (1962). *The Medicinal and Poisonous Plants of Southern and Eastern Africa being an Account of their Medicinal and other Uses, Chemical Composition, Pharmacological Effects and Toxicology in Man and Animal. The Medicinal and Poisonous Plants of Southern and Eastern Africa being an Account of their Medicinal and other Uses, Chemical Composition, Pharmacological Effects and Toxicology in Man and Animal.*, (Edn. 2).

Brodie, C. & Blumberg, P. M. (2003). Regulation of cell apoptosis by protein kinase c δ . *Apoptosis*, 8, 19-27.

- Buzzini, P., Arapitsas, P., Goretti, M., Branda, E., Turchetti, B., Pinelli, P., *et al.* (2008). Antimicrobial and antiviral activity of hydrolysable tannins. *Mini-reviews in medicinal chemistry*, 8(12), 1179.
- Cao, S., Brodie, P., Miller, J. S., Birkinshaw, C., Rakotondrafara, A., Andriantsiferana, R., *et al.* (2009). Antiproliferative compounds of *Helmiopsis sphaerocarpa* from the Madagascar rainforest. *Natural product research*, 23(7), 638-643.
- Carréu, J. P. M. (2020). *Bioactive terpenoids from Euphorbia pubescens: isolation and derivatization* (Masters dissertation). University of Lisbon.
- Carter, S. (1994). A preliminary classification of *Euphorbia* subgenus *Euphorbia*. *Annals of the missouri botanical garden*, 368-379.
- Cataluna, P. & Rates, S. M. K. (1997). The traditional use of the latex from *Euphorbia tirucalli* Linnaeus (*Euphorbiaceae*) in the treatment of cancer in South Brazil. In *II WOCMAP congress medicinal and aromatic plants, part 2: pharmacognosy, pharmacology, phytomedicine, toxicology* 501, 289-296.
- Chaturvedula, V. P., Schilling, J. K., Miller, J. S., Andriantsiferana, R., Rasamison, V. E. & Kingston, D. G. (2004). New cytotoxic terpenoids from the wood of *Vvepris p unctata* from the Madagascar Rainforest. *Journal of natural products*, 67(5), 895-898.
- Chen, J., Wei, S. L. & Gao, K. (2015). Chemical constituents and antibacterial activities of compounds from *Lentinus edodes*. *Chemistry of natural compounds*, 51, 592-594.
- Chen, L., Chen, R. & Wei, K. (1992). Constituents of tannins from *Euphorbia prostrata* Ait. *Zhongguo Zhong yao za zhi= Zhongguo Zhongyao Zazhi= China Journal of Chinese Materia Medica*, 17(4), 225-6.

- Chen, Y. & Li, J. (2021). Glutinol inhibits the proliferation of human ovarian cancer cells via PI3K/AKT signaling pathway. *Tropical journal of pharmaceutical research*, 20(7), 1331-1335.
- Cheng, D., Guo, Z. & Zhang, S. (2015). Effect of β -sitosterol on the expression of HPV E6 and p53 in cervical carcinoma cells. *Contemporary oncology/Współczesna Onkologia*, 19(1), 36-42.
- Cheng, JT, Lin, TC & Hsu, FL (1995). Antihypertensive effect of corilagin in the rat. *Canadian journal of physiology and pharmacology*, 73 (10), 1425-1429.
- Chinsembu, K. C. (2020). Coronaviruses and nature's pharmacy for the relief of coronavirus disease 2019. *Revista brasileira de farmacognosia*, 30, 603-621.
- Choene, M. & Motadi, L. (2016). Validation of the antiproliferative effects of Euphorbia tirucalli extracts in breast cancer cell lines. *Molecular biology*, 50(1), 98–110.
- Chung, K. T., Wong, T. Y., Wei, C. I., Huang, Y. W. & Lin, Y. (1998). Tannins and human health: a review. *Critical reviews in food science and nutrition*, 38(6), 421-464.
- Cock, I. E., Ndlovu, N. & Van Vuuren, S. F. (2021). The use of South African botanical species for the control of blood sugar. *Journal of ethnopharmacology*, 264, 113234.
- Cowan, M. M. (1999). Plant products as antimicrobial agents. *Clinical microbiology reviews*, 12(4), 564-582.
- Csupor-Löffler, B., Hajdú, Z., Zupkó, I., Molnár, J., Forgo, P., Vasas, A., *et al.* (2011). Antiproliferative constituents of the roots of *Conyza canadensis*. *Planta medica*, 77(11), 1183-1188.

Damle, A. A., Pawar, Y. P. & Narkar, A. A. (2013). Anticancer activity of betulinic acid on MCF-7 tumors in nude mice.

de Miranda, A. L. P., Silva, J. R., Rezende, C. M., Neves, J. S., Parrini, S. C., Pinheiro, M. L., *et al.* (2000). Antiinflammatory and analgesic activities of the latex containing triterpenes from *Himatanthus sucuuba*. *Planta medica*, 66(03), 284-286.

de Oliveira, T. L., Munhoz, A. C. M., Lemes, B. M., Minozzo, B. R., Nepel, A., Barison, A., *et al.* (2013). Antitumoral effect of *Synadenium grantii* Hook f.(*Euphorbiaceae*) latex. *Journal of ethnopharmacology*, 150(1), 263-269.

de Souza, L. S., Puziol, L. C., Tosta, C. L., Bittencourt, M. L., Santa Ardisson, J., *et al.* (2019). Analytical methods to access the chemical composition of an *Euphorbia tirucalli* anticancer latex from traditional Brazilian medicine. *Journal of ethnopharmacology*, 237, 255-265.

De, P. T., Urones, J. G., Marcos, I. S., Basabe, P., Cuadrado, M. S. & Moro, R. F. (1987). Triterpenes from *Euphorbia broteri*. *Phytochemistry*, 26(6), 1767-1776.

Demirkapu, M. J. & Yananli, H. R. (2020). Opium Alkaloids. *Bioactive compounds in nutraceutical and functional food for good human health*.

Dinala, M. M., Siwe-Noundoub, X., Augustyna, W. & Tembu, V.J. (2022). Metabolomics and phytochemicals of *Euphorbia rowlandii* with anticancer properties. The 2nd African Traditional and Natural Product Medicine Conference. Poster 6. University of Limpopo.

Ding, Y., Liang, C., Kim, J. H., Lee, Y. M., Hyun, J. H., Kang, H. K., *et al.* (2010). Triterpene compounds isolated from *Acer mandshuricum* and their antiinflammatory activity. *Bioorganic and medicinal chemistry letters*, 20(5), 1528-1531.

Domsalla, A., Görick, C. & Melzig, M. F. (2010). Proteolytic activity in latex of the genus *Euphorbia*—a chemotaxonomic marker?. *Die Pharmazie-An International journal of pharmaceutical sciences*, 65(3), 227-230.

Driedger, P. E. & Blumberg, P. M. (1980). Structure-activity relationships in chick embryo fibroblasts for phorbol-related diterpene esters showing anomalous activities in vivo. *Cancer research*, 40(2), 339-346.

Dudareva, N., Pichersky, E. & Gershenzon, J. (2004). Biochemistry of plant volatiles. *Plant physiology*, 135(4), 1893-1902.

Duong, T. H., Benididir, M. A., Genta-Jouve, G., Nguyen, H. H., Nguyen, D. P., Mac, D. H., *et al.* (2019). Further terpenoids from *Euphorbia tirucalli*. *Fitoterapia*, 135, 44-51.

Dutra, R. C., Claudino, R. F., Bento, A. F., Marcon, R., Schmidt, E. C., Bouzon, Z. L., *et al.* (2011). Preventive and therapeutic euphol treatment attenuates experimental colitis in mice. *PloS one*, 6(11), e27122.

Dutra, R. C., da Silva, K. A. B. S., Bento, A. F., Marcon, R., Paszcuk, A. F., Meotti, F. C., *et al.* (2012). Euphol, a tetracyclic triterpene produces antinociceptive effects in inflammatory and neuropathic pain: The involvement of cannabinoid system. *Neuropharmacology*, 63(4), 593-605.

Ekpo, O. E. & Pretorius, E. 2007. Asthma, *Euphorbia hirta* and its antiinflammatory properties: News and views. *South African journal of science*, 103(5), 201-203.

El Izzi, A., Benie, T., Thieulant, M. L., Le Men-Olivier, L. & Duval, J. (1992). Stimulation of LH release from cultured pituitary cells by saponins of *Petersianthus macrocarpus*: A permeabilizing effect. *Planta medica*, 58(03), 229-233.

El-Fiky, F., Asres, K., Gibbons, S., Hammada, H., Badr, J. & Umer, S. (2008). Phytochemical and antimicrobial investigation of latex from *Euphorbia abyssinica* Gmel. *Natural product communications*, 3(9).

El-Hawary, S. S., Mohammed, R., Tawfike, A. F., Lithy, N. M., AbouZid, S. F., Amin, M. N., *et al.* (2020). Cytotoxic activity and metabolic profiling of fifteen *Euphorbia* Species. *Metabolites*, 11(1), 15.

El-Shanwani, M. A. A. (1996). Al-nibatat al-mustakhdima fi al-tibb al-sha'abi al-Saudi [Plants used in Saudi folk medicine]. *General directorate of research grants program, KACST, Riyadh*.

El-Sherei, M. M., Islam, W. T., El-Dine, R. S., El-Toumy, S. A. & Ahmed, S. R. (2015). Phytochemical investigation of the cytotoxic latex of *Euphorbia cooperi* NE Br. *Australian journal of basic and applied sciences*, 9(11), 488-493.

El-Toumy, S. A., Salib, J. Y., El-Kashak, W. A., Marty, C., Bedoux, G. & Bourgougnon, N. (2018). Antiviral effect of polyphenol rich plant extracts on herpes simplex virus type 1. *Food science and human wellness*, 7(1), 91–101.

Engi, H., Vasas, A., Redei, D., Molnár, J. & Hohmann, J. (2007). New MDR modulators and apoptosis inducers from *Euphorbia* species. *Anticancer research*, 27(5A), 3451-3458.

Erazo, S., Rocco, G., Zaldivar, M., Delporte, C., Backhouse, N., Castro, C., *et al.* (2008). Active metabolites from *Dunalia spinosa* resinous exudates. *Zeitschrift für naturforschung C*, 63(7-8), 492-496.

Esposito, F., Sanna, C., Del Vecchio, C., Cannas, V., Venditti, A., Corona, A., *et al.* (2013). *Hypericum hircinum* L. components as new single-molecule inhibitors of both HIV-1 reverse

transcriptase-associated DNA polymerase and ribonuclease H activities. *Pathogens and disease*, 68(3), 116-124.

Evans, F. J. (1978). The irritant toxins of Blue *Euphorbia* (*Euphorbia coerulescens* Haw.). *Toxicon*, 16(1), 51-57.

Evans, F. J. & Kinghorn, A. D. (1977). A comparative phytochemical study of the diterpenes of some species of the genera *Euphorbia* and *Elaeophorbia* (*Euphorbiaceae*). *Botanical journal of the linnean society*, 74(1), 23-35.

Fatope, M. O., Zeng, L., Ohayaga, J. E., Shi, G. & McLaughlin, J. L. (1996). Selectively cytotoxic diterpenes from *Euphorbia poisonii*. *Journal of medicinal chemistry*, 39(4), 1005-1008.

Fattorusso, E., Lanzotti, V., Taglialatela-Scafati, O., Di Rosa, M. & Ianaro, A. (2000). Cytotoxic saponins from bulbs of *Allium porrum* L. *Journal of agricultural and food chemistry*, 48(8), 3455-3462.

Fernandez-Arche, A., Saenz, M. T., Arroyo, M., De la Puerta, R. & Garcia, M. D. (2010). Topical antiinflammatory effect of tirucallol, a triterpene isolated from *Euphorbia lactea* latex. *Phytomedicine*, 17(2), 146-148.

Foo, J. B., Yazan, L. S., Tor, Y. S., Wibowo, A., Ismail, N., How, C. W., *et al.* (2015). Induction of cell cycle arrest and apoptosis by betulinic acid-rich fraction from *Dillenia suffruticosa* root in MCF-7 cells involved p53/p21 and mitochondrial signalling pathway. *Journal of ethnopharmacology*, 166, 270-278.

- Fraga-Corral, M., Otero, P., Cassani, L., Echave, J., Garcia-Oliveira, P., Carpena, M., *et al.* (2021). Traditional applications of tannin rich extracts supported by scientific data: Chemical composition, bioavailability and bioaccessibility. *Foods*, 10(2), 251.
- Francis, G., Kerem, Z., Makkar, H. P. and Becker, K. (2002). The biological action of saponins in animal systems: a review. *British journal of nutrition*, 88(6), 587-605.
- Fuchs, H., Bachran, D., Panjideh, H., Schellmann, N., Weng, A., Melzig, M. F., *et al.* (2009). Saponins as tool for improved targeted tumor therapies. *Current drug targets*, 10(2), 140-151.
- Fürstenberger, G. & Hecker, E. (1977). New highly irritant *Euphorbia* factors from latex of *Euphorbia tirucalli* L. *Experientia*, 33, 986-988.
- Fürstenberger, G. & Hecker, E. (1985). On the Active Principles of the Spurge Family (*Euphorbiaceae*) XI.[1] the Skin Irritant and Tumor Promoting Diterpene Esters of *Euphorbia tirucalli* L. Originating from South Africa. *Zeitschrift für naturforschung C*, 40(9- 10), 631-646.
- Gaidi, G., Correia, M., Chauffert, B., Beltramo, J. L., Wagner, H. & Lacaille-Dubois, M. A. (2002). Saponins-mediated potentiation of cisplatin accumulation and cytotoxicity in human colon cancer cells. *Planta medica*, 68(01), 70-72.
- Galleggiante, V., De Santis, S., Cavalcanti, E., Scarano, A., De Benedictis, M., Serino, G., *et al.* (2017). Dendritic cells modulate iron homeostasis and inflammatory abilities following quercetin exposure. *Current pharmaceutical design*, 23(14), 2139-2146.
- Gallo, M. B. & Sarachine, M. J. (2009). Biological activities of lupeol. *International journal of pharmaceutical and bio-medical science*, 3(1), 46-66.

- Gildenhuys, S. (2006). The three most abundant tree *Euphorbia* species of the Transvaal (South Africa). *Euphorbia world*, 2(1), 9 -14.
- Glauert, A. M., Dingle, J. T. & Lucy, J. A. (1962). Action of saponin on biological cell membranes. *Nature*, 196, 953-955.
- Goutam, M., Sadhan, K.R. & Jnanojjal, C. (2017). *Euphorbia tirucalli* l.: a review on its potential pharmacological use in chronic diseases. *International journal of scientific research*, 6(8), 241-245.
- Gschwendt, M. & Becker, E. (1970). Tumor promoting compounds from *Euphorbia triangularis*: mono-and diesters of 12-desoxy-phorbol. *Tetrahedron letters*, 10(40), 3509-3512.
- Guillarmod, A.J. (1971). Flora of Lesotho (Basutoland). *Flora of Lesotho (Basutoland)*.
- Gundidza, M., Sorg, B. & Hecker, E. (1992). A skin irritant phorbol ester from *Euphorbia cooperi* NE Br. *Central African journal of medicine*, 38(12), 444-447.
- Guo, Z., Xu, Y., Han, L., Bo, X., Huang, C. & Ni, L. (2011). Antioxidant and cytotoxic activity of the acetone extracts of root of *Euphorbia hylonoma* and its ellagic acid derivatives. *Journal of medicinal plants research*, 5(23), 5584-9.
- Gupta, B., Srivastava, R. S. & Goyal, R. (2007). Phcog Rev.: Plant Review Therapeutic Uses of *Euphorbia thymifolia*: A Review. *Pharmacognosy reviews*, 1(2).
- Gupta, N., Vishnoi, G., Wal, A., & Wal, P. (2013). Medicinal value of *Euphorbia tirucalli*. *Systematic reviews in pharmacy*, 4(1), p. 40.

- Gupta, R. K. & Mahadevan, V. (1967). Chemical examination of the stems of *Euphorbia tirucalli*. *Indian journal of pharmacy*, 29(5), 152-154.
- Gupta, R., Sharma, A. K., Sharma, M. C., Dobhal, M. P. & Gupta, R. S. (2012). Evaluation of antidiabetic and antioxidant potential of lupeol in experimental hyperglycaemia. *Natural product research*, 26(12), 1125-1129.
- Gyebi, G., Ogunyemi, O., Ibrahim, I., Afolabi, S., Ojo, R., Ejike, U., & Adebayo, J. (2022). Inhibitory potentials of phytochemicals from *Ocimum gratissimum* against anti-apoptotic BCL-2 proteins associated with cancer: An integrated computational study. *Egyptian Journal of Basic and Applied Sciences*, 9(1), 588–608.
- Hampson, P., Chahal, H., Khanim, F., Hayden, R., Mulder, A., Assi, L. K., *et al.* (2005). PEP005, a selective small-molecule activator of protein kinase C, has potent antileukemic activity mediated via the delta isoform of PKC. *Blood*, 106(4), 1362-1368.
- Hanson, J. R. (2003). *Natural products: the secondary metabolites* (Vol. 17). Royal society of chemistry.
- Hargreaves, B. J. (1991). The spurges of Botswana. *Botswana notes and records*, 23(1), 115-130.
- Hecker, E. (1977). New toxic, irritant and cocarcinogenic diterpene esters from *Euphorbiaceae* and from *Thymelaeaceae*. *Pure and applied chemistry*, 49(9), 1423-1431.
- Hedberg, I. & Staugård, F. (1989). *Traditional medicinal plants* (Vol. 3). Ipelegeng publishers.
- Heliawati, L., Kurnia, D., Apriyanti, E., Adriansyah, P. N. A. & Ndruru, S. T. C. L. (2023). Natural Cycloartane Triterpenoids from *Corypha utan* Lamk. with Anticancer Activity towards P388 Cell Lines and their Predicted Interaction with FLT3. *Combinatorial chemistry and high throughput screening*, 26, 001-011.

Hirota, M., Suttajit, M., Suguri, H., Endo, Y., Shudo, K., Wongchai., *et al.* (1988). A new tumor promoter from the seed oil of *Jatropha curcas* L., an intramolecular diester of 12- deoxy-16-hydroxyphorbol. *Cancer research*, 48(20), 5800-5804.

Hlengwa, S. S. (2018). *Isolation and characterisation of bioactive compounds from antidesma venosum E. Mey. ex Tul. and Euphorbia cooperi NE Br. ex A. Berger* (Doctoral dissertation). University of KwaZulu-Natal.

Ho, P. L., Yung, R. W. H., Tsang, D. N. C., Que, T. L., Ho, M., Seto., *et al.* (2001). Increasing resistance of *Streptococcus pneumoniae* to fluoroquinolones: results of a Hong Kong multicentre study in 2000. *Journal of antimicrobial chemotherapy*, 48(5), 659-665.

Hua, J., Liu, Y., Xiao, C. J., Jing, S. X., Luo, S. H. & Li, S. H. (2017). Chemical profile and defensive function of the latex of *Euphorbia peplus*. *Phytochemistry*, 136, 56-64.

Imam, S. & Iqbal Azhar, M. (2007). Two triterpenes lupanone and lupeol, isolated and identified from *Tamarindus indica*. Linn. *Pak. Journal of pharmaceutical sciences*, 20(2), 125-127.

Inoue, Y., Shiraishi, A., Hada, T., Hirose, K., Hamashima, H. & Shimada, J. (2004). The antibacterial effects of terpene alcohols on *Staphylococcus aureus* and their mode of action. *FEMS microbiology letters*, 237(2), 325-331.

Iorizzi, M., Lanzotti, V., Ranalli, G., De Marino, S. & Zollo, F. (2002). Antimicrobial furostanol saponins from the seeds of *Capsicum annuum* L. var. acuminatum. *Journal of agricultural and food chemistry*, 50(15), 4310-4316.

Iwalewa, E. O., McGaw, L. J., Naidoo, V. & Eloff, J. N. (2007). Inflammation: the foundation of diseases and disorders. A review of phytomedicines of South African origin used to treat

pain and inflammatory conditions. *African journal of biotechnology*, [online] 6(25).

Jabeen, K., Javaid, A., Ahmad, E. & Athar, M. (2011). Antifungal compounds from *Melia azedarach* leaves for management of *Ascochyta rabiei*, the cause of chickpea blight. *Natural product research*, 25(3), 264-276.

Jannet, S. B., Hymery, N., Bourgou, S., Jdey, A., Lachaal, M., Magné, C. & Ksouri, R. (2017). Antioxidant and selective anticancer activities of two *Euphorbia* species in human acute myeloid leukemia. *Biomedicine and pharmacotherapy*, 90, 375-385.

Jia, WWG, Bu, X., Philips, D., Yan, H., Liu, G., Chen, X., *et al.* (2004). Rh2, a compound extracted from ginseng, hypersensitizes multidrug-resistant tumor cells to chemotherapy. *Canadian journal of physiology and pharmacology*, 82 (7), 431-437.

Jin, F., Cheng, D., Tao, J. Y., Zhang, S. L., Pang, R., Guo, *et al.* (2013). Antiinflammatory and anti-oxidative effects of corilagin in a rat model of acute cholestasis. *BMC gastroenterology*, 13(1), 1-10.

Johann, S., Soldi, C., Lyon, J. P., Pizzolatti, M. G. & Resende, M. A. (2007). Antifungal activity of the amyrin derivatives and in vitro inhibition of *Candida albicans* adhesion to human epithelial cells. *Letters in applied microbiology*, 45(2), 148-153.

Johnson, T. (2019). *CRC ethnobotany desk reference*. CRC press.

Jurberg, P., Cabral Neto, J. B. & Schall, V. T. (1985). Molluscicide activity of the "Avelós" plant (*Euphorbia tirucalli*, L.) on *Biomphalaria glabrata*, the mollusc vector of schistosomiasis. *Memórias do instituto oswaldo cruz*, 80, 423-427.

Kajikawa, M., Yamato, K. T., Fukuzawa, H., Sakai, Y., Uchida, H. & Ohyama, K. (2005). Cloning and characterization of a cDNA encoding β -amyrin synthase from petroleum plant

Euphorbia tirucalli L. *Phytochemistry*, 66(15), 1759-1766.

Kangsamaksin, T., Chaithongyot, S., Wootthichairangsan, C., Hanchaina, R., Tangshewinsirikul, C. & Svasti, J. (2017). Lupeol and stigmasterol suppress tumor angiogenesis and inhibit cholangiocarcinoma growth in mice via downregulation of tumor necrosis factor- α . *PloS one*, 12(12), e0189628.

Kapingu, M. C., Mbwambo, Z. H., Moshi, M. J. & Magadula, J. J. (2005). Brine shrimp lethality of a glutarimide alkaloid from *Croton sylvaticus* Hochst. *East and Central African journal of pharmaceutical sciences*, 8(1), 3-5.

Karalai, C., Wiriyaichitra, P., Sorg, B. & Hecker, E. (1995). Medicinal plants of *Euphorbiaceae* occurring and utilized in Thailand. V. Skin irritants of the daphnane and tiglane type in latex of *Excoecaria bicolor* and the uterotonic activity of the leaves of the tree. *Phytotherapy research*, 9(7), 482-488.

Kazłowska, K., Lin, H. T. V., Chang, S. H. & Tsai, G. J. (2013). In vitro and in vivo anticancer effects of sterol fraction from red algae *Porphyra dentata*. *Evidence-based complementary and alternative medicine*, 2013.

Kedei, N., Lundberg, D. J., Toth, A., Welburn, P., Garfield, S. H. & Blumberg, P. M. (2004). Characterization of the interaction of ingenol 3-angelate with protein kinase C. *Cancer research*, 64(9), 3243-3255.

Kemboi, D., Peter, X., Langat, M. & Tembu, J. (2020). A review of the ethnomedicinal uses, biological activities, and triterpenoids of *Euphorbia* species. *Molecules*, 25(17), p.4019.

Kgosiemang, I. K., Lefojane, R., Direko, P., Madlanga, Z., Mashele, S. & Sekhoacha, M. (2020). Green synthesis of magnesium and cobalt oxide nanoparticles using *Euphorbia tirucalli*: Characterization and potential application for breast cancer inhibition. *Inorganic and*

Khan, A. Q. & Malik, A. (1990). A new macrocyclic diterpene ester from the latex of *Euphorbia tirucalli*. *Journal of natural Products*, 53(3), 728-731.

Khan, A. Q., Ahmed, Z., Kazmi, N. U. H., Malik, A. & Afza, N. (1988b). The structure and absolute configuration of cyclotirucanenol, a new triterpene from *Euphorbia tirucalli* Linn. *Zeitschrift für naturforschung B*, 43(8), 1059-1062.

Khan, A. Q., Rasheed, T., Kazmi, S. N. U. H., Ahmed, Z. & Malik, A. (1988a). Cycloeuphordenol, a new triterpene from *Euphorbia tirucalli*. *Phytochemistry*, 27(7), 2279-2281.

Khan, A., Ahmed, Z., Najam-ul-Hussain Kazmi & Malik, A. (1987). Further triterpenes from the stem bark of *Euphorbia tirucalli*. *Planta medica*, 53(06), 577-577.

Khan, A., Kazmi, S., Ahmed, Z. & Malik, A. (1989). Euphorcinol: A new pentacyclic triterpene from *Euphorbia tirucalli*. *Planta medica*, 55(03), 290-291.

Kinghorn, A. D. (1979). Characterization of an irritant 4-deoxyphorbol diester from *Euphorbia tirucalli*. *Journal of natural products*, 42(1), 112-115.

Kinghorn, A. D. & Kinghorn, A. (1979). Cocarcinogenic irritant *Euphorbiaceae*. *Toxic Plants*. Columbia University press, New York, 137-160.

Kiyohara, H., Ichino, C., Kawamura, Y., Nagai, T., Sato, N., Yamada, H., *et al.* (2012). *In vitro* anti-influenza virus activity of a cardiotonic glycoside from *Adenium obesum* (Forssk.). *Phytomedicine*, 19(2), 111-114.

Koleckar, V., Kubikova, K., Rehakova, Z., Kuca, K., Jun, D., Jahodar, L. & Opletal, L. (2008). Condensed and hydrolysable tannins as antioxidants influencing the health. *Mini reviews in*

Kolodziej, H., Burmeister, A., Trun, W., Radtke, O. A., Kiderlen, A. F., Ito, H., *et al.* (2005).

Tannins and related compounds induce nitric oxide synthase and cytokines gene expressions in *Leishmania major*-infected macrophage-like RAW 264.7 cells. *Bioorganic and medicinal chemistry*, 13(23), 6470-6476.

Komoreng, L., Thekiso, O., Lehasa, S., Tiwani, T., Mzizi, N., Mokoena, N., *et al.* (2017). An ethnobotanical survey of traditional medicinal plants used against lymphatic filariasis in South Africa. *South African journal of botany*, 111, 12-16.

Kose, L. E. S. (2017). *Evaluation of commonly used medicinal plants of Maseru District in Lesotho for their ethnobotanical uses, antimicrobial properties and phytochemical compositions*. [Doctoral dissertation]. University of Johannesburg (South Africa).

Kose, L. S., Moteetee, A. & Van Vuuren, S. (2015). Ethnobotanical survey of medicinal plants used in the Maseru district of Lesotho. *Journal of ethnopharmacology*, 170, 184-200.

Kratz, J. M., Grienke, U., Scheel, O., Mann, S. A., & Rollinger, J. M. (2017). Natural products modulating the hERG channel: Heartaches and hope. *Natural Product Reports*, 34(8), 957–980.

Krstić, G., Anđelković, B., Choi, Y. H., Vajs, V., Stević, T., Tešević, V. & Gođevac, D. (2016). Metabolic changes in *Euphorbia palustris* latex after fungal infection. *Phytochemistry*, 131, 17-25.

Kumar, S., Kumar, V. & Prakash, O. (2013). Enzymes inhibition and antidiabetic effect of isolated constituents from *Dillenia indica*. *BioMed research international*, 2013.

Kumari, A. & Kakkar, P. (2012). Lupeol prevents acetaminophen-induced in vivo hepatotoxicity by altering the Bax/Bcl-2 and oxidative stress-mediated mitochondrial signaling cascade. *Life sciences*, 90(15-16), 561-570.

Lagunin, A. A., Dubovskaja, V. I., Rudik, A. V., Pogodin, P. V., Druzhilovskiy, D. S., Gloriovova, T. A., & Poroikov, V. V. (2018). CLC-Pred: A freely available web-service for in silico prediction of human cell line cytotoxicity for drug-like compounds. *PLOS ONE*, 13(1), e0191838.

Lan, Y. H., Yen, C. H. & Leu, Y. L. (2020). Chemical constituents from the aerial parts of *Euphorbia formosana* Hayata and their chemotaxonomic significance. *Biochemical systematics and ecology*, 88, 103967.

Langenheim, J. H. (1994). Higher plant terpenoids: a phytocentric overview of their ecological roles. *Journal of chemical ecology*, 20, 1223-1280.

Latansio de Oliveira, T., Reder Custodio de Souza, A., Dias Fontana, P., Carvalho Carneiro, M., Beltrame, F. L., de Messias Reason, I. J. & Bavia, L. (2022). Bioactive secondary plant metabolites from *Euphorbia umbellata* (PAX) BRUYNS (*Euphorbiaceae*). *Chemistry and biodiversity*, e202200568.

Latté, K. P. & Kolodziej, H. (2000). Antifungal effects of hydrolysable tannins and related compounds on dermatophytes, mould fungi and yeasts. *Zeitschrift für naturforschung C*, 55(5-6), 467-472.

Letícia, M., Cristiane Pimentel Victório, Costa, H. B., Wanderson Romão, Ricardo Machado Kuster & Cerli Rocha Gattass (2017). Antiproliferative activity of extracts of *Euphorbia tirucalli* L (*Euphorbiaceae*) from three regions of Brazil. *Tropical journal of pharmaceutical research*, 16(5), 1013–1013.

- Lin, K. W., Huang, A. M., Tu, H. Y., Lee, L. Y., Wu, C. C., Hour, T. C., *et al.* (2011). Xanthine oxidase inhibitory triterpenoid and phloroglucinol from guttiferaceous plants inhibit growth and induced apoptosis in human NTUB1 cells through a ROS-dependent mechanism. *Journal of agricultural and food chemistry*, 59(1), 407-414.
- Lin, M. W., Lin, A. S., Wu, D. C., Wang, S. S., Chang, F. R., Wu, Y. C. & Huang, Y. B. (2012). Euphol from *Euphorbia tirucalli* selectively inhibits human gastric cancer cell growth through the induction of ERK1/2-mediated apoptosis. *Food and chemical toxicology*, 50(12), 4333-4339.
- Lipinski, C. A. (2004). Lead- and drug-like compounds: The rule-of-five revolution. *Drug Discovery Today: Technologies*, 1(4), 337–341.
- Lirio, L. G., Hermano, M. L. & Fontanilla, M. Q. (1998). Note antibacterial activity of medicinal plants from the Philippines. *Pharmaceutical biology*, 36(5), 357-359.
- Liu, Q., Tang, J. S., Hu, M. J., Liu, J., Chen, H. F., Gao, H., *et al.* (2013). Antiproliferative cardiac glycosides from the latex of *Antiaris toxicaria*. *Journal of natural products*, 76(9), 1771-1780.
- Loizou, S., Lekakis, I., Chrousos, G. P. & Moutsatsou, P. (2010). β -Sitosterol exhibits anti-inflammatory activity in human aortic endothelial cells. *Molecular nutrition and food research*, 54(4), 551-558.
- Lu, L., Zhang, J., Xie, Y., Gao, F., Xu, S., Wu, X., *et al* (2020). Wearable health devices in health care: narrative systematic review. *Journal of medical internet research*, 8(11), e18907.
- Luseba, D. & Van der Merwe, D. (2006). Ethnoveterinary medicine practices among Tsonga speaking people of South Africa. *Onderstepoort journal of veterinary research*, 73(2), 115-

Lynn, K. R. & Clevette-Radford, N. A. (1986). Lectins from latices of *Euphorbia* and *Elaeophorbium* species. *Phytochemistry*, 25(7), 1553-1557.

Lynn, K. R. & Clevette-Radford, N. A. (1985). Four serine proteases from the latex of *Euphorbia tirucalli*. *Canadian journal of biochemistry and cell biology*, 63 (10), 1093-1096.

Macedo, R., Dulcin Furtado Teixeira, Luiz, A. & Cristina, T. (2021). Analysis of in vitro activity of high dilutions of *Euphorbia tirucalli* L. in human melanoma cells. *International journal of high dilution research*, 10(36), 183–193.

MacNeil, A., Sumba, O. P., Lutzke, M. L., Moormann, A. & Rochford, R. (2003). Activation of the Epstein–Barr virus lytic cycle by the latex of the plant *Euphorbia tirucalli*. *British journal of cancer*, 88(10), 1566-1569.

Mai, Z. P., Ni, G., Liu, Y. F., Li, L., Shi, G. R., Wang, X., *et al.* (2017). Heliosterpenoids A and B, two novel jatrophanes-derived diterpenoids with a 5/6/4/6 ring system from *Euphorbia helioscopia*. *Scientific reports*, 7(1), 4922.

Mali, P. Y. & Panchal, S. S. (2017a). *Euphorbia neriifolia* L.: Review on botany, ethnomedicinal uses, phytochemistry and biological activities. *Asian Pacific journal of tropical medicine*, 10(5), 430-438.

Mali, P. Y. & Panchal, S. S. (2017b). *Euphorbia tirucalli* L.: Review on morphology, medicinal uses, phytochemistry and pharmacological activities. *Asian pacific journal of tropical biomedicine*, 7(7), 603-613.

Maliehe, E. B. (1997). *Medicinal plants and herbs of Lesotho*. Mafeteng development project.

Mampa, S. T. M., Mashele, S. S. & Sekhoacha, M. P. (2020). Applications of Chromatographic

Techniques for Fingerprinting of Toxic and Non-toxic *Euphorbia* Species. *Pakistan journal of biological sciences: PJBS*, 23(4), 552-560.

Marathe, K., Nashikkar, N., Bundale, S. & Upadhyay, A. (2019). Analysis of quorum quenching potential of *Euphorbia trigona* Mill. *International journal of pharmaceutical sciences and research*, 10(3), 1372-1386.

Martínez-Vázquez, M., Apan, T. O. R., Lazcano, M. E. & Bye, R. (1999). Antiinflammatory active compounds from the n-hexane extract of *Euphorbia hirta*. *Journal of the mexican chemical society J. Mex. Chem. Soc*, 43, 103-105.

Martins, C. G., Appel, M. H., Coutinho, D. S., Soares, I. P., Fischer, S., de Oliveira, B. C. & de Souza, L. M. (2020). Consumption of latex from *Euphorbia tirucalli* L. promotes a reduction of tumor growth and cachexia, and immunomodulation in Walker 256 tumor- bearing rats. *Journal of ethnopharmacology*, 255, 112722.

Matsue, M., Mori, Y., Nagase, S., Sugiyama, Y., Hirano, R., Ogai, K., *et al.* (2019). Measuring the antimicrobial activity of lauric acid against various bacteria in human gut microbiota using a new method. *Cell transplantation*, 28(12), 1528-1541.

Mavundza, E. J., Street, R. & Baijnath, H. (2022). A review of the ethnomedicinal, pharmacology, cytotoxicity and phytochemistry of the genus *Euphorbia* in southern Africa. *South African journal of botany*, 144, 403-418.

Mazoir, N., Benharref, A., Bailén, M., Reina, M., González-Coloma, A. & Martínez-Díaz, R. A. (2011). Antileishmanial and antitrypanosomal activity of triterpene derivatives from latex of two *Euphorbia* species. *Zeitschrift für naturforschung C*, 66(7-8), 360-366.

Mbeunkeu, A. B. D., Azebaze, A. G. B., Tala, M. F., Teinkela, J. E. M., Noundou, X. S., Krause, R. W. M., *et al.* (2018). Three new pentacyclic triterpenoids from twigs of

Manniophyton fulvum (Euphorbiaceae). *Phytochemistry letters*, 27, 1-8.

Mbhele, N. (2021). *Ethnobotanical survey, pharmacological evaluation and chemical characterization of selected medicinal plants used in South Africa in the management of wounds* (Doctoral dissertation). University of Johannesburg (South Africa).

Mertens-Talcott, S. U., Noratto, G. D., Li, X., Angel-Morales, G., Bertoldi, M. C. & Safe, S. (2013). Betulinic acid decreases ER-negative breast cancer cell growth in vitro and in vivo: role of Sp transcription factors and microRNA-27a: ZBTB10. *Molecular carcinogenesis*, 52(8), 591-602.

Min, B. S., Na, M. K., Oh, S. R., Ahn, K. S., Jeong, G. S., Li, G., *et al.* (2004). New Furofuran and Butyrolactone Lignans with Antioxidant Activity from the Stem Bark of *Styrax japonica*. *Journal of natural products*, 67(12), 1980-1984.

Mindat. (2023). “*Euphorbiaceae*”. <https://www.mindat.org/taxon-4691.html>. [Accessed June 20, 2023].

Ming, Y., Zheng, Z., Chen, L., Zheng, G., Liu, S., Yu, Y. & Tong, Q. (2013). Corilagin inhibits hepatocellular carcinoma cell proliferation by inducing G2/M phase arrest. *Cell biology international*, 37(10), 1046-1054.

Miranda, R. D. S., de Jesus, B. D. S. M., da Silva Luiz, S. R., Viana, C. B., Adao Malafaia, C. R., Figueiredo, F. D. S. & Martins, R. C. C. (2022). Antiinflammatory activity of natural triterpenes—An overview from 2006 to 2021. *Phytotherapy research*, 36(4), 1459-1506.

Molyneux, R. J., Nash, R. J., & Asano, N. (1996). Alkaloids: Chemical and biological perspectives (Vol. 11, S. W. Pelletier, Ed.).

Morsi, S. R. A. (2015). Phytochemical and Biological Study of Certain *Euphorbia* Species

Cultivated in Egypt. [Doctoral dissertation]. Cairo University.

Moteetee, A., Moffett, R. O. & Seleteng-Kose, L. (2019). A review of the ethnobotany of the Basotho of Lesotho and the Free State Province of South Africa (South Sotho). *South African journal of botany*, 122, 21-56.

Mshvildadze, V., Legault, J., Lavoie, S., Gauthier, C. & Pichette, A. (2007). Anticancer diarylheptanoid glycosides from the inner bark of *Betula papyrifera*. *Phytochemistry*, 68(20), 2531-2536.

Mukherjee, P. K., Saha, K., Das, J., Pal, M. & Saha, B. P. (1997). Studies on the antiinflammatory activity of rhizomes of *Nelumbo nucifera*. *Planta medica*, 63(04), 367-369.

Munro, B., Vuong, Q. V., Chalmers, A. C., Goldsmith, C. D., Bowyer, M. C. & Scarlett, C. J. (2015). Phytochemical, antioxidant and anticancer properties of *Euphorbia tirucalli* methanolic and aqueous extracts. *Antioxidants*, 4(4), 647-661.

Mwine, T. J. & Damme, V. P. (2011). Why do *Euphorbiaceae* tick as medicinal plants? A review of *Euphorbiaceae* family and its medicinal features.

Nashikkar, N., Begde, D., Bundale, S., Mashitha, P., Rudra, J. & Upadhyay, A. (2012). Evaluation of the immunomodulatory properties of *Euphorbia trigona*—An in vitro study. *International journal of institutional pharmacy and life sciences*, 2, 88-105.

National Center for Biotechnology Information (2023). "PubChem Compound Summary for CID 92110, Cycloartenol" PubChem. <https://pubchem.ncbi.nlm.nih.gov/compound/Cycloartenol> . [Accessed March 9, 2023].

National Center for Biotechnology Information (2023). "PubChem Compound Summary for CID 9932254, Glutanol" PubChem. <https://pubchem.ncbi.nlm.nih.gov/compound/Glutinol>

Ndam, L. M., Mih, A. M., Tening, A. S., Fongod, A. G. N., Temenu, N. A. & Fujii, Y. (2016). Phytochemical analysis, antimicrobial and antioxidant activities of *Euphorbia golondrina* LC Wheeler (*Euphorbiaceae* Juss.): an unexplored medicinal herb reported from Cameroon. *SpringerPlus*, 5(1), 1-15.

Nguemfo, E. L., Dimo, T., Dongmo, A. B., Azebaze, A. G. B., Alaoui, K., Asongalem, A. E., *et al.* (2009). Anti-oxidative and antiinflammatory activities of some isolated constituents from the stem bark of *Allanblackia monticola* Staner LC (Guttiferae). *Inflammopharmacology*, 17, 37-41.

Nielsen, P. E., Nishimura, H., Liang, Y. & Calvin, M. (1979). Steroids from *Euphorbia* and other latex-bearing plants. *Phytochemistry*, 18(1), 103-104.

Nielsen, P. E., Nishimura, H., Otvos, J. W. & Calvin, M. (1977). Plant crops as a source of fuel and hydrocarbon-like materials. *Science*, 198(4320), 942-944.

Nirmal, S. A., Pal, S. C., Mandal, S. C. & Patil, A. N. (2012). Analgesic and anti- inflammatory activity of β -sitosterol isolated from *Nyctanthes arbortristis* leaves. *Inflammopharmacology*, 20, 219-224.

Niu, H., Li, X., Yang, A., Jin, Z., Wang, X., Wang, Q., *et al.* (2018). Cycloartenol exerts antiproliferative effects on Glioma U87 cells via induction of cell cycle arrest and p38 MAPK-mediated apoptosis. *Journal of buon*, 23, 1840-1845.

Nothias-Scaglia, L. F., Pannecouque, C., Renucci, F., Delang, L., Neyts, J., Roussi, F., *et al.* (2015). Antiviral activity of diterpene esters on chikungunya virus and HIV replication.

Notka, F., Meier, G. R. & Wagner, R. (2003). Inhibition of wild-type human immunodeficiency virus and reverse transcriptase inhibitor-resistant variants by *Phyllanthus amarus*. *Antiviral research*, 58(2), 175-186.

Ogbourne, S. M., Suhrbier, A., Jones, B., Cozzi, S. J., Boyle, G. M., Morris, M., *et al.* (2004). Antitumor activity of 3-ingenyl angelate: plasma membrane and mitochondrial disruption and necrotic cell death. *Cancer research*, 64(8), 2833-2839.

Ogunnusi, T. A., Oso, B. A. & Dosumu, O. O. (2010). Isolation and antibacterial activity of triterpenes from *Euphorbia kamerunica* Pax. *International journal of biological and chemical sciences*, 4(1).

Ogunyemi, O. M., Gyebi, G. A., Ibrahim, I. M., Esan, A. M., Olaiya, C. O., Soliman, M. M., & Batiha, G. E.-S. (2022). Identification of promising multi-targeting inhibitors of obesity from *Vernonia amygdalina* through computational analysis. *Molecular Diversity*

Öksüz, S., Gürek, F., Lin, L. Z., Gil, R. R., Pezzuto, J. M. & Cordell, G. A. (1996). Aleppicatines A and B from *Euphorbia aleppica*. *Phytochemistry*, 42(2), 473-478.

Öksüz, S., Ulubelen, A., Barla, A. & Voelter, W. (2002). Terpenoids and aromatic compounds from *Euphorbia heteradena*. *Turkish journal of chemistry*, 26(4), 457-464.

Oliveira, A., Marcela Nunes Rosa, Tansini, A., Oliveira, R., Martinho, O., Paulo, J., Pianowski, L. F. & Rui Manuel Reis (2018). In vitro screening of cytotoxic activity of euphol from *Euphorbia tirucalli* on a large panel of human cancer-derived cell lines. *Experimental and therapeutic medicine*.

Oriakhi, K., Uadia, P. O., Shaheen, F., Jahan, H., Ibeji, C. U. & Iqbal, C. M. (2021). Isolation,

characterization, and hepatoprotective properties of betulinic acid and ricinine from *Tetracarpidium conophorum* seeds (*Euphorbiaceae*). *Journal of food biochemistry*, 45(3), e13288.

Ourhzif, E. M., Ricelli, A., Stagni, V., Cirigliano, A., Rinaldi, T., Bouissane, L., *et al.* (2022). Antifungal and Cytotoxic Activity of Diterpenes and Bisnorsesquiterpenoides from the Latex of *Euphorbia resinifera* Berg. *Molecules*, 27(16), 5234.

Ovesnaâ, Z., Lkovaâ, A. V. & Thovaâ, K. H. (2004). Taraxasterol and b-sitosterol: new naturally compounds with chemoprotective/chemopreventive effects Minireview. *Neoplasma*, 51(6), 407.

Panda, S., Jafri, M., Kar, A. & Meheta, B. K. (2009). Thyroid inhibitory, antiperoxidative and hypoglycemic effects of stigmasterol isolated from *Butea monosperma*. *Fitoterapia*, 80(2), 123-126.

Parekh, J. & Chanda, S. (2007). In vitro antimicrobial activity and phytochemical analysis of some Indian medicinal plants. *Turkish journal of biology*, 31(1), 53-58.

Pires, D. E. V., Blundell, T. L., & Ascher, D. B. (2015). pkCSM: Predicting small-molecule pharmacokinetic and toxicity properties using graph-based signatures. *Journal of Medicinal Chemistry*, 58(9), 4066–4072.

Ponsinet, G. & Ourisson, G. (1968). Chemotaxonomic studies in the family *Euphorbiaceae* III: Distribution of triterpenes in the latexes of *Euphorbia*. *Phytochemistry*, 7(1), 89-98.

Popplewell, W. L., Marais, E. A., Brand, L., Harvey, B. H. & Davies-Coleman, M. T. (2010). *Euphorbias* of South Africa: two new phorbol esters from *Euphorbia bothae*. *South African journal of chemistry*, 63, 175-179.

- Priya, C. L. & Rao, K. V. B. (2011). A Review of phytochemical ad pharmacological profile of *Euphorbia tirucalli*. *Pharmacologyonline*, 2, 384-390.
- Ragasa, C. Y. & Cornelio, K. B. (2013). Triterpenes from *Euphorbia hirta* and their cytotoxicity. *Chinese journal of natural medicines*, 11(5), 528-533.
- Rasool, N., Khan, A. Q. & Malik, A. (1989). A taraxerane type triterpene from *Euphorbia tirucalli*. *Phytochemistry*, 28(4), 1193-1195.
- Rédei, D., Forgo, P. & Hohmann, J. (2010). New tiglane diterpenes from *Euphorbia grandicornis*. In *Planta medica*, 76, (12), 1256-1256.
- Rehman, U. U., Shah, J., Khan, M. A., Shah, M. R. & Khan, I. (2013). Molecular docking of taraxerol acetate as a new COX inhibitor. ||| *Bangladesh journal of pharmacology* |||, 8(2), 194-197.
- Rezende, E. L., Bozinovic, F. & Jr, T. G. (2004). Climatic adaptation and the evolution of basal and maximum rates of metabolism in rodents. *Evolution*, 58(6), 1361-1374.
- Rizk, A. F. M. (1987). The chemical constituents and economic plants of the *Euphorbiaceae*. *Botanical journal of the linnean society*, 94(1-2), 293-326.
- Rizk, A. M., Hammouda, F. M., El-Missiry, M. M., Radwan, H. M. & Evans, F. J. (1985). Biologically active diterpene esters from *Euphorbia peplus*. *Phytochemistry*, 24(7), 1605-1606.
- Sakata, K., Kawazu, K. & Mitsui, T. (1971). Studies on a Piscicidal Constituent of *Hura crepitans*: Part II. Chemical Structure of Huratoxin. *Agricultural and biological chemistry*, 35(13), 2113-2126.
- Saleem, M. (2009). Lupeol, a novel anti-inflammatory and anti-cancer dietary

triterpene. *Cancer Letters*, 285(2), 109-115.

Salomé-Abarca, L. F., Godevac, D., Kim, M. S., Hwang, G. S., Park, S. C., Jang, Y. P., *et al.* (2020). The instantaneous multi-pronged defense system of latex against general plant enemies. *bioRxiv*, 2020-06.

Sangeetha, K. N., Sujatha, S., Muthusamy, V. S., Anand, S., Nithya, N., Velmurugan, D., *et al.* (2010). 3β -taraxerol of *Mangifera indica*, a PI3K dependent dual activator of glucose transport and glycogen synthesis in 3T3-L1 adipocytes. *Biochimica et biophysica acta (BBA)-general subjects*, 1800(3), 359-366.

Sawale, J. A., Patel, J. R. & Kori, M. L. (2019). Antioxidant properties of cycloartenol isolated from *Euphorbia neriifolia* leaves. *Indian journal of natural products*, 33(1).

Schmidt, R. J.; Evans, F. J. Candletoxins A and B, 2 new aromatic esters of 12-deoxy-16-hydroxy-phorbol, from the irritant latex of *Euphorbia poissonii* Pax. *Experientia* **1977**, 33, 1197–1198.

Seigler, D. S. & Seigler, D. S. (1998). Alkaloids derived from anthranilic acid. *Plant secondary metabolism*, 568-577

Sekhoacha, M., Campbell, W. & Smith, P. (2009). In vitro and in vivo antimalarial activity of dcm extract of *Agathosma Betulina*. *African Journal of Traditional, Complementary and Alternative Medicines*, 323-324.

Selzer, E., Pimentel, E., Wacheck, V., Schlegel, W., Pehamberger, H., Jansen, B. & Kodym, R. (2000). Effects of betulinic acid alone and in combination with irradiation in human melanoma cells. *Journal of investigative dermatology*, 114(5), 935-940.

Shale, T. L., Stirk, W. A. & van Staden, J. (1999). Screening of medicinal plants used in

Lesotho for antibacterial and antiinflammatory activity. *Journal of ethnopharmacology*, 67(3), 347-354.

Shen, X., Xiong, G. L., Jing, Y., Xiao, H., Cui, Y., Zhang, Y. F., *et al.* (2015). The protein kinase C agonist prostratin induces differentiation of human myeloid leukemia cells and enhances cellular differentiation by chemotherapeutic agents. *Cancer letters*, 356(2), 686- 696.

Shi, Q. W., Su, X. H. & Kiyota, H. (2008). Chemical and pharmacological research of the plants in genus *Euphorbia*. *Chemical reviews*, 108(10), 4295-4327.

Shi, Q., Li, L., Huo, C., Zhang, M. & Wang, Y. (2010). Study on natural medicinal chemistry and new drug development. *Zhongcaoyao= chinese traditional and herbal drugs*, 41(10), 1583-1589.

Shih, M. F., Cheng, Y. D., Shen, C. R. & Cherng, J. Y. (2010). A molecular pharmacology study into the antiinflammatory actions of *Euphorbia hirta* L. on the LPS-induced RAW 264.7 cells through selective iNOS protein inhibition. *Journal of natural medicines*, 64, 330-335.

Shivkumar, S. P. (2015). Ethnopharmacological validation of medicinal plants treating skin diseases in hyderabad karnataka region. <http://hdl.handle.net/10603/37479>

Shlamovitz, G. Z., Gupta, M. & Diaz, J. A. (2009). A case of acute keratoconjunctivitis from exposure to latex of *Euphorbia tirucalli* (pencil cactus). *The Journal of emergency medicine*, 36(3), 239-241.

Si, L., Meng, K., Tian, Z., Sun, J., Li, H., Zhang, Z., *et al.* (2018). Triterpenoids manipulate a broad range of virus-host fusion via wrapping the HR2 domain prevalent in viral envelopes. *Science advances*, 4(11), eaau8408.

Siddique, H. R. & Saleem, M. (2011). Beneficial health effects of lupeol triterpene: a review of preclinical studies. *Life sciences*, 88(7-8), 285-293.

Siddique, N. A., da Silva, J. A. T. & Bari, M. A. (2010). Preservation of indigenous knowledge regarding important and endangered medicinal plants in Rajshahi district of Bangladesh. *Journal of plant sciences*, 5(2), 201-215.

Silva, F. S., Oliveira, P. J. & Duarte, M. F. (2016). Oleanolic, ursolic, and betulinic acids as food supplements or pharmaceutical agents for type 2 diabetes: promise or illusion? *Journal of agricultural and food chemistry*, 64(15), 2991-3008.

Silva, V. A., Rosa, M. N., Miranda-Gonçalves, V., Costa, A. M., Tansini, A., Evangelista, A. F., *et al.* (2019). Euphol, a tetracyclic triterpene, from *Euphorbia tirucalli* induces autophagy and sensitizes temozolomide cytotoxicity on glioblastoma cells. *Investigational new drugs*, 37, 223-237.

Silva, V. A. O., Rosa, M. N., Tansini, A., Oliveira, R. J., Martinho, O., Lima, J. P., ... Reis, R. M. (2018). In vitro screening of cytotoxic activity of euphol from *Euphorbia tirucalli* on a large panel of human cancer-derived cell lines. *Experimental and Therapeutic Medicine*, 16(2), 557–566.

Singh, A. B., Yadav, D. K., Maurya, R. & Srivastava, A. K. (2009). Antihyperglycaemic activity of α -amyrin acetate in rats and db/db mice. *Natural product research*, 23(9), 876- 882.

Singh, B., Sahu, P. M. & Sharma, M. K. (2002). Antiinflammatory and antimicrobial activities of triterpenoids from *Strobilanthes callosus* Nees. *Phytomedicine*, 9(4), 355-359.

Sosath, S., Ott, H. H. & Hecker, E. (1988). Irritant principles of the spurge family (*Euphorbiaceae*) XIII. Oligocyclic and macrocyclic diterpene esters from latices of some *Euphorbia* species utilized as source plants of honey. *Journal of natural products*, 51(6), 1062-

Sousa, G. F. D., Soares, D. C. F., Mussel, W. D. N., Pompeu, N. F. E., Silva, G. D. D. F., Vieira Filho, S. A. & Duarte, L. P. (2014). Pentacyclic triterpenes from branches of *Maytenus robusta* and in vitro cytotoxic property against 4T1 cancer cells. *Journal of the brazilian chemical society*, 25, 1338-1345.

South African National Biodiversity Institute (SANBI) (2023). “*Euphorbia*”. <https://pza.sanbi.org/Euphorbia>. [Accessed June 20, 2023]

Sudhahar, V., Kumar, S. A., Mythili, Y. & Varalakshmi, P. (2007). Remedial effect of lupeol and its ester derivative on hypercholesterolemia-induced oxidative and inflammatory stresses. *Nutrition research*, 27(12), 778-787.

Sultana, A., Hossain, M. J., Kuddus, M. R., Rashid, M. A., Zahan, M. S., Mitra, S., ... Naina Mohamed, I. (2022). Ethnobotanical uses, phytochemistry, toxicology, and pharmacological properties of *Euphorbia neriifolia* Linn. against infectious diseases: A comprehensive review. *Molecules*, 27(14), 4374.

Sytwala, S., Gřnther, F. & Melzig, M. F. (2015). Lysozyme-and chitinase activity in latex bearing plants of genus *Euphorbia*—A contribution to plant defense mechanism. *Plant physiology and biochemistry*, 95, 35-40.

Tada, M. & Seki, H. (1989). Toxic diterpernes from *Euphorbia trigona* (Saiunkaku: an indoor foliage plant in Japan). *Agricultural and biological chemistry*, 53(2), 425-430.

Takasaki, M., Konoshima, T., Tokuda, K., Masuda, K., Arai, Y., Shiojima, K. & Ageta, H. (1999). Anti-carcinogenic activity of Taraxacum plant. II. *Biological and pharmaceutical bulletin*, 22(6), 606-610.

Tamamura, H., Kobayakawa, T. & Ohashi, N. (2018). Springer Briefs in Pharmaceutical Science and Drug Development.

Tan, B., Shi, H. L., Ji, G. & Xie, J. Q. (2011). Effects of taraxerol and taraxerol acetate on cell cycle and apoptosis of human gastric epithelial cell line AGS. *Zhong xi yi jie he xue bao= Journal of chinese integrative medicine*, 9(6), 638-642.

Tanaka, R., Kasubuchi, K., Kita, S. & Matsunaga, S. (1999). Obtusifoliol and related steroids from the whole herb of *Euphorbia chamaesyce*. *Phytochemistry*, 51(3), 457-463.

Tang, Y. Y., He, X. M., Sun, J., Li, C. B., Li, L., Sheng, J. F., *et al.* (2019). Polyphenols and alkaloids in byproducts of longan fruits (*Dimocarpus Longan* Lour.) and their bioactivities. *Molecules*, 24(6), 1186.

Tapas, A. R., Sakarkar, D. M. & Kakde, R. B. (2008). Flavonoids as nutraceuticals: a review. *Tropical journal of pharmaceutical research*, 7(3), 1089 1099.

Tilabi, J. & Upadhyay, R. R. (1983). Adenoma formation by ingenol 3, 5, 20- triacetate. *Cancer letters*, 18(3), 317-320.

Tiwani, T. (2017). *Phytochemical screening, cytotoxicity, antimicrobial and anthelmintic activity of medical plants used in the reatment of lymphatic filariasis in the Eastern Cape, South Africa* [Doctoral dissertation]. University of the Free State.

Tiwari, S., Singh, P. & Singh, A. (2003). Toxicity of *Euphorbia tirucalli* plant against freshwater target and non-target organisms. *Pakistan journal of biological sciences (Pakistan)*.

Tong, Y., Zhang, G., Li, Y., Xu, J., Yuan, J., Zhang, B., *et al.* (2018). Corilagin inhibits breast cancer growth via reactive oxygen species-dependent apoptosis and autophagy. *Journal of cellular and molecular medicine*, 22(8), 3795-3807.

- Tran, Q. L., Tezuka, Y., Banskota, A. H., Tran, Q. K., Saiki, I. & Kadota, S. (2001). New Spirostanol Steroids and Steroidal Saponins from Roots and Rhizomes of *Dracaena angustifolia* and Their Antiproliferative Activity. *Journal of natural products*, 64(9), 1127-1132.
- Traore, F., Faure, R., Ollivier, E., Gasquet, M., Azas, N., Debrauwer, L., *et al.* (2000). Structure and antiprotozoal activity of triterpenoid saponins from *Glinus oppositifolius*. *Planta medica*, 66(04), 368-371.
- Tsai, J. Y., Rédei, D., Hohmann, J. & Wu, C. C. (2020). 12-Deoxyphorbol esters induce growth arrest and apoptosis in human lung cancer A549 cells via activation of PKC- δ /PKD/ERK signaling pathway. *International journal of molecular sciences*, 21(20), 7579.
- Ovesná, Z., Vachálková, A., & Horváthová, K. (2004). Taraxasterol and β -sitosterol: New natural compounds with chemoprotective/chemopreventive effects. *Neoplasma*, 51(6), 407–414.
- Upadhyay, B., Singh, K. P., & Kumar, A. (2010). Ethno-medicinal, phytochemical, and antimicrobial studies of *Euphorbia tirucalli* L. *Journal of Phytology*, 2(4).
- Upadhyaya, C., & Sathish, S. (2017). A review on *Euphorbia neriifolia* plant. *International Journal of Pharmaceutical and Chemical Research*, 3, 149–154.
- ur Rahman, U., Ali, S., Khan, I., Khan, M. A. & Arif, S. (2016). Antiinflammatory activity of taraxerol acetate. *Journal of medical sciences*, 24(4), 216-219.
- Valadares, M. C., Carrucha, S. G., Accorsi, W. & Queiroz, M. L. (2006). *Euphorbia tirucalli* L. modulates myelopoiesis and enhances the resistance of tumor-bearing mice. *International immunopharmacology*, 6(2), 294-299.

- Van Damme, P. (1989). *Studie van Euphorbia tirucalli L.: morfologie, fysiologie, teeltvoorwaarden*. [Doctoral dissertation] Ghent University.
- Van Damme, P. L. (2001). *Euphorbia tirucalli* for high biomass production. *Combating desertification with plants*, 169-187.
- Van den Bosch, C., Griffin, B. E., Kazembe, P., Dziweni, C. & Kadzamira, L. (1993). Are plant factors a missing link in the evolution of endemic Burkitt's lymphoma?. *British journal of cancer*, 68(6), 1232-1235.
- Van Wyk, B. E., Oudtshoorn, B. V. & Gericke, N. (1997). *Medicinal plants of South Africa*. Briza.
- Vazquez, M. M., Apan, T. O. R., Lazcano, M. E. & Bye, R. (1999). Antiinflammatory active compounds from the n-hexane extract of *Euphorbia hirta*. *Journal of the mexican chemical society*, 43(3-4), 103-105.
- Veza, T., Canet, F., de Marañón, A. M., Bañuls, C., Rocha, M. & Víctor, V. M. (2020). Phytosterols: Nutritional health players in the management of obesity and its related disorders. *Antioxidants*, 9(12), 1266.
- Vilahur, G., Ben-Aicha, S., Diaz-Riera, E., Badimon, L. & Padró, T. (2019). Phytosterols and inflammation. *Current medicinal chemistry*, 26(37), 6724-6734.
- Villanueva, J., Quirós, L. M. & Castañón, S. (2015). Purification and partial characterization of a ribosome-inactivating protein from the latex of *Euphorbia trigona* Miller with cytotoxic activity toward human cancer cell lines. *Phytomedicine*, 22(7-8), 689-695.
- Vogg, G., Mattes, E., Rothenburger, J., Hertkorn, N., Achatz, S. & Sandermann Jr, H. (1999). Tumor promoting diterpenes from *Euphorbia leuconeura* L. *Phytochemistry*, 51(2), 289-295.

Voigt, W. E. (2007). *Euphorbia tirucalli* L.(*Euphorbiaceae*). <http://pza.sanbi.org/Euphorbia-tirucalli> .[Accessed March 9, 2023].

Waczuk, E. P., Kamdem, J. P., Abolaji, A. O., Meinerz, D. F., Caeran Bueno, D., do Nascimento Gonzaga, T. K. S., ... & Ávila, D. S. (2015). *Euphorbia tirucalli* aqueous extract induces cytotoxicity, genotoxicity and changes in antioxidant gene expression in human leukocytes. *Toxicology research*, 4(3), 739-748.

Wal, A., Wal, P., Gupta, N., Vishnoi, G. & Srivastava, R. S. (2013). Medicinal value of *Euphorbia tirucalli*. *International journal of pharmaceutical and biological*, 31-40.

Wang, H. B., Wang, X. Y., Liu, L. P., Qin, G. W. & Kang, T. G. (2015). Tiglane diterpenoids from the *Euphorbiaceae* and *Thymelaeaceae* families. *Chemical reviews*, 115(9), 2975-3011.

Wardana, A. P., Abdjan, M. I., Aminah, N. S., Fahmi, M. Z., Siswanto, I., Kristanti, A. N., & Takaya, Y. (2022). 3,4,3'-Tri-O-Methylelagic acid as an anticancer agent: In vitro and in silico studies. *RSC Advances*, 12(46), 29884–29891.

Watt, J. M. & Breyer-Brandwijk, M. G. (1962). The Medicinal and Poisonous Plants of Southern and Eastern Africa being an Account of their Medicinal and other Uses, Chemical Composition, Pharmacological Effects and Toxicology in Man and Animal. *The Medicinal and Poisonous Plants of Southern and Eastern Africa being an Account of their Medicinal and other Uses, Chemical Composition, Pharmacological Effects and Toxicology in Man and Animal.*, (Edn 2).

Wen, S., Gu, D. & Zeng, H. (2018). Antitumor effects of beta-amyrin in Hep-G2 liver carcinoma cells are mediated via apoptosis induction, cell cycle disruption and activation of JNK and P38 signalling pathways. *Journal of buon*, 23, 965-970.

White, R. E. (2017). Role of ADME/PK in drug discovery, safety assessment, and clinical development. In S. Chackalamannil, D. Rotella, & S. E. Ward (Eds.), *Comprehensive medicinal chemistry III* (1–33). Elsevier.

Wu, T. S., Lin, Y. M., Haruna, M., Pan, D. J., Shingu, T., Chen, Y. P., *et al.* (1991). Antitumor agents, 119. Kansuiphorins A and B, two novel antileukemic diterpene esters from *Euphorbia kansui*. *Journal of natural products*, 54(3), 823-829.

Wu, Y., Zhang, H., Zhang, R., Cao, G., Li, Q., Zhang, B., *et al.* (2021). Serum metabolome and gut microbiome alterations in broiler chickens supplemented with lauric acid. *Poultry science*, 100(9), 101315.

Xiao, K., Yi, Y. H., Wang, Z. Z., Tang, H. F., Li, Y. Q. & Lin, H. W. (1999). A cytotoxic triterpene saponin from the root bark of *Aralia dasyphylla*. *Journal of natural products*, 62(7), 1030-1032.

Yadav, A. N., Kour, D., Rana, K. L., Yadav, N., Singh, B., Chauhan, V. S., *et al.* (2019). Metabolic engineering to synthetic biology of secondary metabolites production. In *New and future developments in microbial biotechnology and bioengineering* (279-320). Elsevier.

Yamamoto, Y., Mizuguchi, R. & Yamada, Y. (1981). Chemical constituents of cultured cells of *Euphorbia tirucalli* and *E. millii*. *Plant cell reports*, 1, 29-30.

Yang, J., Fa, J. & Li, B. (2017). Apoptosis induction of epifriedelinol on human cervical cancer cell line. *African journal of traditional, complementary and alternative medicines*, 14(4), 80-86.

Yang, M. H., Baek, S. H., Hwang, S. T., Um, J. Y. & Ahn, K. S. (2022). Corilagin exhibits differential anticancer effects through the modulation of STAT3/5 and MAPKs in human gastric cancer cells. *Phytotherapy research*, 36(6), 2449-2462.

Yang, M. H., Vasquez, Y., Ali, Z., Khan, I. A. & Khan, S. I. (2013). Constituents from *Terminalia* species increase PPAR α and PPAR γ levels and stimulate glucose uptake without enhancing adipocyte differentiation. *Journal of ethnopharmacology*, 149(2), 490-498.

Yang, Z. G., Jia, L. N., Shen, Y., Ohmura, A. & Kitanaka, S. (2011). Inhibitory effects of constituents from *Euphorbia lunulata* on differentiation of 3T3-L1 cells and nitric oxide production in RAW264. 7 cells. *Molecules*, 16(10), 8305-8318.

Yasukawa, K. E. N., Akihisa, T., Yoshida, Z. Y. & Takido, M. (2000). Inhibitory effect of euphol, a triterpene alcohol from the roots of *Euphorbia kansui*, on tumor promotion by 12-O-tetradecanoylphorbol-13-acetate in two-stage carcinogenesis in mouse skin. *Journal of pharmacy and pharmacology*, 52(1), 119-124.

Yokosuka, A., Mimaki, Y. & Sashida, Y. (2002). Spirostanol saponins from the rhizomes of *Tacca chantrieri* and their cytotoxic activity. *Phytochemistry*, 61(1), 73-78.

Yoshida, T., Yokoyama, K. I., Namba, O. & Okuda, T. (1991). Tannins and related polyphenols of *Euphorbiaceae* plants. VII. Tirucallins A, B and Euphorbin F, Monomeric and Dimeric Ellagitannins from *Euphorbia tirucalli* L. *Chemical and pharmaceutical bulletin*, 39(5), 1137-1143.

You, Y. J., Nam, N. H., Kim, Y., Bae, K. H. & Ahn, B. Z. (2003). Antiangiogenic activity of lupeol from *Bombax ceiba*. *Phytotherapy research: An international journal devoted to pharmacological and toxicological evaluation of natural product derivatives*, 17(4), 341-344.

Yu, H.-C., Shen, C., Yi, H.-M., Chen, T.-H., Hsueh, M.-L., Lin, C. & Don, M. (2012). Euphorbiane: A Novel Triterpenoid with an unprecedented skeleton from *Euphorbia tirucalli*. *Journal of the Chinese chemical society*, 60(2), 191–194.

Zare, S., Ghaedi, M., Heiling, S., Asadollahi, M., Baldwin, I. T. & Jassbi, A. R. (2015). Phytochemical investigation on *Euphorbia macrostegia* (Persian wood spurge). *Iranian Journal of pharmaceutical research: IJPR*, 14(1), 243.

Zayed, S., Sorg, B. & Hecker, E. (1984). Structure activity relations of polyfunctional diterpenes of the tiglane type, VI1. *Planta medica*, 50(01), 65-69.

Zhang, D. M., Xu, H. G., Wang, L., Li, Y. J., Sun, P. H., Wu, X. M., *et al.* (2015). Betulinic acid and its derivatives as potential antitumor agents. *Medicinal research reviews*, 35(6), 1127-1155.

Zhang, W. K., Xu, J. K., Zhang, X. Q., Yao, X. S. & Ye, W. C. (2008). Chemical constituents with antibacterial activity from *Euphorbia sororia*. *Natural product research*, 22(4), 353-359.

Zhang, Z. L., Luo, Z. L., Shi, H. W., Zhang, L. X. & Ma, X. J. (2017). Research advance of functional plant pharmaceutical cycloartenol about pharmacological and physiological activity. *Zhongguo zhong yao za zhi= zhongguo zhongyao zazhi= China journal of chinese materia medica*, 42(3), 433-437.

Zheng, Z. Z., Chen, L. H., Liu, S. S., Deng, Y., Zheng, G. H., Gu, Y. & Ming, Y. L. (2016). Bioguided fraction and isolation of the antitumor components from *Phyllanthus niruri* L. *BioMed research international*

CHAPTER 4

***IN VITRO* ANTIPROLIFERATIVE AND SELECTIVE CYTOTOXIC EFFECTS OF SIXTEEN SOUTH AFRICAN *EUPHORBIA* SPECIES AGAINST BREAST CANCER CELL LINES**

Abstract

This study explored medicinal plants used in South Africa, especially in areas with limited access to primary healthcare and where traditional knowledge has been orally passed down for generations. The present study investigated the growth inhibitory effects of sixteen unexplored *Euphorbia* species against breast cancer cells. The methodology involved a systematic approach, including plant selection, sequential extraction with organic solvents, assessment of secondary metabolites and *in vitro* testing using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. High-performance liquid chromatography (HPLC) was employed to profile the secondary metabolites within the extracts. All sixteen plants contained glycosides and triterpenoids. The ethyl acetate (EtOAc) extract from *Euphorbia ledienii*, dichloromethane (DCM) extract from *Euphorbia cooperi* and DCM extract from *Euphorbia clavarioides* demonstrated the highest inhibitory effect on cell proliferation. Specifically, the EtOAc extract from *Euphorbia ledienii* showed a CC₅₀ value of 0.06 µg/mL against the MCF-7 cell line, the DCM extract from *Euphorbia cooperi* showed a CC₅₀ value of 0.006 µg/mL against the MDA-MB231 cell line and the DCM extract from *Euphorbia clavarioides* showed a CC₅₀ value of 1.18 µg/mL against the Vero cell line. Among the extracts tested, the *Euphorbia tirucalli* DCM extract and the *Euphorbia cooperi* Hex extract displayed significant selectivity for MDA-MB231 cells, with selectivity index (SI) values of 49.40 and 84.03; respectively. *Euphorbia trigona*, *Euphorbia*

gorgonis, *Euphorbia ledienii* and *Euphorbia arabica* shared similar major peaks in the HPLC analysis. This study suggests that these selective *Euphorbia* species can be considered for further investigation, as they have shown inhibitory effects on cancer cells while sparing non-cancerous cells.

4.1. Introduction

Chemotherapy is a widely employed cancer treatment that has limitations such as non-specific drug delivery and lack of selectivity, resulting in high toxicity to healthy cells (Conte, 2013; Yuan *et al.*, 2016; Senapati *et al.*, 2018). These limitations hinder effective disease control. To address this, complementary therapeutic strategies like the use of herbs and medicinal plants have gained attention. Many plants possess compounds that can enhance cancer treatment efficacy and mitigate toxicity (Choudhari *et al.*, 2020). The medicinal advantages of plants include their affordability, accessibility and proven success in cancer treatment, prompting global research interest.

Most ethnobotanical assertions, however, have not yet been scientifically examined. Therefore, evaluating these plants for their potential as chemotherapeutic agents becomes crucial. For instance, some researchers have isolated and screened some of these medicinal plants for biological active compounds (Ugboko *et al.*, 2020). In fact, a few approved anticancer agents (vincristine, irinotecan, etoposide and paclitaxel) have been derived from plants and have played a crucial role in chemotherapy (Huang *et al.*, 2012).

Numerous plants within the Euphorbiaceae family are commonly used in various parts of the world as traditional medicinal herbs and some of these plants have shown anticancer properties (Mwine *et al.*, 2011; Hartwell, 1969). It is against this background that the antiproliferative activity and selectivity of the sixteen Euphorbia species: *Euphorbia bupleurifolia* (*E. bupleurifolia*), *Euphorbia trigona* (*E. trigona*), *Euphorbia gorgonis* (*E. gorgonis*), *Euphorbia cooperi* (*E. cooperi*), *Euphorbia horrida* Var (*E. horrida* Var) and *Euphorbia horrida* Indigenous (*E. horrida* Indigenous), *Euphorbia polygona* (*E. polygona*), *Euphorbia enopla* (*E. enopla*), *Euphorbia Arabica* (*E. arabica*), *Euphorbia ammak* (*E. ammak*), *Euphorbia stellate* (*E. stellata*), 192

Euphorbia ferox (*E. ferox*), *Euphorbia clavarioides* (*E. clavarioides*), *Euphorbia coerulescens* (*E. coerulescens*), *Euphorbia tirucalli* (*E. tirucalli*) and *Euphorbia ledienii* (*E. ledienii*) were investigated against MCF-7, MDA-MB231 and Vero cell lines, to evaluate their potential as anticancer agents.

4.2. Materials and Methods

4.2.1. Plant collection and processing

In March of 2017, 16 species of *Euphorbia* plants were procured from Griep Nursery, located in Pretoria, South Africa (GPS coordinates: 25°47'3"S, 28°18'58"E), to be utilized in the current research. Due to the small size of most of the plants, it was not feasible to isolate their roots, stems and leaves, thus the entire plant was utilized for the study. For further details regarding the commercially acquired *Euphorbia* plant species, see Table 4.1.

Table 4.1. List of 16 selected *Euphorbia* plant species used in this study.

Plant species	Abbreviation
<i>Euphorbia ferox</i>	<i>E. ferox</i>
<i>Euphorbia bupleurifolia</i>	<i>E. bupleurifolia</i>
<i>Euphorbia tirucalli</i>	<i>E. tirucalli</i>
<i>Euphorbia clavarioides</i>	<i>E. clavarioides</i>
<i>Euphorbia polygona</i>	<i>E. polygona</i>
<i>Euphorbia gorgonis</i>	<i>E. gorgonis</i>
<i>Euphorbia enopla</i>	<i>E. enopla</i>
<i>Euphorbia cooperi</i>	<i>E. cooperi</i>
<i>Euphorbia ammak</i>	<i>E. ammak</i>

<i>Euphorbia caerulescens</i>	<i>E. caerulescens</i>
<i>Euphorbia trigona</i>	<i>E. trigona</i>
<i>Euphorbia ledienii</i>	<i>E. ledienii</i>
<i>Euphorbia horrida</i> Var	<i>E. horrida</i> Var
<i>Euphorbia horrida</i> Indigenous	<i>E. horrida</i> Ind
<i>Euphorbia stellata</i>	<i>E. stellata</i>
<i>Euphorbia arabica</i>	<i>E. arabica</i>

The plants were rinsed with tap water upon arrival, cut into smaller pieces, air-dried for 14 days and then ground into fine powder. To isolate the different components from the various *Euphorbia* species, 10g of their powdered material was sequentially extracted with hexane, dichloromethane, ethyl acetate, methanol and acetone in increasing order of polarity. The extracts were subjected to a 48-hour shake at 37°C on an OrbiShake shaker (Labotec) before being filtered through Whatman filter paper. This process was repeated twice for each solvent and the resulting filtrates were concentrated under reduced pressure at 45°C using a rotary vacuum evaporator (Buchi Labotech, Switzerland). The percentage yield of each extract was calculated based on the weight of dried extract and ground plant materials.

$$\% \text{ yield} = \frac{\text{Dried extract (g)} - \text{Empty Vial (g)}}{\text{Dried powder (g)}} \times 100$$

The extract concentrates were then dried under the LabAire fume hood and stored at 4°C for future use.

4.2.2. Phytochemical analysis

A standard qualitative screening of the sixteen *Euphorbia* plant species for the presence of secondary metabolites such as alkaloids, glycosides, flavonoids, phenols, saponins, terpenoids and tannins was carried out following previously described protocols by Yadav and Agarwala (2011) with slight modifications. The tests were based on the visual observation of a change in colour or formation of precipitate after the addition of specific reagents.

4.3.1. Antiproliferation and Cytotoxic screening of *Euphorbia* extracts

4.3.1.1. Cell culture

Two cell lines belonging to human breast cancer, namely MCF-7 and MDA-MB231, along with a green monkey kidney cell line named Vero, were procured from Cellonex in South Africa. These cells were cultured in flasks and maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and placed in a humidified 5% CO₂ incubator at a temperature of 37°C. The cells were sub-cultured once they attained a confluency level of 90%.

4.3.1.2. MTT assay

After the cells in the flask had reached the desired confluency of 90% as observed under a microscope, they were rinsed with 10 mL of PBS and harvested using trypsin. The concentration of the live cells was evaluated using typhan blue dye under a cell counter; countess FI life technology. A total of 1×10^5 cells/mL were seeded into each well in a 96-well microtiter plate and incubated under the same condition for 24 hours to allow attachment.

All cells were treated with 100 µL of a series of dilutions (100-0.1 µg/mL) of the extracts, and were replicated five/six times in triplicates for 48 hours. Wells with untreated cells served as negative control. Doxorubicin drug was used as positive control. Cell viability was conducted

based on the tetrazolium dye (MTT assay) method with slight modification (Munro *et al.*, 2015).

4.3.1.3. CC₅₀ determination

Graph Prism 9 software was utilized to analyze the data, with CC₅₀ values being determined through non-linear regression plotting. To improve data visualization, stabilize variances and normalize data distributions, Prism graphs were generated using log-transformed values. The toxicity of the extracts was categorized using the CC₅₀ standard criteria established by Indrayanto *et al.* (2021) (see Table 4.2).

Table 4.2. CC₅₀ criteria for cytotoxicity.

CC ₅₀ (µg/mL)	Status
>100 µg/mL	Inactive
<100 ≥ 50 µg/mL	Low activity
< 50 µg/mL ≥ 10 µg/mL	Moderate activity
< 10 µg/mL ≥ 5 µg/mL	Good activity
<5 µg/mL	Potent activity

4.3.1.4. Selectivity index

The potential of an extract to inhibit growth of cancerous cells without harming normal cells was evaluated by calculating the selectivity index (SI) of the potent extracts. This was determined by comparing the antiproliferative activity (CC₅₀) of the plant extract against two breast cancer cells (MCF-7 and MDA-MB231) with that from the kidney of an African green monkey cell line (Vero). An SI value greater than 3 indicates potential of safer therapy (Mahavorasirikul *et al.*, 2010). For the purpose of calculating SI, extracts that had a growth inhibition of less than 50% and which had an CC₅₀ greater than 100 were taken as 100.

Selectivity index= IC_{50} calculated for normal cells/ CC_{50} calculated for cancer cells

4.3. High-Performance Liquid Chromatography (HPLC)

The HPLC-diode array detection analysis was performed on the extracts using an Agilent 1100 series (Agilent, Waldbronn, Germany) instrument equipped with photo diode array, autosampler, column thermostat and degasser. A Phenomenex: Luna 5 μ m C18 (2) (150 $\times$ 4.6 mm; 5 μ m particle size) column was used as the stationary phase. Water containing 0.1% of formic acid (A) and acetonitrile (B) served as mobile phases at a flow rate of 1 ml/min. Gradient elution was applied as shown on Table 4.3:

Table 4.3. HPLC gradient elution.

Time (min)	% Solvent A (0.1% formic acid in H ₂ O)	% Solvent B (Acetonitrile)
0	95	5
10	90	10
20	70	30
30	50	50
40	95	5
41	STOP	

Temperature was set to 30 °C for column. Extracts were dissolved in HPLC grade methanol (2 mg/mL) and the injection volume was 20.0 μ L. Chromatograms were recorded at 254 nm. The elucidation of the chemical structures of the compounds depicted by peaks on the chromatograms falls outside of the scope of this study.

4.3.1. Statistical analysis

The results are presented as mean $\pm$ SD of three independent experimental measurements that are used to calculate the inhibitory concentrations. Graph Pad prism 9 software was used to calculate the IC₅₀/CC₅₀ values.

4.4. Results

4.4.1. Percentage yield

The results obtained from the extraction method revealed that all solvents (water, hexane, dichloromethane, methanol and ethyl acetate) used in this process were able to extract some constituents from all 16 *Euphorbia* plants. However, the % yields differed from plant to plant. The % yield of *Euphorbia* plants extracted by different solvents is presented in Table 4.4 and 4.5. The yield is based on the weight of dried extract and ground plant materials. The highest yield was extracted from *E. ferox* hexane extract with 59.83% and the lowest yield was from *E. enopla* ethyl acetate extract with 0.09% as seen in Table 4.3. Most hexane (non-polar) and methanol (polar solvent) extracts had higher yields compared to dichloromethane and ethyl acetate.

Table 4.4. Percentage yields of organic extract

Plant Sample	Dried powder (g)	Solvent	Dried extract (g)	% Yield
<i>E. ferox</i>	2.35	Hex	1.41	59.83
		DCM	0.03	1.28
		MeOH	0.19	8.34
		EtOAc	0.40	17.02
<i>E. bupleurifolia</i>	4.41	Hex	1.83	41.49
		DCM	0.03	0.68
		MeOH	0.55	12.47
		EtOAc	0.05	1.13

<i>E. clavarioides</i>	10	Hex	0.41	4.10
		DCM	0.20	2.00
		MeOH	0.91	9.10
		EtOAc	0.03	0.30
<i>E. ammak</i>	10	Hex	0.56	5.60
		DCM	0.17	1.70
		MeOH	0.63	6.30
		EtOAc	0.01	0.10
<i>E. Enopla</i>	10.1	Hex	0.39	3.86
		DCM	0.12	1.19
		MeOH	0.27	2.67
		EtOAc	0.01	0.09
<i>E. cooperi</i>	10	Hex	0.38	3.8
		DCM	0.26	2.6
		MeOH	0.66	6.6
		EtOAc	0.01	0.10
<i>E. gorgonis</i>	7.71	Hex	0.56	7.26
		DCM	0.51	6.61
		MeOH	0.05	0.65
		EtOAc	0.06	0.78
<i>E. arabica</i>	6.28	Hex	0.07	1.11
		DCM	0.06	0.96
		MeOH	0.92	14.65
		EtOAc	0.11	1.75
<i>E. trigona</i>	10.01	Hex	0.20	1.99
		DCM	0.15	1.49
		MeOH	1.23	12.29
		EtOAc	0.09	0.89
<i>E. horrida Var</i>	7.63	Hex	0.66	8.65
		DCM	0.03	0.39
		MeOH	0.24	3.15
		EtOAc	0.01	0.13

<i>E. horrida</i> <i>Indigenous</i>	6.24	Hex	0.34	5.45
		DCM	0.07	1.12
		MeOH	0.24	3.85
		EtOAc	0.01	0.16
<i>E. polygona</i>	9.8	Hex	0.21	2.14
		DCM	0.43	4.39
		MeOH	0.27	2.76
		EtOAc	0.06	0.61
<i>E. caerulescens</i>	10.00	Hex	0.24	2.40
		DCM	0.30	3.00
		MeOH	0.76	7.60
		EtOAc	0.08	0.80
<i>E. ledienii</i>	8.00	Hex	0.38	4.75
		DCM	0.25	3.13
		MeOH	1.54	19.32
		EtOAc	0.27	3.38
<i>E. stellata</i>	6.76	Hex	0.38	5.62
		DCM	0.16	2.37
		MeOH	0.53	7.84
		EtOAc	0.07	1.04

Abbreviations: Hexane, Hex; dichloromethane, DCM; methanol, MeOH; ethyl acetate, EtOAc.

Furthermore, the percentage yield of *E. Ferox* DCM, MeOH, EtOAc extracts ranged from 1.28 to 17.02%; *E. Bupleurifolia* DCM, MeOH, EtOAc extracts yields ranged from 0.68 to 12.47%. The MeOH extract of *E. Clavariodes* had the highest yield of 9.10%, and the other extracts (DCM, hexane, EtOAc) yields ranged from 0.30 to 4.10%; respectively. The MeOH extract of *E. Ammak* had the highest yield of 6.30% and the other extracts (DCM, hexane, EtOAc) ranged from 0.10 to 5.60%. The Hex extract of *E. Enopla* had the highest yield of 3.86% and for the other extracts (DCM, MeOH, EtOAc) yield ranged from 0.09 to 2.67%. The MeOH extract of *E. cooperi* had

the highest yield of 6.60%, while the other extracts (Hex, DCM, EtOAc) resulted in yields ranging from 0.10 to 3.80%. The Hex extract of *E. gorgonis* had the highest yield of 7.26%, whereas other extracts (DCM, MeOH, EtOAc) yields ranged from 0.78 to 6.61%. The MeOH extract of *E. Arabica* had the highest yield of 14.64%, while the other extracts (DCM, hexane, EtOAc) ranged from 0.96 to 1.75%. The MeOH extract of *E. Trigona* had the highest yield of 12.29%, whereas the other extracts (DCM, hexane, EtOAc) yields ranged from 0.89 to 1.99%. The Hex extract of *E. Horrida* Var had the highest yield of 8.65%, while other (DCM, MeOH, EtOAc) extracts yields ranged from 0.13 to 3.15%. The Hex extract of *E. Horrida* Indigenous had the highest yield of 5.45%, and the other extracts yields (DCM, MeOH, EtOAc) ranged from 0.16 to 3.85%. The DCM extract of *E. Polygona* had the highest yield of 4.39%, and other extracts (Hexane, MeOH, EtOAc) yield ranged from 0.61 to 2.76%. The yield of *E. Caerulenscens* with MeOH was 7.60%, while other extracts (DCM, hexane, EtOAc) provided a yield ranging from 0.80 to 3.00%. The MeOH extract of *E. Ledienii* has the highest yield of 19.32%, while other extracts (DCM, hexane, EtOAc) yields ranged from 3.13 and 4.75%. Lastly, the highest yield of *E. Stellata* was found to be 7.84% with MeOH extract, while the other extracts (DCM, hexane, EtOAc) resulted in yields ranging from 1.04 to 5.62% (see Table 4.4).

Table 4.5. Percentage yields of inorganic extracts

Plant Sample	Dried powder (g)	Dried extract (g)	% Yield
<i>E. clavarioides</i>	10.01	2.06	20.40
<i>E. ammak</i>	10.04	1.46	14.54
<i>E. horrida</i> Var	6.77	1.36	20.09
<i>E. polygona</i>	5.50	0.43	7.82

<i>E. trigona</i>	10.00	0.81	8.10
<i>E. caerulenscence</i>	10.00	0.17	1.7

For water extracts, only six species were evaluated due to the limited sample size. Among these, *E. clavariodes* was found to have the highest yield of 20.40%, followed by *E. horrida* Var with 20.09%, *E. ammak* with 14.54%, *E. polygona* 7.82%, *E. trigona* with 8.10% and *E. coerulenscence* with the lowest yield of 1.70% (see Table 4.5).

4.4.2. Phytochemical investigation

Phytochemical analysis of the sixteen *Euphorbia* plant species is summarized in Table 4.6. The results revealed the presence of glycosides and triterpenoids in all sixteen plants. Flavonoids and phytosterols were found in all the plants except *E. tirucalli*. Furthermore, *E. horrida* Var showed the presence all 9 secondary metabolites. Alkaloids were present in 11 plants species namely; *E. ammak*, *E. ferox*, *E. bupleurifolia*, *E. cooperi*, *E. gorgonis*, *E. trigona*, *E. horrida* Var, *E. polygona*, *E. coerulescens*, *E. ledienii* and *E. clavaroides*. Tannins were present in 12 plants namely; *E. bupleurifolia*, *E. tirucalli*, *E. enopla*, *E. cooperi*, *E. arabica*, *E. horrida* Var, *E. horrida* indigenous, *E. polygona*, *E. coerulescens*, *E. ledienii* and *E. clavaroides*.

Table 4.6. Qualitative phytochemical analysis of sixteen selected *Euphorbia* plant species. Legend: (+) presence of phytochemical compounds; (-) absence of phytochemical compound.

Phytoconstituents		<i>Euphorbia</i> sp.															
	<i>E. ferox</i>	<i>E. bupleurifolia</i>	<i>E. tirucalli</i>	<i>E. ammak</i>	<i>E. enopla</i>	<i>E. cooperi</i>	<i>E. gorgonis</i>	<i>E. arabica</i>	<i>E. trigona</i>	<i>E. horrida</i> Var	<i>E. horrida</i> indigenous	<i>E. polygona</i>	<i>E. coerulescens</i>	<i>E. ledienii</i>	<i>E. stellata</i>	<i>E. clavaroides</i>	
Phytosterols	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	
Pentose	+	+	-	-	+	+	+	-	+	+	+	-	+	-	-	-	
Tannins	-	+	+	-	+	+	-	+	-	+	+	+	+	+	-	+	
Glycosides	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Triterpenoids	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	

Anthraquinones	+	-	-	+	+	+	-	+	+	+	+	-	-	-	-	+
Saponins	-	+	+	-	+	-	+	-	-	+	+	+	+	-	-	-
Flavonoids	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+
Alkaloids	+	+	-	+	-	+	+	-	+	+	-	+	+	+	-	+

Saponins were present in 8 plants namely; *E. polygona*, *E. tirucalli*, *E. coerulescens*, *E. bupleurifolia*, *E. enopla*, *E. gorgonis*, *E. horrida* Var and *E. horrida* indigenous. Alkaloids, saponins and terpenoids which were present in *E. bupleurifolia*, *E. gorgonis*, *E. horrida* Var, *E. polygona* and *E. coerulescens* are all believed to have similar pharmacological effects, such as anticancer and antibacterial properties (Berdy, 2005; Hanson, 2003).

4.4.3. Cell growth inhibition

The cytotoxic effect of *Euphorbia* extracts was determined using the MTT assay. All 16 plant species were tested for their ability to inhibit cell growth against MCF-7, MDA-MB231 and Vero cell lines. Each cell was treated with 100 μ l of a series of dilutions, ranging from 100-0.1 μ g/mL, and were replicated five/six times. The results showed that some extracts inhibited the growth of the tested cell lines while others induced cell proliferation. This may be due to the lack of activity of some extracts against the cell lines. Others showed both proliferation at lower concentrations and inhibition at higher concentrations. This is known as a hormetic dose-response, where the extract or treatment substance has a stimulating effect at a low dose but becomes inhibitory at a higher dose (Iavicoli *et al.*, 2014). The CC_{50} values were determined by non-linear regression of MTT assay dose-response data. The CC_{50} value is essential for evaluating the substance's cytotoxicity. Lower CC_{50} values suggest higher potency, while higher CC_{50} values indicate lower toxicity. $CC_{50} > 100$ μ g/mL was considered as 100 μ g/mL.

To calculate the SI value, the study compared the CC_{50} (concentration causing cytotoxicity in 50% of cells) values of the plant extract against two breast cancer cell lines, namely MCF-7 and MDA-MB231, with that

of Vero cells (likely normal, non-cancerous cells). An SI value greater than 3 suggests that the plant extract has a significantly higher toxicity to the breast cancer cells compared to the normal Vero cells, indicating its potential selectivity for cancer cells.

Table 4.7. Cytotoxicity (IC_{50} in $\mu\text{g/mL}$, $n=5/6$) of doxorubicin against MCF-7, MDA-MB231 and Vero cell lines. The dilutions were in triplicates.

Anti-proliferative activity of doxorubicin			
	MCF-7	MDA-MB231	Vero
IC_{50}	4.40	20.80	100.9

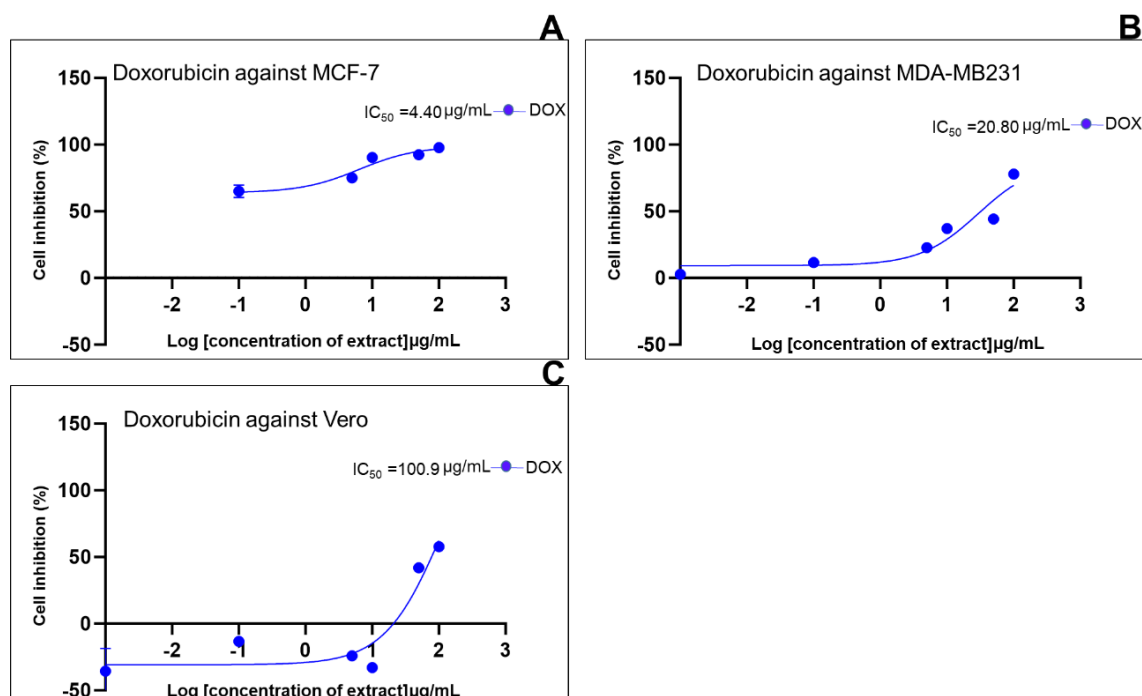


Figure 4.1. Anti-proliferative effect of doxorubicin against MCF-7 (A), MDA-MB231 (B) and Vero (C) cell lines was assessed using MTT assay ($\mu\text{g/mL}$) and IC_{50} values were evaluated. The IC_{50} values of doxorubicin against each cell line were calculated using nonlinear regression analysis. The MTT assay results showed a concentration-dependent inhibitory effect (A and B) and hormetic-dose response (C) of doxorubicin. Values are presented as means $\pm$ standard deviation (SD) from three independent experiments; $n = 5$ for (A), and $n = 6$ (B and C). Doxorubicin (DOX).

Figure 4.1 presents the IC_{50} values for doxorubicin against MCF-7, MDA-MB231 and Vero cell lines. MCF-7 cells were highly sensitive to doxorubicin, with a IC_{50} value of 4.40 $\mu\text{g/mL}$, indicating potent toxicity against this cell line. Doxorubicin displayed moderate cytotoxicity against MDA-MB231 cells, with a IC_{50} value of 20.80 $\mu\text{g/mL}$ (see Table 4.7). In the case of Vero cells, it required a higher doxorubicin concentration of 100.9 $\mu\text{g/mL}$ compared to MCF-7 and MDA-MB231 cells to achieve a 50% inhibition. These IC_{50} values offer valuable insights into the cytotoxic effects of doxorubicin on these cell lines, highlighting variations in its potency and selectivity.

The Hex and DCM extracts from *E. bupleurifolia* were found to inhibit the growth of MCF-7 and Vero cells, with CC_{50} values of 7.74, >100, and 4.67, 1.18 $\mu\text{g/mL}$ (see Table 4.8 and Figure 4.2); respectively. This indicates that the extracts have varying degrees of activity against the cell lines, ranging from no activity to potent activity. However, the cytotoxic selectivity of *E. bupleurifolia* extracts showed that none of the active extracts had selectivity for the breast cancer cell line, as the SI value was lower than 3 (see Table 4.8).

Table 4.8. Cytotoxicity (CC_{50} in $\mu\text{g/mL}$, $n=5/6$) of *E. bupleurifolia* against MCF-7, MDA-MB231 and Vero cell lines. The table illustrates the CC_{50} values ($\mu\text{g/mL}$, $n=5/6$) representing half-maximal cytotoxic concentrations of varying degrees of cytotoxicity, which were in triplicate. Selectivity index (SI) are also presented, highlighting potential candidates for further investigation. Species names and organic solvents are provided. Hexane (Hex), dichloromethane (DCM), methanol (MeOH), ethyl acetate (EtOAc), not determined (ND).

Anti-proliferative activity of <i>E. bupleurifolia</i>				
CC_{50} ($\mu\text{g/mL}$)	Hexane	Dichloromethane	Methanol	Ethyl acetate
MCF-7	7.74	>100	>100	>100
SI	0.60	>100	>100	>100
MDA-MB231	>100	>100	>100	>100
SI	>100	>100	>100	>100
Cytotoxic activity of <i>E. bupleurifolia</i>				
CC_{50} ($\mu\text{g/mL}$)	Hexane	Dichloromethane	Methanol	Ethyl acetate
Vero	4.67	1.18	>100	>100

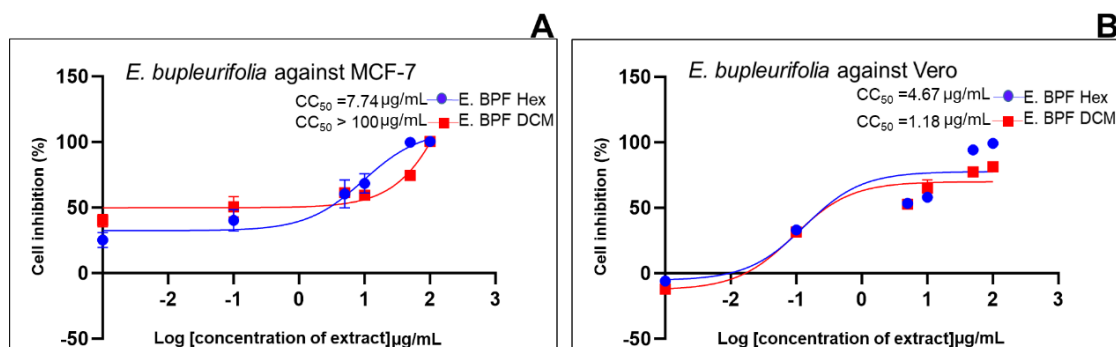


Figure 4.2. Anti-proliferative effect of *E. bupleurifolia* extracts against MCF-7 (A) and Vero (B) cell lines was assessed using MTT assay ($\mu\text{g/mL}$) and CC_{50} values were evaluated. The CC_{50} values of *E. bupleurifolia* extract against each cell line were calculated using nonlinear regression analysis. The percentage concentration of the extracts is plotted on the X-axes in log format. The MTT assay results showed a concentration-dependent inhibitory effect (A) and hormetic dose-response (B) of *E. bupleurifolia* extracts. Values are presented as means $\pm$ SD from three independent experiments; $n = 6$. *E. bupleurifolia* hexane (E. BPF); *E. bupleurifolia* dichloromethane (E.BPF DCM).

The Hex and DCM extracts of *E. trigona* showed cell growth inhibition on MCF-7 cells with CC_{50} values of 7.55 and 10.58 $\mu\text{g/mL}$ (Table 4.9 and Figure 4.3), indicating good to moderate activity against MCF-7. The Hex extract showed cell growth inhibition of Vero cells with CC_{50} value of 12.11 $\mu\text{g/mL}$ (Table 4.9 and Figure 4.3), indicating moderate activity against the normal cell line. The DCM extract of *E. trigona* showed selectivity for the MCF-7 cell line with an SI value of 9.45 (Table 4.9).

Table 4.9. Cytotoxicity assessment of extracts from *E. trigona* on MCF-7, MDA-MB231 and Vero cell lines. The table illustrates the CC_{50} values ($\mu\text{g/mL}$, $n=5/6$) representing half-maximal cytotoxic concentrations of varying degrees of cytotoxicity, which were in triplicate. Selectivity index (SI) are also presented, highlighting potential candidates for further investigation. Species names and organic solvents are provided. Hexane (Hex), dichloromethane (DCM), methanol (MeOH), ethyl acetate (EtOAc), not determined (ND).

Anti-proliferative activity of <i>E. trigona</i> CC_{50} ($\mu\text{g/mL}$)				
	Hexane	Dichloromethane	Methanol	Ethyl acetate
MCF-7	7.55	10.58	>100	>100
SI	1.60	9.45	>100	>100
MDA-MB231	>100	>100	>100	>100
SI	>100	>100	>100	>100
Cytotoxic activity of <i>E. trigona</i> CC_{50} ($\mu\text{g/mL}$)				
Vero	12.11	>100	>100	>100

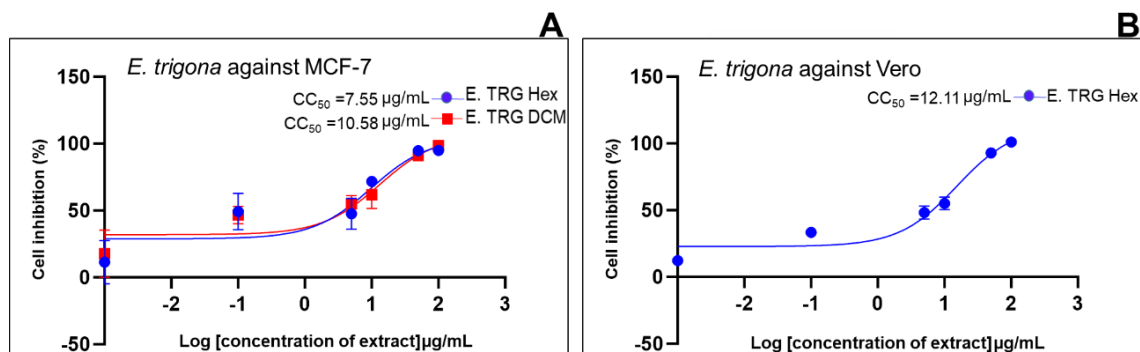


Figure 4.3. Anti-proliferative effect of *E. trigona* extracts against MCF-7 (A) and Vero (B) cell lines was assessed using MTT assay ($\mu\text{g/mL}$) and CC_{50} values were evaluated. The CC_{50} values of *E. trigona* extract against each cell line were calculated using nonlinear regression analysis. The percentage concentration of the extracts is plotted on the X-axes in log format. The MTT assay results showed a concentration-dependent inhibitory effect of *E. trigona* extracts. Values are means $\pm$ SD from three independent experiments; $n = 6$. *E. trigona* hexane (E. TRG Hex); *E. trigona* dichloromethane (E. TRG DCM).

The Hex extract of *E. gorgonis* displayed the highest cell growth inhibition against MCF-7 and MDA-MB231 with CC_{50} values of 1.16 and 2.34 $\mu\text{g/mL}$ (see Table 4.10 and Figure 4.4), demonstrating its potent activity against both cell lines. Moreover, the Hex extract of *E. gorgonis* exhibited selectivity for MCF-7 and MDA-MB231 cells with an SI values of 85.98 and 42.73 (see Table 4.10); respectively, affirming its efficacy and specificity.

Table 4.10. Cytotoxicity assessment of extracts from *E. gorgonis* on MCF-7, MDA-MB231, and Vero cell lines. The table illustrates the CC_{50} values ($\mu\text{g/mL}$, $n=5/6$) representing half-maximal cytotoxic concentrations of varying degrees of cytotoxicity, which were in triplicate. Selectivity index (SI) are also presented, highlighting potential candidates for further investigation. Species names and organic solvents are provided. Hexane (Hex), dichloromethane (DCM), methanol (MeOH), ethyl acetate (EtOAc), not determined (ND).

Anti-proliferative activity of <i>E. gorgonis</i> CC_{50} ($\mu\text{g/ml}$)				
	Hexane	Dichloromethane	Methanol	Ethyl acetate
MCF-7	1.16	>100	>100	>100
SI	85.98	>100	>100	>100
MDA-MB231	2.34	>100	>100	>100
SI	42.73	>100	>100	>100
Cytotoxic activity of <i>E. gorgonis</i> CC_{50} ($\mu\text{g/ml}$)				
Vero	>100	>100	>100	>100

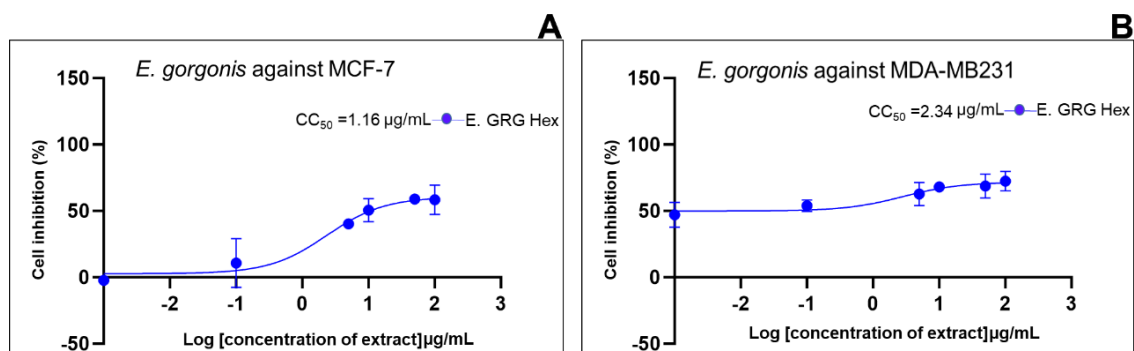


Figure 4.4. Anti-proliferative effect of *E. gorgonis* extracts against MCF-7 (A) and MDA-MB231 (B) cell lines was assessed using MTT assay (µg/mL) and CC₅₀ values were evaluated. The CC₅₀ values of *E. gorgonis* extract against each cell line were calculated using nonlinear regression analysis. The percentage concentration of the extracts is plotted on the X-axes in log format. The MTT assay results showed a concentration-dependent inhibitory effect of *E. gorgonis* extracts. Values are means $\pm$ SD from three independent experiments; n = 6. *E. gorgonis* hexane (E. TRG Hex).

The Hex and DCM extracts of *E. polygona* demonstrated increased cell growth inhibition of MDA-MB231 cells with CC₅₀ value 17.84 and 3.93 µg/mL (see Table 4.11 and Figure 4.5), indicating moderate to potent activity against the cell line; respectively. The Hex extract from *E. polygona* showed selectivity for MDA-MB231 cells, with an SI value of 5.60 and 25.44 (see Table 4.11).

Table 4.11. Cytotoxicity assessment of extracts from *E. polygona* on MCF-7, MDA-MB231, and Vero cell lines. The table illustrates the CC₅₀ values (µg/mL, n=5/6) representing half-maximal cytotoxic concentrations of varying degrees of cytotoxicity, which were in triplicate. Selectivity index (SI) are also presented, highlighting potential candidates for further investigation. Species names and organic solvents are provided. Hexane (Hex), dichloromethane (DCM), methanol (MeOH), ethyl acetate (EtOAc), not determined (ND).

Anti-proliferative activity of <i>E. polygona</i> CC50 (µg/ml)				
	Hexane	Dichloromethane	Methanol	Ethyl acetate
MCF-7	>100	>100	>100	>100
SI	>100	>100	>100	>100
MDA-MB231	17.84	3.93	>100	>100
SI	5.60	25.44	>100	>100
Cytotoxic activity of <i>E. polygona</i> CC50 (µg/ml)				
Vero	>100	>100	>100	>100

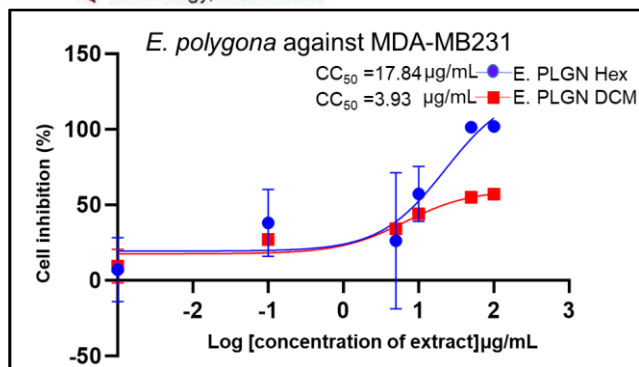


Figure 4.5. Anti-proliferative effect of *E. polygona* extracts against MDA-MB231 cell line was assessed using MTT assay ($\mu\text{g/mL}$) and CC_{50} values were evaluated. The CC_{50} values of *E. polygona* extract against MDA-MB231 cell line was calculated using nonlinear regression analysis. The percentage concentration of the extracts is plotted on the X-axes in log format. The MTT assay results showed a concentration-dependent inhibitory effect of *E. polygona* extracts. Values are means $\pm$ SD from three independent experiments; $n = 6$. *E. polygona* hexane (E. PLGN Hex); *E. polygona* dichloromethane (E. PLGN DCM).

All extracts from *E. horrida* Var showed low cell growth inhibition against MCF-7 and MDA-MB231. However, the DCM extract showed cell growth inhibition of Vero with CC_{50} value of $6.89 \mu\text{g/mL}$ (see Table 4.12), indicating its cytotoxic activity towards the normal healthy cells (see Table 4.12).

Table 4.12. Cytotoxicity assessment of extracts from *E. horrida* Var on MCF-7, MDA-MB231 and Vero cell lines. The table illustrates the CC_{50} values ($\mu\text{g/mL}$, $n=5/6$) representing half-maximal cytotoxic concentrations of varying degrees of cytotoxicity, which were in triplicate. Selectivity index (SI) are also presented, highlighting potential candidates for further investigation. Species names and organic solvents are provided. Hexane (Hex), dichloromethane (DCM), methanol (MeOH), ethyl acetate (EtOAc), not determined (ND).

Anti-proliferative activity of <i>E. horrida</i> Var				
CC_{50} ($\mu\text{g/mL}$)				
	Hexane	Dichloromethane	Methanol	Ethyl acetate
MCF-7	>100	>100	>100	>100
SI	>100	>100	>100	>100
MDA-MB231	>100	>100	>100	>100
SI	>100	>100	>100	>100
Cytotoxic activity of <i>E. horrida</i> Var				
CC_{50} ($\mu\text{g/mL}$)				
Vero	>100	6.89	>100	>100

Furthermore, extracts from *E. horrida* Indigenous showed low inhibitory activity against MCF-7 cells and MDA-231 as well. The Hex and DCM extracts demonstrated cell growth inhibition against Vero cells with CC₅₀ values of 4.24 and 5.42 µg/mL, indicating its cytotoxic activity against the normal cell line; respectively (see Table 4.13).

Table 4.13. Cytotoxicity assessment of extracts from *E. horrida* Indigenous on MCF-7, MDA-MB231 and Vero cell lines. The table illustrates the CC₅₀ values (µg/mL, n=5/6) representing half-maximal cytotoxic concentrations of varying degrees of cytotoxicity, which were in triplicate. Selectivity index (SI) are also presented, highlighting potential candidates for further investigation. Species names and organic solvents are provided. Hexane (Hex), dichloromethane (DCM), methanol (MeOH), ethyl acetate (EtOAc), not determined (ND).

Anti-proliferative activity of <i>E. horrida</i> Indigenous				
CC₅₀ (µg/ml)				
	Hexane	Dichloromethane	Methanol	Ethyl acetate
MCF-7	>100	>100	>100	>100
SI	>100	>100	>100	>100
MDA-MB231	>100	>100	>100	>100
SI	>100	>100	>100	>100
Cytotoxic activity of <i>E. horrida</i> Indigenous				
CC₅₀ (µg/ml)				
Vero	4.24	5.42	>100	>100

The Hex and MeOH extracts of *E. ferox* showed cell growth inhibition of MDA-MB231 with CC₅₀ value of 16.79 and 6.37 µg/mL, indicating good to moderate activity against the cell line (see Table 4.14 and Figure 4.6). Both the Hex and MeOH of *E. ferox* extracts showed selectivity for MDA-MB231 cell line, with an SI values of 5.95 and 15.69; respectively (see Table 4.14).

Table 4.14. Cytotoxicity assessment of extracts from *E. ferox* on MCF-7, MDA-MB231 and Vero cell lines. The table illustrates the CC₅₀ values (µg/mL, n=5/6) representing half-maximal cytotoxic concentrations of varying degrees of cytotoxicity, which were in triplicate. Selectivity index (SI) are also presented, highlighting potential candidates for further investigation. Species names and organic solvents are provided. Hexane (Hex), dichloromethane (DCM), methanol (MeOH), ethyl acetate (EtOAc), not determined (ND).

Anti-proliferative activity of <i>E. ferox</i>				
CC₅₀ (µg/ml)				
	Hexane	Dichloromethane	Methanol	Ethyl acetate
MCF-7	>100	>100	>100	>100
SI	>100	>100	>100	>100
MDA-MB231	>100	16.79	6.37	>100
SI	>100	5.95	15.69	>100

Cytotoxic activity of *E. ferox*

CC₅₀ (µg/ml)

Vero	>100	>100	>100	>100
------	------	------	------	------

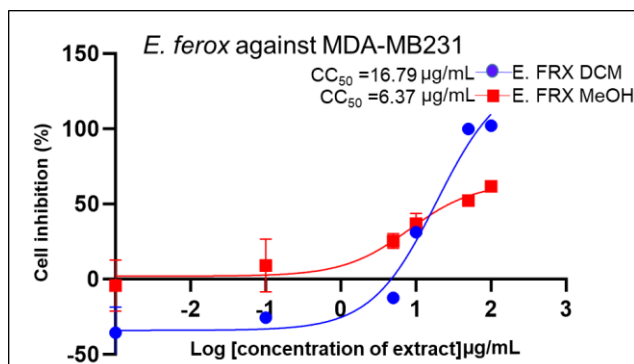


Figure 4.6. Anti-proliferative effect of *E. ferox* extract against MDA-MB231 cell line was assessed using MTT assay (µg/mL) and CC₅₀ values were evaluated. The CC₅₀ values of *E. ferox* extract against MDA-MB231 cell line was calculated using nonlinear regression analysis. The percentage concentration of the extracts is plotted on the X-axes in log format. The MTT assay results showed a concentration-dependent inhibitory effect/ hormetic dose-response of *E. ferox* extracts. Values are means $\pm$ SD from three independent experiments; n = 6. *E. ferox* dichloromethane (E. FRX DCM; *E. ferox* methanol (E. FRX MeOH).

The Hex and DCM extract of *E. clavarioides* exhibited increased cell growth inhibition of MCF-7 and MDA-MB231 with CC₅₀ value of 0.85, 0.23 and 1.26 µg/mL (see Table 4.15 and Figure 4.7), indicating its potent activity against the two breast cancer cell lines; respectively. Furthermore, the DCM extract exhibited inhibitory activity of Vero cells, with CC₅₀ values of 1.82 µg/mL (see Table 4.15 and Figure 4.7), indicating cytotoxic activity against the normal cell line; respectively. The Hex and DCM extracts of *E. clavarioides* displayed higher selectivity for MCF-7 with an SI values of 7.91 and >100; respectively (see Table 4.15).

Table 4.15. Cytotoxicity assessment of extracts from *E. clavarioides* on MCF-7, MDA-MB231 and Vero cell lines. The table illustrates the CC₅₀ values (µg/mL, n=5/6) representing half-maximal cytotoxic concentrations of varying degrees of cytotoxicity, which were in triplicate. Selectivity index (SI) are also presented, highlighting potential candidates for further investigation. Species names and organic solvents are provided. Hexane (Hex), dichloromethane (DCM), methanol (MeOH), ethyl acetate (EtOAc), not determined (ND).

Anti-proliferative activity of <i>E. clavarioides</i>				
CC ₅₀ (µg/ml)				
	Hexane	Dichloromethane	Methanol	Ethyl acetate
MCF-7	0.85	0.23	>100	>100

SI	117.64	7.91	>100	>100
MDA-MB231	>100	>100	>100	>100
SI	>100	>100	>100	>100
Cytotoxic activity of <i>E. clavaroides</i>				
CC₅₀ (µg/ml)				
Vero	>100	1.82	>100	>100

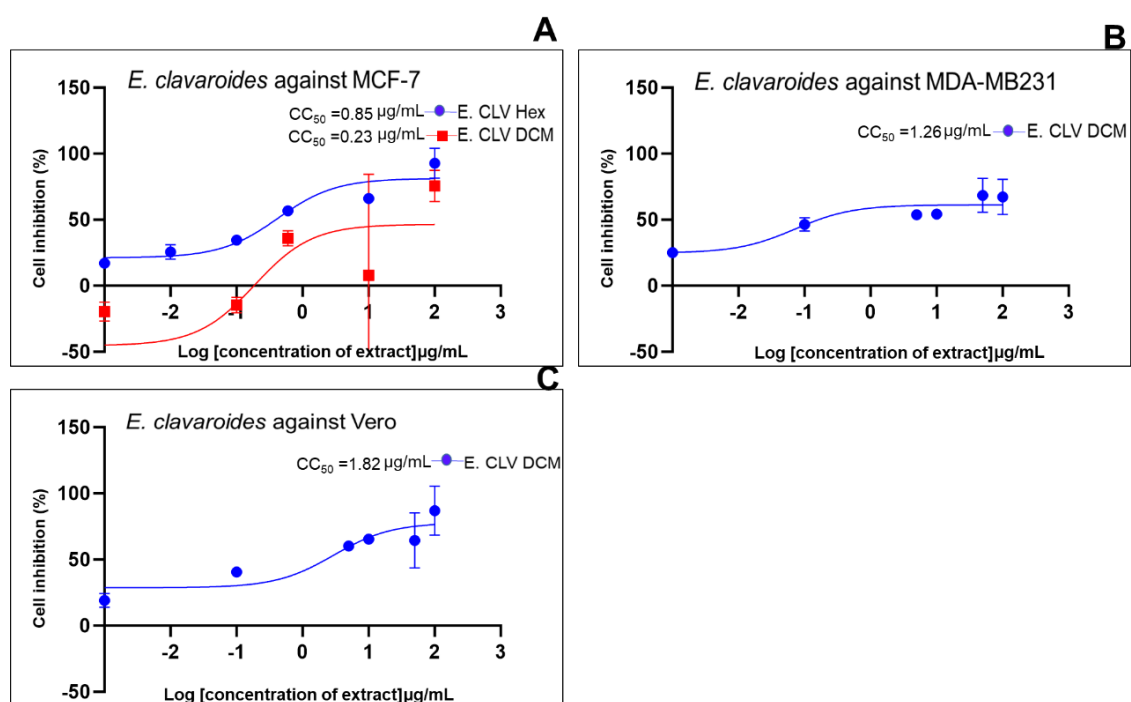


Figure 4.7. Anti-proliferative effect of *E. clavaroides* extracts against MCF-7 (A), MDA-MB231 (B) and Vero (C) cell lines was assessed using MTT assay (µg/mL) and CC_{50} values were evaluated. The CC_{50} values of *E. clavaroides* extract against each cell line were calculated using nonlinear regression analysis. The percentage concentration of the extracts is plotted on the X-axes in log format. The MTT assay results showed a hormetic dose-reponse (A) and concentration-dependent inhibitory effect (B and C) of *E. clavaroides* extracts. Values are presented as means $\pm$ SD from three independent experiments; $n = 6$. *E. clavaroides* hexane (E. CLV Hex); *E. clavaroides* dichloromethane (E. CLV DCM).

The Hex and DCM extracts of *E. ammak* exhibited increased cell growth inhibition of MCF-7 and MDA-MB231 with CC_{50} values of 1.17, 0.27 and 4.32 µg/mL (see Table 4.16 and Figure 4.8), indicating potent activity against the two breast cancer cell lines. Furthermore, the Hex and DCM extract showed cell growth inhibition of Vero with CC_{50} value of 6.49 and >100 µg/mL (see Table 4.16 and Figure 4.8), indicating no cytotoxicity to cytotoxicity activity against the cell line; respectively. Based on the SI values, the Hex and DCM extracts of *E.*

ammak showed selectivity for MCF-7 cells, with an SI values of 5.54 and > 100; respectively (see Table 4.16).

Table 4.16. Cytotoxicity assessment of extracts from *E. ammak* on MCF-7, MDA-MB231 and Vero cell lines. The table illustrates the CC₅₀ values (µg/mL, n=5/6) representing half-maximal cytotoxic concentrations of varying degrees of cytotoxicity, which were in triplicate. Selectivity index (SI) are also presented, highlighting potential candidates for further investigation. Species names and organic solvents are provided. Hexane (Hex), dichloromethane (DCM), methanol (MeOH), ethyl acetate (EtOAc), not determined (ND).

Anti-proliferative activity of <i>E. ammak</i> CC ₅₀ (µg/ml)				
	Hexane	Dichloromethane	Methanol	Ethyl acetate
MCF-7	1.17	0.27	>100	>100
SI	5.54	>100	>100	>100
MDA-MB231	4.32	>100	>100	>100
SI	1.50	>100	>100	>100
Cytotoxic activity of <i>E. ammak</i> CC ₅₀ (µg/ml)				
Vero	6.49	>100	>100	>100

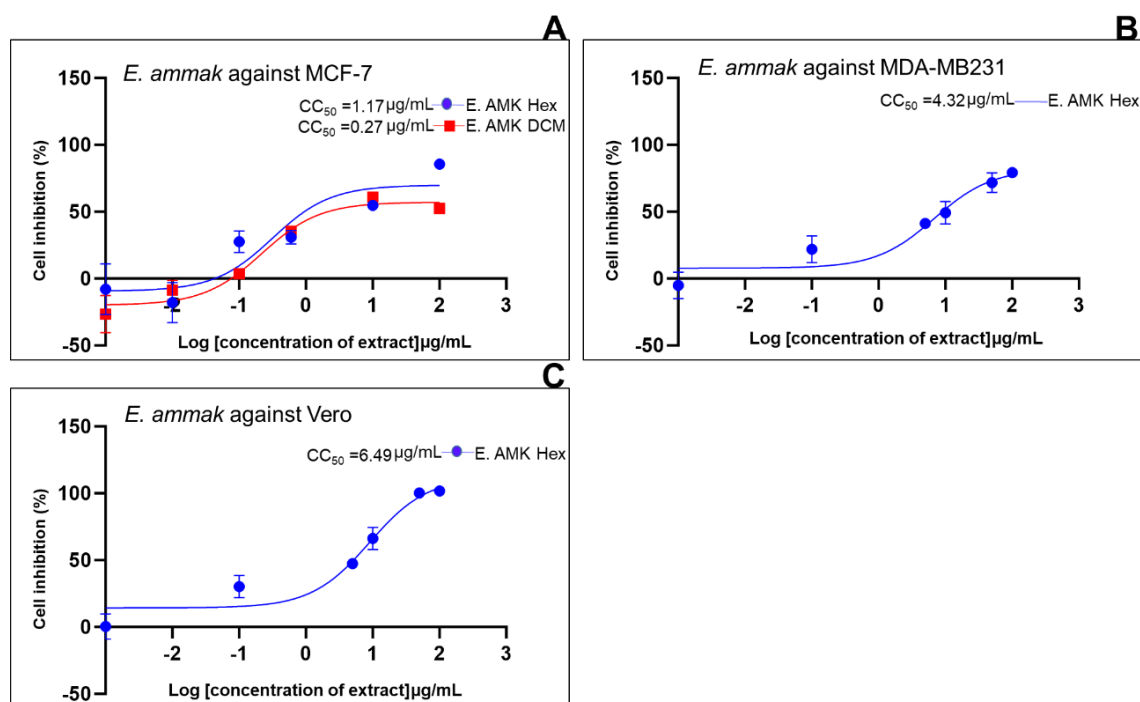


Figure 4.8. Anti-proliferative effect of *E. ammak* extracts against MCF-7 (A), MDA-MB231 (B) and Vero (C) cell lines was assessed using MTT assay (µg/mL) and CC₅₀ values were evaluated. The CC₅₀ values of *E. ammak* extract against each cell line were calculated using nonlinear regression analysis. The percentage concentration of the extracts is plotted on the X- axes in log format. The MTT assay results showed a hormetic dose-response effect (A) and concentration-dependent inhibitory effect (B and C) of *E. ammak* extracts. Values are presented as means ± SD from three independent experiments; n = 6. *E. ammak* hexane (E. AMK Hex); *E. ammak* dichloromethane (E. AMK DCM).

The Hex and DCM extracts from *E. enopla* exhibited cell growth inhibition of MCF-7 and MDA-MB231 with CC₅₀ values of 2.14, 0.07, 1.12 and 3.21 µg/mL (see Table 4.17 and Figure 4.9), indicating potent activity towards both cells; respectively. The Hex and DCM extract exhibited cell growth inhibition of Vero with CC₅₀ value of 8.65 and >100 µg/mL (see Table 4.17 and Figure 4.9), indicating non-toxicity to toxicity activity against Vero; respectively. The Hex and DCM extracts of *E. enopla* showed selectivity for MCF-7 and MDA-MB231 cells, with high SI values of 4.04, > 100, 7.72 and 31.15; respectively (see Table 4.17).

Table 4.17. Cytotoxicity assessment of extracts from *E. enopla* on MCF-7, MDA-MB231 and Vero cell lines. The table illustrates the CC₅₀ values (µg/mL, n=5/6) representing half-maximal cytotoxic concentrations of varying degrees of cytotoxicity, which were in triplicate. Selectivity index (SI) are also presented, highlighting potential candidates for further investigation. Species names and organic solvents are provided. Hexane (Hex), dichloromethane (DCM), methanol (MeOH), ethyl acetate (EtOAc), not determined (ND).

Anti-proliferative activity of <i>E. enopla</i>				
CC₅₀ (µg/ml)				
	Hexane	Dichloromethane	Methanol	Ethyl acetate
MCF-7	2.14	0.70	>100	>100
SI	4.04	>100	>100	>100
MDA-MB231	1.12	3.21	>100	>100
SI	7.72	31.15	>100	>100
Cytotoxic activity of <i>E. enopla</i>				
CC₅₀ (µg/ml)				
Vero	8.65	>100	>100	>100

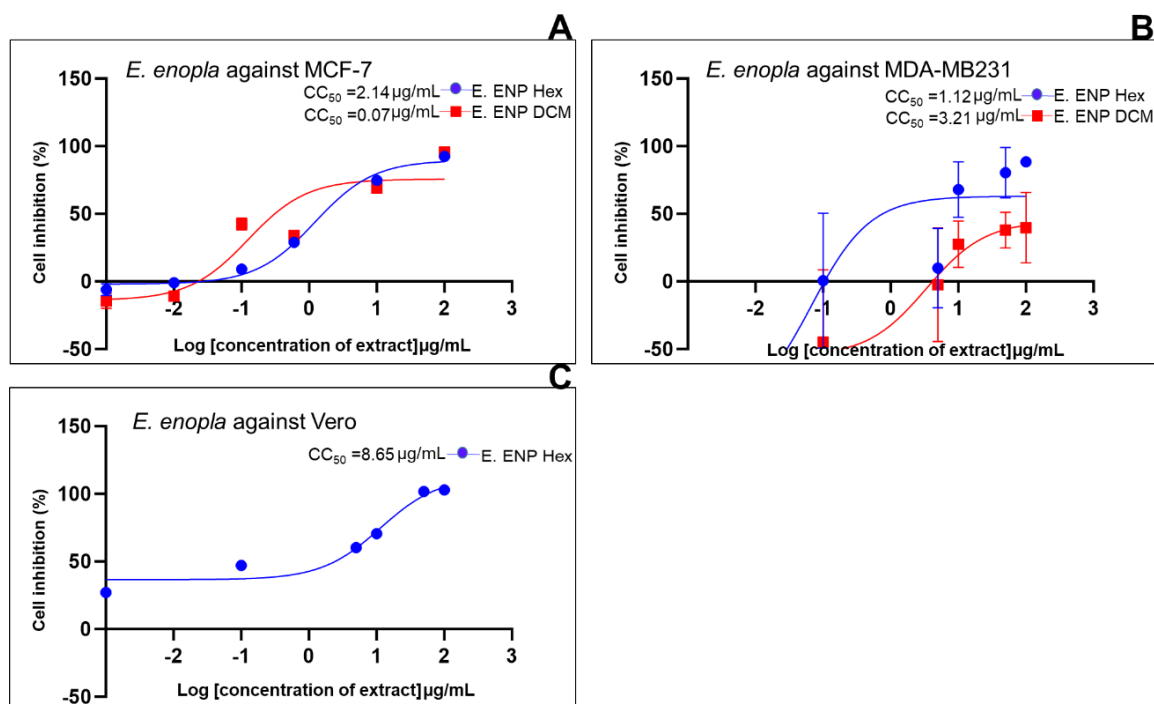


Figure 4.9. Anti-proliferative effect of *E. enopla* extracts against MCF-7 (A), MDA-MB231 (B) and Vero (C) cell lines was assessed using MTT assay ($\mu\text{g/mL}$) and CC_{50} values were evaluated. The CC_{50} values of *E. enopla* extract against each cell line were calculated using nonlinear regression analysis. The percentage concentration of the extracts is plotted on the X- axes in log format. The MTT assay results showed a hormetic dose-response effect (A and B) and concentration-dependent inhibitory effect (C) of *E. enopla* extracts. In order to improve the normality of data distribution, the outliers for Graph B were removed. Values are presented as means $\pm$ SD from three independent experiments; $n = 6$. *E. enopla* hexane (E. ENP Hex); *E. enopla* dichloromethane (E. ENP DCM).

The DCM extract of *E. stellata* displayed cell growth inhibition of MCF-7 and MDA-MB231 with CC_{50} values of 9.06 and 3.92 $\mu\text{g/mL}$ (see Table 4.18 and Figure 4.10), indicating good to potent activity against the breast cancer cell lines; respectively. Even though DCM extract displayed considerable inhibitory activity of MCF-7 and MDA-MB231, this extract did not demonstrate any selectivity for any of the breast cancer cell lines.

Table 4.18. Cytotoxicity assessment of extracts from *E. stellata* on MCF-7, MDA-MB231, and Vero cell lines. The table illustrates the CC_{50} values ($\mu\text{g/mL}$, $n=5/6$) representing half-maximal cytotoxic concentrations of varying degrees of cytotoxicity, which were in triplicate. Selectivity index (SI) are also presented, highlighting potential candidates for further investigation. Species names and organic solvents are provided. Hexane (Hex), dichloromethane (DCM), methanol (MeOH), ethyl acetate (EtOAc), not determined (ND).

Anti-proliferative activity of <i>E. stellata</i> CC ₅₀ (µg/ml)				
	Hexane	Dichloromethane	Methanol	Ethyl acetate
MCF-7	>100	9.06	>100	>100
SI	>100	0.91	>100	>100
MDA-MB231	>100	3.92	>100	>100
SI	>100	2.10	>100	>100
Cytotoxic activity of <i>E. stellata</i> CC ₅₀ (µg/ml)				
Vero	>100	8.26	>100	>100

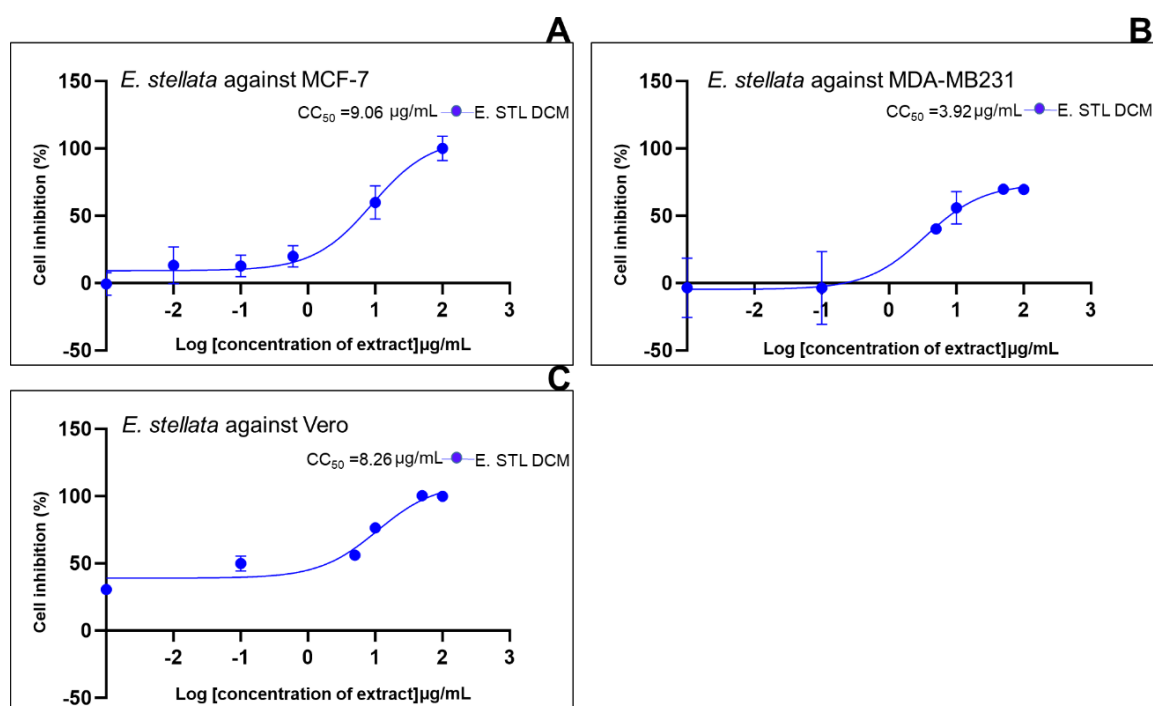


Figure 4.10. Anti-proliferative effect of *E. stellata* extracts against MCF-7 (A), MDA-MB231 (B) and Vero (C) cell lines was assessed using MTT assay (µg/mL) and CC₅₀ values were evaluated. The CC₅₀ values of *E. stellata* extract against each cell line were calculated using nonlinear regression analysis. The percentage concentration of the extracts is plotted on the X-axes in log format. The MTT assay results showed a concentration-dependent inhibitory effect of *E. stellata* extracts. Values are presented as means ± SD from three independent experiments; n = 6. *E. stellata* dichloromethane (E. STL DCM).

The Hex and EtOAc extracts of *E. ledienii* showed cell growth inhibition of MCF-7 with CC₅₀ values of 0.90 and 0.07 µg/mL (see Table 4.19 and Figure 4.11), indicating potent activity towards MCF-7; respectively. The Hex and EtOAc extracts demonstrated cell growth inhibition of Vero cells with CC₅₀ value of 4.23 and >100 µg/mL (see Table 4.19 and Figure

4.11); respectively. The Hex and EtOAc extracts of *E. ledienii* showed selectivity for MCF-7, with an SI value of 4.7 and > 100; respectively (see Table 4.19).

Table 4.19. Cytotoxicity assessment of extracts from *E. ledienii* on MCF-7, MDA-MB231 and Vero cell lines. The table illustrates the CC₅₀ values (µg/mL, n=5/6) representing half-maximal cytotoxic concentrations of varying degrees of cytotoxicity, which were in triplicate. Selectivity index (SI) are also presented, highlighting potential candidates for further investigation. Species names and organic solvents are provided. Hexane (Hex), dichloromethane (DCM), methanol (MeOH), ethyl acetate (EtOAc), not determined (ND).

Anti-proliferative activity of <i>E. ledienii</i>				
CC₅₀ (µg/ml)				
	Hexane	Dichloromethane	Methanol	Ethyl acetate
MCF-7	0.90	>100	>100	0.068
SI	4.70	>100	>100	>100
MDA-MB231	>100	>100	>100	>100
SI	>100	>100	>100	>100
Cytotoxic activity of <i>E. ledienii</i>				
CC₅₀ (µg/ml)				
Vero	4.23	>100	>100	>100

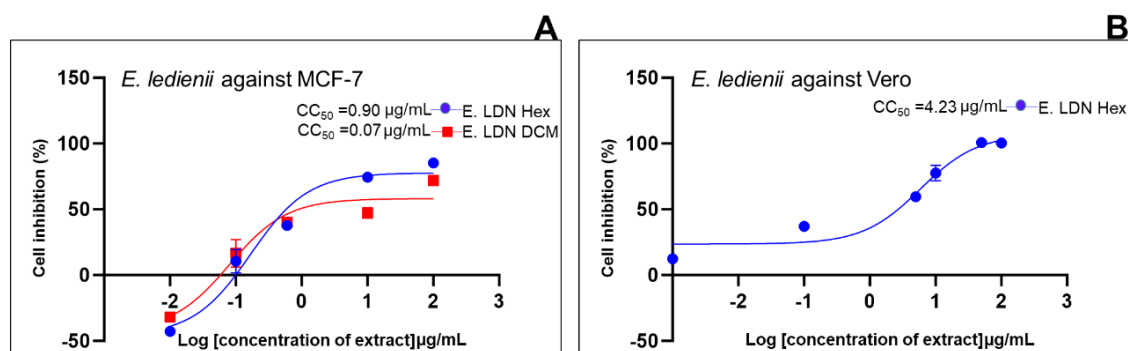


Figure 4.11. Anti-proliferative effect of *E. ledienii* extracts against MCF-7 (A) and Vero (B) cell lines was assessed using MTT assay (µg/mL) and CC₅₀ values were evaluated. The CC₅₀ values of *E. ledienii* extract against each cell line were calculated using nonlinear regression analysis. The percentage concentration of the extracts is plotted on the X-axes in log format. The MTT assay results showed a hormetic dose-reponse effect (A) and concentration-dependent inhibitory effect (B) of *E. ledienii* extracts. Values are means ± SD from three independent experiments; n = 5 for (A), and n = 6 (B). *E. ledienii* hexane (E. LDN Hex); *E. ledienii* dichloromethane (E. LDN DCM).

All tested extracts of *E. coerulescenes* exhibited low cell growth inhibition of MCF-7 and MDA-MB231, while the DCM extract inhibited the cell growth of Vero cells (see Table 4.20). None of the extracts from *E. coerulescenes* demonstrated selectivity for MCF-7 and MDA-MB231.

Table 4.20. Cytotoxicity assessment of extracts from *E. coerulescenes* on MCF-7, MDA-MB231 and Vero cell lines. The table illustrates the CC₅₀ values (µg/mL, n=5/6) representing half-maximal cytotoxic concentrations of varying degrees of cytotoxicity, which were in triplicate. Selectivity index (SI) are also presented, highlighting potential candidates for further investigation. Species names and organic solvents are provided. Hexane (Hex), dichloromethane (DCM), methanol (MeOH), ethyl acetate (EtOAc), not determined (ND).

Anti-proliferative activity of <i>E. coerulescenes</i>				
CC₅₀ (µg/ml)	Hexane	Dichloromethane	Methanol	Ethyl acetate
MCF-7	>100	>100	>100	>100
SI	>100	>100	>100	>100
MDA-MB231	>100	>100	>100	>100
SI	>100	>100	>100	>100
Cytotoxic activity of <i>E. caerulescenes</i>				
CC₅₀ (µg/ml)				
Vero	>100	>100	>100	>100

The DCM extract of *E. arabica* demonstrated cell growth inhibition of MCF-7 with a CC₅₀ value of 2.70 µg/mL (see Table 4.21 and Figure 4.12), indicating potent activity against MCF-7 cells. Furthermore, the Hex and DCM extracts exhibited cell growth inhibition of Vero with CC₅₀ values of 51.34 and 13.01, indicating moderate to low cytotoxic activity against the normal healthy cells (see Table 4.21 and Figure 4.12); respectively. The DCM extract of *E. arabica* showed selectivity for MCF-7 cells with an SI value of 4.81 (see Table 4.21).

Table 4.21. Cytotoxicity assessment of extracts from *E. arabica* on MCF-7, MDA-MB231 and Vero cell lines. The table illustrates the CC₅₀ values (µg/mL, n=5/6) representing half-maximal cytotoxic concentrations of varying degrees of cytotoxicity, which were in triplicate. Selectivity index (SI) are also presented, highlighting potential candidates for further investigation. Species names and organic solvents are provided. Hexane (Hex), dichloromethane (DCM), methanol (MeOH), ethyl acetate (EtOAc), not determined (ND).

Anti-proliferative activity of <i>E. arabica</i>				
CC₅₀ (µg/ml)	Hexane	Dichloromethane	Methanol	Ethyl acetate
MCF-7	>100	2.70	>100	>100
SI	>100	4.81	>100	>100
MDA-MB231	>100	ND	>100	>100
SI	>100	ND	>100	>100
Cytotoxic activity of <i>E. arabica</i>				
CC₅₀ (µg/ml)				
Vero	51.34	13.01	>100	>100

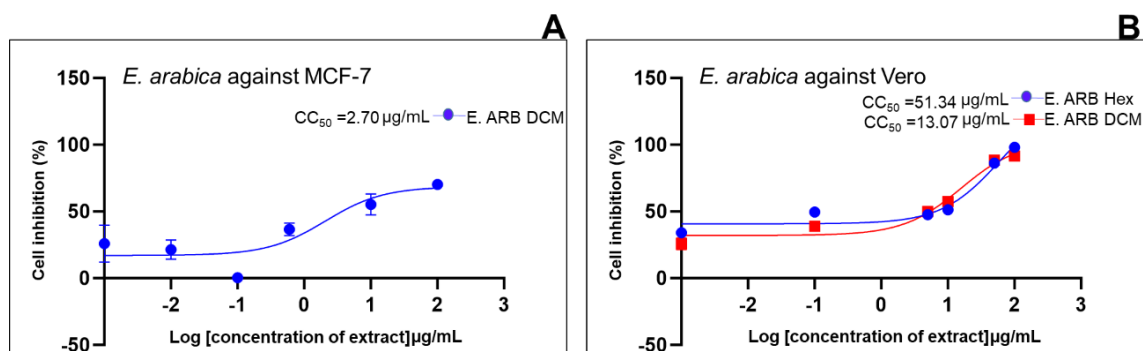


Figure 4.12. Anti-proliferative effect of *E. arabica* extracts against MCF-7 (A) and Vero (B) cell lines was assessed using MTT assay (µg/mL) and CC₅₀ values were evaluated. The CC₅₀ values of *E. arabica* extract against each cell line were calculated using nonlinear regression analysis. The percentage concentration of the extracts is plotted on the X-axes in log format. The MTT assay results showed a concentration-dependent inhibitory effect of *E. arabica* extracts. Values are means ± SD from three independent experiments; n = 6. *E. arabica* hexane (E. ARB Hex); *E. arabica* dichloromethane (E. ARB DCM).

The Hex and DCM extract of *E. cooperi* showed cell growth inhibition of MCF-7 and MDA-MB231 cells with CC₅₀ values of 4.80, 15.57, 2.02 and 0.006 µg/mL (see Table 4.22 and Figure 4.13), indicating moderate to potent activity against the breast cancer cell lines; respectively. Moreover, the Hex and DCM extracts of *E. cooperi* was selectivity for MCF-7 and MDA-MB231 with SI values of 20.83, 6.42, 49.40 and >100 (see Table 4.22 and Figure 4.13); respectively. These SI values show considerable selectivity.

Table 4.22. Cytotoxicity assessment of extracts from *E. cooperi* on MCF-7, MDA-MB231 and Vero cell lines. The table illustrates the CC₅₀ values (µg/mL, n=5/6) representing half-maximal cytotoxic concentrations of varying degrees of cytotoxicity, which were in triplicate. Selectivity index (SI) are also presented, highlighting potential candidates for further investigation. Species names and organic solvents are provided. Hexane (Hex), dichloromethane (DCM), methanol (MeOH), ethyl acetate (EtOAc), not determined (ND).

Anti-proliferative activity of <i>E. cooperi</i>				
CC₅₀ (µg/ml)				
	Hexane	Dichloromethane	Methanol	Ethyl acetate
MCF-7	4.80	15.57	>100	>100
SI	20.83	6.42	>100	>100
MDA-MB231	2.02	0.006	>100	>100
SI	49.40	>100	>100	>100
Cytotoxic activity of <i>E. cooperi</i>				
CC₅₀ (µg/ml)				
Vero	>100	>100	>100	>100

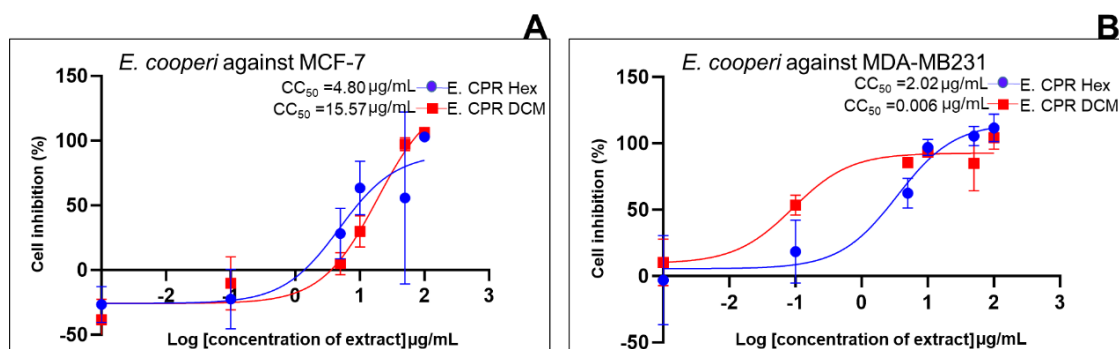


Figure 4.13. Anti-proliferative effect of *E. cooperi* extracts against MCF-7 (A) and MDA-MB231 (B) cell lines was assessed using MTT assay ($\mu\text{g/mL}$) and CC_{50} values were evaluated. The CC_{50} values of *E. cooperi* extract against each cell line were calculated using nonlinear regression analysis. The percentage concentration of the extracts is plotted on the X- axes in log format. The MTT assay results showed a hormetic dose-response effect (A) and concentration-dependent inhibitory effect (B) of *E. cooperi* extracts. In order to improve the normality of data distribution, the outliers for Graph A were removed. Values are means $\pm$ SD from three independent experiments; $n = 6$. *E. cooperi* hexane (E. CPR Hex); *E. cooperi* dichloromethane (E. CPR DCM).

The Hex and DCM extracts of *E. tirucalli* exhibited cell growth inhibition against MCF-7 and MDA-MB231 cells with CC_{50} values of 9.77, 7.19, 0.94 and 1.19 $\mu\text{g/mL}$ (see Table 4.23 and Figure 4.14), demonstrating potent to good activity against the breast cancer cell lines; respectively. The Hex and DCM extract of *E. tirucalli* displayed significantly higher selectivity for MCF-7 and MDA-MB231 with SI values of 10.23, 13.90, 84.03 and >100 ; respectively (see Table 4.23).

Table 4.23. Cytotoxicity assessment of extracts from *E. tirucalli* on MCF-7, MDA-MB231 and Vero cell lines. The table illustrates the CC_{50} values ($\mu\text{g/mL}$, $n=5/6$) representing half-maximal cytotoxic concentrations of varying degrees of cytotoxicity, which were in triplicate. Selectivity index (SI) are also presented, highlighting potential candidates for further investigation. Species names and organic solvents are provided. Hexane (Hex), dichloromethane (DCM), methanol (MeOH), ethyl acetate (EtOAc), not determined (ND).

Anti-proliferative activity of <i>E. tirucalli</i> CC_{50} ($\mu\text{g/mL}$)				
	Hexane	Dichloromethane	Methanol	Ethyl acetate
MCF-7	9.77	7.19	>100	>100
SI	10.23	13.90	>100	>100
MDA-MB231	0.94	1.19	>100	>100
SI	>100	84.03	>100	>100
Cytotoxic activity of <i>E. tirucalli</i> CC_{50} ($\mu\text{g/mL}$)				
Vero	>100	>100	>100	>100

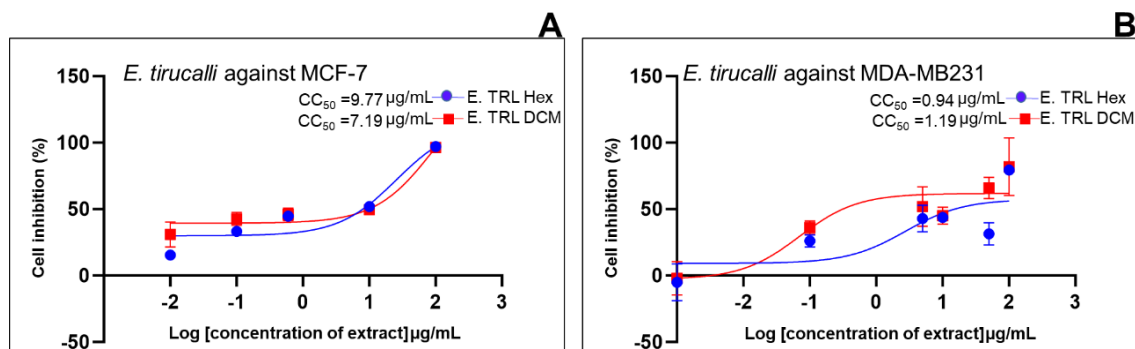


Figure 4.14. Anti-proliferative effect of *E. tirucalli* extracts against MCF-7 (A) and MDA-MB231 (B) cell lines was assessed using MTT assay ($\mu\text{g/mL}$) and CC_{50} values were evaluated. The CC_{50} values of *E. tirucalli* extract against each cell line were calculated using nonlinear regression analysis. The percentage concentration of the extracts is plotted on the X- axes in log format. The MTT assay results showed a concentration-dependent inhibitory effect of *E. tirucalli* extracts. Values are means $\pm$ SD from three independent experiments; $n = 5$ for (A), and $n = 6$ (B). *E. tirucalli* hexane (E. TRL Hex); *E. tirucalli* dichloromethane (E. TRL DCM).

4.3.4. Chromatographic profiling of key metabolites from eleven selective *Euphorbia* extracts

The utilization of HPLC has proven to be instrumental in the identification of secondary metabolites. The eleven plant extracts that demonstrated selectivity for MCF-7 and MDA-MB231 were run on HPLC. The chromatograms were derived from the dissolution of 2 mg/ml of each active extract in MeOH and run using the method described in section 4.2.3. These are depicted in Figures 4.15 through 4.24. Within each active plant extract, distinctive characteristic peaks of significance were readily visible. It is worth noting, however, that no standards were used in this study because the focus was not on isolating individual compounds but rather on identifying classes of phytochemicals. Additionally, resource constraints hindered the isolation of observed peaks for structural elucidation.

The outcome of the analysis revealed that the two extracts from *E. clavarioides* exhibited dissimilar constituents across varying retention times (ranging from 1.51 to 17.52 minutes).

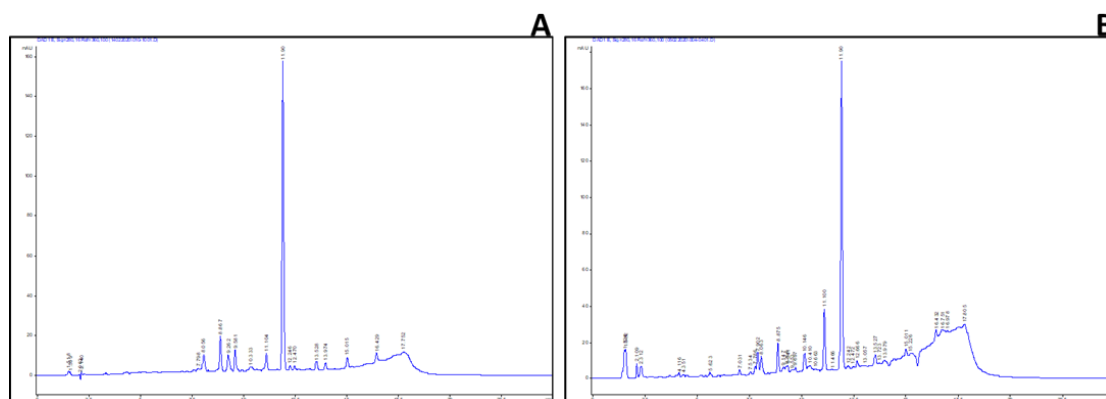


Figure 4.15. HPLC chromatograms of selective extracts of *E. clavarioides* (A) Hex and (B) DCM for MCF-7.

Notably, major peaks surfaced at 8.86, 11.10 and 11.90 minutes (Figure 4.15). Similarly, *E. trigona* DCM, *E. gorgonis* Hex, *E. ledienii* Hex and *E. arabica* DCM exhibited parallel peaks spanning a retention time of 1.50 to 22.76 minutes, with prominent peaks at 1.58, 9.83, 10.45 and 22.76 minutes for all four extracts (Figure 4.16 - 4.19). This shared profile highlights the presence of common phytochemicals such as phytosterols, glycosides, triterpenoids and flavonoids among these *Euphorbia* species.

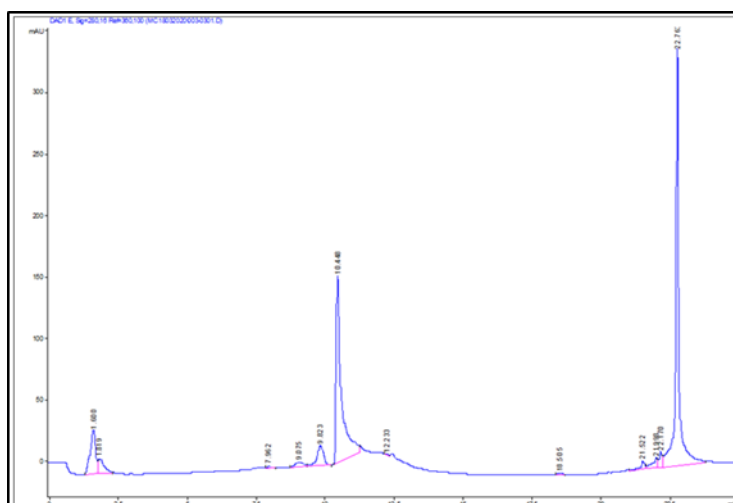


Figure 4.16. HPLC chromatograms of selective extract of *E. trigona* DCM for MCF-7.

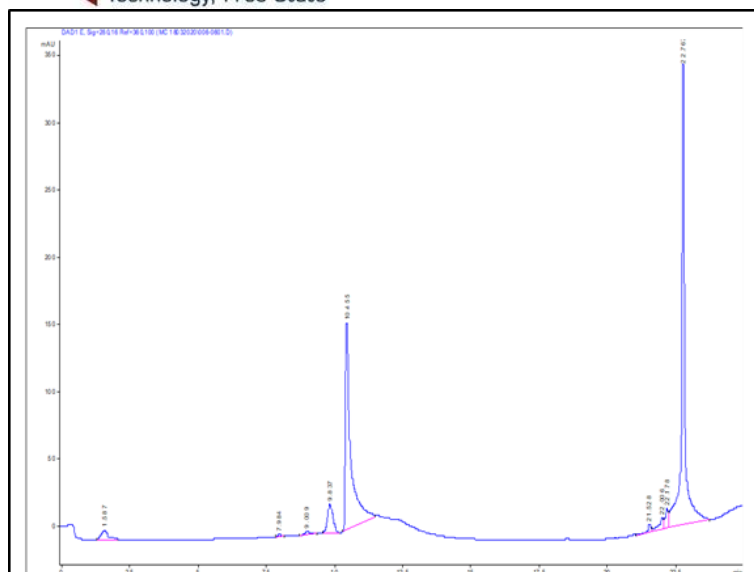
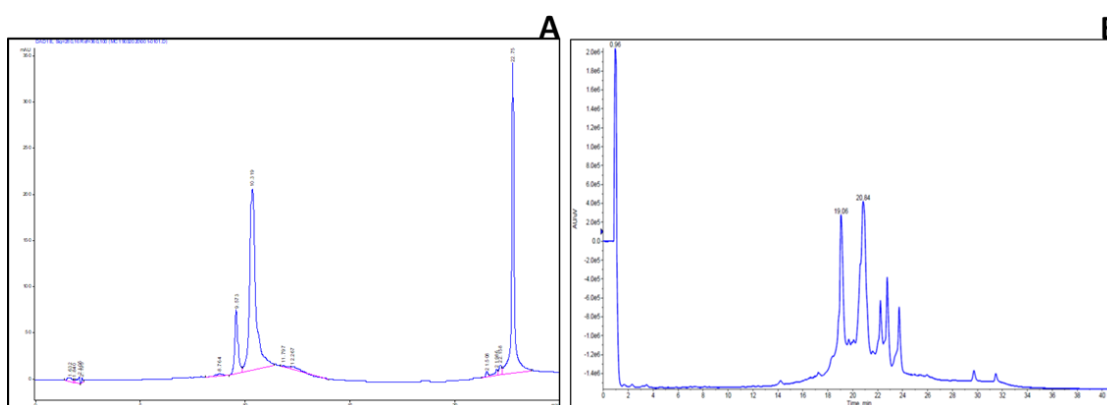


Figure 4.17. HPLC chromatograms of selective extract of *E. gorgonis* Hex for MCF-7 and MDA-MB231.



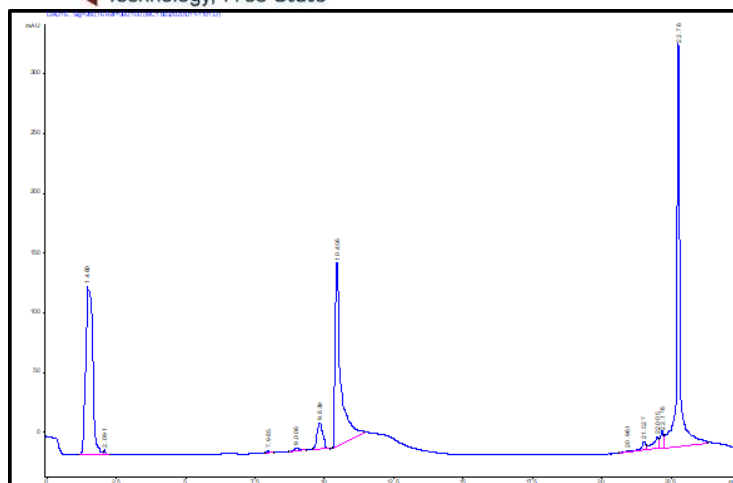


Figure 4.19. HPLC chromatograms of selective extract *E. arabica* DCM for MFC-7 and MDA-MB231.

Noteworthy observations were made in the extracts from *E. cooperi*, with peaks manifesting across a range of 1.80 to 32.90 minutes. Specifically, significant peaks were evident at 20.70, 26.54, 28.6, 29.04 and 32.90 minutes, with a more noticeable presence in the *E. cooperi* DCM extract (Figure 4.20). Similarly, *E. ammak* Hex exhibited peaks within a retention time of 1.55 to 17.89 minutes, with major peaks recorded at 1.55, 2.40, 3.66, 7.73 and 14.88 minutes. Conversely, *E. ammak* DCM featured peaks from 0.99 to 31.43 minutes, with major peaks at 0.99, 21.30, 23.36 and 29.50 minutes (Figure 4.21).

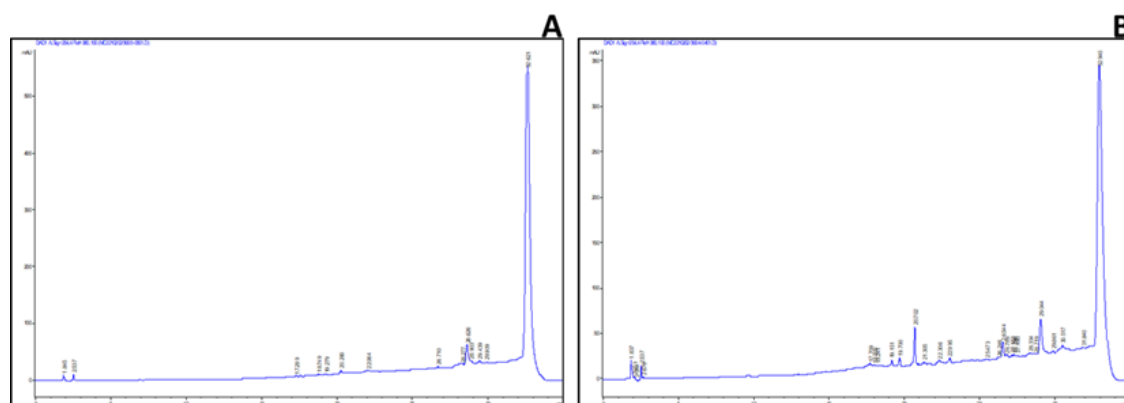


Figure 4.20. HPLC chromatograms of selective extracts of *E. cooperi* (A) Hex and (B) DCM for MCF-7 and MDA-MB231.

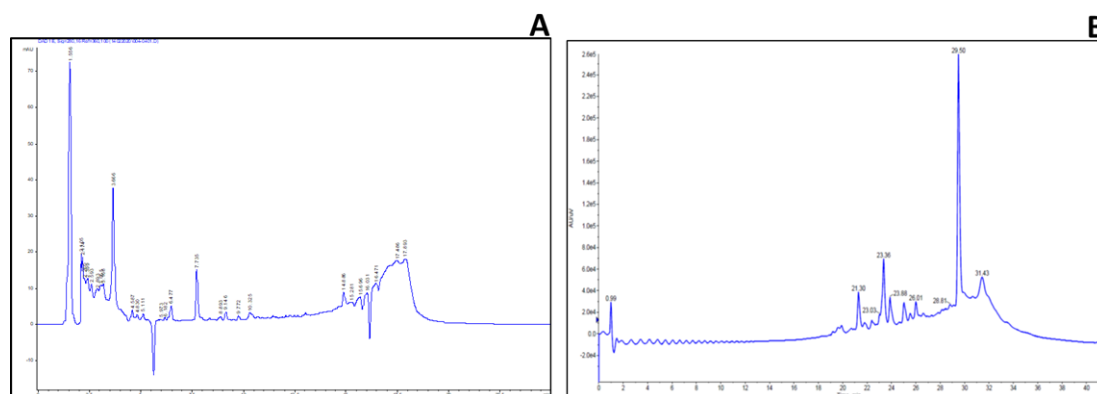


Figure 4.21. HPLC chromatograms of selective extracts of *E. ammak* (A) Hex and (B) DCM for MCF-7.

Further investigation unveiled that *E. ferox* displayed peaks over a retention time range of 0.92 to 28.88 minutes, with major peaks at 0.92, 20.51, 25.47, 28.45 and 28.88 minutes (Figure 4.22). *E. polygona* Hex displayed peaks from 0.93 to 32.60 minutes, prominently at 0.93, 21.91, 22.78, 25.58 and 31.95 minutes. In contrast, the DCM extract of *E. polygona* displayed peaks spanning 1.53 to 17.28 minutes, with major peaks observed at 1.53, 4.03, 7.74, 9.15, 11.46 and 13.54 minutes (Figure 4.23).

Remarkably similar profiles occurred for *E. enopla* Hex and *E. enopla* DCM extracts. The former displayed peaks from 0.91 to 32.66 minutes, featuring major peaks at 0.91, 18.43, 22.58, 24.40 and 29.03 minutes. Similarly, the DCM extract of *E. enopla* displayed peaks within a retention time of 0.95 to 30.41 minutes, notably at 0.95, 21.10, 22.54, 23.88, 26.71 and 30.41 minutes (Figure 4.24).

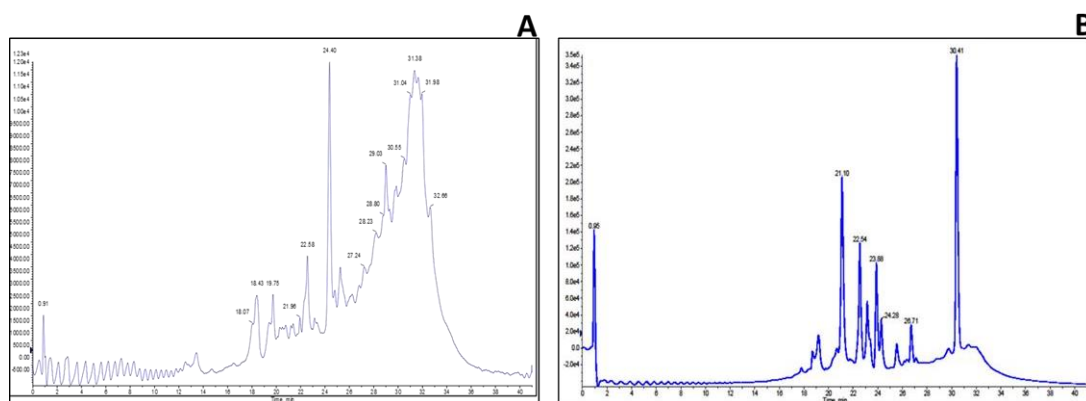


Figure 4.24. HPLC chromatograms of selective extract *E. enopla* (A) Hex and (B) DCM for MFC-7 and MDA-MB231.

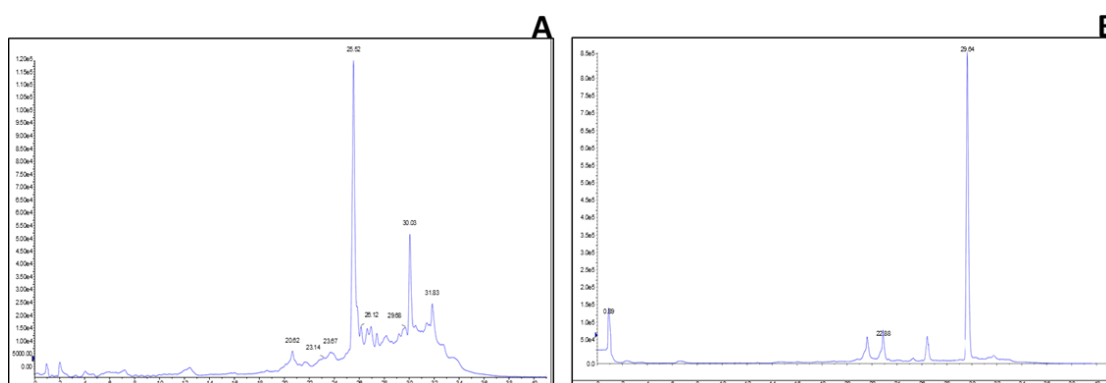


Figure 4.25. HPLC chromatograms of selective extract *E. tirucalli* (A) and Hex (B) DCM for MFC-7 and MDA-MB231.

In the context of *E. tirucalli* Hex, peaks emerged between 20.62 and 31.83 minutes, with major peaks at 20.62, 25.52 and 30.02 minutes. *E. tirucalli* DCM, on the other hand, exhibited peaks over a retention time span of 0.89 to 29.64 minutes, featuring major peaks at 0.89, 22.88 and 29.64 minutes (Figure 4.25).

4.5. Discussion

The study presented focused on evaluating the phytochemical profiles, cytotoxic activity and selectivity of sixteen *Euphorbia* plant species against breast cancer cell lines MCF-7 and MDA-MB231. The results offer valuable insights into the potential anticancer properties of these plant extracts. The phytochemical analysis revealed the presence of various secondary metabolites across these *Euphorbia* species.

Glycosides and triterpenoids were found ubiquitously, indicating their wide distribution within the *Euphorbia* family. The presence of these phytochemicals aligns with studies by Shi *et al.* (2008), which noted the common secondary metabolites found in the Euphorbiaceae family that were also present in all the selected plants in this study. It has also been shown that these two phytochemicals also possess anticancer properties (Liu *et al.*, 2013). Furthermore, triterpenoid has proven to possess medicinal properties such as anticarcinogenic, antimalarial, anti-ulcer, hepaticidal, antimicrobial activity, antimalarial and anti-viral (Sema & Waktola, 2022; Si *et al.*, 2018; Dudareva *et al.*, 2004; Langenheim, 1994). Munro *et al.* (2015) demonstrated that the tetracyclic triterpene euphol derived from *E. tirucalli* latex has anticancer properties. Arnidiol is a compound that belongs to the class of pentacyclic triterpenoid diols. It is a natural substance that has been the subject of several studies in the field of cancer research. A study conducted by Hu *et al.* (2020) to investigate the effects of arnidiol on human breast cancer cells known as MDA-MB-231 cells. The study found that arnidiol was able to induce apoptosis in these cells. The researchers also observed a correlation between the induction of apoptosis and the reduction of PARP expression,

which is a protein involved in DNA repair, and the increase in caspase-3 cleavage, which is a marker of apoptosis.

Stevioside is a naturally occurring diterpenoid glycoside that is commonly found in the leaves of the *Stevia rebaudiana* (Bertoni) Bertoni plant. It has gained significant attention in recent years due to its potential health benefits (Paul *et al.*, 2012).

Flavonoids and phytosterols were almost universally present, except in *E. tirucalli*. This suggests that these compounds play a significant role in the biological activity of these plants. Flavonoids were used to treat various ailments as they possess medicinal properties such as anti-inflammatory activity, enzyme inhibition, antimicrobial activity, estrogenic activity, antiallergic activity and antioxidant activity (Munro *et al.*, 2015; Abdel-Hameed *et al.*, 2013; Tapas *et al.*, 2008; Cowan, 1999). Mai *et al.* (2017) reported that *E. helioscopia* which had a high concentration of flavonoids exhibited cytotoxic activity against a triple negative breast cancer cell line. Zhu and Xue (2019) conducted a study where they investigated the effect of kaempferol, a flavonoid, on MDA-MB-231 cells. They found that kaempferol induces apoptosis in these cells through a mechanism that involves increasing the expression of cleaved caspase-9/3 and p-ATM. Moreover, in a study conducted by Zhang *et al.* (2018), three flavonoids were isolated from *Tephrosia kirilowii* (Turcz. ex DC.): Holub-isorhamnetin, genkwanin and acacetin. These flavonoids were found to have a significant impact on MDA-MB-231 cells. They suppressed cell growth, induced apoptosis and downregulated the PI3K/AKT/mTOR/p70S6K/ULK signaling pathway. They did this by stimulating the apoptotic pathways, leading to the programmed death of the MDA-MB-231 cancer cells. The downregulation of the PI3K/AKT/mTOR/p70S6K/ULK signaling pathway by these flavonoids is also significant (Zhang *et al.* 2018). This pathway is known to play a crucial role in cell

survival and growth. The flavonoids were able to suppress this pathway, leading to decreased cell growth and increased sensitivity to apoptosis.

Additionally, phytosterols are considered to have a number of bioactive properties that have a variety of health benefits, including anti-inflammatory, antioxidative and anticarcinogenic properties, as well as the ability to reduce cholesterol (Vezza *et al.*, 2020; Vilahur *et al.*, 2019). A mixture of phytosterols were able to inhibit tumor development in various cancer forms, including cholangiocarcinoma and breast cancer, at physiological doses (Kangsamaksin *et al.*, 2017; Kaziłowska *et al.*, 2013). Alkaloids were detected in a considerable number of species, further emphasizing the chemical diversity within the *Euphorbia* genus. It is not surprising that alkaloids were found in almost all selected plants, because the presence of alkaloids in *Euphorbiaceae* species has been documented in many studies (Al-Saraireh *et al.*, 2021; Ndam *et al.*, 2016; Tarek *et al.*, 2010; Kapingu *et al.*, 2005). Extracts from plants containing alkaloids have been used as prescription medicines for many years and due to the presence of alkaloids they owe their potent effects. Morphine which is currently used as an analgesic was the first alkaloid to be obtained from opium poppy (Demirkapu & Yananli, 2020). Furthermore, other alkaloids such as vinblastine, quinine, morphine, atropine, nicotine, caffeine, ephedrine and strychnine are also used in production of medicine (Mfengwana, 2019). Alkaloids have a number of physiological effects on humans, such as antibacterial, antimitotic, anti-inflammatory, local anesthetic, hypnotic, antitumor activity and several others (Molyneux *et al.*, 1996). Furthermore, they are also of great interest to organic chemists, biologists, biochemists, pharmacologists and pharmacists, typically from plants rather than animals (Kurek, 2019). Berberine is a naturally occurring isoquinoline alkaloid isolated from *Cotridis rhizoma*. Zhao *et al.* (2017) investigated the effect of berberine on triple-negative breast cancer (TNBC) cells. Their findings revealed that

berberine activated caspase-9/CytoC-mediated apoptosis in TNBC cells, leading to a significant suppression of cell proliferation both *in vitro* and *in vivo*. Tetrandrine is a naturally occurring alkaloid that has shown great potential as an anticancer agent. A study conducted by Chandrashekar *et al.* (2019) found that tetrandrine, which was extracted from *Cyclea peltata* (Lam.) Hook. f. and Thomson, demonstrated cytotoxic and apoptotic effects on MDA-MB-231 cells. The mechanism of action was attributed to an increase in the levels of reactive oxygen species (ROS) and caspase-8/9/3.

Tannins, known for their antioxidant properties, were also detected in several species. The presence of these diverse compounds aligns with the potential bioactivity of *Euphorbia* plants against cancer cells. The presence of these phytochemicals is in agreement with Aleksandrov *et al.* (2019), who reported larger number of tannins in *E. hirta*. Fraga-Corral *et al.* (2021) reported that topical tannin treatments help to flush all the irritants out of the skin, can also be used as an anti-inflammatory agent and their antihemorrhagic and antiseptic ability is used in the treatment of burns and other wounds. In other studies, tannins were utilized in the treatment of different diseases, as they have been reported to possess high antioxidants, free radical scavenging activity, antimicrobial, antivirals and also in cancer chemotherapy (Koleckar *et al.*, 2008; Buzzini *et al.*, 2008; Amarowicz *et al.*, 2004; Ho, 2000; Chung *et al.*, 1998). Saponins were also detected in some plants investigated. Bigoniya and Rana (2009) reported the presence of saponins in *E. neriifolia*. Furthermore, saponins were also reported in *E. paralias* and *E. terracina* (Jannet *et al.*, 2017). The presence of saponins in the above plants is unsurprising, as most of the Euphorbiaceae have them. Saponins possess pharmacological and medicinal properties such as cell membrane permeability, hemolytic activity, antiviral, antifungal, anti-inflammatory and antiallergic (Francis *et al.*, 2002; El Izzi *et al.*, 1992; Glauert *et al.*, 1962). Furthermore, they have promising uses in the

treatment of cancer based on their ability to suppress angiogenesis, decreased invasiveness of cells, cell cycles arrest and apoptosis induction (Fuchs *et al.*, 2009). Moreover, saponins can be added to other antitumor medicines to enhance their cytotoxicity in tumor treatment (Jia *et al.*, 2004; Gaidi *et al.*, 2002).

The cytotoxicity assays, performed using the MTT assay, yielded intriguing findings. The extracts from various *Euphorbia* species displayed a spectrum of effects on the tested cell lines. Some extracts exhibited potent cell growth inhibition, while others demonstrated little or no activity. This variability could be attributed to differences in chemical composition, concentrations of active compounds, and their underlying mechanisms of action. Extracts from *E. clavarioides*, *E. gorgonis* and *E. tirucalli* showed notably low CC₅₀ values, indicating strong inhibition of cancer cell growth. The current findings are in agreement with those of a study conducted by Choene and Motadi (2016), who reported significant antiproliferative activity of *E. tirucalli* butanol extract against MCF-7 and MDA-MB231. These findings highlight the potential of these extracts as promising candidates for further investigation and potential development into therapeutic agents. This similarity supports the reliability of the current findings, implying that these extracts possess potent antiproliferative potential against the specific cancer cell lines. The co-occurrence between the two studies strengthens the notion that these extracts could serve as promising candidates for further exploration and potential development into therapeutic agents for breast cancer treatment. However, the differences between the current study and the research of El-Hawary *et al.* (2020) regarding the activity of *E. tirucalli* extracts against MCF-7 cells raise questions about potential contributing factors. Variability in aspects such as plant sourcing, extraction techniques, sample preparation, cell culture conditions and experimental methodologies can all introduce variability into cytotoxicity assay outcomes. Furthermore, the study also assessed the

potential toxicity of *E. tirucalli* on HEPG2 and CACO2 cell lines. The findings indicated that there was no noteworthy impact observed on these two cell lines (El-Hawary *et al.*, 2020). The current study's observation of moderate antiproliferative activity of *E. trigona* extracts against MCF-7 cells, which aligns with the mild cytotoxic effect noted by El-Hawary *et al.* (2020), presents consistency regarding the cytotoxic potential of *E. trigona* extracts against breast cancer cells. This consistency supports the theory that *E. trigona* extracts may indeed have biologically relevant activities against breast cancer cells. Moreover, it was observed that *E. trigona* exhibited a cytotoxic effect on the CACO2 cell line (El-Hawary *et al.*, 2020). Additionally, the absence of antiproliferative effects in *E. horrida* extracts against all tested cell lines in the current study is consistent with the findings reported by El-Hawary *et al.* (2020), indicating that *E. horrida* extracts might lack significant cytotoxic effects against the selected breast cancer cell lines. Additionally, *E. horrida* did not demonstrate any notable cytotoxic effects on HEPG2 and CACO2 cells (El-Hawary *et al.*, 2020). This uniformity in results, despite the differences between the studies, highlights the strength of the conclusions. Furthermore, several extracts demonstrated a high SI, indicating a degree of selectivity for cancer cells. Notably, *E. gorgonis* extracts exhibited remarkable selectivity for both MCF-7 and MDA-MB231 cells, while extracts from *E. tirucalli* and *E. cooperi* were particularly selective for MDA-MB231 cells, which represent triple negative breast cancer cells. These findings suggest that the complex mixtures of various compounds within these extracts may possess distinct mechanisms of action that specifically target breast cancer cells. The observed selectivity in these extracts provides further evidence that *Euphorbia* species could be a valuable source for developing anticancer agents with reduced side effects.

The HPLC analysis further revealed the complexity of the phytochemical profiles within the tested *Euphorbia* extracts. Common peaks observed across multiple species indicate the presence of shared compounds. Although specific compounds cannot be conclusively identified as representing entire classes, the analysis suggests the presence of compounds such as phytosterols, glycosides, triterpenoids and flavonoids. This suggests a certain level of structural similarity in these phytochemicals, which may contribute to their observed bioactivities. However, the presence of distinct peaks in specific species indicates individuality in their chemical composition. This variation might explain the differential activities observed in the cytotoxicity assays.

4.6. Conclusions

This is the first study to report on antiproliferative and selectivity of *Euphorbia* species investigated against MCF-7, MDA-MB231 and Vero cell lines. The findings of this study contribute to the understanding of *Euphorbia* plant species as potential sources of anticancer agents. The presence of various bioactive compounds suggests their pharmacological relevance. Further research could involve isolating and characterizing these compounds to identify key bioactive constituents responsible for the observed effects. Mechanistic studies could elucidate how these compounds interact with cancer cells and potentially modulate critical pathways involved in cell growth and proliferation. Correlating the identified metabolites with their observed cytotoxic and selective effects could pave the way for targeted drug development. This information might also guide the selection of appropriate extraction methods, yielding higher concentrations of active compounds.

This study highlights the diverse chemical composition and potential anticancer activities of *Euphorbia* plant species. The combination of phytochemical analysis and cytotoxicity assays



provides a valuable foundation for further research aimed at connecting the therapeutic potential of these plants in breast cancer treatment.

4.7. References

- Abdel-Hameed, E. -S. S., Bazaid, S. A., & Salman, M. S. (2013). Characterization of the phytochemical constituents of Taif rose and its antioxidant and anticancer activities. *BioMed Research International*, 2013, 1–13.
- Aleksandrov, M., Maksimova, V. & Koleva Gudeva, L. (2019). Review of the Anticancer and Cytotoxic Activity of some Species from Genus Euphorbia. *Agriculturae Conspectus Scientificus*, 84(1), 1–5.
- Al-Saraireh, Y. M., Youssef, A. M. M., Za'al Alsarayreh, A., Al Hujran, T. A., Al-Sarayreh, S., Al-Shuneigat, J. M. & Alrawashdeh, H. M. (2021). Phytochemical and anti-cancer properties of Euphorbia hierosolymitana Boiss. crude extracts. *Journal of Pharmacy & Pharmacognosy Research*, 9(1), 13–23.
- Amarowicz, R., Troszynska, A., Barylko-pikielna, N. & Shahidi, F. (2004). Polyphenolics extracts from legume seeds: correlations between total antioxidant activity, total phenolics content, tannins content and astringency. *Journal of Food Lipids*, 11 (4), 278–286.
- Arnold, M., Morgan, E., Rungay, H., Mafra, A., Singh, D., Laversanne, M., Vignat, J., Gralow, J. R., Cardoso, F., Siesling, S., & Soerjomataram, I. (2022). Current and future burden of breast cancer: Global statistics for 2020 and 2040. *The Breast*, 66, 15-23.
- Bérdy, J. (2005). Bioactive microbial metabolites. *The Journal of Antibiotics*, 58(1), 1–26.

Bhagya, N., Chandrashekar, K. R., Prabhu, A. & Rekha, P. D. (2019). Tetrandrine isolated from *Cyclea peltata* induces cytotoxicity and apoptosis through ROS and caspase pathways in breast and pancreatic cancer cells. *In Vitro Cellular & Developmental Biology – Animal*, 55(5), 331–340.

Bigoniya, P., & Rana, A. (2010). Radioprotective and in-vitro cytotoxic sapogenin from *Euphorbia neriifolia* (Euphorbiaceae) leaf. *Tropical Journal of Pharmaceutical Research*, 8(6), 521-530.

Boudiar, T., Lakhal, H., Khalfallah, A., Kabouche, A., Kabouche, Z., Brouard, I., Bermejo, J., & Bruneau, C. (2010). A new alkaloid and flavonoids from the aerial parts of *Euphorbia guyoniana*. *Natural Product Communications*, 5(1), 1934578X1000500- 1934578X1000500.

Buzzini, P., Arapitsas, P., Goretti, M., Branda, E., Turchetti, B., Pinelli, P., Ieri, F., & Romani, A. (2008). Antimicrobial and antiviral activity of hydrolysable tannins. *Mini-Reviews in Medicinal Chemistry*, 8(12), 1179–1187.

Choene, M. & Motadi, L. (2016). Validation of the antiproliferative effects of *Euphorbia tirucalli* extracts in breast cancer cell lines. *Molecular Biology*, 50(1), 98–110.

Choudhari, A. S., Mandave, P. C., Deshpande, M., Ranjekar, P. & Prakash, O. (2020). Phytochemicals in Cancer Treatment: From Preclinical Studies to Clinical Practice. *Frontiers in Pharmacology*, 10.

Chung, K. -T., Wong, T. Y., Wei, C. -I., Huang, Y. -W. & Lin, Y. (1998). Tannins and Human Health: A Review. *Critical Reviews in Food Science and Nutrition*, 38(6), 421–464.

Conte, C. (2013). Core-shell sustained release nanosystems for the intravenous delivery of

anticancer drugs (Ph.D. thesis). University of Naples Federico II, Italy.

Cowan, M. M. (1999). Plant products as antimicrobial agents. *Clinical Microbiology Reviews*, 12(4), 564–582.

Demirkapu, M. J., & Yananli, H. R. (2020). Opium alkaloids. In K. Sharma, K. Mishra, K. K. Senapati, & C. Danciu (Eds.), *Bioactive compounds in nutraceutical and functional food for good human health* (15-15). IntechOpen.

Done, S. (ed.) (2011). Breast cancer: recent advances in biology, imaging and therapeutics. Toroto, Canada.190-196.

Dudareva, N., Pichersky, E. & Gershenzon, J. (2004). Biochemistry of plant volatiles. *Plant Physiology*, 135(4),1893–1902.

El Izzi, A., Benie, T., Thieulant, M. L., Le Men-Olivier, L., & Duval, J. (1992). Stimulation of LH release from cultured pituitary cells by saponins of *Petersianthus macrocarpus*: A permeabilizing effect. *Planta Medica*, 58(3), 229–233.

El-Hawary, S. S., Mohammed, R., Tawfike, A., Lithy, N. M., AbouZid, S., Amin, M. N., Usama, R. A., & Amin, E. (2020). Cytotoxic activity and metabolic profiling of fifteen Euphorbia species. *Metabolites*, 11(1), 15–15.

Fraga-Corral, M., Otero, P., Cassani, L., Echave, J., Garcia-Oliveira, P., Carpena, M., Chamorro, F., Lourenço-Lopes, C., Prieto, M. A., & Simal-Gandara, J. (2021). Traditional applications of tannin rich extracts supported by scientific data: Chemical composition, bioavailability and bioaccessibility. *Foods*, 10(2), 251.

Francis, G., Kerem, Z., Makkar, H. P. S. & Becker, K. (2002). The biological action of saponins in animal systems: a review. *British Journal of Nutrition*, 88(6), 587–605.

Fuchs, H., Bachran, D., Panjideh, H., Schellmann, N., Weng, A., Melzig, M., Sutherland, M., & Bachran, C. (2009). Saponins as tool for improved targeted tumor therapies. *Current Drug Targets*, 10(2), 140–151.

Gaidi, G., Correia, M., Chauffert, B., Beltramo, J.-L., Wagner, H., & Lacaille-Dubois, M.-A. (2002). Saponins-mediated potentiation of cisplatin accumulation and cytotoxicity in human colon cancer cells. *Planta Medica*, 68(1), 70-72.

George, B. P., Chandran, R., & Abrahamse, H. (2021). Role of phytochemicals in cancer chemoprevention: Insights. *Antioxidants*, 10(9), 1455.

Giuliano, A. E., Connolly, J. L., Edge, S. B., Mittendorf, E. A., Rugo, H. S., Solin, L. J., Weaver, D. L., Winchester, D. J., & Hortobagyi, G. N. (2017). Breast cancer—Major changes in the American Joint Committee on Cancer eighth edition cancer staging manual. *CA: A Cancer Journal for Clinicians*, 67(4), 290-303.

Glauert, A. M., Dingle, J. T., & Lucy, J. A. (1962). Action of saponin on biological cell membranes. *Nature*, 196(4858), 953–955).

Hanson, J. (2003). The classes of natural product and their isolation. In *Natural products: The secondary metabolites* (1-21). Royal Society of Chemistry.

Hartwell, J. L. (1969). Plants used against cancer. A survey. *Lloydia* 1969, 32(1), 79-107.

Ho, P. L. (2001). Increasing resistance of *Streptococcus pneumoniae* to fluoroquinolones: results of a Hong Kong multicentre study in 2000. *Journal of Antimicrobial Chemotherapy*, 48(5), 659–665.

Hu, J., Zhang, H., Li, J., Jiang, X., Zhang, Y., Wu, Q., Shen, L., Shi, J. & Gao, N. (2020). ROCK1 activation-mediated mitochondrial translocation of Drp1 and cofilin are required

for arnidiol-induced mitochondrial fission and apoptosis. *Journal of Experimental & Clinical Cancer Research*, 39(1).

Huang, M., Lu, J. -J., Huang, M. -Q., Bao, J. -L., Chen, X. -P. & Wang, Y. -T. (2012). Terpenoids: natural products for cancer therapy. *Expert Opinion on Investigational Drugs*, 21(12), 1801–1818.

Iavicoli, I., Fontana, L., Leso, V. & Calabrese, E. J. (2014). Hormetic dose–responses in nanotechnology studies. *Science of The Total Environment*, 487, 361–374.

Indrayanto, G., Putra, G. S. & Suhud, F. (2021). Validation of in-vitro bioassay methods: Application in herbal drug research. *Profiles of Drug Substances, Excipients and Related Methodology*, 46, 273–307.

Ismail, N. Z., Md Saad, S., Adebayo, I. A., Md Toha, Z., Abas, R., Mohamad Zain, N. N., & Arsad, H. (2022). The antiproliferative and apoptotic potential of *Clinacanthus nutans* against human breast cancer cells through targeted apoptosis pathway. *Environmental Science and Pollution Research*, 29(54), 81685–81702.

Jannet, S. B., Hymery, N., Bourgou, S., Jdey, A., Lachaal, M., Magné, C. & Ksouri, R. (2017). Antioxidant and selective anticancer activities of two Euphorbia species in human acute myeloid leukemia. *Biomedicine & Pharmacotherapy*, 90, 375–385.

Jia, W. W. -G., Bu, X., Philips, D., Yan, H., Liu, G., Chen, X., Bush, J. A. & Li, G. (2004). Rh2, a compound extracted from ginseng, hypersensitizes multidrug-resistant tumor cells to chemotherapy. *Canadian Journal of Physiology and Pharmacology*, 82(7), 431–437.

Kangsamaksin, T., Chaithongyot, S., Wootthichairangsan, C., Hanchaina, R., Tangshewinsirikul, C., & Svasti, J. (2017). Lupeol and stigmasterol suppress tumor angiogenesis and inhibit cholangiocarcinoma growth in mice via downregulation of tumor necrosis factor- α . *PLOS ONE*, 12(12), e0189628.

Kapingu, M. C., Mbwambo, Z. H., M, M. J., & Magadula, J. J. (2005). Brine shrimp lethality of a glutarimide alkaloid from *Croton sylvaticus* Hochst. *The East and Central African Journal of Pharmaceutical Sciences*, 8(1), 3–5.

Kazłowska, K., Lin, H.-T. V., Chang, S.-H., & Tsai, G.-J. (2013). In vitro and in vivo anticancer effects of sterol fraction from red algae *Porphyra dentata*. *Evidence-Based Complementary and Alternative Medicine*, 2013, 1–10.

Koleckar, V., Kubikova, K., Rehakova, Z., Kuca, K., Jun, D., Jahodar, L., & Opletal, L. (2008). Condensed and hydrolysable tannins as antioxidants influencing the health. *Mini-Reviews in Medicinal Chemistry*, 8(5), 436–447.

Kurek, J. (Ed.). (2019). *Alkaloids: Their importance in nature and human life*. BoD–Books on Demand. IntechOpen Limited. *Intechopen limited.*: London, UK, 1-15.

Langenheim, J. H. (1994). Higher plant terpenoids: A phytocentric overview of their ecological roles. *Jornal of Chemical Ecology*, 20(6), 1223–1280.

Mahavorasirikul, W., Viyanant, V., Chaijaroenkul, W., Itharat, A. & Na-Bangchang, K. (2010). Cytotoxic activity of Thai medicinal plants against human cholangiocarcinoma,

laryngeal and hepatocarcinoma cells in vitro. *BMC Complementary and Alternative Medicine*, 10(1).

Liu, Q., Tang, J., Hu, M., Liu, J., Chen, H., Gao, H., Wang, G., Li, S.-L., Hao, X.-J., Zhang, X., & Yao, X.-S. (2013). Antiproliferative cardiac glycosides from the latex of *Antiaris toxicaria*. *Journal of Natural Products*, 76(9), 1771–1780.

Mai, Z.-P., Ni, G., Liu, Y., Li, L., Shi, G.-R., Wang, X., Li, J., & Yu, D.-Q. (2017). Heliosterpenoids A and B, two novel jatrophone-derived diterpenoids with a 5/6/4/6 ring system from *Euphorbia helioscopia*. *Scientific Reports*, 7(1), Article 12345.

Mfengwana, P. M. A. H. (2019). *Evaluation of Pharmacological Properties of Traditional Medicinal Plants Used for the Treatment of Cancer by South African and Lesotho Communities* (Doctoral dissertation, Bloemfontein: Central University of Technology, Free State).

Molyneux, R. J., Nash, R. J., & Asano, N. (1996). Alkaloids: Chemical and biological perspectives. In S. W. Pelletier (Ed.), *Alkaloids: Chemical and biological perspectives* (155-299). Pergamon.

Munro, B., Vuong, Q., Chalmers, A., Goldsmith, C., Bowyer, M., & Scarlett, C. (2015). Phytochemical, antioxidant and anti-cancer properties of *Euphorbia tirucalli* methanolic and aqueous extracts. *Antioxidants*, 4(4), 647–661.

Mwine, J. & Patrick Van Damme. (2011). Why do Euphorbiaceae tick as medicinal plants? A review of Euphorbiaceae family and its medicinal features. *Journal of Medicinal Plants Research*, 5(5), 652–662.

Ndam, L. M., Mih, A. M., Tening, A. S., Fongod, A. G. N., Temenu, N. A., & Fujii, Y. (2016). Phytochemical analysis, antimicrobial and antioxidant activities of *Euphorbia*

golondrina L.C. Wheeler (Euphorbiaceae Juss.): An unexplored medicinal herb reported from Cameroon. *SpringerPlus*, 5(1), Article 264.

Paul, S., Sengupta, S., Bandyopadhyay, T. K., & Bhattacharyya, A. (2012). Stevioside induced ROS-mediated apoptosis through mitochondrial pathway in human breast cancer cell line MCF-7. *Nutrition and Cancer*, 64(7), 1087–1094.

Sema, Y. A., & Waktola, T. A. (2022). Anti-malarial plants in Ethiopia and their activities on drug-resistant malaria. *FEMS Microbes*, 3, xtac001.

Senapati, S., Mahanta, A. K., Kumar, S., & Maiti, P. (2018). Controlled drug delivery vehicles for cancer treatment and their performance. *Signal Transduction and Targeted Therapy*, 3(1), Article 7.

Shi, Q.-W., Su, X.-H., & Kiyota, H. (2008). Chemical and pharmacological research of the plants in genus *Euphorbia*. *Chemical Reviews*, 108(10), 4295–4327.

Si, L., Meng, K., Tian, Z. -Y., Sun, J., Li, H., Zhang, Z., Soloveva, V., Li, H., Fu, G., Xia, Q., Xiao, S., Zhang, L. & Zhou, D. (2018). Triterpenoids manipulate a broad range of virus-host fusion via wrapping the HR2 domain prevalent in viral envelopes. *Science Advances*, 4(11).

Siegel, R. L., Miller, K. D., & Jemal, A. (2019). Cancer statistics, 2019. *CA: A Cancer Journal for Clinicians*, 69(1), 7–34.

Tapas, A., Sakarkar, D., & Kakde, R. (2008). Flavonoids as nutraceuticals: A review. *Tropical Journal of Pharmaceutical Research*, 7(3), 1089-1099.

Tewari, D., Priya, A., Bishayee, A., & Bishayee, A. (2022). Targeting transforming growth factor- β signalling for cancer prevention and intervention: Recent advances in developing small molecules of natural origin. *Clinical and Translational Medicine*, 12(4).

Ugboko, H. U., Nwinyi, O. C., Oranusi, S. U., Fatoki, T. H., & Omonhinmin, C. A. (2020). Antimicrobial importance of medicinal plants in Nigeria. *The Scientific World Journal*, 2020, 1–10.

Vezza, T., Canet, F., de Marañón, A. M., Bañuls, C., Rocha, M., & Víctor, V. M. (2020). Phytosterols: Nutritional health players in the management of obesity and its related disorders. *Antioxidants*, 9(12), 1266.

Vilahur, G., Ben-Aicha, S., Diaz-Riera, E., Badimon, L. & Padró, T. (2019). Phytosterols and Inflammation. *Current Medicinal Chemistry*, 26(37), 6724–6734.

Villanueva, J., Quirós, L. M. & Castañón, S. (2015). Purification and partial characterization of a ribosome-inactivating protein from the latex of *Euphorbia trigona* Miller with cytotoxic activity toward human cancer cell lines. *Phytomedicine*, 22(7-8), 689–695.

Vogg, G. (1999). Tumor promoting diterpenes from *Euphorbia leuconeura* L. *Phytochemistry*, 51(2), 289–295.

Yadav, R. N. S., & Agarwala, M. (2011). Phytochemical analysis of some medicinal plants. *Journal of Phytology*, 3(12), 10-14.

Yuan, Y., Cai, T., Xia, X., Zhang, R., Chiba, P. & Cai, Y. (2016). Nanoparticle delivery of anticancer drugs overcomes multidrug resistance in breast cancer. *Drug Delivery*, 23(9), 3350–3357.

Zhang, H.-W., Hu, J.-J., Fu, R.-Q., Liu, X., Zhang, Y.-H., Li, J., Liu, L., Li, Y.-N., Deng, Q., Luo, Q.-S., Ouyang, Q., & Gao, N. (2018). Flavonoids inhibit cell proliferation and induce apoptosis and autophagy through downregulation of PI3K γ mediated PI3K/AKT/mTOR/p70S6K/ULK signaling pathway in human breast cancer cells. *Scientific Reports*, 8(1), 1–13.

Zhao, Y., Jing, Z., Lv, J., Zhang, Z., Lin, J., Cao, X., Zhao, Z., Liu, P. & Mao, W. (2017). Berberine activates caspase-9/cytochrome c -mediated apoptosis to suppress triple-negative breast cancer cells in vitro and in vivo. *Biomedicine & Pharmacotherapy*, 95, 18–24.

Zhu, L. & Xue, L. (2018). Kaempferol suppresses proliferation and induces cell cycle arrest, apoptosis, and DNA damage in breast cancer cells. *Oncology Research Featuring Preclinical and Clinical Cancer Therapeutics*.

CHAPTER 5

GREEN SYNTHESIS OF MAGNESIUM, COBALT, IRON OXIDE AND IRON DIOXIDE NANOPARTICLES USING *EUPHORBIA TIRUCALLI*: CHARACTERIZATION AND ANTIPROLIFERATIVE EVALUATION AGAINST BREAST CANCER CELL LINES

Abstract

In this study, magnesium oxide (MgONPs), cobalt oxide (CoONPs), iron oxide (FeO) and iron dioxide (FeO₂) nanoparticles were synthesized simultaneously using the aerial parts of *Euphorbia tirucalli* as a reducing agent. The nanoparticles were characterized using multiple techniques, including Ultraviolet-visible spectrophotometry (UV-Vis), Fourier-transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), energy-dispersive spectroscopy (EDS), X-ray photoelectron spectroscopy (XPS), scanning electron microscopy (SEM), and transmission electron microscopy (TEM). UV-Vis analysis confirmed the formation of FeO, FeO₂, MgONPs and CoONPs, with absorbance peaks observed between 220–390 nm for Fe-based nanoparticles and at 305 and 508 cm⁻¹ for CoONPs. A colour change from brown to black confirmed MgONPs formation, while pink indicated CoONPs and a light brown color to black for FeONPs. EDS analysis revealed the presence of magnesium, cobalt, iron and oxygen in their respective nanoparticles, and TEM confirmed nanoparticle sizes ranging from 1 nm to 100 nm. The antiproliferative effects of these nanoparticles were evaluated against MCF-7, SK-BR-3, MDA-MB231 and Vero cell lines using the MTT assay. The hexane (Hex) and dichloromethane (DCM) extracts of *E. tirucalli* exhibited growth inhibition of MCF-7 cells, with IC₅₀ values of 10 mg/mL. CoONPs demonstrated enhanced cell growth inhibition, with IC₅₀ values <10 mg/mL. FeO nanoparticles showed over 80% antiproliferative activity against MCF-7 cells at 10 µg/mL. Fe-based nanoparticles exhibited similar activity against the MDA-

MB231 cell line at 10 and 1 $\mu\text{g/mL}$ concentrations. However, decreased antiproliferative effects were observed against Vero and SK-BR-3 cell lines.

This study highlights the environmentally friendly synthesis of MgONPs, CoONPs and Fe-based nanoparticles using *E. tirucalli*, and their potential applications in cancer therapy, particularly against MCF-7 and MDA-MB231 cell lines. The results emphasize the significant antiproliferative properties of the nanoparticles, contributing to the development of plant-based nanomedicine for cancer treatment.

5.1. Introduction

Breast cancer, characterized by its heterogeneity, presents a diverse range of characteristics and clinical features, necessitating tailored treatment approaches (Siegel *et al.*, 2021; Polyak, 2007). Current treatments, though effective to some extent, exhibit adverse side effects, lack selectivity and suffer from poor pharmacokinetics (Nurgali *et al.*, 2022; Pucci *et al.*, 2019; Zugazagoitia *et al.*, 2016). In light of these limitations, there is a pressing need for innovative therapeutic strategies in cancer treatment.

Nanoparticles (NPs) have emerged as a promising avenue in cancer research, offering versatility and unique properties. Researchers globally have explored their applications in tumor imaging, cancer biomarker profiling and targeted drug delivery (Rocha *et al.*, 2017). Notably, the use of green chemistry for NP synthesis, particularly utilizing plant extracts, has gained attention for its eco-friendly, economical and sustainable approach (Mittal *et al.*, 2013; Iravani, 2011; Huang *et al.*, 2007; Chandran *et al.*, 2006).

Euphorbia tirucalli, a shrubby succulent belonging to the Euphorbiaceae family, has been utilized in traditional medicine for various purposes, including the treatment of asthma, cough and rheumatism (Ernst *et al.*, 2015). The plant's latex, rich in bioactive compounds, catalyzes the reduction of metal precursors into metal oxide nanoparticles, providing a unique avenue for green synthesis (Saravanakumar *et al.*, 2019; Singh *et al.*, 2018; Chandrasekhar *et al.*, 2014).

Metal and metal oxide nanoparticles are multifunctional and crucial in research due to their physio-chemical and optoelectronic properties (Thema *et al.*, 2016; Hao *et al.*, 2010). Recently, the synthesis of CoONPs, FeONPs and MgONPs has attracted interest in the biomedical field due to their application as antibacterial treatments, antacids for heartburn, bone regeneration, drug delivery systems and cancer therapy (Gupta *et al.*, 2005; Huang *et al.*, 2005; Khan *et al.*, 2015; Ezhilarasi *et al.*, 2016; Kavitha *et al.*, 2017; Cai *et al.*, 2018; Cai *et al.*, 2018; Dailey *et al.*, 1999).

Among the various metal oxides, iron oxide NPs have garnered significant attention due to their low toxicity, superparamagnetic properties, surface area and volume ratio, protein immobilization, potential use in diagnostic magnetic resonance imaging (MRI), thermal therapy and drug delivery (Kulpa-Koterwa *et al.*, 2021). In addition, they have demonstrated effectiveness in inhibiting MCF-7 cell lines in prior research (Sulaiman *et al.*, 2017). Furthermore, inorganic metal oxides such as MgONPs and CoONPs have also shown some advantages: they are stable under extreme conditions, possess a higher adsorption ability, contain a large surface area and exhibit interesting physical and chemical properties (Raman *et al.*, 2016). CoONPs, for instance, have size-dependent structures and demonstrate energy storage, magnetic properties and catalytic activities (Rasheed *et al.*, 2019; Lutsev *et al.*, 2009). MgONPs, on the other hand, are less toxic, relatively easy to obtain and have been reported to induce apoptosis in cancer cells, making them potential candidates for cancer therapy (Behzadi *et al.*, 2019; Krishnamoorthy *et al.*, 2012; Martinez-Boubeta *et al.*, 2010).

Despite the extensive research on MgONPs, CoONPs, and FeONPs, no comparative study has been conducted to assess the anti-proliferative effects of these metal oxides against MCF-7 and MDA-MB231 cell lines, and their selectivity for these cell lines.

Therefore, this study aims to synthesize MgONPs, CoONPs and FeONPs using the aerial plant extract of *E. tirucalli* as a reducing agent, characterize the NPs and compare their anti-proliferative effect against two different subtypes of breast cancer cell lines. Our research focuses on investigating the potential anti-cancer properties of *E. tirucalli* against breast carcinoma.

5.2. Materials and Methods

In March 2017, the *E. tirucalli* plant species was commercially acquired from Gariep Nursery in Pretoria, South Africa (GPS coordinates 25°47'3"S, 28°18'58"E). Dr. Nolubabalo Matinise of iTHEMBA LABS,

South Africa generously donated ferric nitrate nonahydrate salts. The extraction solvents, namely Hex, DCM and methanol (MeOH), were purchased from Merck in South Africa. MCF-7, MDA-MB231 and Vero cells were purchased from Cellonex in South Africa. Reagents including DMEM, trypan blue dye and MTT salt were purchased from Sigma Aldrich in South Africa.

5.2.1. Plant extraction and preparation

Upon arrival at the laboratory, the aerial parts of the plant were washed with tap water and chopped into smaller pieces. The pieces were then air-dried and ground into a fine powder and stored at 4°C until further use.

5.2.2. Phytochemical Screening

A phytochemical analysis, as detailed in Chapter 4, was conducted on the aerial part of *E. tirucalli* using Yadav and Agarwala's (2011) protocol with slight modifications. Various tests were carried out to determine the presence or absence of alkaloids, glycosides, flavonoids, phenols, saponins, terpenoids and tannins.

5.2.3. Preparation of MgONPs, CoONPs and FeONPs

In order to synthesize NPs, the *E. tirucalli* finely ground powder weighing 30g was boiled in distilled water at 80°C for a duration of 2 hours. Following this, the extract was divided into three beakers and mixed with 5g of magnesium nitrate hexahydrate, cobalt (III) sulfate heptahydrate and ferric nitrate nonahydrate. These solutions were then placed on a magnetic stirrer, maintained at 60°C for a period of 12 hours and closely observed for any changes in colour which would indicate the formation of NPs. The resulting NP pellets were obtained by subjecting the solutions to centrifugation at 5000rpm for 20 minutes; after which they were washed with double distilled water to remove any excess plant extract. These pellets were then dried in an oven at 80°C for 2 hours. Once dried, the NPs were annealed at 500°C for a duration of 2 hours to eliminate impurities and were subsequently stored at room temperature for characterization.

5.2.3.1. Preparation of *Euphorbia tirucalli* aerial extracts for cell culture

To extract compounds from the plant, a 10g fine powder was sequentially extracted using various organic solvents (hexane, dichloromethane, methanol, and ethyl acetate) in 150 mL portions. The extraction process was carried out for 48 hours on a shaker at room temperature. The resulting extracts were filtered and concentrated under reduced pressure at 45°C using a rotary vacuum evaporator from Buchi labotech, Switzerland. Finally, the extracts were dried under a fume hood and stored at 4°C for future use.

5.2.4. Characterization of synthesized nanoparticles

Characterization of MgONPs, CoONPs and FeONPs was conducted using the established protocol by Shunmugadevi and Palanisamyb (2016), with slight modifications. UV spectroscopy was utilized to investigate the absorption patterns, employing the Multiskan Go 1.01.10 spectrophotometer. Fourier Transform Infrared Spectroscopy (FTIR) was used to identify the functional groups involved in the capping process, with the Thermo Nicolet Nexus 670 spectrometer measuring the spectrum. The structural properties of the NPs were examined using the Bruker D8 X-ray diffractometer. The chemical state of the NPs was determined by using XPS with the Versa Probe XPS V1.4. The morphology and size of the particles were determined using SEM and TEM; respectively. The JEOL JEM 2010 UHR and Philips 100 transmission electron microscope were used for this purpose. Lastly, EDS was used to detect the nano constituents present in MgO, CoO and FeO NPs. The Philips 100 transmission electron microscope was used for this process.

5.2.5. Anti-proliferation screening of *Euphorbia* extracts

5.2.5.1. Cell culture

The MCF-7, SK-BR-3 and MDA-MB231 cell lines were cultured using Dulbecco's modified eagle medium (DMEM), DMEM: HAMS F12 (1:1) and Mc-Coy media; respectively. Each medium was supplemented

with 10% fetal bovine serum (FBS) and the cells were kept in an environment at 37°C temperature and 5% CO₂ humidity in an incubator. When the cells reached 80% confluency, they were sub-cultured.

5.2.5.2. Anti-proliferative assay

To facilitate easy harvesting, the cells were treated with Trypsin. An automated cell counter (Countess FI, life technology) was used to count the viable cells with trypan blue dye. 1×10^5 cells/ml were then seeded into each well of a 96-well microtiter plate and incubated under the same conditions for 24 hours. The cells were treated with unannealed and annealed NPs along with crude *E. tirucalli* at concentrations of 100, 10 and 1 µg/mL in triplicate for 48 hours. Wells containing untreated cells were used as a negative control, while Doxorubicin drug was used as a positive control. Cell growth activity was measured using the tetrazolium dye (MTT assay) (Munro *et al.*, 2017). Cell viability and growth inhibition percentage were calculated and graphs were plotted using Microsoft Excel 2008.

5.3. Results

5.3.1. Phytochemical analysis

The findings of the study are briefly presented in Table 1. Phytochemical analysis of *E. tirucalli* demonstrated the presence of tannins, glycosides, triterpenoids and saponins.

Table 5.1. Phytochemical profiling of *E. tirucalli*. Presence of phytochemical (+), absence of phytochemicals (-).

<i>E. tirucalli</i> sp	
Phytochemical	Presence/absence
Phytosterols	-
Pentose	-
Tannins	+
Glycosides	+
Triterpenoids	+
Anthroquines	-
Saponins	+
Flavonoids	-
Alkaloids	-

5.3.2. Synthesis of Iron, magnesium and cobalt Oxide NPs

The utilization of the aerial part of *E. tirucalli* as a reducing agent resulted in a discernible colour shift from light brown to black when Ferric nitrate nonahydrate salt was introduced to the *E. tirucalli* extract. This alteration in color signifies the stimulation of electrons and the subsequent formation of FeONPs. The involvement of plant secondary metabolites, particularly polyphenols, played a crucial role in reducing metal ions and stabilizing both FeONPs. Notably, these nanoparticles exhibited the attachment of phytochemicals to their surface, featuring hydrophilic hydroxyl groups that facilitate their uniform dispersion in aqueous solutions (Kiwumulo *et al.*, 2022).

During the agitation process, the *E. tirucalli* extract exhibited a colour change from light brown to black, indicating the phytochemical reduction of the magnesium nitrate precursors to MgONPs. Additionally, the introduction of cobalt sulfate to the *E. tirucalli* extract resulted in a colour change from light brown to pink,

revealing the reduction process facilitated by the secondary metabolites present in the aerial parts of the plant.

5.3.3. Characterization of synthesized NPs

5.3.3.1. UV-VIS

To analyze the formation of iron, magnesium and cobalt NPs, a UV-vis spectrophotometer was employed to measure absorbance in the range of 200-1000 nm. The results, illustrated in Fig 5.1, reveal two absorption peaks at 220 and 390 nm for FeONPs. These absorption peaks are indicative of the formation of iron nanoparticles.

An absorption peak at 220 nm was observed in the MgONPs sample. However, this peak did not align with the absorption spectrum peak of MgO, which typically ranges between 260 and 280 nm (see Figure 5.2a). The observed peak at 220 nm may be attributed to the presence of different compounds interacting with magnesium oxide ions and surface oxide ions during the reduction of metal ions (Sharma *et al.*, 2017).

For CoONPs, a robust peak was observed at 508 nm, accompanied by a weaker peak at 305 nm (Figure 5.2b). These peaks are indicative of the formation of CoONPs. The two peaks correspond to the charge transfers of O_2^- to Co^{2+} and O_2^- to Co^{3+} ; respectively. This observation aligns with the findings reported by Bibi *et al.* (2017).

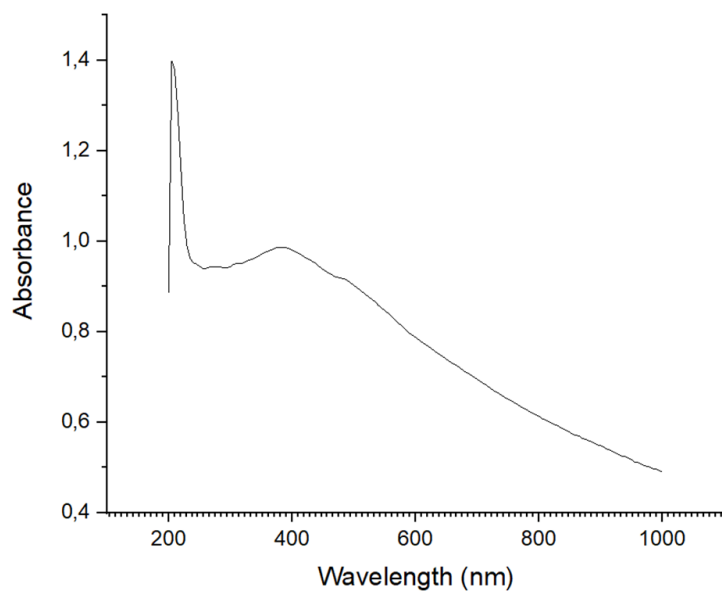


Figure 5.1. Ultraviolet-visible spectroscopy of FeO from *E. tirucalli*.

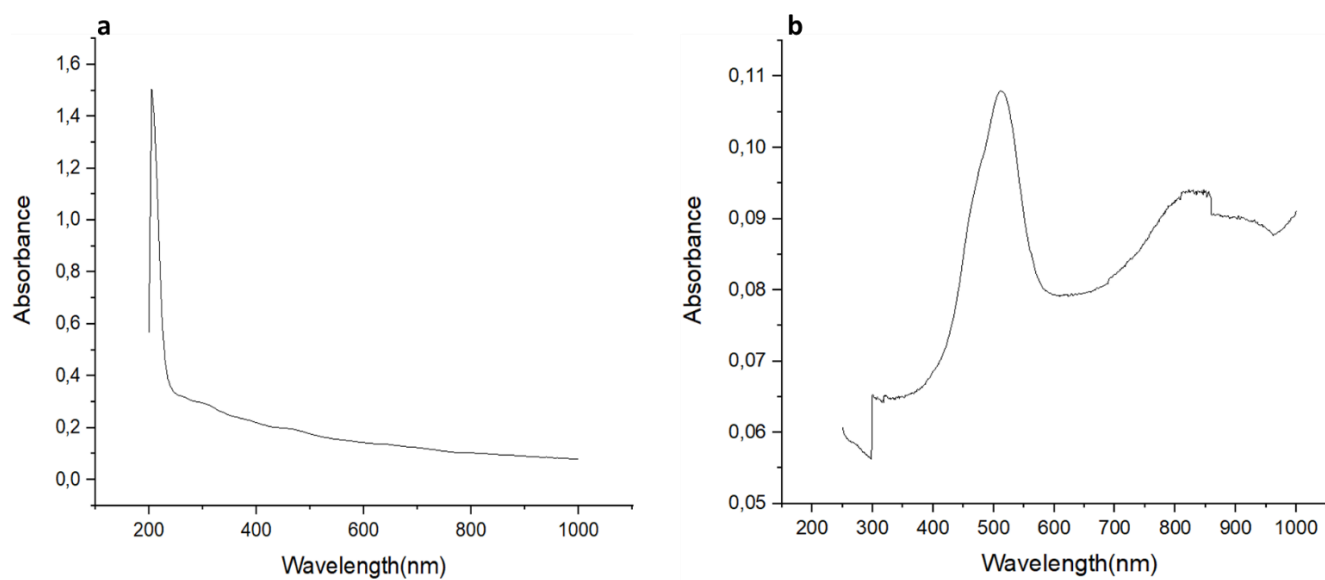


Figure 5.2. Ultraviolet-visible spectroscopy of MgO (a) and CoO (b).

5.3.3.2. FTIR analysis

FTIR spectroscopy was employed to investigate the functional groups within *E. tirucalli* and the synthesized iron, magnesium and cobalt NPs. The FTIR spectra of the iron NPs is shown in Figure 5.3. The figure displayed peaks in the 500-4000 cm^{-1} range, specifically at 3350 cm^{-1} , 1650 cm^{-1} , 1150-1050 cm^{-1} , 750 cm^{-1} , and 514 cm^{-1} . The 3350 cm^{-1} peak signifies the presence of O-H groups, potentially originating from phenols or water in the *E. tirucalli* plant. At 1650 cm^{-1} , the peak suggests hydrogen bonding between oxygen molecules in the FeONP and hydrogen molecules in *E. tirucalli*, representing C=O stretching vibrations of amide-I. The absorption peaks at 1150-1050 cm^{-1} are attributed to C-O groups, aligning with previous research confirming the formation of iron oxide nanoparticles. The 750 cm^{-1} peak is due to CH groups present in aromatic compounds within *E. tirucalli*. Furthermore, the presence of a peak at 514 cm^{-1} confirms the formation of both iron oxide and dioxide nanoparticles, corroborating findings from earlier studies (Sulaiman *et al.*, 2017; Pal, 2014; Kumar *et al.*, 2014).

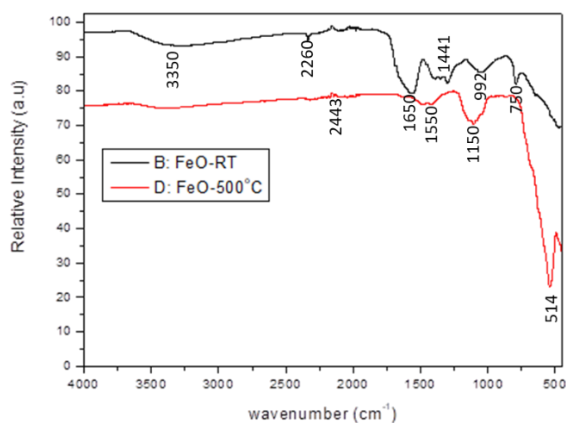


Figure 5.3. FTIR spectrum analysis of iron oxide (FeO) NPs reduced using *E. tirucalli*. Black line (B:FeO-RT) represents NPs at room temperature and red (D:FeO-500°C) represent the annealed NPs at 500°C.

The magnesium and cobalt oxide nanoparticles displayed FTIR spectra within the wavenumber range of 500 to 4000 cm^{-1} ; respectively. In the spectrum of CoO (Figure 5.4a), a broad peak at 3300 cm^{-1} indicated the presence of the O-H group from water. Additionally, peaks were observed at 2211 cm^{-1} (indicating a cyanide group), 1560 cm^{-1} (indicating alkenes) and 1441 cm^{-1} (assigned to the carboxylate group). Peaks at 1150 cm^{-1} , 1050 cm^{-1} and 1000 cm^{-1} were assigned to the aromatic group, while IR spectra at 650 cm^{-1} and 559 cm^{-1} suggested Co–O vibrations.

The FTIR spectrum of magnesium oxide nanoparticles (Figure 5.4b) revealed a broad peak at 3441 cm^{-1} , indicating the stretching vibration of the hydroxyl group from water. Absorption peaks at 2249 cm^{-1} and 1661 cm^{-1} were observed on the unannealed sample (MgO-RT), representing the amine group, which were absent in the annealed sample (MgO-500°C). The peak at 1441 cm^{-1} was attributed to symmetric stretching of carboxylic groups, while peaks at 1150 cm^{-1} suggested the presence of ester bonds. Absorption peaks between 754 cm^{-1} and 801 cm^{-1} were associated with the formation of MgO, consistent with prior research findings.

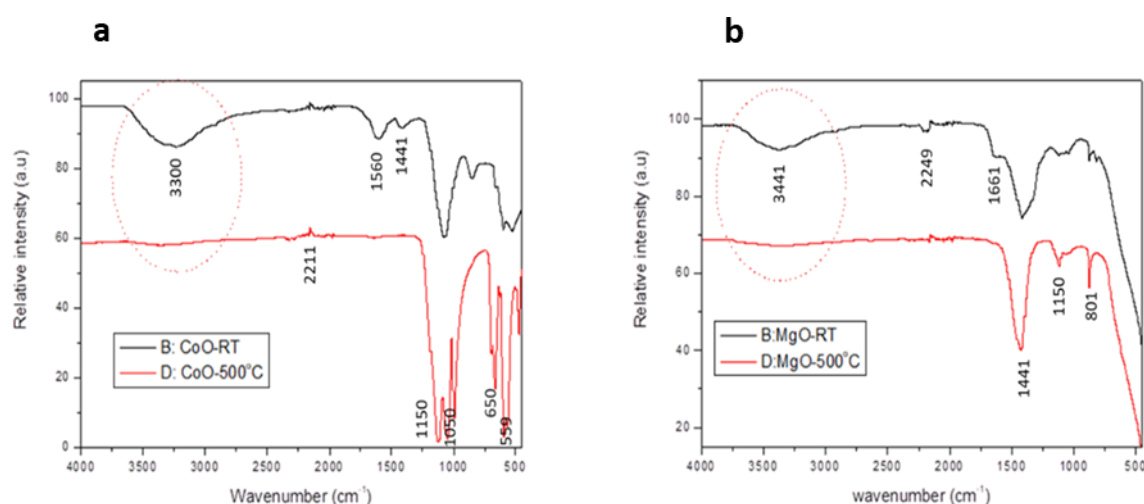


Figure 5.4. FTIR spectrum analysis of MgONPs and CoONPs reduced through the *E. tirucalli* plant at different temperatures. (a) CoONPs (b) MgONPs. Black lines represent NPs at room temperature and red represent the NPs annealed at 500°C, respectively.

5.3.3.3. XRD analysis

The XRD spectra analysis of FeONPs synthesized using a green method, illustrated in Figure 5.5, revealed distinct diffraction peaks at 2θ values of 30.91° , 35.70° , 43.14° , 57.46° and 62.90° , corresponding to the planar reflections of (220), (311), (222), (400), (511) and (440) of a cubic phase for both types of nanoparticles; respectively. These peaks were attributed to the magnetite structure of iron oxide as corroborated by previous studies and data from the JCPDS card (77-1545). However, additional peaks were detected in both samples, indicating the presence of impurities. The XRD analysis of CoO and MgO nanoparticles also displayed various peaks confirming the formation of metal-ion crystals. The XRD patterns for MgO nanoparticles (Figure 5.6a) exhibited peaks at 2θ values of 36.8° , 42.9° , 62.0° , 74.0° and 78.1° , corresponding to metal crystalline indices (111), (200), (220), (311) and (222); respectively, with the calculated particle size found to be <10 nm. The presence of other peaks in the samples indicated the presence of impurities (Manigandan *et al.*, 2013; Howli *et al.*, 2019).

The XRD analysis of cobalt oxide nanoparticles (Figure 5.6b) revealed numerous weak peaks positioned at 2θ values of 21.16° , 30.93° , 34.24° , 36.45° , 44.57° , 51.76° and 59.12° , corresponding with the plane metallic cobalt indices of (111), (220), (311), (222), (400), (422) and (511), respectively. However, the peak at 77.50° nm could not be detected, possibly due to impurities in the sample.

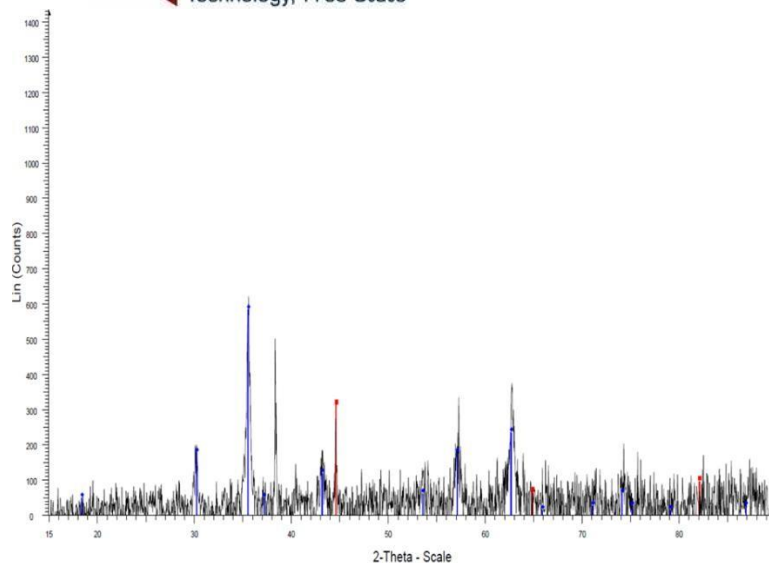


Figure 5.5. X-ray diffraction analysis of reduced FeO NPs from *E. tirucalli* aerial parts after annealing.

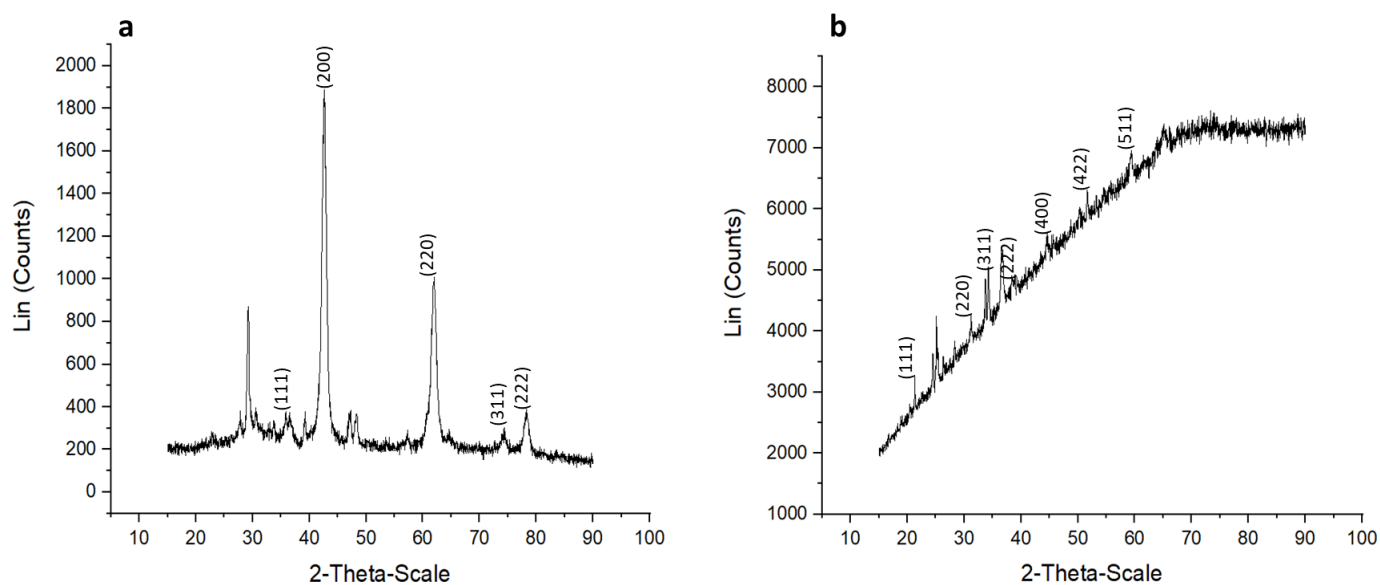


Figure 5.6 X-ray diffraction analysis of reduced (a) MgO and (b) CoONPs from *E. tirucalli* aerial parts after annealing.

5.3.3.4. XPS analysis

The XPS technique was employed to analyze the elemental composition and electron surface state of various components in FeO, MgO and CoO nanoparticles (NPs). The resulting analysis, as shown in Figure 5.7 - 5.9, revealed the presence of iron (Fe), magnesium (Mg), oxygen (O), carbon (C) and calcium (Ca) in FeO NPs, thereby providing valuable insights into the chemical composition of the material. These findings were further affirmed through EDS analysis, which corroborated the presence of these elements in FeO NPs. The peaks observed at 712.3 eV, 710.3 eV and 708.5 eV were attributed to Fe 2p₁, Fe 2p and Fe p₃; respectively, providing evidence of the presence of Fe³⁺ octahedral species in the NP. Additionally, the peaks observed at 530.5 eV and 532 eV were attributed to O 1s in the NPs and the presence of hydroxyl groups; respectively. Moreover, the peak observed at 529.2 eV indicated the presence of O on the surface of *E. tirucalli* FeO NP, associated with O–H, O–C and O=C compounds. These findings suggest that the electronic properties of FeO NPs are heavily influenced by the presence of O on the surface of the plant. Overall, the valence states of Fe and O in FeO NPs were found to be +3 and -2; respectively, as observed in the attributes of Fe 2p and O 1s core levels.

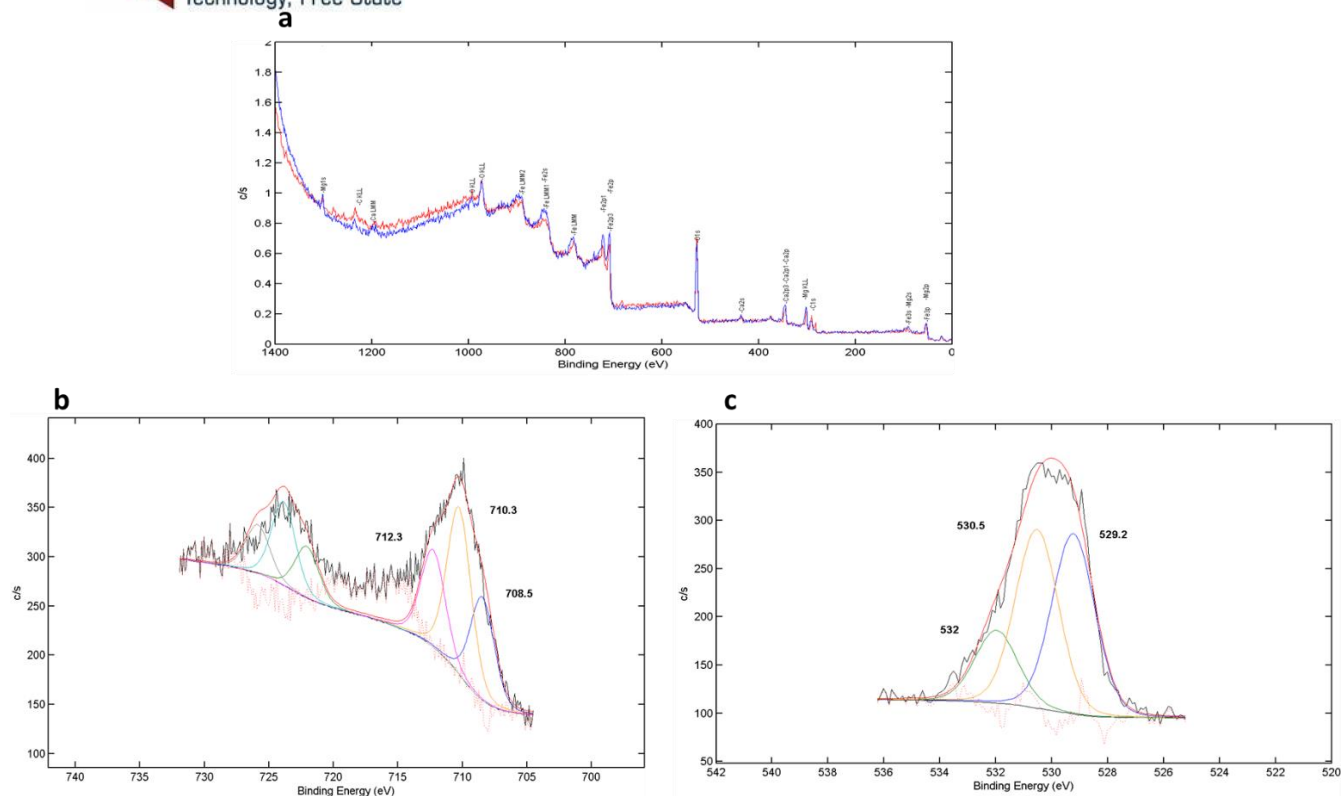


Figure 5.7. XPS analysis of FeO NPs: high resolution FeO₂ scan (a), FeO-2p₁ scan (b), and FeO-O1s scan (c).

The XPS technique was further utilized to observe the chemical and surface state of different constituents found in MgO and CoO nanoparticles. The spectrum scan revealed the presence of magnesium (Mg), carbon (C), oxygen (O) and argon (Ar) in MgO nanoparticles. The Mg (1s) spectrum confirmed the binding energy reported in previous studies, and the O (1s) spectrum indicated the presence of MgO both before and after calcination. Additionally, peaks corresponding to hydroxide groups were observed, confirming the successful reduction of magnesium nitrate to MgO.

In the case of CoO nanoparticles, peaks corresponding to cobalt (Co), carbon (C), oxygen (O) and sulfur (S) were observed. The C (1s) peak illustrated sp² carbon, while the O (1s) spectrum exhibited a shift in peaks after calcination, indicating changes in the chemical environment. Peaks corresponding to cobalt

bound to oxygen (CoO) were observed, with characteristics of 2p_{3/2}, further supporting the presence of CoO in the nanoparticles. However, peaks in the range of 785.4eV to 788.8eV were challenging to characterize, suggesting non-uniform oxidation processes occurring at the shell of the nanoparticles. These findings provide valuable insights into the chemical composition and surface state of MgO and CoO nanoparticles, highlighting their potential applications in various fields.

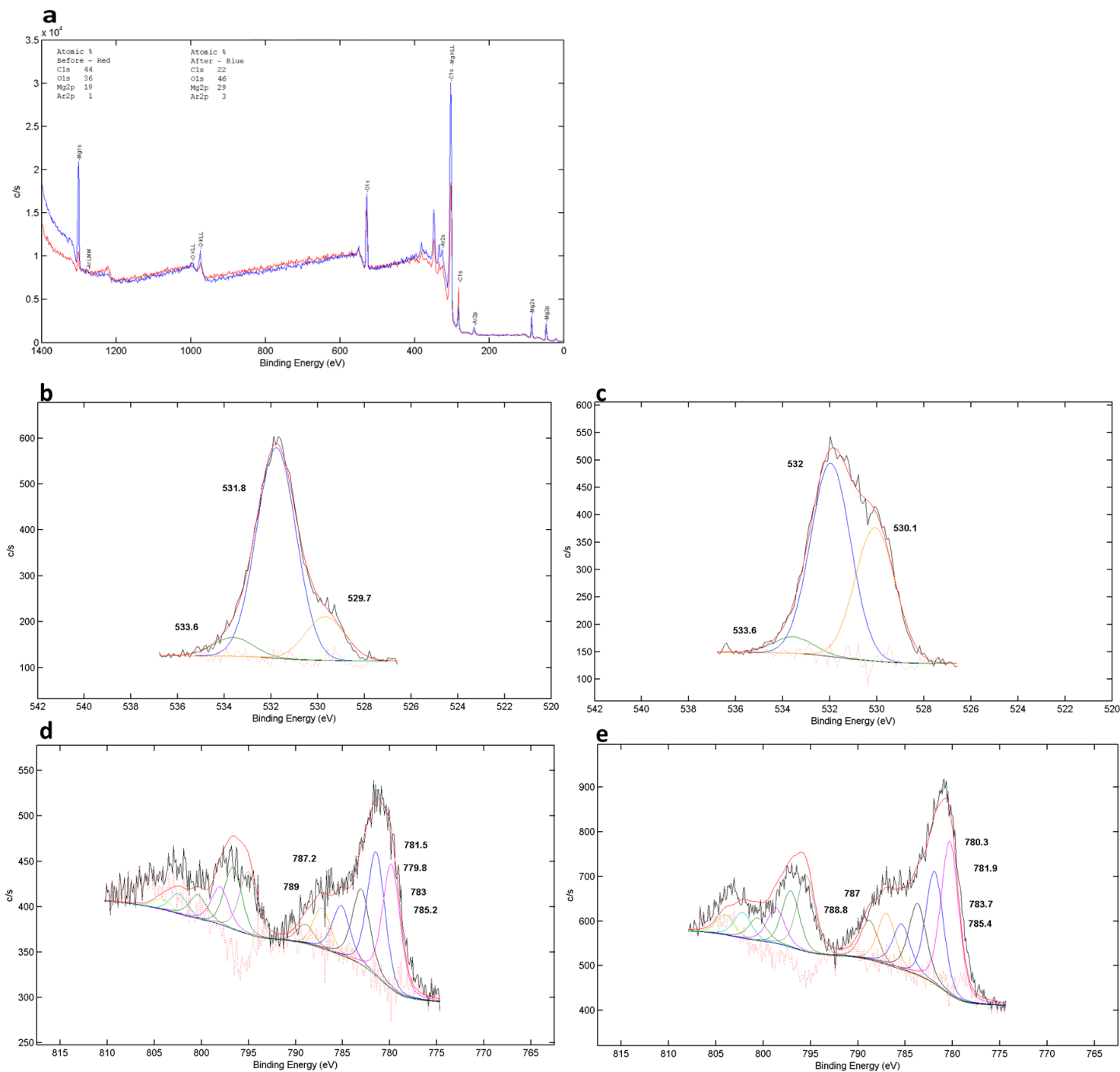


Figure 5.8. XPS analysis of MgONPs: high resolution MgO scan (a); MgO₂p scan before (b) and after (c), MgOO₁s scan before annealing (d) and after annealing (e).

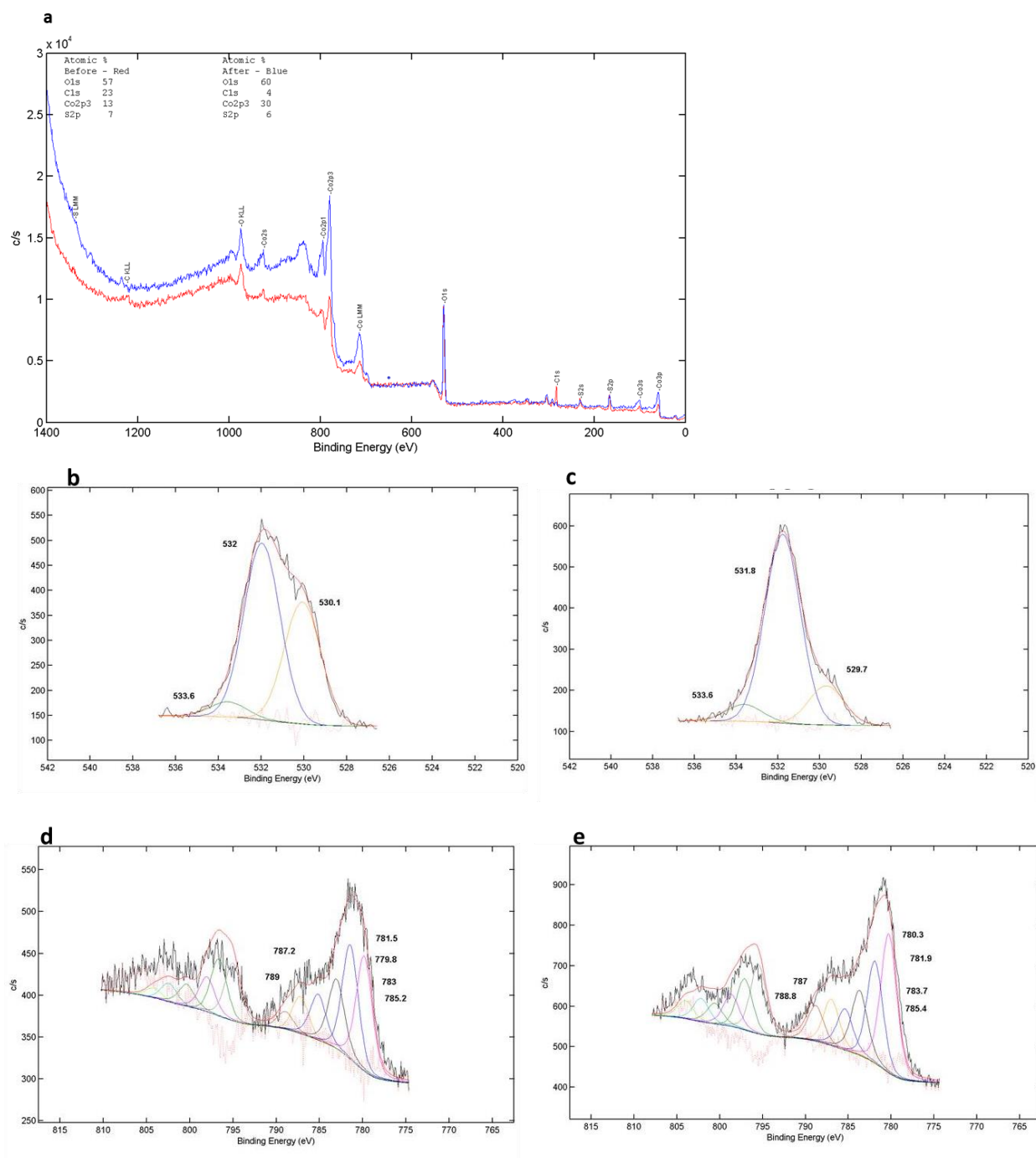


Figure 5.9. XPS analysis of CoONPs high resolution CoO scan (a); CoO₂p scan before (b) and after annealing (c), CoO O₁s scan before (d) and after (e) annealing.

5.3.3.5. SEM analysis

The morphology and size of iron oxide, magnesium oxide and cobalt oxide nanoparticles were examined using scanning electron microscopy (SEM). The nanoparticles, measuring approximately 100 nm in size, were depicted. In Figure 5.10, the FeONPs exhibited a mesoporous and matrix-like arrangement, with a flat plate shape that could bend into a rod shape. Figure 5.11 displayed the distribution of MgO nanoparticles in a uniform network-like agglomerated form. The observed agglomeration in MgO nanoparticles could potentially be attributed to factors such as poor dispersion of nanoparticles, strong magnetic fields, or the presence of impurities within the nanoparticle samples.

Conversely, Figure 5.12 illustrated CoO nanoparticles with a smooth, agglomerated, irregular and spherical shape. The particle size observed in both types of nanoparticles ranged between 1 μm to 100 nm; respectively. Notably, the presence of larger particles in cobalt nanoparticles under SEM analysis may be due to the aggregation or overlapping of smaller particles during sample preparation.

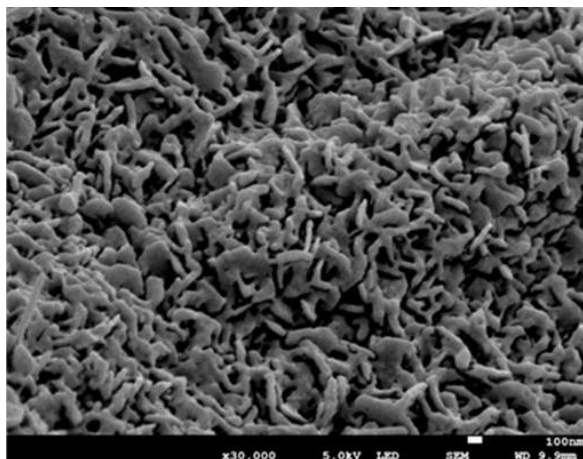


Figure 5.10. SEM imaging of FeO observed at 100nm.

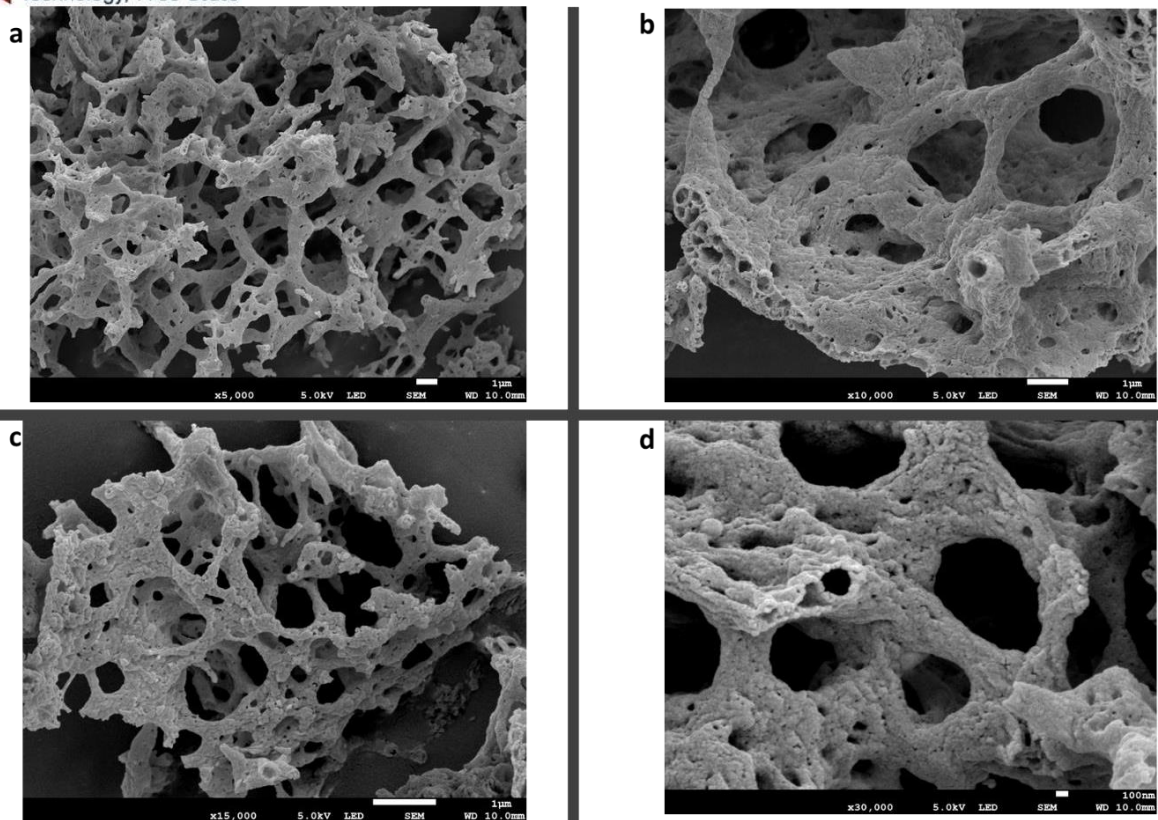


Figure 5.11. SEM imaging of MgONPs at different magnifications (a) 1 000 X; (b) 5 000 X; (c) 15 000 X; (d) 30 000 X.

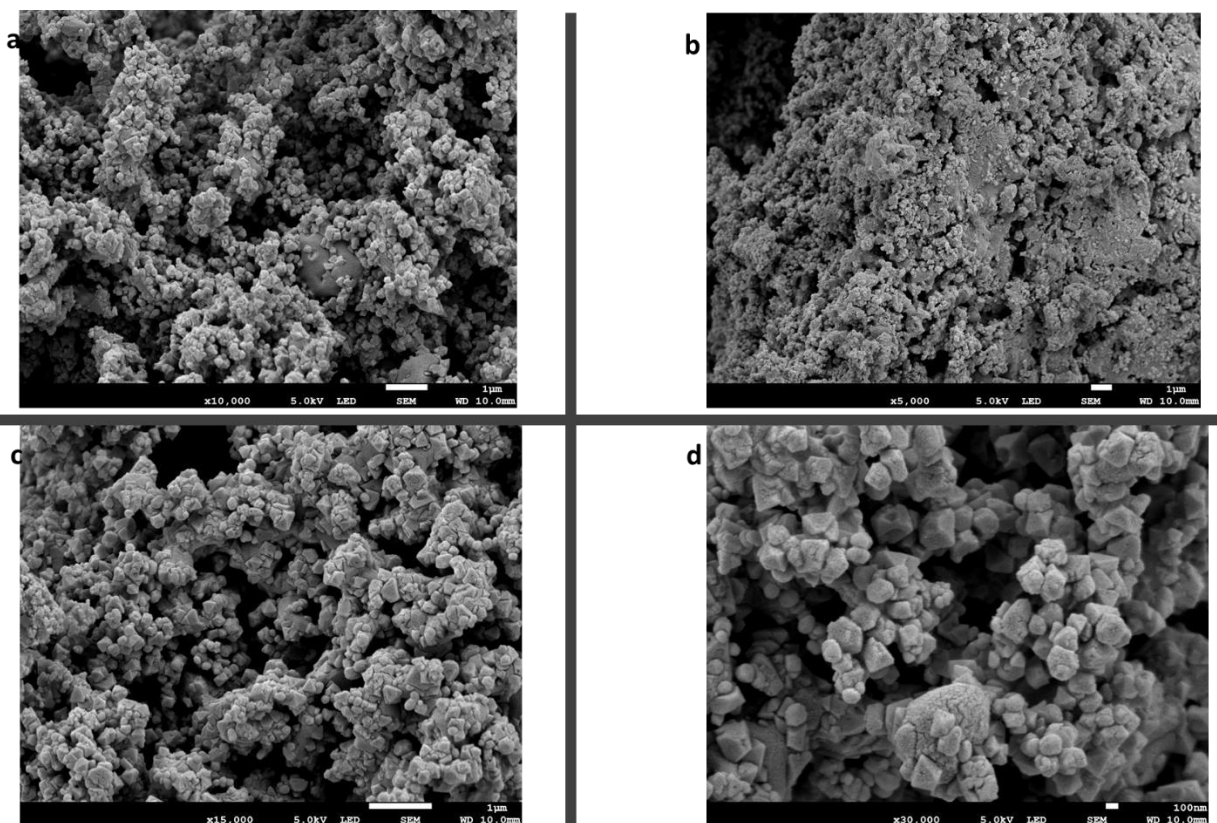


Figure 5.12. SEM imaging of CoONPs at different magnifications (a) 1 000 X; (b) 5 000 X; (c) 15 000 X; (d) 30 000 X.

5.3.3.6. TEM and EDS analysis

The TEM images presented in Figures 5.13a-b showcase the synthesized iron nanoparticles (NPs), revealing hexagonal agglomerated NPs with a size of 100 nm. EDS profiling of all nanocomposites obtained after calcination indicated peaks, with a high characteristic peak of oxygen followed by carbon, iron, calcium, chloride, magnesium, potassium, sodium, sulfur and phosphorus. The presence of iron and oxygen confirmed the formation of iron oxide NPs. The high oxygen and carbon peaks detected suggest that polyphenols were involved in the synthesis of iron nanoparticles. Furthermore, the oxygen and carbon peaks may represent the polyphenols present in the plant. Additional weak composite peaks observed of calcium, sodium and magnesium may arise from the glass that holds the sample during synthesis. The phosphorus, potassium, sulfur and chloride peaks observed were due to improper washing of the sample. The composition analysis revealed that the iron oxide NPs comprised 53.4% iron, 22.1% carbon, 17.0% oxygen and all other composites were less than 5% (Figure 5.13). The presence of oxygen and carbon is crucial as they stabilize and reduce the two metal oxides. Although there were impurities present in the samples, they were in low concentrations, as shown in the results.

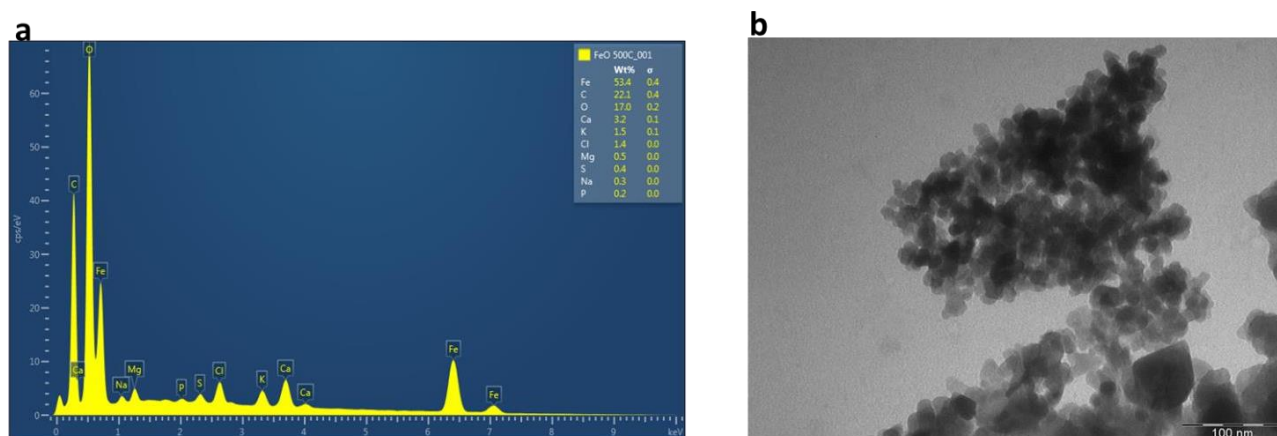


Figure 5.13. EDS analysis of (a) FeONPs and TEM imaging of (b) FeONPs synthesized using aerial parts

of *E. tirucalli*.

The morphological and structural characteristics of MgO and CoO nanoparticles showcase the MgO nanoparticles with a size of 200 nm and pores in Figure 5.14a-b. EDS profiling revealed peaks of all nanocomposites obtained after calcination, with a high characteristic peak of magnesium followed by oxygen, calcium, carbon, potassium, sulfur, phosphorus and chloride. The elemental percentages of composites present in the nanoparticles were 39.7% oxygen, 36.4% magnesium and all other composites were less than 9%. Conversely, Figures 5.15a-b present the CoO nanoparticles with a size of 200 nm. EDS profiling demonstrated peaks of nanocomposites obtained after calcination, revealing characteristic peaks of cobalt followed by carbon, oxygen, sulfur, calcium, potassium and magnesium. The atomic percentages of composites present in the nanoparticles were 41.2% cobalt, 27.1% carbon, 20.2% oxygen and all other composites were less than 9%. The carbon detected in both nanoparticles might be from the carbon coating of the TEM grid. These findings provide valuable insights into the composition and structural characteristics of MgO and CoO nanoparticles, which are essential for understanding their properties and potential applications in various fields.

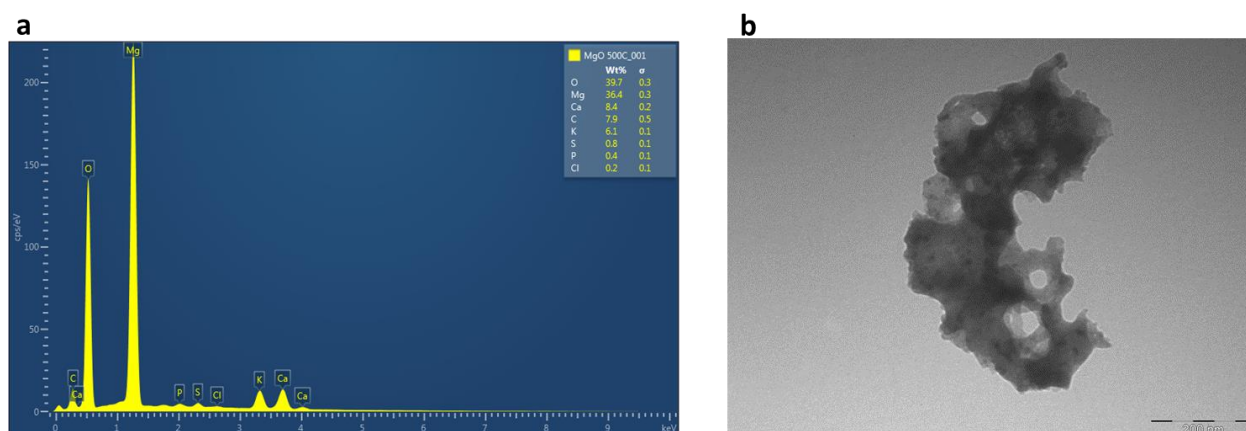


Figure 5.14. EDS analysis of (a) MgONPs and TEM imaging of (b) MgONPs synthesized using aerial parts of *E. tirucalli*.

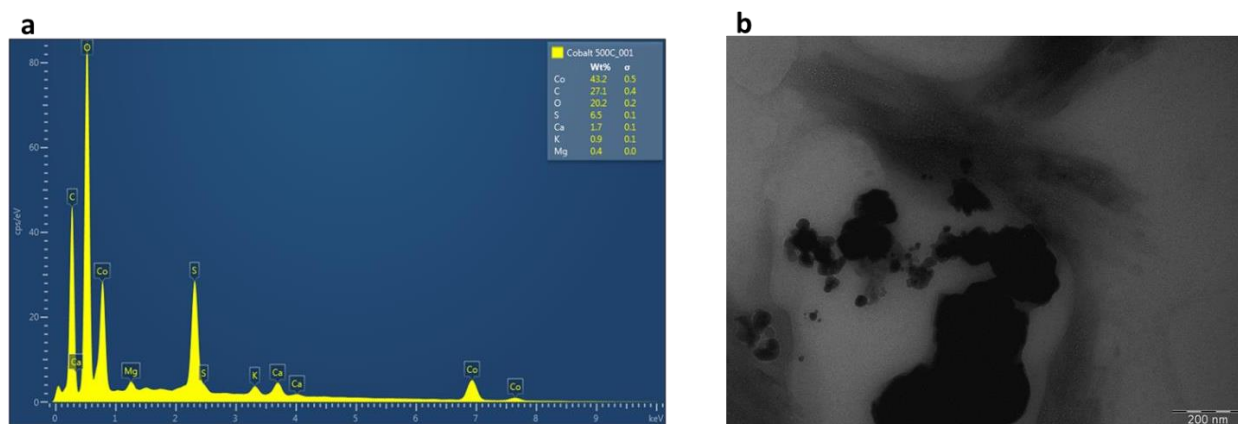


Figure 5.15. EDS analysis of (a) CoONPs and TEM imaging of (b) CoONPs synthesized using aerial parts of *E. tirucalli*.

5.3.4. MTT Assay

The present investigation utilised the MTT assay to evaluate the effectiveness of *Euphorbia tirucalli* crude extracts, magnesium oxide nanoparticles (MgONPs) and cobalt oxide nanoparticles (CoONPs) in inhibiting the proliferation of MCF-7 breast cancer cells as seen in Figure 5.16. Initially, MCF-7 cells were seeded at a density of 1×10^5 cells/ml and exposed to varying concentrations of the tested substances (100, 10, and 1 $\mu\text{g/ml}$; respectively). IC_{50} values, indicative of the concentration required for a 50% reduction in cell proliferation, were calculated using Microsoft Excel 2008. The potency of the test samples was classified based on the IC_{50} values obtained, following the cytotoxicity guidelines outlined by The Council for Scientific and Industrial Research (CSIR), with concentrations above 100 $\mu\text{g/ml}$ deemed inactive, those between 100 $\mu\text{g/ml}$ and 30 $\mu\text{g/ml}$ classified as weak, between 30 $\mu\text{g/ml}$ and 5 $\mu\text{g/ml}$ as moderate and below 5 $\mu\text{g/ml}$ as potent (Mariri *et al.*, 2017).

Additionally, the study investigated the antiproliferative effects of crude extracts, annealed and unannealed iron oxide and iron dioxide across multiple cell lines—MCF-7, SKBR-3, MDA-231 and Vero—using concentrations of 1, 10 and 100 $\mu\text{g/mL}$ in triplicate experiments. These experiments, which included doxorubicin as a standard drug, revealed a concentration-dependent toxicity trend, with the highest toxicity

observed at the highest concentration tested. Comprehensive details of our findings are presented in Figures 5.17a-d.

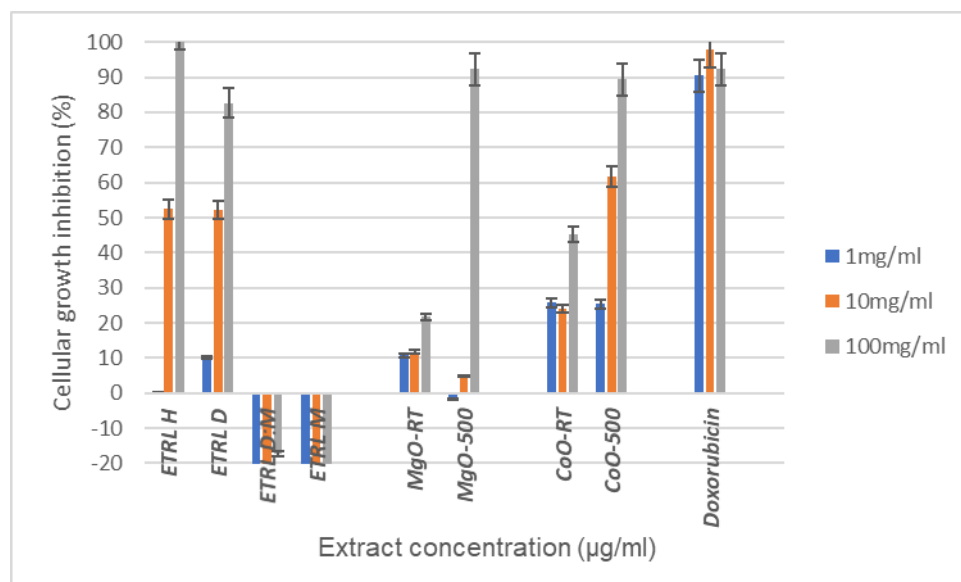


Figure 5.16. Cell growth inhibitory activity of MgONPs, CoONPs and four *Euphorbia* crude extracts: *E. tirucalli* Hex, DCM, DCM:MeOH and MeOH extracts against MCF-7 cells. Doxorubicin (standard drug) served as positive control.

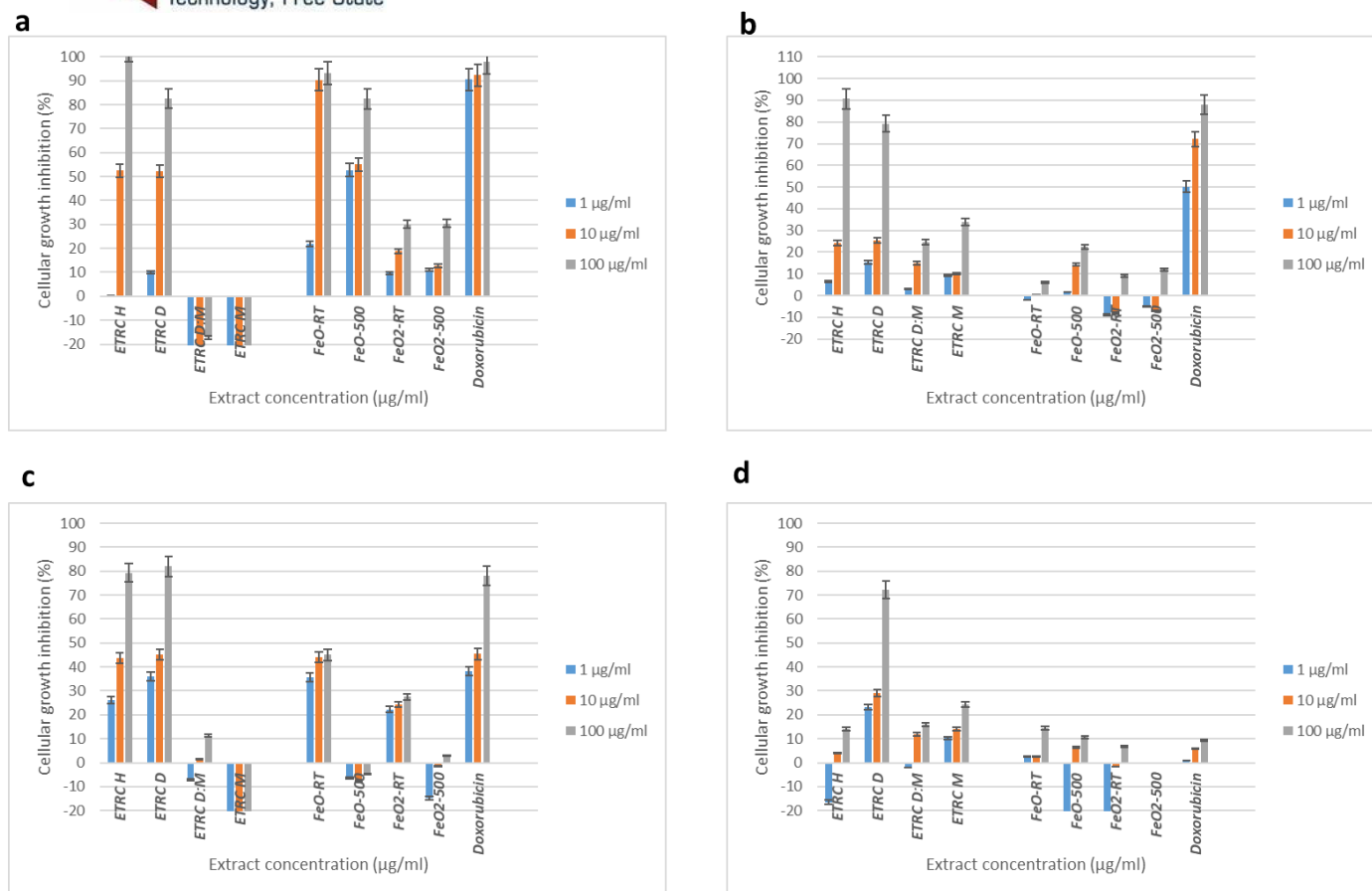


Figure 5.17. Cell growth inhibition % of four *Euphorbia* crude extracts, FeO-RT and FeO-500 NPs against MCF-7 (A), SK-BR-3 (B), MDA-MB231 (C) and Vero (D). *E. tirucalli* hexane (ETRC Hex) extract, *E. tirucalli* Dichloromethane (ETRC DCM), *E. tirucalli* Dichloromethane: Methanol (ETRC DCM: MeOH), *E. tirucalli* methanol (ETRC MeOH), Iron oxide unannealed NPs (FeO-RT), Iron oxide annealed NPs (FeO-500). Doxorubicin (standard drug).

5.4. Discussion

The detected phytochemicals in *E. tirucalli*, including tannins, glycosides, triterpenoids, saponins, flavonoids and steroids align with the plant's known pharmacological activities reported in various studies (Jyothi *et al.*, 2008). The presence of these compounds suggests potential bioactive properties, such as anti-cancer and antioxidant effects. The variation in phytochemical profiles between studies emphasizes the

importance of considering geographical and environmental factors in phytochemical investigations (Borokini & Ayodele, 2012). This discrepancy could also be attributed to different extraction methods and the plant parts analyzed. Further studies should explore the impact of these variables on the therapeutic potential of *E. tirucalli*.

The observed colour changes suggest successful reduction processes leading to the formation of iron oxide nanoparticles (FeONPs), magnesium oxide nanoparticles (MgONPs) and cobalt nanoparticles (CoONPs). The distinct colour alterations serve as visual indicators of the involvement of plant secondary metabolites in the reduction of metal ions (Anjitha *et al.*, 2021). The observed colour alteration aligns with the findings of Katata-Seru *et al.* (2018), who reported a similar change from reddish-brown to black, indicative of the presence of Fe NPs. This consistency in results strengthens the credibility of the study's outcomes. The role of polyphenols in reducing metal ions and stabilizing nanoparticles underscores the potential of *E. tirucalli* as a valuable source for the green synthesis of metal oxide nanoparticles (Latif *et al.*, 2019). The even dispersion of these nanoparticles in aqueous solutions further enhances their applicability in various fields, including medicine and industry (Mabrouk *et al.*, 2021). Future research should delve into optimizing the synthesis process and exploring the specific bioactive properties of the synthesized FeONPs.

The UV-vis spectrophotometric analysis provides strong evidence for the successful synthesis of iron nanoparticles and offers insights into their characteristics. The observed absorption peaks at 220 and 390 nm align with previous studies. The peak at 220 nm is attributed to the presence of tannins and triterpenoids in the *E. tirucalli* extract (Kiwumulo *et al.*, 2022). This finding is consistent with Pattanayak and Nayak (2013), which suggests the presence of surface plasmon vibrations in metallic iron nanoparticle solutions, corresponding to the peaks at 220 and 230 nm. Furthermore, the peak at 390 nm is in agreement with the study by Kumar and Prem (2018), which identified the formation of iron oxide nanoparticles. The distinctive absorption peaks in the ultraviolet spectrum provide valuable insights into the characteristics

and formation of MgONPs and CoONPs. The differences in peak positions for MgONPs suggest unique interactions during the reduction process, while the specific peaks for CoONPs correspond with charge transfers, as reported in the literature.

The FTIR analysis provided valuable insights into the chemical composition and interactions present within *E. tirucalli* and the synthesized iron nanoparticles. The detected functional groups, including O-H, C=O and C-O, indicate potential chemical bonding between the plant extract and the FeONPs (Jubb *et al.*, 2018; Wang *et al.*, 2016; Elumalai & Velmurugan, 2015; Pal, 2014). The presence of aromatic compounds and phenols in *E. tirucalli* suggests their involvement in the synthesis process, possibly acting as reducing agents or stabilizers for the nanoparticle formation (Kgosiemang *et al.*, 2023). The observed peaks at specific wavenumbers further validate the successful synthesis of FeONPs and highlight the structural characteristics of the nanoparticles (Sulaiman *et al.*, 2017; Kumar *et al.*, 2014; Pal, 2014).

The observed peaks corresponding to various functional groups such as hydroxyl, cyanide, alkenes, carboxylate and aromatic groups indicating the complexity of the organic molecules within the plant extract (Kuthirummal *et al.*, 2016). These organic compounds likely play a crucial role in the reduction and stabilization of metal ions during nanoparticle formation (Abdelmigid *et al.*, 2022). The absence of specific peaks in the annealed samples suggests structural changes induced by the annealing process, highlighting the sensitivity of FTIR spectroscopy in detecting subtle chemical alterations (Vijayarangamuthu & Rath, 2014). Furthermore, the presence of specific characteristic peaks further validates the successful synthesis of FeO, CoO and MgO NP (Ai, 2012).

The XRD spectra analysis provided crucial insights into the crystallographic structures and compositions of the synthesized nanoparticles. The observed diffraction peaks corresponding to specific crystal planes confirmed the presence of the iron oxide phase, consistent with previous studies, affirming the success of the green synthesis approach (Perumal *et al.*, 2022). However, the presence of additional peaks in the

spectra suggested the possible incorporation of impurities during the synthesis process, emphasizing the importance of stringent purification methods (Kgosiemang *et al.*, 2019). The XRD analysis of CoO and MgO nanoparticles further validated the formation of metal-ion crystals, although the absence of certain peaks indicated deviations from the ideal crystal structure, possibly due to impurities (Rao *et al.*, 2014; He *et al.*, 2015). These findings underscored the necessity for comprehensive characterization techniques to ensure the purity and integrity of synthesized nanoparticles. Additionally, the determination of particle size for MgO nanoparticles <10 nm highlighted the potential for fine-tuning synthesis conditions to achieve desired nanoparticle properties.

The XPS technique offers a powerful tool for observing the chemical composition and surface state of various constituents in nanoparticles. In the analysis of FeO nanoparticles, the presence of iron, magnesium, oxygen, carbon and calcium was confirmed, providing valuable information about the composition of the material. The detected peaks corresponding to Fe 2p, O 1s, and hydroxyl groups shed light on the electronic properties of FeO nanoparticles, indicating the involvement of Fe³⁺ octahedral species and surface oxygen compounds (Sridevi *et al.*, 2023). These findings align with previous studies and underscore the significance of Fe and O in influencing the properties of FeO nanoparticles (Ba-Abbad *et al.*, 2022; Miri *et al.*, 2021). Similarly, in the analysis of MgO and CoO nanoparticles, the presence of magnesium, carbon, oxygen and sulfur was observed, with peaks corresponding to specific chemical bonds providing insights into the surface state of the nanoparticles. The shifts in peaks after calcination suggest changes in the chemical environment, highlighting the dynamic nature of nanoparticle synthesis processes (Alayoglu *et al.*, 2016). The presence of metallic magnesium and cobalt bound to oxygen in MgO and CoO nanoparticles further adds to the understanding of their chemical properties.

The morphological analysis through SEM imaging provides valuable insights into the structural characteristics and size distribution of the synthesized nanoparticles. The mesoporous and matrix-like

arrangement observed in FeONPs indicate the morphology achievable through the green synthesis approach employed. Moreover, the agglomerated form of MgO nanoparticles suggests potential challenges in achieving uniform dispersion, which could impact their applications. Conversely, the smooth and spherical morphology of CoO nanoparticles underscores their potential suitability for various applications requiring well-defined shapes (Khan *et al.*, 2021). The observed particle sizes ranging from 1 μm to 100 nm highlight the nanoscale dimensions of the synthesized nanoparticles, essential for their enhanced properties and applications in fields such as catalysis, sensing and biomedical applications.

The characterization results obtained through TEM imaging and EDS profiling provide valuable insights into the composition and structural properties of the synthesized iron nanoparticles. The observed hexagonal agglomerated morphology and the elemental composition analysis confirm the successful synthesis of FeONPs (Alayoglu *et al.*, 2016). The significant presence of oxygen and carbon peaks in both nanoparticles suggests the involvement of polyphenols from the plant extract in the synthesis process, highlighting the green and eco-friendly nature of the fabrication method.

The observed size and porous structures of the MgO nanoparticles suggest their potential applications in various fields such as catalysis and biomedical engineering, where large surface areas are desirable. The EDS profiling results revealed the elemental composition of the nanoparticles, with magnesium and oxygen being the predominant components in MgO nanoparticles. Similarly, CoO nanoparticles exhibited characteristic peaks of cobalt, indicating their successful synthesis. The elemental percentages further confirmed the composition of the nanoparticles, with carbon likely originating from the TEM grid coating.

In this study, doxorubicin served as a benchmark drug, demonstrating expected anti-proliferative effects against MCF-7 with an IC_{50} value of $<1 \mu\text{g/ml}$. Extracts obtained from the aerial parts of *E. tirucalli* in DCM: MeOH and MeOH did not exhibit any anti-proliferative effects across the tested concentrations. However, the Hexane and DCM extracts displayed a 50% inhibition of MCF-7 cell growth at concentrations

of 10 µg/ml. These results imply that compounds causing cell growth inhibition possess low polarity. CoONPs displayed heightened cell growth inhibition of MCF-7 cells with an IC₅₀ of <10 µg/ml >1 µg/ml, consistent with previous studies by Akhtar *et al.* (2017) and Sawant *et al.* (2014). Conversely, MgONPs showed inhibition only at the highest concentration of 100 µg/ml, aligning with literature suggesting their minimal toxicity, particularly at lower concentrations (Krishnamoorthy *et al.*, 2012; Sharma *et al.*, 2017; Ge *et al.*, 2011). The IC₅₀ values of *E. tirucalli* Hex and DCM extracts and CoONPs were below 30 µg/ml, indicating moderate activity against MCF-7 cells.

Additionally, *E. tirucalli* extracts, particularly Hex and DCM, demonstrated moderate antiproliferative effects against MCF-7 and MDA-MB231 cells. These extracts inhibited cell growth by over 50% and 40%; respectively, at concentrations of 100 and 10 µg/mL. However, their activity against SK-BR-3 and Vero cells was weaker, with inhibition rates of less than 40%, suggesting differing susceptibility among cell lines. Previous research has highlighted higher cytotoxicity of certain compounds against MDA-MB231 and MCF-7 compared to SK-BR-3, potentially due to variations in receptor expression. Moreover, Fe₃O₄NPs, Fe₂O₃NPs and IONPs have shown significant cytotoxicity against various cancer cell lines, including breast cancer, with minimal impact on normal cells, indicating their potential as therapeutic agents.

Furthermore, unannealed FeO-RT NPs exhibited potent activity against MCF-7 cells, attributed to impurities and compounds present before calcination, with activity decreasing post-calcination due to phytochemical degradation (Thakur & Arora, 2023). Both annealed and unannealed FeO NPs displayed higher inhibitory activity against MCF-7 than Hex and DCM extracts, comparable to the standard drug doxorubicin. Conflicting findings regarding mesoporous silica NPs and pure iron oxide NPs suggest differences in cytotoxicity among various nanoparticles.

5.5. Conclusion

The development of magnesium, cobalt, iron oxide and iron dioxide nanoparticles using *Euphorbia tirucalli* offers a promising path for constructing nanostructures with potential medical applications. This study employs a comprehensive methodology involving plant extraction, nanoparticle synthesis and detailed characterization techniques to illustrate the creation of stable nanoparticles with unique physical and chemical characteristics. The utilization of *E. tirucalli* as a reducing agent streamlines the nanoparticle synthesis, with observable colour changes indicating the reduction processes.

A variety of characterization techniques, such as UV-vis spectroscopy, FTIR spectroscopy, XRD analysis, XPS analysis, SEM imaging and TEM imaging, provide valuable insights into the structural, chemical and morphological attributes of the synthesized nanoparticles. These analyses validate the successful synthesis of iron, magnesium and cobalt nanoparticles, highlighting the contribution of phytochemicals from *E. tirucalli* to both the formation and stabilization of these nanoparticles. The nanoparticles showcase distinct absorption spectra, chemical bonds, crystallographic structures and elemental compositions, which are essential for comprehending their properties and potential applications.

Furthermore, the assessment of *E. tirucalli* extracts and synthesized nanoparticles against breast cancer cell lines reveals promising cytotoxic effects, especially for cobalt nanoparticles. The notable activity exhibited by these nanoparticles suggests their potential as therapeutic agents for cancer treatment, offering a more environmentally sustainable option compared to conventional chemotherapeutic drugs. In summary, this study adds to the expanding field of environmentally friendly nanoparticle synthesis and their medical uses. Future research could focus on further optimizing synthesis parameters, investigating the specific mechanisms underlying nanoparticle-induced cytotoxicity and exploring additional therapeutic applications in cancer treatment and beyond.

5.6. References

- Abdelmigid, H. M., Hussien, N. A., Alyamani, A. A., Morsi, M. M., AlSufyani, N. M. & Kadi, H. A. (2022). Green synthesis of zinc oxide nanoparticles using pomegranate fruit peel and solid coffee grounds vs. chemical method of synthesis, with their biocompatibility and antibacterial properties investigation. *Molecules*, 27(4), 1236.
- Akhtar, M. J., Ahamed, M., Alhadlaq, H. A. & Alshamsan, A. (2017). Nanotoxicity of cobalt induced by oxidant generation and glutathione depletion in MCF-7 cells. *Toxicology in Vitro*, 40, 94-101.
- Alayoglu, S., Rosenberg, D. J. & Ahmed, M. (2016). Hydrothermal synthesis and characterization under dynamic conditions of cobalt oxide nanoparticles supported over magnesium oxide nanoplates. *Dalton Transactions*, 45(24), 9932-9941.
- Anjitha, K. S., Sameena, P. P. & Puthur, J. T. (2021). Functional aspects of plant secondary metabolites in metal stress tolerance and their importance in pharmacology. *Plant Stress*, 2, 100038.
- Behzadi, E., Sarsharzadeh, R., Nouri, M., Attar, F., Akhtari, K., Shahpasand, K. & Falahati, M. (2019). *International Journal of Nanomedicine*. 2019, 14, 257.
- Bibi, I., Nazar, N., Iqbal, M., Kamal, S., Nawaz, H., Nouren, S., ... & Abbas, M. (2017). Green and eco-friendly synthesis of cobalt-oxide nanoparticle: Characterization and photo-catalytic activity. *Advanced Powder Technology*, 28(9), 2035-2043.
- Borokini, T. I. & Ayodele, A. E. (2012). Phytochemical screening of *Tacca leontopetaloides* (L.) Kuntze collected from four geographical locations in Nigeria. *International Journal of Modern Botany*, 2(4), 97-102.

Cai, L., Chen, J., Liu, Z., Wang, H., Yang, H. & Ding, W. (2018). Magnesium oxide nanoparticles: effective agricultural antibacterial agent against *Ralstonia solanacearum*. *Frontiers in microbiology*, 9, 335574.

Chandran, S. P., Chaudhary, M., Pasricha, R., Ahmad, A. & Sastry, M. (2006). Synthesis of Gold Nanotriangles and Silver Nanoparticles Using Aloe vera Plant Extract. *Biotechnology Progress*, 22(2), 577–583.

Chandrasekhar, M., Nagabhushana, H., Sharma, S. C., Dhananjaya, N., Sunitha, D. V., Shivakumara, C., & Nagabhushana, B. M. (2014). Particle size, morphology and color tunable ZnO: Eu³⁺ nanophosphors via plant latex mediated green combustion synthesis. *Journal of Alloys and Compounds*, 584, 417-424.

Dailey, J. P., Phillips, J. P., Li, C., & Riffle, J. S. (1999). Synthesis of silicone magnetic fluid for use in eye surgery. *Journal of Magnetism and Magnetic Materials*, 194(1-3), 140-148.

Ernst, M., Grace, O. M., Saslis-Lagoudakis, C. H., Nilsson, N., Simonsen, H. T. & Rønsted, N. (2015). Global medicinal uses of *Euphorbia* L.(*Euphorbiaceae*). *Journal of ethnopharmacology*, 176, 90-101.

Ezhilarasi, A. A., Vijaya, J. J., Kaviyarasu, K., Maaza, M., Ayeshamariam, A. & Kennedy, L. J. (2016). Green synthesis of NiO nanoparticles using *Moringa oleifera* extract and their biomedical applications: Cytotoxicity effect of nanoparticles against HT-29 cancer cells. *Journal of Photochemistry and Photobiology B: Biology*, 164, 352-360.

Ge, S., Wang, G., Shen, Y., Zhang, Q., Jia, D., Wang, H., ... & Yin, T. (2011). Cytotoxic effects of MgO nanoparticles on human umbilical vein endothelial cells in vitro. *IET nanobiotechnology*, 5(2), 36-40.

Gupta, A. K. & Gupta, M. (2005). Cytotoxicity suppression and cellular uptake enhancement of surface modified magnetic nanoparticles. *Biomaterials*, 26(13), 1565-1573.

Hao, R., Xing, R., Xu, Z., Hou, Y., Gao, S. & Sun, S. (2010). Synthesis, functionalization, and biomedical applications of multifunctional magnetic nanoparticles. *Advanced materials*, 22(25), 2729-2742.

He, X., Song, X., Qiao, W., Li, Z., Zhang, X., Yan, S., ... & Du, Y. (2015). Phase-and size-dependent optical and magnetic properties of CoO nanoparticles. *The Journal of Physical Chemistry C*, 119(17), 9550-9559.

Howli, P., Panigrahi, K., Mitra, A., Das, N. S. & Chattopadhyay, K. K. (2019). Textile-based RGO-muffled cobalt (II, III) oxide hybrid nano-architectures for flexible energy storage device. *Applied Surface Science*, 485, 238-246.

Huang, J., Li, Q., Sun, D., Lu, Y., Su, Y., Yang, X., Wang, H., Wang, Y., Shao, W., He, N., Hong, J., & Chen, C. (2007). Biosynthesis of silver and gold nanoparticles by novel sundried *Cinnamomum camphora* leaf. *Nanotechnology*, 18(10), 105104.

Huang, L., Li, D. Q., Lin, Y. J., Wei, M., Evans, D. G. & Duan, X. (2005). Controllable preparation of Nano-MgO and investigation of its bactericidal properties. *Journal of inorganic biochemistry*, 99(5), 986-993.

Iravani, S. (2011). Green synthesis of metal nanoparticles using plants. *Green Chemistry*, 13(10), 2638.

Yothi, T. M., Shankariah, K., Prabhu, K., Lakshminarasu, S., Srinivasa, G. M., & Ramachandra, S. S. (2008). Hepatoprotective and antioxidant activity of *Euphorbia tirucalli*.

Katata-Seru, L., Moremedi, T., Aremu, O. S., & Bahadur, I. (2018). Green synthesis of iron nanoparticles using *Moringa oleifera* extracts and their applications: Removal of nitrate from water and antibacterial activity against *Escherichia coli*. *Journal of Molecular Liquids*, 256, 296–304.

Kavitha, T., Haider, S., Kamal, T. & Ul-Islam, M. (2017). Thermal decomposition of metal complex precursor as route to the synthesis of Co₃O₄ nanoparticles: antibacterial activity and mechanism. *Journal of Alloys and Compounds*, 704, 296-302.

Kgosiemang, I. K. R., Adegoke, A. M., Mashele, S. S. & Sekhoacha, M. P. (2023). Green synthesis of Iron oxide and Iron dioxide nanoparticles using *Euphorbia tirucalli*: characterization and antiproliferative evaluation against three breast cancer cell lines. *Journal of Experimental Nanoscience*, 18(1), 2276276.

Khan, M. A., Ali, F., Faisal, S., Rizwan, M., Hussain, Z., Zaman, N., ... & Bibi, N. (2021). Exploring the therapeutic potential of *Hibiscus rosa sinensis* synthesized cobalt oxide (Co₃O₄-NPs) and magnesium oxide nanoparticles (MgO-NPs). *Saudi Journal of Biological Sciences*, 28(9), 5157-5167.

Khan, S., Ansari, A. A., Khan, A. A., Ahmad, R., Al-Obaid, O. & Al-Kattan, W. (2015). *In vitro* evaluation of anticancer and antibacterial activities of cobalt oxide nanoparticles. *Journal of Biological Inorganic Chemistry*, 20, 1319-1326.

- Kiwumulo, H. F., Muwonge, H., Ibingira, C., Lubwama, M., Kirabira, J. B. & Ssekitoleko, R. T. (2022). Green synthesis and characterization of iron-oxide nanoparticles using *Moringa oleifera*: a potential protocol for use in low and middle income countries. *BMC Research Notes*, 15(1), 1-8.
- Krishnamoorthy, K., Moon, J. Y., Hyun, H. B., Cho, S. K., & Kim, S. J. (2012). *Journal of Materials Chemistry*, 22, 24610-24617.
- Kulpa-Koterwa, A., Ossowski, T., & Niedziałkowski, P. (2021). Functionalized Fe₃O₄ nanoparticles as glassy carbon electrode modifiers for heavy metal ions detection—A mini review. *Materials*, 14(24), 7725.
- Kumar Das, A., Marwal, A. & Verma, R. (2014). Bio-Reductive Synthesis and Characterization of Plant Protein Coated Magnetite Nanoparticles. *Nano Hybrids*, 7, 69–86.
- Latif, M. S., Abbas, S., Kormin, F. & Mustafa, M. K. (2019). Green synthesis of plant-mediated metal nanoparticles: The role of polyphenols. *Asian J. Pharm. Clin. Res.* v2020, 75-84.
- Lutsev, L. V., Stognij, A. I. & Novitskii, N. N. (2009). *Physical Review B*. 2009, 80, 184423.
- Mabrouk, M., Das, D. B., Salem, Z. A. & Beherei, H. H. (2021). Nanomaterials for biomedical applications: Production, characterisations, recent trends and difficulties. *Molecules*, 26(4), 1077.
- Manigandan, R., Giribabu, K., Suresh, R., Vijayalakshmi, L., Stephen, A. & Narayanan, V. (2013). *Chemical Science Transactions*. 2013, 2(Supplement 1), S47-S50.

Mariri, N. G. (2017). In Vitro Evaluation of the Bioactivity of *Gnidia Polycephala* and *Senecio Serratuloides* (Doctoral dissertation, Bloemfontein: Central University of Technology, Free State).

Martinez-Boubeta, C., Balcells, L., Cristòfol, R., Sanfeliu, C., Rodríguez, E., Weissleder, R., Lope-Piedrafita, S., Simeonidis, K., Angelakeris, M., Sandiumenge, F., & Calleja, A. (2010). *Nanomedicine: Nanotechnology, Biology and Medicine*, 6, 362-370.

Mittal, A. K., Chisti, Y. & Banerjee, U. C. (2013). Synthesis of metallic nanoparticles using plant extracts. *Biotechnology Advances*, 31(2), 346–356.

Munro, B., Vuong, Q., Chalmers, A., Goldsmith, C., Bowyer, M., & Scarlett, C. (2015). Phytochemical, antioxidant and anti-cancer properties of *Euphorbia tirucalli* methanolic and aqueous extracts. *Antioxidants*, 4(4), 647–661.

Nurgali, K., Rudd, J. A., Was, H. & Abalo, R. (2022). Editorial: Cancer therapy: The challenge of handling a double-edged sword. *Frontiers in Pharmacology*, 13.

Pal, S. (2014). Antimicrobial activity of iron oxide nanoparticles (Doctoral dissertation). National Institute of Technology, India. Available at: <http://ethesis.nitrkl.ac.in/5598/> [Accessed 4 Oct. 2023].

Pattanayak, M., & Nayak, P. L. (2013). Green synthesis and characterization of zero valent iron nanoparticles from the leaf extract of *Azadirachta indica* (Neem). *World Journal of Nano Science and Technology*, 2(1), 6-9.

Polyak, K. (2007). Breast cancer: origins and evolution. *Journal of Clinical Investigation*, [online] 117(11), 3155–3163.

- Pucci, C., Martinelli, C. & Ciofani, G. (2019). Innovative approaches for cancer treatment: current perspectives and new challenges. *Ecancermedicalscience*, [online] 13(1).
- Rao, K. G., Ashok, C. H., Rao, K. V. & Chakra, C. S. (2014). Structural properties of MgO nanoparticles: synthesized by co-precipitation technique. *International Journal of Science and Research*, 3(12), 43-46.
- Raman, V., Suresh, S., Savarimuthu, P. A., Raman, T., Tsatsakis, A. M., Golokhvast, K. S., & Vadivel, V. K. (2016). Experimental and therapeutic medicine. *Experimental and Therapeutic Medicine*, 11, 553-560.
- Rasheed, T., Nabeel, F., Bilal, M. & Iqbal, H. M. (2019). *Biocatalysis and Agricultural Biotechnology*. 2019, 19, 101154.
- Rocha, M., Chaves, N. & Báo, S. (2017). Nanobiotechnology for Breast Cancer Treatment. *Breast Cancer – From Biology to Medicine*. [online] doi:<https://doi.org/10.5772/66989>.
- Saravanakumar, K., Shanmugam, S., Varukattu, N. B., Mubarak Ali, D., Kathiresan, K. & Wang, M. H. (2019). Biosynthesis and characterization of copper oxide nanoparticles from indigenous fungi and its effect of photothermolysis on human lung carcinoma. *Journal of Photochemistry and Photobiology B: Biology*, 190, 103-109.
- Sawant, V. J., Bamane, S. R., Shejwal, R. V. & Patil, S. B. (2016). Comparison of drug delivery potentials of surface functionalized cobalt and zinc ferrite nanohybrids for curcumin in to MCF-7 breast cancer cells. *Journal of Magnetism and Magnetic Materials*, 417, 222-229.
- Sharma, G., Soni, R. & Jasuja, N. D. (2017). Phytoassisted synthesis of magnesium oxide nanoparticles with *Swertia chirayita*. *Journal of Taibah University for Science*, 11(3), 471-477.

Shunmugadevi, C. & Palanisamy, P. (2016). Comparative Study of Silver Nanoparticles: Green Synthesis, Characterization and Biological Evaluation of Carica papaya and Andrographis paniculata Leaf Extract.[online] *Semantic Scholar*. Available at:

<https://www.semanticscholar.org/paper/Comparative-Study-of-Silver-Nanoparticles%3A-Green-of-Shunmugadevi-Palanisamy/190cc92dad896af989591149ebcea4857cc3ca65> [Accessed 4 Oct. 2023].

Siegel, R. L., Miller, K. D., Fuchs, H. E. & Jemal, A. (2021). Cancer Statistics, 2021. *CA: A Cancer Journal for Clinicians*, 71(1), 7–33.

Singh, J., Dutta, T., Kim, K. H., Rawat, M., Samddar, P., & Kumar, P. (2018). 'Green' synthesis of metals and their oxide nanoparticles: Applications for environmental remediation. *Journal of Nanobiotechnology*, 16, 1-24.

Sulaiman, G. M., Tawfeeq, A. T. & Naji, A. S. (2017). Biosynthesis, characterization of magnetic FeONP and evaluations of the cytotoxicity and DNA damage of human breast carcinoma cell lines. *Artificial Cells, Nanomedicine, and Biotechnology*, 46(6), 1215–1229.

Thakur, R. & Arora, V. (2023). Comprehensive review on polymeric and metal nanoparticles: Possible therapeutic avenues. *International Journal of Polymeric Materials and Polymeric Biomaterials*, 72(18), 1489-1509.

Thema, F. T., Manikandan, E., Gurib-Fakim, A., & Maaza, M. (2016). Single phase Bunsenite NiO nanoparticles green synthesis by Agathosma betulina natural extract. *Journal of Alloys and Compounds*, 657, 655-661.

Viju Kumar, V. G., & Prem, A. (2018). Green synthesis and characterization of FeONP using Phyllanthus niruri extract. *Oriental Journal of Chemistry*, 34(5), 2583–2589.

Yadav, R. N. S. & Agarwala, M. (2011). Phytochemical analysis of some medicinal plants. *Journal of Phytology*. [online] Available at: <https://updatepublishing.com/journal/index.php/jp/article/view/2737>.

Zugazagoitia, J., Guedes, C., Ponce, S., Ferrer, I., Molina-Pinelo, S., & Paz-Ares, L. (2016). Current challenges in cancer treatment. *Clinical Therapeutics*, 38(7), 1551–1566.

Sridevi, H., Bhat, M. R., Kumar, P. S., Kumar, N. M., & Selvaraj, R. (2023). Structural characterization of cuboidal α -Fe₂O₃ nanoparticles synthesized by a facile approach. *Applied Nanoscience*, 13(8), 5605–5613.

Miri, A., Najafzadeh, H., Darroudi, M., Miri, M. J., Kouhbanani, M. A. J. & Sarani, M. (2021). Iron Oxide Nanoparticles: Biosynthesis, Magnetic Behavior, Cytotoxic Effect. *ChemistryOpen*.

Ba-Abbad, M. M., Benamour, A., Ewis, D., Mohammad, A. W., & Mahmoudi, E. (2022). Synthesis of Fe₃O₄ nanoparticles with different shapes through a co-precipitation method and their application. *JOM*, 74(9), 3531–3539.

Vijayarangamuthu, K. & Rath, S. (2014). Nanoparticle size, oxidation state, and sensing response of tin oxide nanopowders using Raman spectroscopy. *Journal of Alloys and Compounds*, 610, 706- 712.

CHAPTER 6

SYNERGISTIC POTENTIAL OF ELECTROACUPUNCTURE AND SUPERPARAMAGNETIC IRON OXIDE NANOPARTICLES IN INHIBITING PROLIFERATION OF A TRIPLE-NEGATIVE BREAST CANCER TUMOR IN A MOUSE XENOGRAFT MODEL

Abstract

This study investigates the anti-proliferative efficacy of the superparamagnetic iron oxide nanoparticles (SPIONs-EA) technique in BALB/c nude mice bearing 4T1 tumors. The mice were divided into four groups; control group receiving PBS and three treatment groups receiving iron oxide nanoparticles-electroacupuncture (IONP-EA), *Euphorbia tirucalli* extract and doxorubicin. They were subjected to alternate-day treatments. The mice were inoculated with half a million 4T1 cells on day 0 and the tumor was allowed 10-14 days to grow. Initial uniformity in tumor sizes shifted, with distinct trends observed by day 12, revealing larger tumors in doxorubicin and PBS groups. On day 15, treatment commenced; PBS, doxorubicin and the extract were administered interperitoneally, while the IONPs-EA was delivered using electroacupuncture. By day 17, IONPs-EA, extract and doxorubicin exhibited comparable tumor growth, differing from the PBS group. On day 19, IONPs-EA and extract groups showed slower tumor growth compared to PBS and doxorubicin. Notably, by day 23, IONPs-EA-treated mice exhibited reduced tumors than all other groups, emphasizing its effectiveness in slowing 4T1 cell proliferation *in vivo* compared to the extract and doxorubicin. Body weight stability in the IONPs-EA and extract groups, contrasting with significant changes in the PBS and doxorubicin groups, underscored the lower toxicity of the

SPIONs-EA technique. Histopathological examinations indicated tumor metastasis in the liver and lungs, with a higher incidence observed in the liver at the end of the study. This comprehensive assessment suggests the promising anti-proliferative potential of the IONPs-EA technique, offering insights into its efficacy and safety compared to standard treatments.

6.1. Introduction

Cancer is the second leading cause of death globally, making it a major public health concern (Siegel *et al.*, 2022). Approximately 19.3 million people were diagnosed with cancer in 2020, and 10 million people died from the disease (Sung *et al.*, 2021). Lung cancer is the leading cause of cancer death among males, while breast cancer is the most common cause of cancer deaths among females (WHO, 2022).

Cancer treatment has evolved significantly over the years, offering various approaches to target and manage cancer cells. These approaches include surgery, chemotherapy, radiation therapy, immunotherapy, hormone therapy and stem cell transplantation (Hashemzadeh *et al.*, 2021; Miller *et al.*, 2019). However, each treatment method comes with its own benefits and drawbacks. One major drawback is that the drugs used in chemotherapy can damage healthy cells in the body, just as they harm cancer cells. This is because the drugs are not aimed at a specific target and thus enter the body without any particular destination. Additionally, these drugs face the challenge of lacking selectivity, meaning they cannot differentiate between cancer cells and normal body cells (Senapati *et al.*, 2018). Therefore, these limitations hinder the success of cancer treatment and highlight the need for the development of new treatment strategies.

In recent years, the quest for innovative and effective therapeutic strategies against aggressive forms of cancer has driven researchers to explore synergistic approaches that combine conventional treatments with novel interventions (Zhang *et al.*, 2019). Triple-negative breast cancer (TNBC), characterized by its aggressive behaviour and the lack of therapeutic targets, requires innovative therapeutic interventions that can effectively inhibit tumor progression

(Newton *et al.*, 2022). In this context, the combination of acupuncture, an ancient medical practice rooted in traditional Chinese medicine (TCM), with modern nanotechnology involving superparamagnetic iron oxide nanoparticles (SPIONs), offers a unique way for tackling TNBC (Zeng *et al.*, 2022).

Acupuncture is a component of TCM and has been used for thousands of years to treat a wide range of health conditions (Zeng *et al.*, 2022). It involves inserting thin, sterile needles into specific points on the body. These needles are typically made of stainless steel and are very fine, which minimizes discomfort during insertion. During acupuncture treatment, the needles can be stimulated either by the practitioner's gentle manual manipulation or by the use of electrical stimulation (Zeng *et al.*, 2022). The manual technique involves the practitioner applying gentle pressure and movement to the needles to create a therapeutic effect (Xu *et al.*, 2020). In contrast, electrical stimulation involves attaching electrodes to the needles and delivering a low electric current to enhance the therapeutic effect of the treatment (Dharmananda, 2002). This approach is relatively new and is used in specific cases where manual manipulation is not sufficient. Electroacupuncture (EA) has gained recognition for its potential to modulate various physiological processes, including chronic pain management, neural regulation, muscle relaxation, immune responses and cancer-related pain (Yu *et al.*, 2020; Duanmu *et al.*, 2020; Zhang *et al.*, 2014a). This approach can provide more consistent and controlled stimulation to the acupuncture points compared to manual manipulation, which may lead to more effective treatment for certain conditions (Yang *et al.*, 2022). Additionally, recent investigations have suggested that EA holds promise in cancer therapy by exerting inhibitory effects on tumor growth and metastasis. The inherent ability of EA to engage complex signalling pathways makes it a compelling candidate for combination therapies targeting aggressive cancer subtypes such as TNBC (Jiang *et al.*, 2019).

The use of nanoparticles (NPs) in cancer therapy is an active area of research and offers great promise for improving the precision and effectiveness of cancer treatment (Mundekkad & Cho, 2022). However, one major challenge in their use is the accumulation of NPs at the target site (Wang *et al.*, 2018). To enhance the accumulation within tumors for effective cancer therapy, a range of strategies have been employed. These strategies include surface functionalization, which involves the addition of ligands or antibodies that target tumor-specific receptors. This form of strategy functions by specifically binding the receptor or antigen to the overexpressed tumor cells and is the most commonly used strategy (Bajracharya *et al.*, 2022; Xu *et al.*, 2023). The tailored size and shape of the NPs, as well as leveraging the Enhanced Permeability and Retention (EPR) effect have been utilized to improve accumulation. In this context, smaller NPs may pass through leaky tumor blood vessels more effectively, while specific shapes facilitate cellular uptake (Xu *et al.*, 2023). Furthermore, the NPs will normally accumulate in the tumors due to their leaky vasculature and poor lymphatic draining (Xu *et al.*, 2023; Fan *et al.*, 2023). The use of active transport mechanisms and responsive NPs, such as mediated endocytosis, target NPs specifically to cancer cells. The strategy often involves attaching ligands that bind to receptors on the cell surface (Bajracharya *et al.*, 2022; Xu *et al.*, 2023). Tailored treatment with NPs and their surface modification to the specific characteristics of a patient's tumor, such as genetic mutations or antigen expression, have also been considered for more precise targeting (Bajracharya *et al.*, 2022). The interaction of nanoparticles with the immune system has been considered by developing NPs that are able to suppress the immune response in the tumor microenvironment (Perez-Potti *et al.*, 2023). More clinical trials are conducted to validate the preclinical studies on the effectiveness of the NP-based therapies (Yao *et al.*, 2020; Nirmala *et al.*, 2023). Combinatorial therapies, utilizing NPs in conjunction with other treatment

modalities such as chemotherapy and radiation, are another avenue that have been explored in order to improve accumulation (Chehelgerdi *et al.*, 2023). Additionally, administering NPs directly to the tumor site using techniques such as intratumoral injection also enhances accumulation (Yun *et al.*, 2023; Yakubova *et al.*, 2023). Another recent strategy involves the use of magnetic NPs and an external magnet to guide the NPs to the tumor site to provide better tumor selectivity (Stanicki *et al.*, 2022). This strategy has shown to be more effective compared to the other strategies mentioned. The external magnetic field gradient has the ability to attract magnetic NP complexes to desired locations, thereby boosting drug accumulation within tumors (Stanicki *et al.*, 2022). This magnetic targeting method eliminates the need for carriers to bind to receptors found on tumor cells, making it a promising option for a wide range of solid tumors, unlike traditional targeting methods (Wang *et al.*, 2018).

Complementing the unique attributes of EA, SPIONs have emerged as versatile tools in cancer research and treatment (Yan *et al.*, 2023). These nanoparticles possess inherent magnetic properties that enable targeted delivery and accumulation within tumor sites using external magnetic fields (Kong *et al.*, 2023). When combined with conventional therapeutic agents or physical interventions, SPIONs have demonstrated the potential to enhance treatment efficacy while minimizing off-target effects (Rahman, 2023). In the context of TNBC, the synergistic interplay between EA and SPIONs holds the promise of not only restraining tumor proliferation but also offering insights into novel mechanisms underlying TNBC inhibition (Jiang *et al.*, 2019).

This research aims to develop a novel technique that combines EA with SPIONs to effectively inhibit tumor growth and assess the safety of NPs. The integration of these

methods seeks to introduce an innovative approach with the potential to advance cancer treatment significantly.

6.2. Materials and Methods

An EA Treatment Device (SDZ-II, Hwato Brand) was obtained from Suzhou Medical Appliance Factory in China, while sterile acupuncture needles with dimensions of 0.18 mm in diameter and 15 mm in length were sourced from Suzhou Dong Bang Medical Co., Ltd., also based in Suzhou, China. Doxorubicin was procured from Teva Pharmaceuticals in South Africa. The 4T1 TNBC cells from mice tumor were obtained from Separation Scientific South Africa (Pty) Ltd. and Roswell Park Memorial Institute (RPMI) 1640 medium was purchased from Cellonex South Africa. Fetal bovine serum (FBS), amino acids and antibiotics were procured from Sigma-aldrich, South Africa. It's important to note that all of these items, including equipment and reagents, were acquired from well-established and reputable suppliers, ensuring the quality and reliability of the products.

6.2.1. Breast cancer cell preparation

These cells were maintained in RPMI 1640 medium, supplemented with 10% heat- inactivated fetal bovine serum, 0.1% nonessential amino acids, 100 µg/mL penicillin and 100 unit/mL streptomycin; incubated in a humidified incubator at 37 °C with 5% CO₂. After reaching 80% confluency, the cells were stained with trypan blue and counted using an automated hemocytometer to assess their viability.

6.2.2. *In vivo* model

Female BALB/c nude mice (6-8 weeks old; 17-24 g) were purchased from the University of the North West (South Africa). The experiments were conducted in compliance with the

protocols approved by the University of the Free State and University of the North West Animal Research Ethics Committees (UFS-AED2019/0135 and NWU-00791-22-A5; respectively). The experiments were conducted at two animal facilities (first at the University of the Free State, then at the North West University). The mice were housed under pathogen-free conditions in the animal facility in the University of the Free State/ University of the North West, South Africa. The holding room were kept at temperatures ranging between 22-24°C and 40-60% humidity.

6.2.3. *In vivo* TNBC xenograft mouse model

The experiment adhered to the methodology of Krause *et al.* (2013), with minor adjustments. All animals, except for the normal positive controls, were inoculated with 4T1 cells (5×10^5 cells/mice in 150 μ L ice-cold PBS) on their left 4th nipple, Figure 1. Following the emergence of tumors, the animals were randomly allocated into four groups, with five animals in each group. The size of the tumors was measured with a digital caliper and when the tumor volume reached between 80 and 100 mm³ the animals received treatment.



Figure 6.1. Mouse under anaesthesia with nose cone during 4T1 Cell Inoculation.

6.2.4. Treatment grouping

Administration of 4 distinct therapeutic agents: doxorubicin, *Euphorbia tirucalli* dichloromethane (DCM) crude extract and Iron oxide NPs was carried out. Additionally phosphate-buffered saline (PBS) was also administered. Our recent research publication (Kgosiemang *et al.*, 2023) contains detailed findings on the characterization and *in vitro* activity of the extract and iron oxide NPs.

The current study consisted of five groups of mice, each group comprising of five mice. The first group, Group A, served as the control group and received neither cancer cells nor any form of treatment. The second group, Group B, also served as a control group but consisted of tumor-bearing mice treated with 0.9% PBS. The third group, Group C, was the positive control group of tumor-bearing mice treated with 0.04mg/kg of doxorubicin (2mg/mL). The fourth group, Group D, was the experimental group of tumor-bearing mice treated with 5.26mg/kg of the active *Euphorbia* crude DCM extract (0.1mg/mL). Finally, the fifth group, Group E, was also an experimental group of tumor-bearing mice treated with 5.26mg/kg of

magnetic nanoparticles. Unlike the other groups that received treatment interperitoneally, Group E mice received electroacupunctural therapy and were anaesthetized during treatment. The treatment was guided by an external magnetic field, for three minutes, to the breast cancer.

6.2.5. Electroacupuncture intervention

The EA procure was conducted following an established protocol by Yang *et al.* (2019) with minor changes. In the study, sterile acupuncture needles of size 0.18×15 mm were used for electro-acupuncture interventions. Before inserting the needles, the acupunctural site was disinfected using 70% ethanol, following aseptic techniques. Under anaesthesia, four acupuncture needles were inserted towards the tumor, precisely targeting the 4th mammary gland fat pad (see Figure 2). The acupuncture site was marked approximately 5 mm from the boundary of the tumor on the mammary fat pad, with an acupuncture depth of around 5 mm horizontally. The EA interventions were conducted using the SDZ-II electronic acupuncture treatment device. The longitudinal wave settings were configured at 15Hz for 5 seconds and then transitioned to 20Hz for a duration of 3 minutes, all at a current intensity of 1mA. During the procedure, a magnet was strategically positioned at the tumor site. After each treatment session, each mouse was promptly transferred to its respective cage and placed on a heat pad to prevent hypothermia. Vigilant monitoring ensued, with close observation intervals at 30 minutes, 1 hour and subsequently at least twice more until each mouse had fully recuperated from the intervention. Furthermore, after each treatment each mouse was subcutaneously administered with 0.05-0.1 mg/mL of analgesic injection to alleviate any pain resulting from the procedures. After the two-week (14 days) treatment period, all mice were

ethanized once tumors in group B reached 2cm in diameter (Wang *et al.*, 2018). Their organs were harvested for histopathological analysis.



Figure 6.2. Electroacupuncture intervention on the xenograft model.

6.2.6. Histological studies

After the two-week (14 days) treatment period, the animals were euthanized with CO₂. The tumor, liver and lung organs were harvested, washed with PBS, sectioned into pieces, and then placed in a 10% buffered formaldehyde solution. To check for any metastasis, the sectioned pieces were stained using hematoxylin and eosin solution.

6.2.7. Statistical analysis

The results are presented as the mean $\pm$ standard deviation (SD). To perform statistical analysis, a one-way analysis of variance (ANOVA) was utilized, followed by Kruskal-Wallis comparison tests. The statistical significance level was set at $p < 0.05$.

6.3. Results

In this investigation, the anti-proliferative efficacy of the SPIONs-EA technique using BALB/c nude mice bearing 4T1 tumors was assessed. The mice were categorized into four groups: those treated with PBS, extract (*Euphorbia tirucalli*), doxorubicin and a group subjected to IONP-EA needle stimulation. Tumor-bearing mice with comparable average tumor sizes were randomly distributed across these groups. Despite maintaining uniform conditions for the tumor model, housing and feeding, there were slight variations in tumor sizes owing to individual differences among the mice. Once the tumors reached a size of 80 to 100 mm³, treatments were administered on alternate days and these therapeutic regimens were repeated six times to optimize the desired outcomes. Tumor sizes were measured a day prior to each treatment and the mice's body weights were recorded on the day of the treatment. The efficacy of the treatment was evaluated by comparing the control groups with the therapeutic groups and the results presented in Figures 3, 4 and 5.

The results revealed that on the ninth day (day 9), a successful 4T1 breast tumor mouse model was established through the injection of 4T1 cells, as depicted in Figure 3. However, out of the 20 mice that were inoculated with 4T1 cells, four mice did not develop any tumors.

6.3.1 Tumor size

Treatment was initiated on day 12 for mice whose tumors had reached the desired size by that day. For the remaining mice, treatment began later, once their tumors had also reached the

target size. Although trends in tumor growth became apparent on day 12, with mice treated with doxorubicin and PBS showing larger tumors than those treated with IONPs-EA and extract, all mice had begun treatment by day 15. By day 17, IONPs-EA, extract and doxorubicin continued to demonstrate reduced tumor growth compared to the PBS-treated group ($p < 0.05$), affirming their effectiveness. However, no significant differences ($p \geq 0.05$), indicating comparable results between IONPs-EA, extract and doxorubicin in their tumor-reducing capabilities by Day 17 was observed. On Day 19, the IONPs-EA and extract treatment groups exhibited almost similar tumor sizes, growing at a slower pace compared to the PBS and doxorubicin-treated groups. Significant reductions in tumor size were observed in all treatments (IONPs-EA, extract and doxorubicin) compared to the control (PBS) ($p < 0.05$), indicating that all treatments remained effective at this stage. However, on day 21, the same trend persisted whereby tumors in the PBS group were significantly larger than those treated with IONPs-EA, extract and doxorubicin ($p < 0.05$), indicating the continued effectiveness of these treatments while maintaining a steady tumor growth. However, on the same day, no significant differences were observed between the treatments ($p \geq 0.05$), suggesting similar performance across the treatments at this point, as seen in Figure 6.3. On Day 23, the IONPs-EA-treated group exhibited reduced tumor growth compared to the PBS-treated group. However, the tumor sizes of the IONPs-EA-treated group were almost similar to those in the extract and doxorubicin-treated groups. As a result, no statistically significant differences between the treatment groups were observed ($p \geq 0.05$). After four days of observation (Day 27) post-treatment, the IONPs-EA tumors continued to remain steady, showing significantly reduced growth compared to the PBS group, indicating a promising therapeutic potential. This trend was further supported by the harvested tumor weight. The average tumor weight in the IONPs-EA-treated group was 0.56667g, while the PBS group had a significantly higher average weight of 1.16667g ($p < 0.05$). Additionally, the extract-treated

group had an average tumor weight of 0.83333g and the doxorubicin-treated group had a weight of 0.60000g, both of which were lower than the PBS group but showed no significant difference from the IONPs-EA group ($p \geq 0.05$), as demonstrated in Figure 4. These results suggest that the IONPs-EA treatment significantly slowed down proliferation of 4T1 cells *in vivo* compared to the extract and doxorubicin.

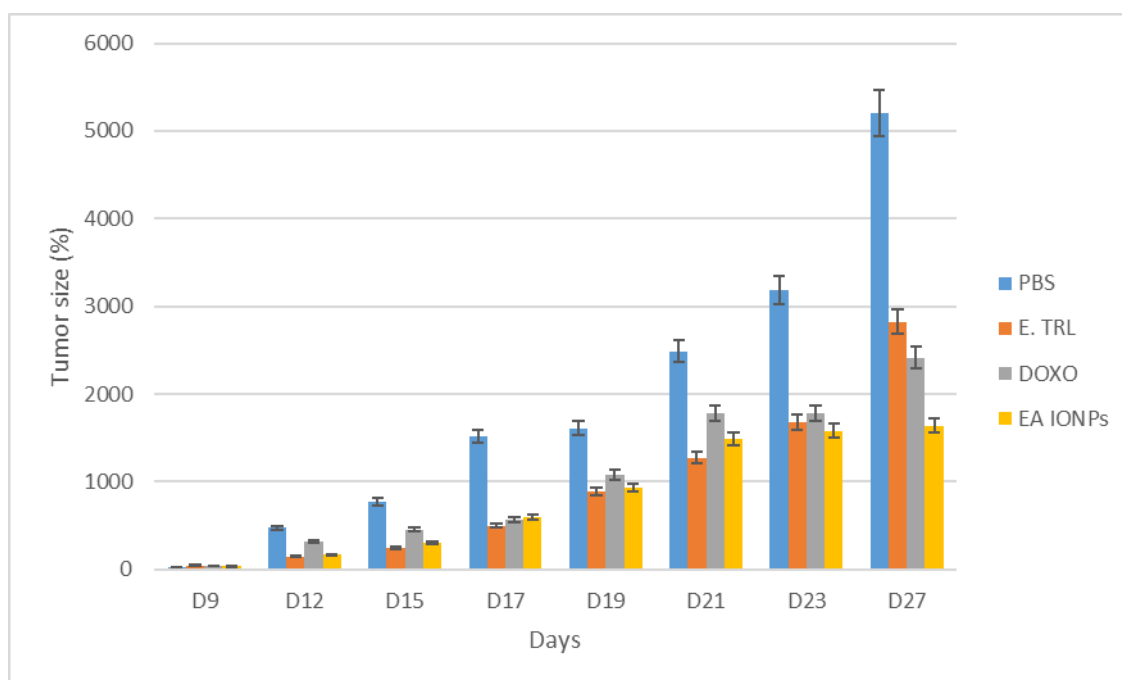


Figure 6.3. Anti-proliferative effect of the EA IONPs, *E. tirucalli*, doxorubicin and PBS treatment groups.

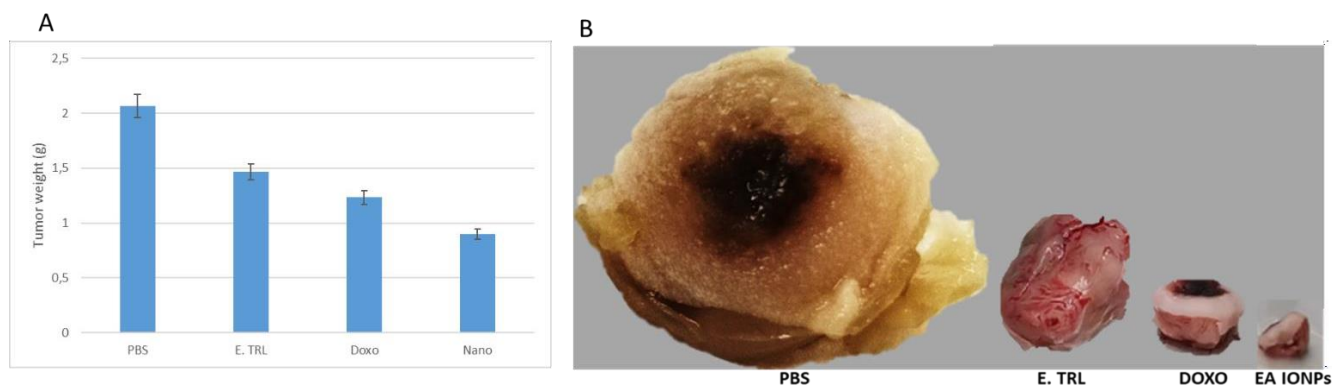


Figure 6.4. Differential Tumor Size Variation Across Treatment Modalities. (A) Tumor weight (g) of the PBS-treated group, E. TRL-treated group, Doxorubicin-treated group and EA IONPs-treated group. (B) Tumor images harvested from the PBS-treated group, E. TRL- treated group, Doxorubicin-treated group and EA IONPs-treated group. (The deviation in tumor color was due to prolonged immersion in formaldehyde solution).

6.3.2 Body weight

During the 8-day treatment and subsequent 4-day physical observation, the body weight of the IONPs-EA group, extract group and positive control group remained relatively stable, suggesting low toxicity of the EA technique (Figure 6.5). However, the PBS group and doxorubicin-treated group experienced significant changes in body weight, reflecting the known toxicity of the standard drug. While body weight is an important indicator, it was not the only parameter used to assess toxicity. Additional health signs including posture, alertness, food consumption, grooming behaviour and the presence of residues around the eyes. Necrosis or skin lesions were observed in the PBS, IONPs and doxorubicin-treated groups (Figure 6.6). However, other severe clinical signs, such as poor posture, paralysis or decreased alertness, were not observed in the IONPs-EA group.

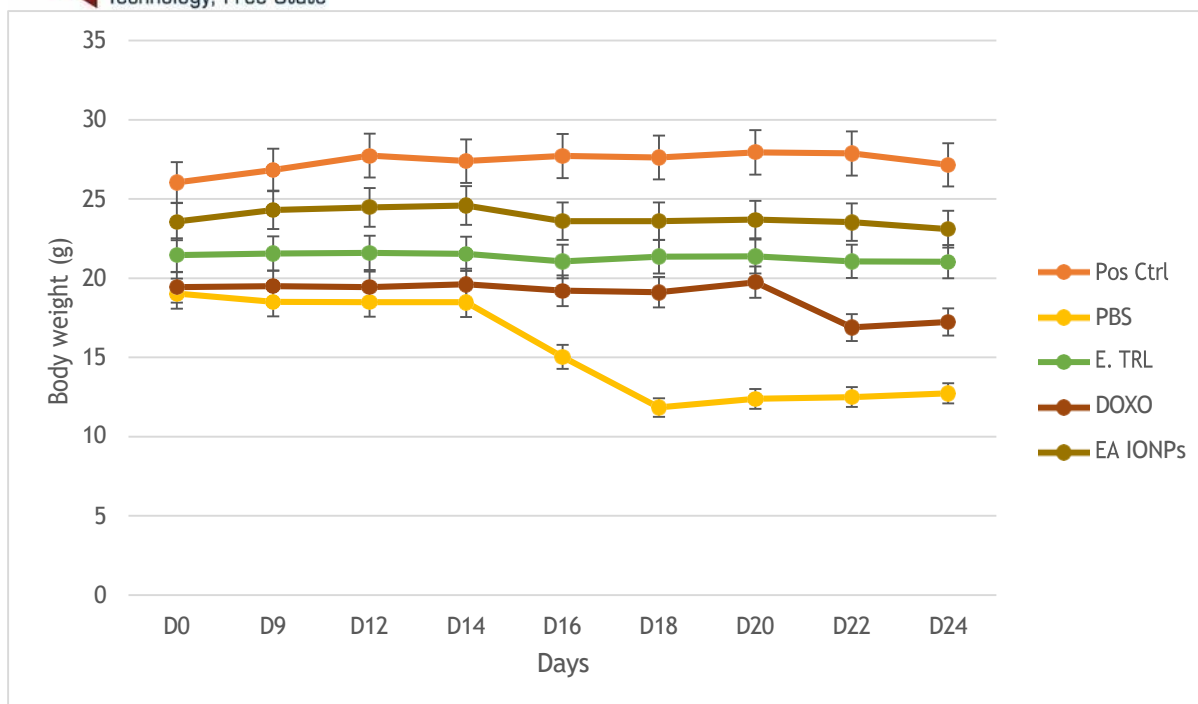


Figure 6.5. Body weights of mice before treatment (D0) and during treatment (D9-D24) with phosphate buffer solution (PBS), Electroacupuncture iron oxide nanoparticles (EA IONPs), *Euphorbia tirucalli* (E. TRL) extract and doxorubicin (DOXO) from day 0 to day 24. Body weights are presented as mean $\pm$ SD in grams from 9 independent experiments, n=5 mice per group.

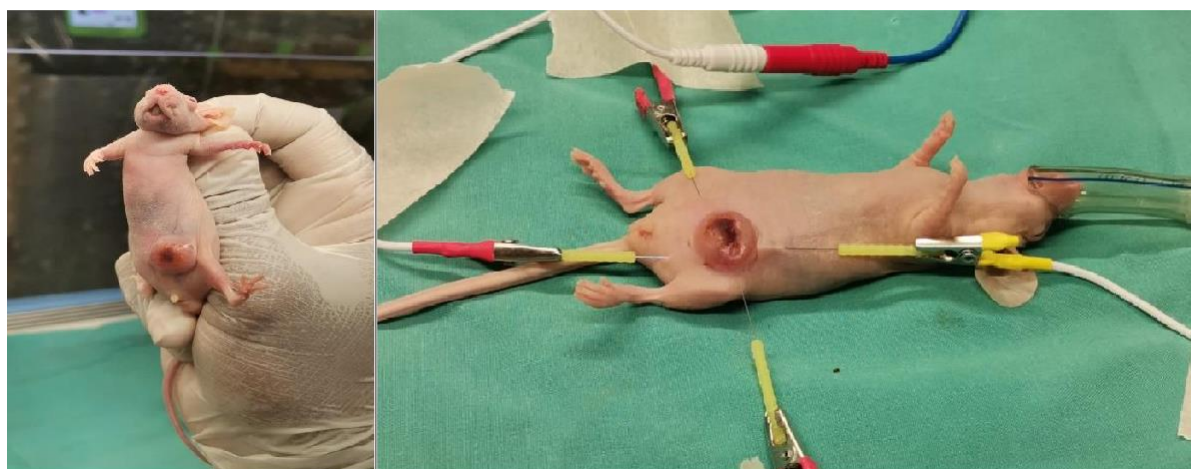


Figure 6.6. Image of mice with skin necrosis on the tumor.

6.3.3 Histopathology

To evaluate the efficacy of the treatment, tumor tissues from various groups of mice were collected and subjected to hematoxylin and eosin staining assays. Furthermore, histopathological examinations were conducted on the liver and lungs following the *in vivo* experiments. The staining of lung and liver tissues using hematoxylin and eosin revealed the presence of tumor metastasis in both organs, with a higher incidence of metastasis observed in the liver of most mice.

The experimental results reveal distinct patterns in the effectiveness of various treatments on cancer metastasis in both the lung and liver as shown in Table 1. Doxorubicin treatment demonstrates inhibition of lung metastasis, as evidenced by the absence of metastasis in all treated lung samples, but its effectiveness in the liver is limited, with only one out of three samples showing inhibition. Nanoparticles exhibit some efficacy in preventing lung metastasis but are ineffective in the liver, where all treated samples show metastasis. Plant extract treatment yields mixed results, with all treated lung samples showing metastasis and two out of three liver samples exhibiting metastatic growth. The negative control consistently leads to metastasis in both organs. These findings underscore the need for tailored treatment approaches targeting specific metastatic sites and highlight the potential of certain treatments, such as doxorubicin, in inhibiting lung metastasis.

Table 6.1. Summary of cancer metastasis results in lung and liver samples following different treatments.

Treatment	Lung	liver
-----------	------	-------

PBS	+	-
PBS	+	-
PBS	+	+
Nanoparticles	-	+
Nanoparticles	-	+
Nanoparticles	+	+
Doxorubicin	-	+
Doxorubicin	-	-
Doxorubicin	-	-
Plant extract	+	+
Plant extract	+	+
Plant extract	-	-

6.4. Discussion

Electroacupuncture, an evolved form of traditional acupuncture, has emerged as a potential tool in cancer therapy (Jiang *et al.*, 2019). This non-invasive technique employs low- frequency electrical currents to stimulate specific acupuncture points, offering benefits in pain management, stress reduction, digestive health, immune system support and overall well-being (Luo *et al.*, 2020). When integrated with SPIONs, EA takes on a new dimension in cancer treatment (Yang *et al.*, 2019). SPIONs enhance targeted drug delivery and localized hyperthermia therapy by leveraging their magnetic properties and controlled heat generation. The current study sheds light on the synergistic effects of EA and SPIONs in effectively slowing down the proliferation of TNBC within a mouse xenograft model. The analysis and interpretation of these results unveil the promising implications of this innovative therapeutic approach for one of the most aggressive forms of breast cancer, by slowing down the growth of tumors in xenograft models offering new avenues for potential advancements in cancer treatment. A prior study documented a significant decrease in bone tumor growth following electroacupuncture treatment (Smeester *et al.*, 2013). Similarly, Jiang *et al.* (2019) illustrated the capability of electroacupuncture to reduce TNBC tumor size and impede tumor progression.

In other studies, the finding implies that electroacupuncture may enhance the accumulation of chemotherapeutic agents within the tumor vicinity (Yang *et al.*, 2019). Moreover, previous research by Zhang *et al.* (2009) demonstrated acupuncture's influence on the targeting and distribution of Dox, and further indicated elevated paclitaxel concentrations in organs post-acupuncture, with the multi-needle method showing superior targeting efficacy compared to the single-needle approach (Zhang *et al.*, 2014b).

According to research, acupuncture has the potential to improve blood circulation in specific parts of the body (Gagliardi *et al.*, 2023). However, the irregular distribution of blood flow within tumors can hinder the efficient delivery of the drug, particularly to areas that are not well-perfused (Jain *et al.*, 2011). Therefore, it is possible that the increased concentration of chemotherapeutic drugs within tumors after acupuncture treatment is due to changes in the microvasculature induced by acupuncture.

The combination of EA and SPIONs explored in the current study holds promise for more precise and effective cancer treatment while minimizing side effects. The experimental results reveal distinct trends in the effectiveness of treatments on cancer metastasis between the lung and liver. Notably, doxorubicin exhibits greater efficacy in preventing lung metastasis compared to the liver, with all lung samples showing presence of tumors while only one liver sample displays similar results. Nanoparticles showed limited efficacy, being effective in preventing lung metastasis but ineffective in the liver. This is in agreement with the study by Park *et al.* (2019), who reported significant lung metastases reduction after treatment with SPIONs. In another study, SPIONs delayed lung metastasis by 45 days after treatment (Mu *et al.*, 2021). Plant extract treatment yields mixed outcomes in both organs,

with some samples showing inhibition of metastasis while others do not. Furthermore, the PBS-treated group consistently leads to more metastasis in the lung compared to the liver; this is in agreement with the study by Yang *et al.* (2020) who revealed that 4T1 mostly metastasizes to the lungs, indicating the reliability of the experimental setup.

Breast cancer metastasis, particularly to the lung and liver, is a well-documented phenomenon in the literature (Rashid *et al.*, 2021). The experimental results align with existing knowledge regarding the organ-specific patterns of breast cancer metastasis. Studies have shown that breast cancer commonly metastasizes to distant organs, with the lung and liver being among the most frequent sites of metastatic spread. This aligns with the observed trend in the experimental results, where the liver consistently showed a higher incidence of metastasis compared to the lung across all treated samples. Furthermore, the differential response to treatments between the lung and liver mirrors the heterogeneous nature of breast cancer metastasis and highlights the importance of considering organ-specific responses when developing treatment strategies.

6.5. Conclusion

This investigation assessed the efficacy of the SPIONs-EA technique in inhibiting tumor proliferation and metastasis in a 4T1 breast tumor mouse model. Despite minor variations in tumor sizes, the successful establishment of the tumor model was confirmed. Distinct trends in tumor growth were observed among treatment groups, with the IONPs-EA-treated group showing promising results in slowing tumor proliferation while maintaining low toxicity. Histopathological examinations revealed tumor metastasis in lung and liver tissues, with varied responses to treatments in these organs. Overall, this study highlights the potential of

the SPIONs-EA technique as a promising therapeutic approach for breast cancer, emphasizing the need for further research to optimize treatment strategies and to improve patient outcomes.

6.6. References

- Bajracharya, R., Song, J. G., Patil, B. R., Lee, S. H., Noh, H. M., Kim, D. H., Kim, G. L., Seo, S. H., Park, J. W., Jeong, S. H. and & Lee, C. H., (2022). Functional ligands for improving anticancer drug therapy: current status and applications to drug delivery systems. *Drug Delivery*, 29(1), 1959-1970.
- Chehelgerdi, M., Chehelgerdi, M., Allela, O. Q. B., Pecho, R. D. C., Jayasankar, N., Rao, D. P., Thamaraikani, T., Vasanthan, M., Viktor, P., Lakshmaiya, N. and & Saadh, M. J., (2023). Progressing nanotechnology to improve targeted cancer treatment: overcoming hurdles in its clinical implementation. *Molecular Cancer*, 22(1), 169.
- Dharmananda, S., (2002). *Electro-acupuncture*. ITM.
- Duanmu, C.L., Wang, X. Y., Zhang, X. N., He, W., Su, Y. S., Wan, H. Y., Hu, L., Wang, Y. and & Jing, X. H., (2020). Electroacupuncture and transcutaneous electrical acupoint stimulation with different intensities relieve muscular of inflammatory pain of the rats. *Zhen ci yan jiu= Acupuncture Research*, 45(11), 902-907.
- Fan, D., Cao, Y., Cao, M., Wang, Y., Cao, Y. and & Gong, T., (2023). Nanomedicine in cancer therapy. *Signal Transduction and Targeted Therapy*, 8(1), 293.
- Gagliardi, V., Gagliardi, G., Ceccherelli, F. and & Lovato, A., (2023). Effect of low-and high-frequency auricular stimulation with electro-acupuncture on cutaneous microcirculation: A cross-over study in healthy subjects. *Medicines*, 10(2), 17.

Hashemzadeh, N., Dolatkhah, M., Adibkia, K., Aghanejad, A., Barzegar-Jalali, M.,

Omidi, Y., & Barar, J. (2021). Recent advances in breast cancer immunotherapy:

The promising impact of nanomedicines. *Life Sciences*, 271, 119110.

Jain, A. V. I. R. A. L., Jain, A. B. H. I. S. H. E. K., Gulbake, A. R. V. I. N. D., Hurkat, P.

O. O. J. A. and Jain, S. K., (2011). Solid tumors: a review. *Int J Pharm Pharm*

Sci, 3(Suppl 5), 45-51.

Jiang, X., Tian, Y., Xu, L., Zhang, Q., Wan, Y., Qi, X., Li, B., Guo, J., Sun, W., Luo, A.

and Huang, J., (2019). Inhibition of triple-negative breast cancer tumor growth by

electroacupuncture with encircled needling and its mechanisms in a mice xenograft

model. *International Journal of Medical Sciences*, 16(12),1642.

Kgosiemang, I. K. R., Adegoke, A. M., Mashele, S. S. and Sekhoacha, M. P., (2023).

Green synthesis of Iron oxide and Iron dioxide nanoparticles using *Euphorbia tirucalli*:

characterization and antiproliferative evaluation against three breast cancer cell

lines. *Journal of Experimental Nanoscience*, 18(1),2276276.

Kong, X., Gao, P., Wang, J., Fang, Y. and Hwang, K. C., (2023). Advances of medical

nanorobots for future cancer treatments. *Journal of Hematology & Oncology*, 16(1),

74.

Krause, S., Brock, A. and Ingber, D. E., (2013). Intraductal injection for localized drug

delivery to the mouse mammary gland. *Journal of visualized experiments: JoVE*, (80).

- Luo, M., Song, B. and & Zhu, J., (2020). Electroacupuncture: a new approach for improved postoperative sleep quality after general anesthesia. *Nature and science of sleep*, 583-592.
- Miller, K. D., Nogueira, L., Mariotto, A. B., Rowland, J. H., Yabroff, K. R., Alfano, C. M., Jemal, A., Kramer, J. L., Siegel, R. L. (2019). Cancer treatment and survivorship statistics, 2019. *CA: a cancer journal for clinicians*. 2019 Sep; 69(5):363-85.
- Mu, Q., Lin, G., Jeon, M., Wang, H., Chang, F. C., Revia, R. A., Yu, J. and & Zhang, M., (2021). Iron oxide nanoparticle targeted chemo-immunotherapy for triple negative breast cancer. *Materials Today*, 50, 149-169.
- Mundekkad, D. and & Cho, W. C., (2022). Nanoparticles in clinical translation for cancer therapy. *International Journal of Molecular Sciences*, 23(3), 1685.
- Newton, E. E., Mueller, L. E., Treadwell, S. M., Morris, C. A., Machado, H.L. (2022). Molecular targets of triple-negative breast cancer: where do we stand?. *Cancers*. 2022 Jan 18; 14(3):482.
- Nirmala, M. J., Kizhuveetil, U., Johnson, A., Balaji, G., Nagarajan, R. and & Muthuvijayan, V., (2023). Cancer nanomedicine: a review of nano-therapeutics and challenges ahead. *RSC advances*, 13(13), 8606-8629.
- Park, J., Park, J., Castanares, M. A., Collins, D. S. and & Yeo, Y., (2019). Magnetophoretic delivery of a tumor-priming agent for chemotherapy of metastatic murine breast cancer. *Molecular pharmaceuticals*, 16(5), 1864-1873.

Perez-Potti, A., Rodríguez-Pérez, M., Polo, E., Pelaz, B. and & Del Pino, P., (2023).

Nanoparticle-based immunotherapeutics: from the properties of nanocores to the differential effects of administration routes. *Advanced Drug Delivery Reviews*, 114829.

Rahman, M. (, 2023). Magnetic Resonance Imaging and Iron-oxide Nanoparticles in the era of Personalized Medicine. *Nanotheranostics*, 7(4), 424.

Rashid, N. S., Grible, J. M., Clevenger, C. V. and & Harrell, J. C. (, 2021). Breast cancer liver metastasis: current and future treatment approaches. *Clinical & experimental metastasis*, 38, 263-277.

Senapati, S., Mahanta, A. K., Kumar, S., & Maiti, P. (2018). Controlled drug delivery vehicles for cancer treatment and their performance. *Signal Transduction and Targeted Therapy*, 3(1), 7.

Siegel, R. L., Miller, K. D., Fuchs, H. E., & Jemal, A. (2022). Cancer statistics, 2022. *CA: A Cancer Journal for Clinicians*, 72(1), 7-33.

Smeester, B. A., Al-Gizawiy, M., O'Brien, E. E., Ericson, M. E., Triemstra, J. L. and & Beitz, A. J., (2013). The effect of electroacupuncture on osteosarcoma tumor growth and metastasis: analysis of different treatment regimens. *Evidence-Based Complementary and Alternative Medicine*, 2013.

Stanicki, D., Vangijzegem, T., Ternad, I. and & Laurent, S., (2022). An update on the applications and characteristics of magnetic iron oxide nanoparticles for drug delivery. *Expert Opinion on Drug Delivery*, 19(3), 321-335.

Sung, H., Ferlay, J., Siegel, R.L., Laversanne, M., Soerjomataram, I., Jemal, A., Bray, F. (2020). Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: a cancer journal for clinicians*. 2021 May; 71(3):209-49.

Wang, G., Gao, S., Tian, R., Miller-Kleinhenz, J., Qin, Z., Liu, T., Li, L., Zhang, F., Ma, Q. and & Zhu, L., (2018). Theranostic hyaluronic acid–iron micellar nanoparticles for magnetic-field-enhanced in vivo cancer chemotherapy. *ChemMedChem*, 13(1), 78-86.

WHO. (Cancer factsheet), (2022). <https://www.who.int/news-room/factsheets/detail/cancer>

Xu, M., Han, X., Xiong, H., Gao, Y., Xu, B., Zhu, G. and & Li, J., (2023). Cancer Nanomedicine: Emerging Strategies and Therapeutic Potentials. *Molecules*, 28(13), 5145.

Xu, S., Yu, L., Luo, X., Wang, M., Chen, G., Zhang, Q., Liu, W., Zhou, Z., Song, J., Jing, H. and & Huang, G., (2020). Manual acupuncture versus sham acupuncture and usual care for prophylaxis of episodic migraine without aura: multicentre, randomised clinical trial. *Bmj*, 368.

Yakubova, A. A., Mitusova, K. A., Darwish, A., Rogova, A., Ageev, E.I ., Brodskaia, A., Muslimov, A. R., Zyuzin, M. V. and & Timin, A. S., (2023). Calcium carbonate

nanoparticles tumor delivery for combined chemo-photodynamic therapy: Comparison of local and systemic administration. *Journal of Controlled Release*, 359, 400-414.

Yan, Y., Liu, Y., Li, T., Liang, Q., Thakur, A., Zhang, K., Liu, W., Xu, Z. and & Xu, Y., (2023). Functional roles of magnetic nanoparticles for the identification of metastatic lymph nodes in cancer patients. *Journal of Nanobiotechnology*, 21(1), 337.

Yang, L., Yong, L., Zhu, X., Feng, Y., Fu, Y., Kong, D., Lu, W. and & Zhou, T. Y., (2020). Disease progression model of 4T1 metastatic breast cancer. *Journal of pharmacokinetics and pharmacodynamics*, 47, 105-116.

Yang, M., Wan, Y., Jiang, X., Qi, X., Wang, L., Liu, Z., Song, X., Pan, L., Sun, W., Zhao, W. and & Huang, J., (2019). Electro-acupuncture promotes accumulation of paclitaxel by altering tumor microvasculature and microenvironment in breast cancer of mice. *Frontiers in oncology*, 9, 576.

Yang, N. N., Tan, C. X., Lin, L. L., Su, X. T., Li, Y. J., Qi, L. Y., Wang, Y., Yang, J. W. and & Liu, C. Z., (2022). Potential mechanisms of acupuncture for functional dyspepsia based on pathophysiology. *Frontiers in Neuroscience*, 15, 781215.

Yao, Y., Zhou, Y., Liu, L., Xu, Y., Chen, Q., Wang, Y., Wu, S., Deng, Y., Zhang, J. and & Shao, A., (2020). Nanoparticle-based drug delivery in cancer therapy and its role in overcoming drug resistance. *Frontiers in molecular biosciences*, 7, 193.

Yu, M. L., Wei, R. D., Zhang, T., Wang, J. M., Cheng, Y., Qin, F. F., Fu, S. P., Lu, Z. G. and & Lu, S. F., (2020). Electroacupuncture relieves pain and attenuates inflammation

progression through inducing IL-10 production in CFA-induced mice. *Inflammation*, 43, 1233-1245.

Yun, W. S., Kim, J., Lim, D. K., Kim, D. H., Jeon, S. I. and & Kim, K., (2023). Recent Studies and Progress in the Intratumoral Administration of Nano-Sized Drug Delivery Systems. *Nanomaterials*, 13(15), 2225.

Zeng, M., Guo, D., Fernández-Varo, G., Zhang, X., Fu, S., Ju, S., Yang, H., Liu, X., Wang, Y. C., Zeng, Y. and & Casals, G., (2022). The Integration of Nanomedicine with Traditional Chinese Medicine: Drug Delivery of Natural Products and Other Opportunities. *Molecular Pharmaceutics*, 20(2), 886-904.

Zhang, H., Han, G., & Litscher, G. (2019). Traditional acupuncture meets modern nanotechnology: Opportunities and perspectives. *Evidence-Based Complementary and Alternative Medicine*, 2019, Article 2019.

Zhang, R., Lao, L., Ren, K. and & Berman, B. M., (2014a). Mechanisms of acupuncture–electroacupuncture on persistent pain. *Anesthesiology*, 120(2), 482-503.

Zhang, Z. X., Cheng, Z. D., Li, C. R., Ke, A. J., Chen, J. L. and & Chen, Y. G., (2014b). Effects of acupuncture on distribution taxis of paclitaxel in mice with lung cancer. *Zhongguo Zhen jiu= Chinese Acupuncture & Moxibustion*, 34(12), 1208- 1213.

Summary

7.1. Introduction

Cancer is a complex global health challenge and traditional treatments such as chemotherapy have been instrumental in managing cancer. However, they are not without limitations. It is, therefore, crucial to explore innovative and effective treatment strategies. In this regard, medicinal plants, particularly those belonging to the Euphorbiaceae family, have shown promising anti-cancer properties. Additionally, the application of magnetic nanoparticles in nanotechnology has made targeted drug delivery possible, thereby minimizing adverse effects on healthy cells. When combined with Traditional Chinese Medicine (TCM), such as acupuncture, these approaches offer a comprehensive and well-rounded approach to cancer treatment. This study aimed to develop a system that leverages magnetic nanoparticles and acupuncture to treat breast cancer while addressing the limitations of conventional treatments. The study's objectives included screening plant extracts for anti-cancer activity, synthesizing metal nanoparticles and developing a targeted delivery system for *in vivo* testing.

7.2. Results summary

The comprehensive evaluation of sixteen Euphorbia plant species in Chapters 4, 5 and 6 illuminates their multifaceted potential in cancer therapy. In **Chapter 4** a comprehensive evaluation of sixteen Euphorbia plant species sheds light on their phytochemical profiles, cytotoxic activity and selectivity against breast cancer cell lines MCF-7 and MDA-MB231. The presence of secondary metabolites such as glycosides, triterpenoids, flavonoids, alkaloids, tannins and saponins across the species suggests their potential significance in cancer therapy. Cytotoxicity assays reveal varying degrees of inhibition among extracts, with

notable efficacy observed in certain species like *E. clavarioides*, *E. gorgonis* and *E. tirucalli* extracts. Additionally, certain extracts including those from *E. gorgonis*, *E. tirucalli* and *E. cooperi* demonstrate selectivity for the MDA-MB231 cell line, known for its aggressiveness as triple-negative breast cancer cells. The findings underscore the potential of Euphorbia plants as sources of bioactive compounds for breast cancer treatment.

The exploration of *Euphorbia tirucalli*'s potential in cancer therapy, detailed in **Chapter 5**, extends beyond its phytochemical composition to encompass its role in synthesizing metal nanoparticles with medicinal applications. Notably, successful synthesis of iron oxide (FeONPs), magnesium oxide (MgONPs) and cobalt nanoparticles (CoONPs) was achieved using *E. tirucalli* extracts. The involvement of plant secondary metabolites, particularly polyphenols, in the reduction of metal ions highlights *E. tirucalli*'s potential as a green synthesis agent. These nanoparticles exhibit distinct colour changes and characteristic absorption peaks, indicative of their formation and properties. Further characterization through techniques like FTIR, XRD, XPS, SEM, TEM and EDS provides insight into their composition, structure and morphology, highlighting their potential applications in various fields, including medicine and industry. Additionally, antiproliferative assays demonstrate moderate activity of *E. tirucalli* extracts against breast cancer cell lines MCF-7 and MDA-MB231, with selectivity for cancer cells.

Antiproliferative assays demonstrated moderate activity of *E. tirucalli* extracts against breast cancer cell lines MCF-7 and MDA-MB231, with selectivity for cancer cells. CoONPs exhibited heightened cell growth inhibition, while MgONPs showed minimal toxicity at lower concentrations. Unannealed FeO nanoparticles displayed potent activity against MCF-7 cells, highlighting their potential as therapeutic agents. Furthermore, the unannealed FeO nanoparticles and doxorubicin also showed similar activity against MDA-MB231 cell lines.

This comprehensive analysis underscores the multifaceted potential of *E. tirucalli* as both a source of bioactive compounds and a green synthesis agent for metal nanoparticles with biomedical applications. Further research should focus on optimizing synthesis processes and exploring the specific bioactive properties of synthesized nanoparticles for targeted cancer therapy.

In **Chapter 6**, the exploration expands to innovative treatment modalities like electroacupuncture combined with superparamagnetic iron oxide nanoparticles (SPIONs) for cancer therapy, particularly targeting triple-negative breast cancer (TNBC). The synergistic effects of electroacupuncture and SPIONs in slowing down TNBC proliferation within a mouse xenograft model offer promising results. Notably, acupuncture's potential to improve blood circulation within specific body parts complements enhanced drug delivery facilitated by SPIONs, showcasing a more precise and effective cancer treatment approach.

Experimental findings on cancer metastasis between the lung and liver reveal distinct treatment efficacy trends, with doxorubicin exhibiting greater effectiveness in preventing lung metastasis compared to the liver. The observed organ-specific patterns of breast cancer metastasis underscore the importance of considering organ-specific responses in treatment strategies.

7.3. Conclusion

This thesis delves into a multifaceted approach to cancer treatment by combining traditional acupuncture techniques with innovative nanoparticle-based drug delivery systems synthesized from *Euphorbia tirucalli*. The comprehensive review of medicinal properties of the *Euphorbia* species reveals the presence of diverse bioactive compounds with significant anti-cancer properties. Compounds such as triterpenoids, tannins, diterpene esters and sterols

show cytotoxic effects, offering potential avenues for cancer therapy. In this study, acupuncture techniques are integrated with synthesized iron nanoparticles to enhance drug delivery mechanisms. Acupuncture, known for its holistic benefits in pain management and overall well-being, complements the pharmacological approach, providing a holistic framework for cancer treatment. Additionally, the synthesis of iron nanoparticles from *E. tirucalli* offers environmentally sustainable therapeutic options, demonstrating promising cytotoxic effects against breast cancer cell lines. Furthermore, the evaluation of the SPIONs- EA technique demonstrates its efficacy in inhibiting tumor proliferation and metastasis in breast tumor mouse models. This integrated approach, combining acupuncture, nanoparticle- based drug delivery and traditional medicinal knowledge, represents a promising direction in cancer therapy. However, further optimization and research are needed to enhance efficacy and elucidate underlying mechanisms. Overall, this thesis highlights the importance of a multidisciplinary approach that combines traditional practices with modern innovations to address the complex challenges of cancer treatment. By integrating acupuncture techniques and nanoparticle-based drug delivery systems, this study offers new insights into potential synergies for enhancing therapeutic outcomes in cancer patients.

7.4. Limitations and recommended further research

- (i) In the evaluation of the *Euphorbia* plant species in Chapter 4, limitations were observed where selectivity for cancer cells was demonstrated by certain extracts. The mechanisms underlying this selectivity were not fully elucidated, suggesting a need for further investigation into the specific compounds responsible for this effect.

- (ii) In Chapter 5, limitations included the specific mechanisms driving the potent activity of FeO nanoparticles against MDA-MB231 cells, comparable to doxorubicin - this was not fully understood, highlighting the complexity of nanoparticle-cell interactions and the need for further mechanistic studies.
- (iii) Limitations were observed in the HPLC analysis of *Euphorbia* extracts. The complexity of phytochemical profiles across different plant species and within the same species poses challenges in accurately identifying and quantifying individual compounds.
- (iv) Additionally, the lack of comprehensive standards for all phytochemicals hinders identification and quantification. This was due to the fact that the standards were costly to obtain and even to synthesize, especially for the wide range of compounds present in complex plant extracts. Therefore, it was important to prioritize research objectives and budget constraints. Complementary techniques like MS or NMR spectroscopy may be necessary for definitive compound characterization.
- (v) Furthermore, the elucidation of compounds identified could not be analyzed due to a lack of funds to carry out the experiments.
- (vi) In Chapter 6, limitations were observed in the experimental results regarding the efficacy of treatments on cancer metastasis between different organs, particularly in the liver and lung. While doxorubicin exhibited greater efficacy in preventing lung metastasis compared to the liver, nanoparticles showed limited efficacy in both organs. This discrepancy in treatment outcomes highlights the complexity of organ-specific responses to therapeutic interventions and suggests the need for further research to elucidate the underlying mechanisms.

- (vii) The combination of electroacupuncture (EA) and superparamagnetic iron oxide nanoparticles (SPIONs) showed promising results in slowing down triple-negative breast cancer (TNBC) proliferation within a mouse xenograft model, however, the specific mechanisms driving this synergistic effect were not fully understood. Further studies are needed to elucidate the molecular pathways involved in the interaction between EA, SPIONs and cancer cells, as well as their effects on tumor growth and metastasis.
- (viii) Acupuncture's potential to improve blood circulation within specific body parts may contribute to increased drug concentration within tumors, however, the exact impact of acupuncture on drug delivery and distribution needs to be fully characterized. Addressing these limitations will be crucial for optimizing the therapeutic potential of EA and SPIONs in cancer treatment and improving their clinical applicability.

Animal Research Ethics

15-Jun-2020

Dear Miss Ipeleng Kgosiemang

Student Project Number: UFS-AED2019/0135

Project Title: Targeted delivery of iron nanoparticles incorporating anticancer constituents of Euphorbia species for treatment of breast cancer.

Department: External Affiliation

You are hereby kindly informed that, at the meeting held on 15-Jun-2020, the Interfaculty Animal Ethics Committee approved the above project.

Kindly take note of the following:

1.
A progress report with regard to the above study has to be submitted Annually and on completion of the project. Reports are submitted by logging in to RIMS and completing the report as described in SOP AEC007: Submission of Protocols, Modifications, Amendments, Reports and Reporting of Adverse Events which is available on the UFS intranet.
2.
Researchers that plan to make use of the Animal Experimentation Unit must ensure to request and receive a quotation from the Head, Mr. Seb Lamprecht.
3.
Fifty (50%) of the quoted amount is payable when you receive the letter of approval.

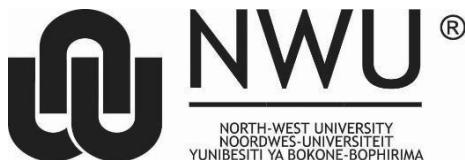
Yours Sincerely



Mr. Gerhard Johannes van Zyl

Chair: Animal Research Ethics Committee

Apply



Private Bag X1290, Potchefstroom
South Africa 2520

Tel: 086 016 9698
Web: <http://www.nwu.ac.za/>

North-West University Animal Care, Health and Safety Research Ethics Committee (NWU-AnimCareREC)

Tel: 018 299-1208
Email: Ethics-AnimCare@nwu.ac.za (for animal studies)

15 April 2023

ETHICS APPROVAL LETTER OF STUDY

Based on approval by the North-West University Animal Care, Health and Safety Research Ethics Committee (NWU-AnimCareREC) on 15/04/2023, the NWU-AnimCareREC hereby approves your study as indicated below. This implies that the NWU-AnimCareREC grants its permission that, provided the general conditions specified below are met and pending any other authorisation that may be necessary, the study may be initiated, using the ethics number below.

Study title: Targeted delivery of iron, cobalt and magnesium nanoparticles incorporating anticancer constituents of Euphorbia species for treatment of breast cancer																		
Principal Investigator/Study Supervisor/Researcher: Prof R Hayeshi																		
Student: -																		
Ethics number:	<table border="1"> <tr> <td>N</td><td>W</td><td>U</td><td>-</td><td>0</td><td>0</td><td>7</td><td>9</td><td>1</td><td>-</td><td>2</td><td>2</td><td>-</td><td>A</td><td>5</td> </tr> </table>			N	W	U	-	0	0	7	9	1	-	2	2	-	A	5
N	W	U	-	0	0	7	9	1	-	2	2	-	A	5				
	<i>Institution Study Number Year Status</i> <u>Status:</u> S = Submission; R = Re-Submission; P = Provisional Authorisation; A = Authorisation																	
Application Type: Single study	Risk:	<table border="1"> <tr> <td>Category 3</td> </tr> </table>		Category 3														
Category 3																		
Commencement date: 15/04/2023																		
Expiry date: 30/04/2024																		
Approval of the study is provided for a year, after which continuation of the study is dependent on receipt and review of an annual monitoring report and the concomitant issuing of a letter of continuation. A monitoring report is due at the end of April annually until completion of the study.																		

General conditions:

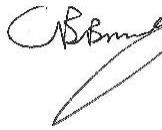
While this ethics approval is subject to all declarations, undertakings and agreements incorporated and signed in the application form, the following general terms and conditions will apply:

- *The principal investigator/study supervisor/researcher must report in the prescribed format to the NWU-AnimCareREC:*
 - *annually on the monitoring of the study, whereby a letter of continuation will be provided annually, and upon completion of the study; and*
 - *without any delay in case of any adverse event or incident (or any matter that interrupts sound ethical principles) during the course of the study.*
- *The approval applies strictly to the proposal as stipulated in the application form. Should any amendments to the proposal be deemed necessary during the course of the study, the principal investigator/study supervisor/researcher must apply for approval of these amendments at the NWU- AnimCareREC, prior to implementation. Should there be any deviations from the study proposal without the necessary approval of such amendments, the ethics approval is immediately and automatically forfeited.*
- *Annually a number of studies may be randomly selected for active monitoring.*

- *The date of approval indicates the first date that the study may be started.*
- *In the interest of ethical responsibility, the NWU-AnimCareREC reserves the right to:*
 - *request access to any information or data at any time during the course or after completion of the study;*
 - *to ask further questions, seek additional information, require further modification or monitor the conduct of your research or the informed consent process;*
 - *withdraw or postpone approval if:*
 - *any unethical principles or practices of the study are revealed or suspected;*
 - *it becomes apparent that any relevant information was withheld from the NWU-AnimCareREC or that information has been false or misrepresented;*
 - *submission of the annual monitoring report, the required amendments, or reporting of adverse events or incidents was not done in a timely manner and accurately; and/or*
 - *new institutional rules, national legislation or international conventions deem it necessary.*
- *NWU-AnimCareREC can be contacted for further information via Ethics-AnimCare@nwu.ac.za or 018 299 1208*

The NWU-AnimCareREC would like to remain at your service and wishes you well with your study. Please do not hesitate to contact the NWU-AnimCareREC for any further enquiries or requests for assistance.

Yours sincerely,



Digitally signed by
Christiaan B Brink
(Tiaan)
Date: 2023.04.18
10:44:34 +02'00'

Chairperson: NWU-AnimCareREC

Current details:(23239522) G:\My Drive\9. Research and Postgraduate Education\9.1.5.4 Templates\9.1.5.4.2_NWU-AC_EAL.docm 20
August 2019

File Reference: 9.1.5.4.2

Green synthesis of magnesium and cobalt oxide nanoparticles using *Euphorbia tirucalli*: Characterization and potential application for breast cancer inhibition

Ipeleng Kopano Kgosiemang^a , Relebohile Lefojane^a, Paballo Direko^a, Zandile Madlanga^a, Samson Mashele^a, and Mamello Sekhoacha^b

^aUnit for Drug Discovery Research, Department of Health Sciences, Faculty of Health and Environmental Sciences, Central University of Technology, Free State, Bloemfontein, South Africa; ^bDepartment of Pharmacology, Faculty of Health Sciences, University of the Free State, Bloemfontein, South Africa

ABSTRACT

The current study reports on the synthesis of magnesium oxide (MgONPs) and cobalt oxide (CoONPs) nanoparticles using the aerial parts of *Euphorbia tirucalli* and further characterizing and evaluating the anti-proliferative effect of the nanoparticles (NPs) on MCF-7 breast cancer cell line. The synthesized NPs were characterized using ultraviolet-visible spectrophotometry (UV-vis), Fourier-transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), energy-dispersive spectroscopy (EDS), X-ray photoelectron spectroscopy (XPS), scanning electron microscopy (SEM) and transmission electron microscopy (TEM). Anti-proliferative activity of *E. tirucalli* extracts and synthesized NPs was assessed using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. Formation of the NPs was confirmed by a color change from brown to black (MgONPs) and pink (CoONPs), respectively. Two UV absorbance peaks were observed at 305 and 508 nm⁻¹ confirming the formation of CoONPs. Traces of cobalt and oxygen as well as magnesium and oxygen were detected by EDS. The NPs sizes ranging between 1 µm and 100 nm was confirmed by TEM for both NPs. The hexane (Hex) and dichloromethane (DCM) extracts of *E. tirucalli* exhibited cell growth inhibition of MCF-7 cells with IC₅₀ values of 10 µg/ml; while CoONPs showed increased cell growth inhibition with an IC₅₀ values <10 µg/ml compared to the crude extracts. The current study contributes to green approach and biomedical application of CoONPs in the development of anti-proliferative agents for cancer.

ARTICLE HISTORY

Received 1 October 2019
Accepted 15 February 2020

KEYWORDS

Euphorbia tirucalli;
magnesium oxide
nanoparticles; cobalt oxide
nanoparticles; anti-
proliferation; breast cancer
MCF-7

Introduction

Green synthesis of nanoparticles utilizing plant extracts has been successfully used by researchers globally.^[1,2] This method is convenient, easy to use, and produces nanoparticles at larger scale compared to the known conventional preparation methods.^[3] *Euphorbia tirucalli* belonging to the Euphorbiaceae family, is a shrubby succulent with red, orange or yellow stems. *E. tirucalli* is used in traditional medicine to treat asthma, cough, earache, neuralgia, rheumatism, toothache, warts and muscle spasms.^[4–6] This plant species contains a latex which possess constituents such as proteins, carbohydrates, lipids, vitamins, resins and various phytochemicals, which are known to be responsible for its biological activity. The phytochemicals also catalyze the reduction of metal precursors into metal oxide nanoparticles, and stabilize the nanoparticles during the redox reaction.^[7–9]

Metal and metal oxide nanoparticles are multifunctional and are considered crucial in research due to their physiochemical and optoelectronic properties.^[10,11] Recently, the synthesis of CoONPs and MgONPs has attracted interest in

the biomedical field due to their application as antibacterial, antacid for heartburn, bone regeneration, drug delivery systems and cancer therapy.^[12–18] Among the known different inorganic metal oxides, MgONPs and CoONPs have some advantages: they are stable under extreme conditions, possess a higher adsorption ability, contain a large surface area, and have interesting physical and chemical properties.^[19] CoONPs have a size-dependent structure, energy storage, magnetic as well as catalytic properties.^[20,21] MgONPs on the other hand, are less toxic and relatively easy to obtain. They have been reported to induce apoptosis in cancer cells and are used as heating mediators for cancer therapy.^[22–24] Although studies have been carried out on MgONPs and CoONPs, not much research has been done on the toxicity of CoONPs toward breast cancer cell lines.

Breast cancer is the most invasive form of cancer among women and remains the leading cause of death in female cancer-related cases.^[25] More than 2 million new cases were reported in 2018 and approximately 626 679 woman died from breast cancer globally; while it claimed 4 690 lives in South Africa in 2018.^[26] These statistics emphasize the need

CONTACT Mamello Patience Sekhoacha  SekhoachaMP@ufs.ac.za  Department of Pharmacology, Faculty of Health Sciences, University of the Free State, PO Box 339, Bloemfontein 9300, South Africa.

 Supplemental data for this article can be accessed at the publisher's website.

© 2020 Taylor & Francis Group, LLC

Green synthesis of Iron oxide and Iron dioxide nanoparticles using *Euphorbia tirucalli*: characterization and antiproliferative evaluation against three breast cancer cell lines

Ipeleng Kopano Rosinah Kgosiemang^a, Ayodeji Mathias Adegoke^{b,c}, Samson Sitheni Mashele^a and Mamello Patience Sekhoacha^b

^aUnit for Drug Discovery Research, Department of Health Sciences, Faculty of Health and Environmental Sciences, Central University of Technology, Bloemfontein, South Africa; ^bDepartment of Pharmacology, Faculty of Health Sciences, University of the Free State, Bloemfontein, South Africa; ^cCancer Research and Molecular Biology Laboratories, Department of Biochemistry, College of Medicine, University of Ibadan, Ibadan, Nigeria

ABSTRACT

Researchers have become increasingly interested in nanoparticles made from plants because of their stability and large surface area. In the current study, iron oxide and iron dioxide nanoparticles were synthesized using aerial parts of the *E. tirucalli* as a reducing agent. The nanoparticles were analyzed using various techniques, including Ultraviolet-visible spectroscopy, Fourier Transform Infrared spectroscopy, X-ray diffractometer, X-ray photoelectron spectroscopy, X-ray energy dispersive spectroscopy, Scanning electron Microscopy, and Transmission Electron Microscopy. The nanoparticles were then investigated for their antiproliferative effect against MCF-7, SK-BR-3, MDA-MB231, and Vero cell lines. The results confirmed the formation of FeO and FeO₂ nanoparticles by color change and a UV absorbance peak between 220–390nm. EDS analysis showed traces of Fe and O, while TEM confirmed the nanoparticle size of 100nm. FTIR showed a peak at 514nm. The FeO-RT NPs demonstrated over 80% antiproliferative activity against the MCF-7 cell line at a concentration of 10µg/mL while doxorubicin, FeO-RT NPs, and DCM extract showed similar activity against the MDA-MB231 cell line at 10 and 1g/mL concentrations. However, Vero and SK-BR-3 cell lines showed decreased antiproliferative activity. This study highlights the environmentally friendly use and safe application of iron oxide NPs in cancer therapy.

ARTICLE HISTORY

Received 28 August 2023
Accepted 23 October 2023

KEYWORDS

Euphorbia tirucalli; iron oxide NPs; iron dioxide NPs; antiproliferation; breast cancer cells

1. Introduction

Cancer is a significant public health concern worldwide, ranking second only to cardiovascular disease as a leading cause of death [1]. Breast cancer is now the most commonly

CONTACT Mamello Sekhoacha  SekhoachaMP@ufs.ac.za  Department of Pharmacology, Faculty of Health Sciences, University of the Free State (UFS), Bloemfontein, Free State, South Africa.

 Supplemental data for this article can be accessed online at <https://doi.org/10.1080/17458080.2023.2276276>.

© 2023 The Author(s). Published by Informa UK Limited, trading as Taylor & Francis Group.

This is an Open Access article distributed under the terms of the Creative Commons Attribution-NonCommercial License (<http://creativecommons.org/licenses/by-nc/4.0/>), which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited. The terms on which this article has been published allow the posting of the Accepted Manuscript in a repository by the author(s) or with their consent.