MEDICINAL PROPERTIES OF *HERMANNIA DEPRESSA* N.E.Br.

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BLOEMFONTEIN
September 2025

DECLARATION OF INDEPENDENT WORK

DECLARATION WITH REGARD TO INDEPENDENT WORK

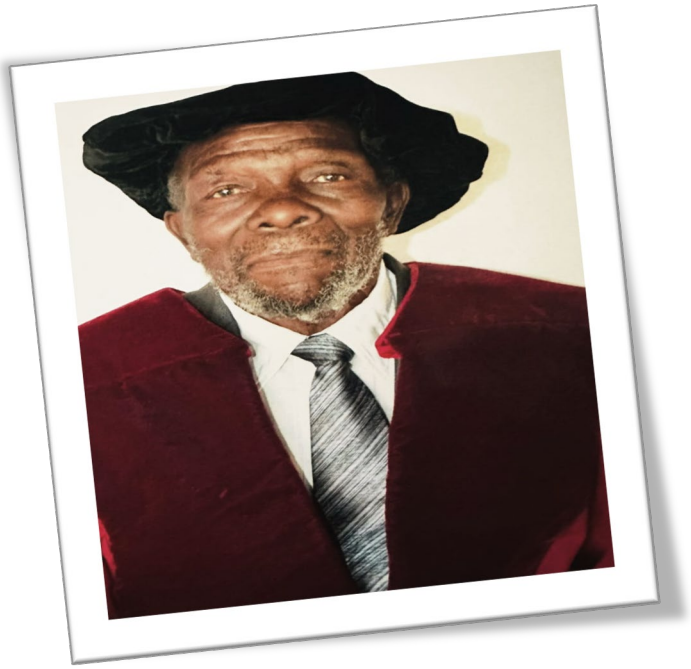
I, **MFUNDISI NHLAPO**, identity number _____ and student number _____, do hereby declare that this research project submitted to the Central University of Technology, Free State for the Degree MASTER OF HEALTH SCIENCES IN BIOMEDICAL TECHNOLOGY, is my own independent work; and complies with the Code of Academic Integrity, as well as other relevant policies, procedures, rules and regulations of the Central University of Technology, Free State; and has not been submitted before to any institution by myself of any other person in fulfilment (or partial fulfilment) of the requirements for the attainment of any qualification.

_____ **26th September 2025**

SIGNATURE OF STUDENT **DATE**

DEDICATION

I dedicate this work to the memory of my father, Dr. (honoris causa) Abel N. Nhlapo. I am eternally grateful for your unwavering support, your love, wisdom, and belief in me. This accomplishment is a testament to the values you instilled in me: perseverance, courage, and relentless pursuit of knowledge. Rest in Peace.



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SUMMARY

Microbial infections, inflammation, and oxidative stress constitute a pathological triad that is challenging to manage effectively, resulting in millions of mortalities and morbidities while burdening healthcare systems and declining economic growth globally. This research aimed to investigate the medicinal properties of *Hermannia depressa* leaves and stems and to validate their use in ethnomedicine to treat various ailments. Acetone, methanol, and hot water extracts of *H. depressa* were used in the study. The broth microdilution method was used to determine antimicrobial activity against bacterial and fungal pathogens. Scanning and Transmission electron microscopy (SEM and TEM) were used to observe ultrastructural changes in microbial cells following extract treatment. Anti-inflammatory activity was evaluated through nitric oxide inhibition assay in lipopolysaccharide (LPS)-stimulated RAW 264.7 macrophages. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay and the ferric reducing antioxidant power (FRAP) assay were used to measure the antioxidant potential of the extracts. Cytotoxicity was assessed using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay on Vero and RAW 264.7 cells. Phytochemical characterisation was conducted through gas chromatography-mass spectrometry (GC–MS), with compound identification based on the Wiley and National Institute of Standards and Technology (NIST) spectral databases. After maceration, the methanol extract showed the highest extraction yield (29%), followed by the acetone extract (26%) and the aqueous extract (6.28%). Antimicrobial evaluation revealed potent activity against certain pathogenic strains, particularly against *Candida albicans* and *Staphylococcus aureus*, for both acetone (MIC = 0.31 mg/mL) and methanol (MIC = 0.63 mg/mL) extracts. The aqueous extract inhibited *S. aureus* (MIC = 2.5 mg/mL) and *Streptococcus pyogenes* (MIC = 2.5 mg/mL), showing weaker activity. The aqueous extract demonstrated the highest selectivity (SI = 37.2) for Vero and RAW 264.7 cells against *S. aureus* and *S. pyogenes*. Acetone extracts also showed selective activity (SI = 2.1 against *C. albicans* and SI = 1.0 against S. aureus), while methanol extracts exhibited moderate selectivity (SI = 2.2

against *C. albicans* and SI = 1.1 against *S. aureus*). Scanning and transmission electron microscopy studies of the effects of acetone extracts *of H. depressa* on *C. albicans* cells confirmed morphological changes, including microbial damage to cellular structures such as the cell membranes and cell wall, as well as possible disruption of reproductive processes. The anti-inflammatory assessment revealed potent dose-dependent inhibition of nitric oxide (NO) production (89% at 250 µg/mL) by the acetone extract, whereas the aqueous and methanol extracts showed insignificant NO inhibition. The anti-inflammatory specificity was promising for the acetone extracts (SI >1), suggesting that the anti-inflammatory activity was likely due to specific NO inhibition rather than cytotoxicity. *H. depressa* extracts showed weak FRAP antioxidant results (Trolox equivalents <1) but potent DPPH activity (78% - 100% DPPH free radical scavenging across all extracts), indicating pathway-dependent antioxidant effects. The aqueous and methanol extracts showed no significant toxicity against Vero and RAW 264.7 cell lines, while the acetone extracts exhibited notable cytotoxicity against RAW 264.7 cells and no significant toxicity against Vero cells. The acetone and methanol extracts contained a diverse array of bioactive compounds after GC-MS analysis, including terpenoids (neophytadiene, phytol, trans-β-ionone), phenolic compounds (scopoletin, 2,4-di-tert-butylphenol), and other pharmacologically active compounds, such as benzothiazole and Vitamin E, and these phytochemicals are likely responsible for the biological activities observed in *H. depressa*. The results of this study demonstrate the therapeutic potential of *H. depressa* in managing diseases associated with microbial infections, chronic inflammation, and oxidative stress. The cytotoxicity results highlight the suitability of using aerial parts of *H. depressa* over roots in the preparation of traditional remedies, a practice that would likely enhance safety in the medicinal use of the plant. Furthermore, the aerial parts of *H. depressa* contain valuable bioactive compounds with significant antimicrobial, anti-inflammatory, and antioxidant properties, which can provide essential leads to the discovery of novel therapeutic agents. Therefore, rigorous scientific research involving the isolation and elucidation of pure compounds, in vivo biosafety studies, and standardised product development are recommended.

RESEARCH OUTPUTS

Publications

- Nhlapo M, Ngobeni B, Manduna I. A Review: Medicinal Uses, Phytochemistry and Pharmacological Properties of Plants from the Hermannia Genus. *Pharmacognosy Journal*. 2025;17(3):384-393. DOI: 10.5530/pj.2025.17.48

- Ngobeni B, Nhlapo M, Manduna IT. In-vitro assessment of the antimicrobial and ultrastructural effects induced by *Hermannia depressa* extracts on microbial cells. (Submitted for publication at the South African Journal of Botany)

Conference Presentations

- Nhlapo M, Ngobeni B, Manduna IT. In-vitro assessment of the antimicrobial and ultrastructural effects induced by *Hermannia depressa* extracts on microbial cells. The 9th International Conference of the Society for Medicinal Plants and Economic Development Conference, Johannesburg, South Africa, 26-28 August 2025.

ABBREVIATIONS AND SIGNS

%	Percent
OH^-	hydroxyl radical
=	Equal to
>	Greater than
°C	Degree Celsius
µg/mL	micrograms per millilitre
AG+	Aminoguanidine
ATCC	American Type Culture Collection
cells/mL	Cells per millilitre
CFU	Colony Forming Unit
CFU/mL	Colony Forming Unit per millimetre
CO_2	Carbon dioxide
COPD	Chronic obstructive pulmonary disease
DMSO	dimethylsulfoxide
DPPH	2,2-Diphenyl-1-Picrylhydrazyl
FRAP	Ferric Reducing Antioxidant Power
g	Gram
GC-MS	Gas-chromatography Mass-spectrometry
H_2O_2	hydrogen peroxide

HIV/AIDS	Human Immunodeficiency Virus and Acquired Immunodeficiency Syndrome
HPLC	High Performance Liquid Chromatography
IC_{50}	Half Maximal Inhibitory Concentration
INT	Iodonitrotetrazolium chloride
LC-MS/MS	Liquid Chromatography Tandem Mass Spectrometry
LPS	Lipopolysaccharide
mg/mL	Milligrams per millilitre
MHA	Mueller Hinton Agar
MHB	Mueller Hinton Broth
MIC	Minimum inhibitory concentration
ml	millimetre
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NED	N-1-naphthyl ethylenediamine dihydrochloride
NIST	National Institute of Standards and Technology
nm	nanometre
NO	Nitric oxide
NSAIDs	non-steroidal anti-inflammatory drugs
O^{-}_{2}	superoxide anion
$ONOO^{-}$	peroxynitrite

RNS	Reactive nitrogen species
ROS	Reactive oxygen species
rpm	Rotations per minute
RPMI	Roswell Park Memorial Institute
SEM	Scanning Electron Microscopy
SI	Selectivity index
TEM	Transmission Electron Microscopy
TLC	Thin-layer chromatography
TPTZ	2,4,6-tripyridyl-s-triazine
Trolox	6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid
VLC	Vacuum Liquid Chromatography
WHO	World Health Organisation
µL	microliter
µM	micrometres

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CHAPTER ONE

INTRODUCTION

1.1. Study Overview

Microbial infections are still a significant cause of mortalities and morbidities, especially in developing nations. Approximately 3.83 million deaths were caused by microbial infections in the World Health Organisation (WHO) African region in 2019 (Sartorius *et al.,* 2024). Bacteria, fungi, viruses, and parasites cause microbial infections and illicit immune responses. Inflammation is the body's immune response to invasion and injury caused by pathogens and other disorders, aimed at repairing damage. In this process, immune cells are activated to release proinflammatory mediators that alleviate infections and restore tissue homeostasis (Iwalewa *et al.,* 2007). Persistent microbial infections lead to prolonged inflammation, resulting in pathological tissue damage due to excessive immune cell infiltration and the continuous release of toxic chemical mediators, such as reactive oxygen species (ROS), which leads to oxidative stress. Oxidative stress is a condition where the production of ROS exceeds the body's ability to scavenge them through physiological antioxidants (Forman and Zhang, 2021). Oxidative stress exacerbates cellular injury, perpetuates inflammatory cycles, and is linked to the progression of chronic diseases such as cardiovascular disorders, neurodegenerative conditions or cancer (Pooja *et al.,* 2025). The coexistence of infection, inflammation, and oxidative stress creates a pathological triad that is difficult to manage effectively, broadening the scope of therapeutic interventions required.

The current treatment of microbial infections, inflammation, and oxidant stress is primarily achieved through synthetic drugs. Synthetic medical drugs are often formulated to be potent and produce quick effects; however, while these have proven efficacy, they

present notable challenges and limitations in the long run. Antibiotic resistance, driven by the overuse and misuse of antimicrobial agents, has begun to render some pathogens untreatable, causing a global health crisis (Lewis, 2013; Workowski *et al.*, 2021). Anti-inflammatory drugs such as non-steroidal anti-inflammatory drugs (NSAIDs) and corticosteroids are linked with serious adverse effects, including gastrointestinal bleeding, immunosuppression, and cardiovascular risks (Rahim *et al.*, 2021). Similarly, antioxidant drugs such as N-acetylcysteine or sulforaphane have limitations, including reduced effectiveness when oxidative stress is secondary to an underlying condition, low bioavailability, and potential risk of carcinogenicity (Forman and Zhang, 2021). Furthermore, other limitations include the inaccessibility of healthcare facilities to rural areas, high costs and general distrust of modern medicine, especially by indigenous populations (Schmidt-Sane *et al.*, 2023). These shortcomings underline the pressing need for alternative or complementary therapies that are effective, safe, and sustainable.

Medicinal plants have been used for centuries worldwide to treat diseases, offering a promising solution to the shortcomings associated with conventional synthetic drugs. In developing countries, such as South Africa, nearly 80% of the people depend on traditional medicine as their primary form of healthcare (Ouoba et al., 2022; Oyebode et al., 2016; Tesfahuneygnand Gebreegziabher, 2019). Moreover, over 115 South African plants are traditionally known to treat various infections, inflammatory disorders and oxidative stress (Iwalewa *et al.*, 2007). Medicinal plants are mainly utilised because they are effective, readily available, cheap and deemed to be healthier or safer than synthetic drugs (Hosseinzadeh *et al.*, 2015). The medicinal effects of plants are primarily ascribed to their phytochemical content, which comprises flavonoids, tannins, curcumin, saponins, terpenoids, alkaloids, and many other compounds (Adebayo *et al.,* 2015). Phytochemicals exhibit potent pharmacological properties, including antimicrobial, anti-inflammatory, antioxidant, and anticancer activities, and often act through multiple mechanisms, thereby enhancing their therapeutic potency. Moreover, medicinal plants

with notable therapeutic properties include *Artemisia annua* (wormwood) yields artemisinin, a powerful antimalarial with antimicrobial properties; *Catharanthus roseus* (rosy periwinkle) provides vinblastine and vincristine, which have anti-inflammatory and anticancer effects; and *Taxus brevifolia* (Pacific yew) is the source of paclitaxel, known for its antioxidant and anticancer activities (Davis and Choisy, 2024). These discoveries validate medicinal plants as an inexhaustible source of potent pharmacological compounds in the ongoing search for novel drugs to treat infections, inflammation, and oxidative stress.

According to ethnobotanical surveys, *Hermannia depressa* is a significant plant in Southern African traditional medicine, with diverse therapeutic applications among indigenous communities, including the amaZulu, Basotho and amaXhosa. It is an important traditional medicine for respiratory ailments, such as tuberculosis and persistent coughs, gastrointestinal disorders, including stomachaches and diarrhoea, and microbial infections like gonorrhoea (Hlongwane, 2008; Molefe, 2013; Ngobeni *et al.*, 2023, 2024; Nhlapo *et al.,* 2025; Reid *et al.,* 2005). This multifaceted use in ethnomedicine has urged scientific interest in *H. depressa*, leading to extensive phytochemical and pharmacological investigations aimed at validating its traditional effectiveness. Phytochemical profiling of *H. depressa* has revealed a diverse range of bioactive compounds that contribute to its medicinal properties. The identified phytochemicals comprise Tannins, flavonoids, phenolics, fatty acids, and alkaloids, all of which contribute to its antioxidant, anti-inflammatory, antimicrobial, and wound-healing properties (Ngobeni *et al.,* 2024; Reid *et al.,* 2005). The phytochemical analysis of *H. depressa* root extracts through LC-MS/MS provided critical insight into the bioactive compounds found in this plant (Ngobeni *et al.,* 2024). The presence of phytochemicals supports the ethnopharmacological relevance of *H. depressa* and highlights its potential value in disease treatment, potentially providing significant leads in the discovery of novel drugs.

1.2. Research Objectives

1.2.1. Problem Statement

A WHO study estimated about 3.83 million deaths from infections in Africa alone (Sartorius et al., 2024). The situation is further worsened by the increasing number of immunosuppressed individuals, due to human immunodeficiency virus and autoimmune deficiency syndrome (HIV & AIDS), as well as chemotherapy for cancer treatment, which heightens the risk of opportunistic infections. Furthermore, inflammation and oxidative stress are associated with the progression of many life-threatening disorders that burden healthcare systems around the globe, such as chronic infections, degenerative diseases such as cancer, cardiovascular disorders, and neurodegenerative conditions (Iwalewa *et al.,* 2007). Additionally, adverse drug reactions are a major concern; a study at a South African hospital discovered that 16% of all mortalities were attributable to adverse drug effects (Mouton *et al.*, 2015).

The modern healthcare system plays a significant role in disease prevention, but also has critical limitations, such as inaccessibility of healthcare facilities to remote areas in developing countries, as well as high costs of medical care (Lewis, 2013; Workowski *et al.,* 2021). The gradual increase in pathogens with antimicrobial resistance, due to inappropriate antibiotic use, has made infections that were once easily treatable more challenging to curb and, therefore, heightened the threat of death from microbial infections. Additionally, synthetic antibiotics can disrupt the balance in gut microbiota, causing diarrhoea, yeast infections, or secondary infections, such as *Clostridium difficile*. Common adverse effects of anti-inflammatory and antioxidant drugs include gastrointestinal ulcers from NSAIDs, immune suppression from corticosteroids, and kidney or liver toxicity, ineffectiveness due to poor bioavailability, and carcinogenic potential (Forman and Zhang, 2021; Tai and McAlindon, 2021).

Plant medicines are presumed to have a gentler effect and support the body's natural healing processes (Hassen *et al.,* 2013). Developing countries, such as South Africa, still have a notable fraction of their population, estimated at 80%, who rely on ethnomedicine primarily for their healthcare owing to ready availability, low cost; the assumption that they are natural and therefore safe as well as deep rooted belief and trust in traditional medicine (Nixon, 2022; Oyebode *et al.,* 2016; WHO, 2013). Medicinal plants used in ethnobotany are an inexhaustible potential source of therapeutic agents, especially those with novel mechanisms of action and excellent biosafety profiles. Therefore, this study will evaluate and document the medicinal properties of the leaf and stem extracts of *H. depressa*.

1.2.2. Aim of Research

To scientifically evaluate the medicinal properties of *Hermannia depressa* to confirm its efficacy in the treatment of various diseases in ethnomedicine.

1.2.3. Objectives

- To evaluate the antimicrobial potential of *H. depressa* leaf and stem extracts using the broth microdilution method.
- To investigate the anti-inflammatory potential of *H. depressa* leaf and stem extracts using the *in vitro* nitric oxide inhibition assay.
- To evaluate the antioxidant activity of *H. depressa* leaf and stem extracts using *in vitro* Ferric Reducing Antioxidant Power (FRAP) and 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) assays.
- To evaluate the biosafety of *H. depressa* leaf and stem extracts employing the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay.
- To identify the phytochemicals that are present in *H. depressa* leaf and stem extracts through Gas Chromatographic Mass Spectrometry (GC-MS).

1.3. Research Layout

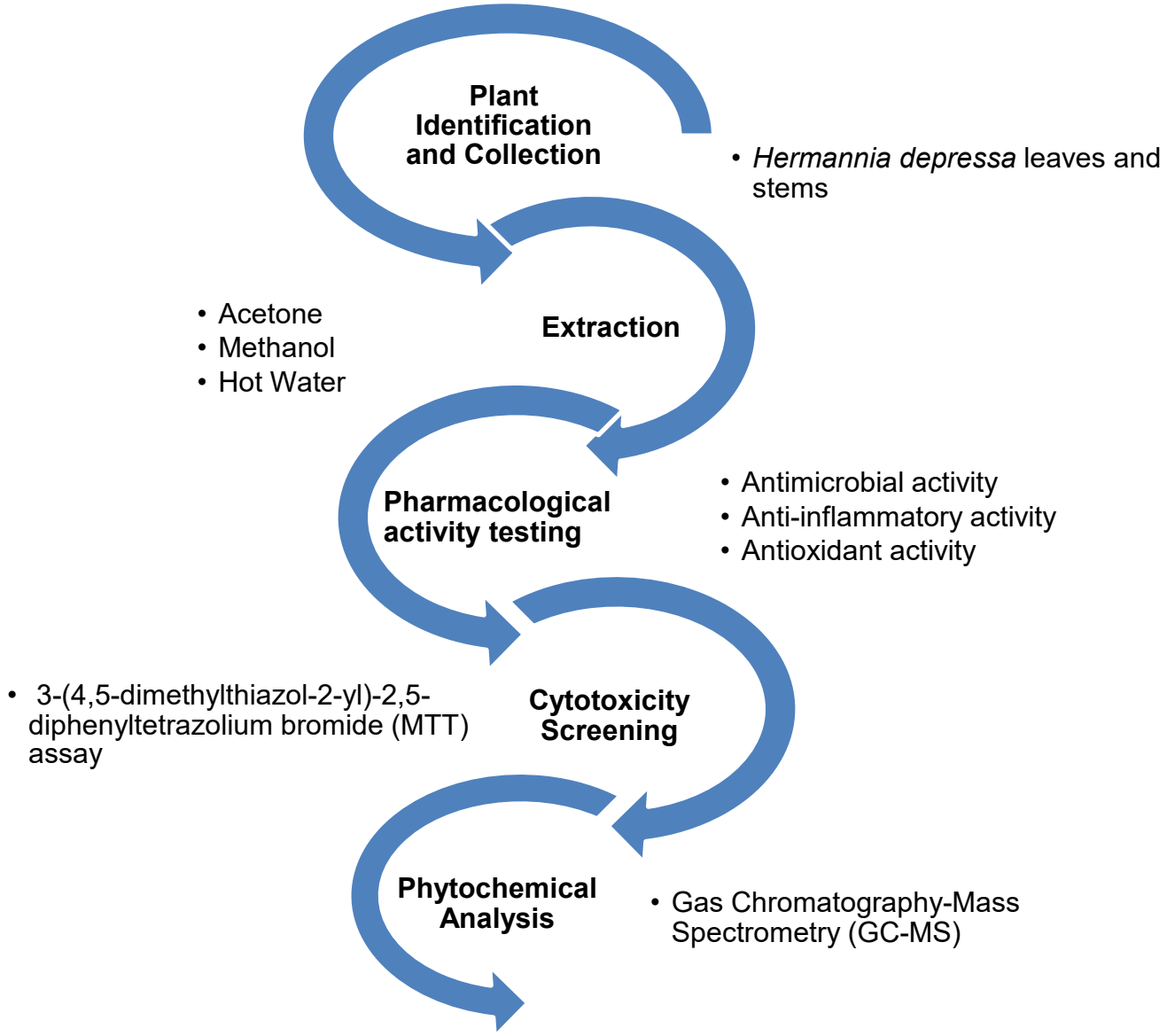

Figure 1. 1: Summary of the methods utilised to achieve the aim of this study

1.4. Overview of Chapters

This study achieved its objectives through a structured approach, starting with **Chapter Two**, which reviewed the literature around the main pathological drivers of disease and limitations of current treatments, as well as *H. depressa* as a potential novel therapeutic candidate. This review identified critical knowledge gaps and substantiated the need for further research. The experimental phase included antimicrobial activity and ultrastructural analyses (**Chapter Three**); evaluation of anti-inflammatory and antioxidant activities (**Chapter Four**); in-vitro biotoxicity assessment (**Chapter Five**); phytochemical profiling (**Chapter Six** and Lastly, **Chapter Seven** integrates key findings, outlines study limitations, and provides future research directions, offering a comprehensive understanding of *H. depressa*'s potential as a novel therapeutic agent.

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CHAPTER TWO

LITERATURE REVIEW

2.1. The interlinked burden of major pathophysiological drivers of chronic diseases: Microbial Infections, Inflammation, and Oxidative Stress

Globally, the burden of infectious diseases is still immense and continues to cause millions of deaths each year. The death toll due to infectious diseases is estimated at 18.7 million deaths worldwide (Bloom, 2025). Approximately 14.7 million deaths were recorded in developing countries, with Sub-Saharan Africa alone reporting 6.8 million deaths, highlighting the high prevalence of mortality in resource-limited countries due to a lack of healthcare infrastructure (Luo, 2024; Zhang *et al.,* 2024). The infectious diseases with the most prevalent mortalities include lower respiratory tract infections (3.4 million deaths), HIV / AIDS (2.7 million deaths), gastrointestinal tract infections (1.8 million deaths), tuberculosis (1.6 million deaths), malaria (1.1 million deaths) and fungal infections (3.8 million deaths), which cumulatively have a critical impact on the death toll in the world. (Avan *et al.,* 2019; Bloom, 2025; Michaud, 2009; Ouoba *et al.,* 2022).

Chronic inflammation and oxidative stress contribute significantly to the global burden of disease by developing as direct consequences of infections or acting as primary causes of many non-communicable conditions. Inflammation-related chronic diseases are even more prominent causes of global mortality, including diseases such as diabetes, which is responsible for 4 million deaths (Chew *et al.,* 2023). Hypertension, a key inflammatory driver, contributes to over 8.5 million deaths, and cardiovascular diseases alone accounted for 20.5 million deaths in 2021 (Luo, 2024). Immune-mediated diseases, including asthma, inflammatory bowel disease, psoriasis, and rheumatoid arthritis, add further to disability-adjusted life years, with their increased global prevalence.

Oxidative stress–mediated conditions interlink significantly with inflammatory disorders and act as underlying mechanisms across multiple chronic diseases. These processes are strongly implicated in cardiovascular disease, diabetes, obesity, chronic kidney disease, and neurodegenerative disorders, conditions that collectively cause over 20 million deaths annually worldwide (Seyedsadjadi and Grant, 2020). For example, oxidative stress accelerates endothelial dysfunction in cardiovascular disease, amplifies complications in diabetes, and contributes to progressive neuronal loss in Parkinson's disease and Alzheimer's (Pooja *et al.,* 2025). Through these pathways, oxidative stress not only drives premature mortality but also sustains long-term disability and socioeconomic burden.

The economic burden of health challenges is overwhelming, especially in Africa, where it is estimated to have cost 3.1 trillion South African Rands in lost income in 2015 (Li *et al.,* 2024). This burden falls heavily on low-income countries, where diseases account for over 23% of total disability-adjusted life years, compared to just 2% in high-income nations, highlighting the significant health equity gap between developed and developing countries (Niohuru, 2023). Moreover, socioeconomic conditions such as lack of healthcare resources, poor sanitation, and malnutrition substantially worsen the effects of disease. This creates a vicious cycle where illness perpetuates poverty. Disease inflicts substantial socioeconomic costs, including an increase in disability-adjusted life years due to chronic disease, premature deaths, reduced productivity, and rising healthcare costs that exhaust public finances. Furthermore, disease outbreaks weaken tourism, delay foreign investment, and disrupt supply chains, ultimately leading to a decline in economic growth (Pizzino *et al.,* 2017; Santos *et al.,* 2024). These interlinked major pathophysiological drivers of chronic diseases result in increased dependency, immense pressure on public resources, and a fundamental burden on human development and economic stability worldwide, especially in Africa.

2.2. The pathophysiological interrelation between Microbial Infections, Inflammation, and Oxidative Stress in perpetuating chronic diseases

Infections occur when pathogenic microorganisms, such as bacteria, fungi, viruses, and protozoa, enter the human body and disrupt its physiology (Iwalewa *et al.,* 2007). Infections trigger inflammation, a crucial process that aims to eliminate pathogens and facilitate tissue repair. Inflammation is characterised by the release of proinflammatory mediators from activated immune cells, such as reactive oxygen species (ROS), nitric oxide, cytokines, prostaglandins, and leukotrienes (Forman and Zhang, 2021). Prolonged inflammation leads to oxidative stress, especially when pathogens persist in a latent or chronic state, leading to overproduction of ROS. Oxidative stress occurs when the production of ROS and other reactive proinflammatory mediators exceeds the capacity of physiological antioxidants to neutralise or adequately repair the resulting tissue damage. ROS include free radicals like superoxide anion (O^{-}_{2}) or hydroxyl radical (OH^{-}), and non-radical derivatives such as hydrogen peroxide (H_2O_2) and reactive nitrogen species (RNS) like peroxynitrite ($ONOO^{-}$), all of which have the potential to injure lipids, proteins, and nucleic acids through oxidation or nitration reactions. Oxidative stress worsens tissue damage and leads to ongoing inflammation, contributing to the progression of chronic diseases such as cancer, cardiovascular disorders, and neurodegenerative conditions (Iwalewa *et al.,* 2007; Pooja *et al.,* 2025). Infection, inflammation, and oxidative stress form a self-perpetuating pathological loop that persists for years and worsens progressively, even when the original infection is controlled or resolved. This triad is challenging to treat effectively, broadening the scope of therapeutic interventions required.

2.3. Drawbacks associated with the conventional treatment of infections, inflammation and oxidative stress with synthetic drugs

Conventional synthetic drugs used to combat infections, inflammation, and oxidative stress have significant drawbacks. Antimicrobials such as vancomycin or ceftriaxone are increasingly undermined by antimicrobial resistance (e.g., Methicillin-Resistant *Staphylococcus aureus*, or super gonorrhoea), poor penetration into biofilms or intracellular pathogens, and collateral microbiome disruption leading to complications such as *Clostridium difficile* infection (CDC, 2019). Anti-inflammatory agents reduce symptoms but often fail to break chronic inflammation cycles. Nonsteroidal anti-inflammatory drugs (NSAIDs) cause gastrointestinal bleeding, kidney damage, and immunosuppression. Glucocorticoids also lead to immunosuppression that increases infection and metabolic risks, and inhaled corticosteroids in Chronic obstructive pulmonary disease (COPD) are associated with the risk of raising pneumonia susceptibility (Pizzino et *al.,* 2017). Antioxidant therapies like N-acetylcysteine offer temporary ROS neutralisation, have poor bioavailability and limited efficacy in established oxidative damage, as shown by inconsistent clinical outcomes in COPD and other oxidative stress–linked diseases (Forman and Zhang, 2021; Pinto *et al.,* 2020). Collectively, these limitations highlight that conventional monotherapies often suppress only one arm of the infection–inflammation–oxidative stress triad, leaving the cycle unresolved and highlight the need for continuous search for diverse therapeutic agents with novel mechanisms of action.

Many synthetic conventional drugs, especially newer, specialised medicines like biological agents, have high costs that create significant socioeconomic disadvantages, worsening health inequities. These costs significantly increase national pharmaceutical expenditures, forcing healthcare systems to make strict reimbursement decisions that can limit, delay, or deny access, particularly in socialised systems with universal coverage.

The limited availability of these medicines in the public sector, a well-documented problem across Africa, where median availability of essential drugs can be as low as 40%, shifts the financial burden to patients (Wahlster *et al.,* 2015; Yenet *et al.,* 2023). As a result, high out-of-pocket costs for these medicines can be devastating, consuming a large portion of household spending and deepening poverty cycles when illness reduces income and disrupts education (Yenet *et al.,* 2023). This ultimately leads to worse health outcomes for socioeconomically disadvantaged populations and places immense strain on both family finances and fragile healthcare systems.

2.4. Pharmacognosy: A potential alternative solution in addressing disease treatment concerns in modern healthcare

2.4.1. Background: Historical review of medicinal plant use and ethnomedicinal foundations to pharmacognosy

The historical use of medicinal plants dates to prehistoric times, when early human societies relied on practical observation to identify and use flora for treating diseases. Ancient civilisations laid the groundwork for therapeutic practices seen across Mesopotamian, Egyptian, Indian, Chinese, Greek, Roman, and African cultures (Haider., 2023). Furthermore, traditional medical systems used local flora and were deeply connected with social and spiritual beliefs, helping to preserve indigenous knowledge of medicinal plants across generations (Jamshidi-Kia *et al.,* 2018). As this ancient knowledge was maintained, expanded, and passed down to younger generations, it contributed to the gradual development of structured medical texts and systems.

Sumerians documented therapeutic species such as poppy and mandrake. In ancient China (c. 2500 BC), 365 plant remedies were recorded, including ginseng and ephedra. Egyptians catalogued hundreds of plant-based treatments, including garlic and aloe, in

the “Ebers Papyrus” around 1550 BC. In the Greco-Roman period, figures such as Hippocrates, Theophrastus, Dioscorides, and Galen advanced the classification and clinical application of medicinal plants by compiling the “*De Materia Medica*”, which served as a primary pharmacological reference for centuries. During the Middle Ages, monastic gardens preserved herbal medicines, and Arab scholars, such as Avicenna, expanded medical texts (Dias *et al.,* 2012; Jamshidi-Kia *et al.,* 2018; Kovalyova *et al.,* 2024). The Age of Exploration introduced New World botanicals to Europe, enriching the Materia medica. The Scientific Revolution in the 16th and 17th centuries promoted a shift towards chemical extraction and formal taxonomy, with Paracelsus emphasising active principles and Linnaeus establishing a systematic naming system. The 19th century saw the isolation of pure compounds, such as morphine from *Papaver somniferum* and quinine from *Cinchona officinalis*, which led to a widespread preference for antibiotics and chemically synthesised single-compound drugs due to the perception that they are more potent and easier to standardise and prescribe (Haider., 2023; Zunic *et al.,* 2017).

In the late 19th and early 20th centuries, pharmacognosy declined, dismissed mainly due to the lack of scientific standardisation in traditional medicine, which also relied on rituals, witchcraft, mysticism, and non-rational practices that could not be scientifically validated (Jamshidi-Kia *et al.,* 2018; Newman and Cragg, 2007). However, since the 1980s, the field has regained importance, driven by renewed global interest in natural products as an inexhaustible reservoir of pharmacological compounds for developing novel drugs; increased respect for indigenous knowledge, and transformative advances in analytical and molecular research (Ferreira *et al.,* 2014). Furthermore, traditional indigenous healing systems, known as ethnomedicine, have gained recognition, including from the World Health Organisation (WHO, 2013; Zunic *et al.,* 2017). Ethnomedicine today provides a crucial basis for systematically exploring biodiversity for new pharmacologically active compounds, a process called bioprospecting. This process directly contributes to modern pharmacognosy (Zunic *et al.,* 2017). Traditional knowledge systems influence plant

selection, preparation methods, and combinations for synergistic effects, forming the foundation of hypothesis-driven drug discovery in pharmacognosy. For instance, the traditional use of *Digitalis purpurea* for heart conditions ultimately led to the isolation of digitoxin, while *Artemisia annua*, used in Traditional Chinese Medicine, produced the potent antimalarial compound artemisinin (Haider, 2023). Such examples demonstrate how ethnobotanical heritage can inform contemporary pharmacology.

Pharmacognosy, a branch of pharmacology, explores the connection between traditional medicinal knowledge and modern scientific research (Kovalyova *et al.,* 2024). Methods in ethnopharmacology often prioritise holistic effects and long-term safety, which are sometimes overlooked in the development of synthetic drugs. Merging this knowledge with robust scientific validation, the therapeutic potential of natural products can be boosted, thereby helping to preserve ethnomedicine. However, the growing interest in ethnobotanical resources has also raised significant ethical concerns, particularly regarding bioprospecting, benefit-sharing, intellectual property rights, and ensuring the conservation and sustainability of medicinal plants (Cahlíková *et al.,* 2020; Elufioye and Badal, 2017). Extracting medicinal knowledge from indigenous communities without proper compensation or recognition is a form of exploitation. This highlights the need for ethical frameworks that promote fair collaboration, transparency, and recognition of the source communities. Initiatives to establish a collaborative platform for integrating ethnopharmacological knowledge with modern healthcare, research methodologies, and guiding policy and regulation in natural product drug development are imperative (WHO, 2013). Therefore, ethnopharmacology remains a dynamic and ethically sound contributor to the ongoing progress of pharmacognosy, providing pathways to new treatments while protecting cultural and biological resources. The ethical duty to protect indigenous knowledge must be paired with strategies that conserve medicinal plant species. Sustainable use should focus on harvesting aerial parts, such as leaves and stems, rather than roots or bark, which are often deadly to plants. In South Africa, leaves are the most

commonly used plant part (29%) for treating childhood diseases (Ndhlovu *et al.*, 2021). Aerial parts possess numerous bioactive compounds, posing a lower risk of overexploitation of plants than roots or whole plants (Chen *et al.,* 2016). Conservation requires a comprehensive approach, combining on-site measures, such as reserves and nurseries, to preserve plants in their natural habitat, and off-site measures, like botanic gardens and seed banks, to safeguard genetic diversity (Chen *et al.,* 2016). Cultivating high-demand species under Good Agricultural Practices (GAP) can alleviate pressure on wild stocks, guarantee quality supply, and provide income for local communities. Hence, conservation frameworks must be collaborative, delivering fair benefits to indigenous communities who hold the rightful authority over this knowledge (Ndhlovu *et al.,* 2021).

2.4.2. Scientific validation of the claimed ethnomedicinal efficacy of plants

“Phyto” is a Greek-derived word meaning plant; therefore, in the literal sense, phytochemicals are plant chemicals (Kumar *et al.,* 2023). The main categories of phytochemicals are primary and secondary metabolites, which are classified based on their function in plant metabolism (Kalimuthu and Prabakaran, 2013). Primary metabolites are essential compounds that are vital for basic cell functions, particularly those associated with growth and energy metabolism. They include carbohydrates, proteins, amino acids, nucleosides, and chlorophylls. Secondary metabolites play specific roles in plants, supporting development through hormonal activity and photosynthesis, aiding reproduction and survival in nature, and actively contributing to their medicinal properties (Böttger *et al.,* 2018; Kalimuthu and Prabakaran, 2013).

Globally, approximately 35,000 medicinal plants have been discovered (Khan, 2014). Moreover, approximately 214,000 secondary metabolites and 7,000 specific compounds with pharmacological properties have been identified in medicinal plants (Hussein and El-Anssary, 2019; Tshibangu *et al.,* 2002). Secondary metabolites have been shown to possess pharmacological properties, providing the scientific basis for the use and

medicinal efficacy claimed in ethnomedicine. Their diverse medicinal characteristics include antioxidant, anti-inflammatory, antimicrobial, anticancer agents, analgesic, and immunomodulatory properties. Furthermore, humans harness secondary metabolites for a variety of purposes, including medications, recreational drugs, flavourings, and colourings (Kabera *et al.,* 2014). These compounds are typically classified based on their chemical structure, biosynthesis, and functions. The main categories include alkaloids, terpenoids, steroids, polyphenols, fatty acid-derived compounds, non-ribosomal polypeptides, and enzyme cofactors, as shown in Table 2.1 below.

Table 2. 1: Pharmacological properties of the main classes of secondary metabolites

Class	Structural Features	Pharmacological Activities	Examples	References
Phenolics	Contains ≥1 phenol group; range from simple (1 ring) to polymers	Antioxidant, anti-inflammatory, antimicrobial, phytoestrogenic, insecticidal	Quercetin, Silybin, Genistein, Arbutin, Salicylates	(Pereira *et al.,* 2009)
Simple phenolics	Hydroxyl, aldehydic or carboxylic groups	Antimicrobial, anti-inflammatory, analgesic, diuretic	Gallic acid, Vanillin, Eugenol, Arbutin,	(Marchiosi *et al.,* 2020)
Tannins	Polyphenols: hydrolysable (gallic / ellagic acid) & condensed (flavonoid oligomers)	Astringent, antidiarrheal, antidote, antiangiogenic	Epigallocatechin gallate, Geraniin, Ellagitannins	(Sieniawska and Baj, 2017)
Coumarins	Benzo-α-pyrone derivatives	Anti-inflammatory, anticoagulant, anticancer, anti-Alzheimer's	Scopoletin, Aesculetin	(Kostova *et al.,* 2011)
Flavonoids	Chroman ring + aromatic ring	Anti-inflammatory, anti-allergic, vasoprotective, anticancer	Quercetin, Liquorice flavonoids, Ginkgo flavonoids	(Kumar and Pandey, 2013; Panche *et al.,* 2016)
Chromones & Xanthones	Benzo-γ-pyrone derivatives	Antifungal, spasmolytic	Eugenin, Xanthones	(Hussein and El-Anssary, 2019)
Stilbenes	Aromatic stilbene skeleton	Estrogenic, antioxidant	Resveratrol	(Hussein and El-Anssary, 2019)
Lignans	Dimers of phenylpropane derivatives	Antimicrobial, antifungal, cytotoxic	Wikstromol, Matairesinol	(Hussein and El-Anssary, 2019)

Alkaloids	N-containing heterocyclic compounds	Analgesic, anaesthetic, antineoplastic, antimicrobial, toxic	Nicotine, Caffeine, Vinblastine, Morphine	(Bribi, 2018; Kukula-Koch and Widelski, 2017)
Saponins	Steroidal / triterpenoid aglycone + sugar chain	Expectorant, anti-inflammatory, anticancer, spermicidal, molluscicidal	Glycyrrhizin (licorice), Aescin (horse chestnut)	(Desai *et al.,* 2009)
Terpenes	Built from 5-C isoprene units	Antimicrobial, analgesic, anti-inflammatory, anticancer, insecticidal	Menthol, Farnesol, Vitamin A & K, Boswellic acids, Resins	(Ninkuu *et al.,* 2021)
Lipids	Fixed oils, waxes, sterols, essential oils	Antioxidant, anti-inflammatory, skin healing, antimicrobial	Linseed oil, Jojoba wax, Essential oils (menthol, thymol, carvacrol)	(Hussein and El-Anssary, 2019)
Carbohydrates	Monosaccharides, polysaccharides; also glycosides, gums, mucilage	Demulcent, emollient, anti-inflammatory	Cellulose, Mucilage (flax seeds, marshmallow root, slippery elm)	(Hussein and El-Anssary, 2019)

2.4.3. Main classes of secondary metabolites

2.4.3.1. Phenolics

Phenolics are a broad and diverse class of plant secondary metabolites characterised by one or more phenol groups in their structures (Hussein and El-Anssary, 2019). They range from simple single-ring compounds to complex polymeric molecules and are found across many plant species. These compounds influence the taste, colour, and aroma of numerous herbs, foods, and beverages, while also playing protective roles against herbivores and pathogens. Pharmacologically, phenolics exhibit various pharmacological effects, including hepatoprotective, antioxidant, anti-inflammatory, insecticidal, and phytoestrogenic actions. Key examples of bioactive phenolics include quercetin, silybin, genistein, and naringenin. The classification of phenolics depends on their structure and biosynthesis, encompassing flavonoids, stilbenes, coumarins, lignans, chromones,

simple phenolics, xanthones, and tannins, making this group central to pharmacognosy and natural product research.

2.4.3.1.1. Simple Phenolics

Simple phenolics, particularly phenolic acids, are abundant in plants and include compounds such as vanillin, caffeic acid, gallic acid, eugenol and salicylic acid (Hussein and El-Anssary, 2019). Gallic acid serves as the parent compound for gallotannins and is known for its astringent, antibacterial, antiviral, antifungal, and anti-inflammatory effects. Many simple phenolics act through diverse mechanisms, such as inhibiting insulin degradation or relaxing smooth muscle. Hydroquinone, often present as the glycoside arbutin, exhibits urinary antiseptic activity, while salicylates display anti-inflammatory effects. Glycosidic forms, such as coniferin, serve as lignin precursors, underscoring their structural significance. Importantly, all phenols share antimicrobial activity, with phenol itself historically recognised as one of the first surgical antiseptics.

2.4.3.1.2. Tannins

Tannins are polyphenols that can bind and precipitate proteins, thereby protecting plant tissues from microbial degradation (Hussein and El-Anssary, 2019). They are divided into hydrolysable tannins, based on gallic or ellagic acid derivatives, and condensed tannins, derived from flavonoid precursors. Gallotannins, ellagitannins (such as geraniin), and proanthocyanidins are key representatives. Tannin-containing plants such as Camellia sinensis (tea) and Hamamelis virginiana are used as antidiarrheals and antidotes against alkaloid or heavy metal poisoning. Their pharmacological activities include antiangiogenic, antioxidant, and antimicrobial properties. Epigallocatechin-3-gallate from tea has demonstrated notable anti-cancer potential, while cranberry juice has been used for centuries as a urinary antiseptic, supported by controlled clinical studies.

2.4.3.1.3. Coumarins

Coumarins are derivatives of benzo-α-pyrone, occurring either in free form or as glycosides in many medicinal plants (Hussein and El-Anssary, 2019). Over 1000 coumarins have been identified, with coumarin itself present in around 150 plant species, including *Dipteryx odorata* and *Galium odoratum*. Other important coumarins include aesculetin, umbelliferone, and scopoletin. These compounds are abundant in species such as *Atropa belladonna* and *Datura stramonium*. Coumarins exhibit a diverse range of biological activities, including anti-inflammatory, anticoagulant, anticancer, and anti-Alzheimer's effects, rendering them significant for both traditional and modern medicine.

2.4.3.1.4. Flavonoids

Flavonoids represent the largest subclass of phenolics, with over 2000 identified compounds, nearly 500 of which naturally occur in free form. (Hussein and El-Anssary, 2019). Structurally, they are characterised by a chroman nucleus linked to an aromatic ring and are classified into subgroups such as anthocyanins, flavones, and flavonols based on oxidation patterns. Flavonoids are pharmacologically crucial for their anti-inflammatory, antiallergic, antithrombotic, vasoprotective, and tumour-inhibiting properties. They also protect the gastric mucosa and act as potent antioxidants, neutralising free radicals. Their diverse presence in fruits, vegetables, and medicinal herbs contributes not only to plant defence but also to significant human health benefits.

2.4.3.1.5. Chromones, Stilbenes and Lignans

Chromones and xanthones are structural derivatives of benzo-γ-pyrone, found in limited numbers but with some therapeutic importance (Hussein and El-Anssary, 2019). Examples include khellin from *Ammi visnaga* and antifungal xanthones from the

Gentianaceae family. Stilbenes are less common but widely distributed, with resveratrol being the most studied due to its estrogen-like, cardioprotective, and antioxidant activities. Lignans, formed from the dimerisation of phenylpropanoid units, occur in plant families such as Asteraceae and Rutaceae. They exhibit antimicrobial, antifungal, cytotoxic, and anticancer effects. Collectively, these phenolic subclasses expand the pharmacological scope of phenolics, supporting their critical role in natural medicine research.

2.4.3.2. Alkaloids

Alkaloids are compounds that contain nitrogen within heterocyclic structures and diverse functions, making them difficult to define under a single category (Hussein and El-Anssary, 2019; Zandavar and Afshari Babazad, 2023). They are classified into structural types, including indoles, isoquinolines, quinolines, pyridines, pyrrolizidines, and purines, among others. Alkaloids are unevenly distributed in nature but are particularly abundant in higher plants, where they function as defence molecules against herbivores and pathogens. Their historical importance is profound, as many alkaloids were isolated in the 19th century and became foundational drugs in pharmacology. Alkaloids occur in several plant families such as Papaveraceae, Solanaceae and Apocynaceae, as well as in fungi and gymnosperms. Alkaloids have a vast range of pharmacological activities, including analgesic, anaesthetic, antihypertensive, stimulant, relaxant, and anticancer properties. They may also act as cytotoxic, mutagenic, or insecticidal compounds, highlighting their medicinal potential. Examples of clinically significant alkaloids include morphine (analgesic), atropine (anticholinergic) and quinine (antimalarial). The structural diversity and potent bioactivity of alkaloids make them one of the most pharmacologically relevant groups of plant secondary metabolites.

2.4.3.3. Saponins

Saponins are amphiphilic compounds with a hydrophobic sapogenin linked to sugars, which gives them soap-like properties, such as lowering surface tension, producing foam, and lysing erythrocytes (Hussein and El-Anssary, 2019; Kabera *et al.,* 2014). Found in various plant parts, notably in *Panax ginseng* roots*, Glycyrrhiza glabra,* and *Dioscorea villosa,* their structural diversity interacts with biological membranes, explaining many of their effects. Pharmacologically, saponins have antitumour, molluscicidal, piscicidal, spermicidal, sedative, expectorant, and analgesic properties. Glycyrrhizin from licorice is used as an expectorant, cough suppressant, and hepatoprotective agent, while aescin from horse chestnut has strong anti-inflammatory effects, reducing oedema. Their membrane activity drives ongoing research as vaccine adjuvants and therapeutic leads.

2.4.3.4. Terpenes

Terpenes are a diverse group of compounds derived from isoprene units (C_5H_8), classified by the number of units: hemiterpenes (C_5), monoterpenes (C_{10}), sesquiterpenes (C_{15}), diterpenes (C_{20}), sesterterpenes (C_{25}), triterpenes (C_{30}), tetraterpenes (C_{40}), and polyterpenes (Hussein and El-Anssary, 2019; Kabera *et al.,* 2014). Monoterpenes, like menthol and camphor, are the main essential oil components used as analgesics and decongestants. Sesquiterpenes have antibacterial, antifungal, and antiparasitic properties; diterpenes include vitamin A and K_1, which are essential for nutrition and medicine. Triterpenes are steroid precursors with anti-inflammatory effects, exemplified by boswellic acids from Boswellia. Sesterterpenes, though rare, are cytotoxic. Tetraterpenes like carotenoids are key in photoprotection and antioxidants. Terpenes are utilised in pharmacology for their analgesic, antimicrobial, anti-inflammatory, and anticancer properties, rendering them highly valuable in drug discovery.

2.4.3.5. Glycosides

Glycosides are compounds made up of a sugar part (glycone) attached to a non-sugar part (aglycone), which can be phenolic, terpenoid, or steroidal. In plants, glycosides usually exist as inactive storage forms that are hydrolysed by enzymes to release the active aglycone (Hussein and El-Anssary, 2019; Kabera *et al.,* 2014). This property enables glycosides to act as natural prodrugs, which are activated only under specific physiological conditions. The aglycone determines the pharmacological activity, while the sugar component affects solubility and transport. Glycosides are classified based on the type of aglycone, including anthraquinone, cardiac, cyanogenic, thioglucoside, flavonoid, and saponin glycosides. Their pharmacological effects are diverse, including cardiotonic, laxative, anticancer, anti-inflammatory, sedative, and digestive actions. For instance, cardiac glycosides such as digoxin and digitoxin from Digitalis enhance cardiac contractility, while anthraquinone glycosides from Senna act as laxatives. The therapeutic versatility of glycosides makes them an important group of secondary metabolites in both traditional and modern medicine.

2.5. Biosafety of plant medicines

Toxicity in plants has been acknowledged for centuries; for instance, cardiac glycosides have been used as both lethal arrow poisons and therapeutic heart tonics (Botha & Penrith, 2008). Toxicity from traditional medicine is common in regions where herbal remedies are the main form of healthcare, particularly affecting children and pregnant women (Botha and Penrith, 2008; Nasri and Shirzad, 2013). In South Africa, approximately 80% of the population uses medicinal plants, and plant-based remedies lead to acute poisoning, causing around 20,000 deaths annually, along with significant morbidity and mortality (Popat *et al.,* 2001). A study at a hospital in South Africa (1987–1992) discovered that traditional medicines caused 7.8% of poisoning cases, and traditional medicines were associated with the highest mortality among poisoning cases.

Children under the age of five years constituted 79% of the mortalities caused by traditional medicines (Scott, 2003). Therefore, the biosafety of medicinal plants is a matter of grave importance. Nonetheless, there is a general belief that because plant medicines are natural, they are safe and have minimal side effects (Philomena, 2011). However, biosafety concerns can stem from misidentification of plant species, improper preparation techniques, or incorrect dosage administration, particularly when carried out by individuals lacking appropriate training (Van Wyk, 2002). The hazards of using medicinal plants are well-documented, despite their therapeutic advantages, and measures must be taken to reduce poisoning from these plants.

Adverse health effects from plant medicines arise due to toxic compounds in plants, which are naturally produced as a defence against microorganisms, insects, herbivores, and environmental stress (Ndhlala *et al.,* 2013; Philomena, 2011). Medicinal plant toxicity affects various organs depending on the compounds, manifesting as neurotoxicity, gastrointestinal issues, genotoxicity, mutagenic and cytotoxic effects, damaging vital organs like the liver, kidneys, heart, and lungs. Extremely toxic plants can cause systemic failure, seizures, coma, and death. The severity depends on plant species, part used, dose, exposure frequency, and individual susceptibility. As a result, initiatives to ensure the safe administration of plant medicines must include the regulated production and quality control of plant medicines, as well as the isolation and characterisation of pure compounds with medicinal effects, which allows them to be distinguished from toxic ones (Butterweck and Nahrstedt, 2012; Jamshidi-Kia *et al.,* 2018; Nworu *et al.,* 2014; Sabotič *et al.,* 2024). Therefore, research must prioritise the development of methods and processes for validation, standardisation, and quality control in the manufacturing and administration of plant-based therapeutic agents.

2.6. *Hermannia depressa*: extensive description of the plant, classification, morphology and habitat

2.6.1. Botanical classification and nomenclature of *H. depressa*

Hermannia depressa N.E.Br. is a significant plant used in Southern African ethnomedicine, and its common names include “Seletjana” in Sesotho or “Rooi-opslag” in Afrikaans or creeping red Hermannia (Ngobeni *et al.,* 2023; Sachse, 2007). It is classified under the Kingdom Plantae, Phylum Streptophyta, and Class Equisetopsida. It belongs within Subclass Magnoliidae, Order Malvales, and Family Malvaceae, Genus Hermannia, with the specific epithet depressa (Germishuizen and Meyer, 2003; Gwynne-Evans, 2015).

2.6.2. Botanical morphological description of *H. depressa*

H. depressa is a small, ground-hugging perennial herb, with a spreading pattern as demonstrated in Figure 2.1. Its stems extend outward from a central woody taproot and remain pressed against the soil surface, allowing the plant to form low mats that help it survive in heavily grazed or disturbed grassland and marsh environments (Reid *et al.,* 2005). The stems are thin, slightly woody near the base, and covered with fine, short hairs that give them a rough texture. These stems carry small to medium-sized leaves, which are attached by short petioles. The leaves are broadly ovate to slightly lobed, with toothed edges and a reddish to purplish tinge, especially in stressed conditions (Gwynne-Evans, 2015). As seen in the images, the leaf surfaces are lightly hairy and show a noticeable star-shaped (stellate) hair pattern, which helps reduce water loss and may deter herbivores. Flowers are usually borne singly or in pairs on short, erect stalks that emerge from the leaf axils. They drop slightly and occur just above the ground, which may help protect them from wind, rain, and grazing. The petals are reddish-purple to mauve, often with dark veins running through them. The flower has five petals, which are fused at the

base and flare outward with a slightly twisted appearance. The interior of the flower is marked with a yellow throat, which may act as a guide for pollinators. One of the key identifying features of the flower is the filament structure. The filaments, which hold the anthers, are swollen at the base and then narrow just below the anther, a trait that places the species within the subgenus Mahernia. The outer parts of the flower, including the sepals and flower stalks, are finely hairy and slightly glandular.

Figure 2. 1: Morphological appearance of *H. depressa* (Hyde *et al.,* 2004)

2.6.3. Natural habitat and distribution

H. depressa has a broad geographical range and significant ecological diversity, demonstrating its resilience to various environmental conditions across different habitats. *H. depressa* mainly grows in the summer-rainfall bioclimatic zones, where annual rainfall

ranges from moderate to high. It thrives in terrestrial environments, especially within grasslands and at the edges of seasonal wetlands, such as marshes and ephemeral swamps. The species is notably widespread across the Highveld, a plateau characterised by grassland ecosystems and scattered rocky outcrops. Specific habitats include the Rocky Highveld Grassland (Deutschlander and Bredenkamp, 1999) and various moist to moderately dry grassland biomes (Parrish and Fitzpatrick, 2017). These areas often experience seasonal droughts and periodic fires. Geographically, *H. depressa* is found throughout all nine provinces of South Africa but is more prevalent in the Free State and the Cape region, and extends into neighbouring countries, including Lesotho, Zimbabwe, and Namibia, with occasional isolated populations reported as far as Madagascar and eastern Africa (Molefe, 2013).

2.6.4. Ethnobotanical and cultural relevance: applications and uses in traditional medicine

This species holds substantial ethnobotanical importance among several indigenous groups in Southern Africa, where it is employed in diverse traditional medicinal and cultural contexts. Documented ethnomedical applications highlight *H. depressa* as a multipurpose plant used in the treatment of numerous ailments. The roots, frequently prepared as a decoction and administered orally, are used to alleviate abdominal pain during pregnancy, breast cancer-related symptoms, nausea, and to stimulate appetite, particularly in pregnant women (Seleteng Kose *et al.,* 2015). Topical applications of root preparations are traditionally employed for the management of burns, inflammatory skin conditions, wounds, and localised swellings (Xaba, 2016). Furthermore, the plant has been used in the treatment of headaches and respiratory conditions, including coughs, wherein decoctions are ingested for symptomatic relief (Reid *et al.,* 2005; Molefe, 2013). The leaf sap, diluted in water, is traditionally administered as a purgative and diaphoretic agent for the treatment of gastrointestinal disturbances such as stomach-ache, colic,

heartburn, diarrhoea, and nausea (Reid *et al.,* 2005). Among Zulu communities, *H. depressa* is also used as an emetic, further underscoring its therapeutic use in cleansing the digestive system and promoting detoxification.

Beyond its pharmacological relevance, *H. depressa* is embedded in socio-cultural practices. Among the Basotho, the plant is reportedly used in spiritual divination to identify various diseases and guide the selection of appropriate treatments, indicating its role in traditional health systems that integrate spiritual and biomedical interpretations of disease (Sobiecki, 2008). Ethnographic accounts also describe the application of *H. depressa* leaves during male initiation rites, where pounded leaves are used to dress wounds resulting from frenulum (moqaqa) piercing; a culturally sensitive procedure conducted in secrecy and intended to facilitate improved genital hygiene (Letsie, 2007). Recent ethnobotanical surveys have expanded the documented therapeutic scope of *H. depressa* to include its use in the treatment of sexually transmitted infections (STIs) such as gonorrhoea, and as an antidote for food poisoning and tuberculosis (Ngobeni *et al.,* 2023). In some cases, the plant is reportedly used as a protective charm against spiritual afflictions such as witchcraft, suggesting its additional role within the metaphysical domain of traditional medicine (Hlongwane, 2008).

2.6.5. Scientific evidence supporting the traditional use of *H. depressa*

H. depressa has long been utilised in traditional medicine and has shown significant pharmacological potential. Phytochemical studies of this species have used various techniques, from classical qualitative tests such as the froth test and ferric chloride test to modern analytical methods including vacuum liquid chromatography (VLC), thin layer chromatography (TLC), high-performance liquid chromatography (HPLC), and liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) (Molefe, 2013; Ngobeni *et al.,* 2024). These studies have consistently identified critical secondary metabolites such as tannins, saponins, phenols, terpenoids, and cardiac glycosides,

which likely contribute to the plant's therapeutic effects (Reid *et al.,* 2005; Molefe, 2013; Hlongwane, 2016). More advanced LC-MS/MS analyses revealed the presence of alkaloids and flavones, including Waltherione D and quercetin in acetone extracts, and tricin in both aqueous and acetone extracts. In contrast, methanol extracts contained steroids, fatty acids, and lignans (Ngobeni *et al.,* 2024). Quantitative evaluations further showed that acetone extracts have the highest phenolic content at 8.45 mg gallic acid equivalent per gram (GAE/g), whereas flavonoid levels are most abundant in aqueous extracts at 0.97 mg quercetin equivalent per gram (QE/g), supporting their potential for bioactivity. The antimicrobial activity of *H. depressa* has been widely documented, with ethanolic and ethyl acetate extracts from roots, leaves and stems showing efficacy against bacterial pathogens such as *Bacillus subtilis*, *Escherichia coli*, and *Klebsiella pneumoniae* (Reid *et al.,* 2005). Further studies highlighted activity against *Mycobacterium tuberculosis* (Hlongwane, 2008). Methanol and acetone extracts demonstrated antimicrobial effects against a broader range of thirteen microorganisms, including *Staphylococcus aureus, Candida parapsilosis, Pseudomonas aeruginosa, Streptococcus pyogenes, Enterococcus faecalis, Neisseria gonorrhoeae, Bacillus cereus, Candida krusei, Staphylococcus epidermidis, Streptococcus pneumoniae, Clostridium perfringens, Candida albicans, and Escherichia coli*, with the strongest activity against *C. albicans* and *B. cereus* at minimum inhibitory concentrations (MICs) of 0.1 and 0.3 mg/mL, respectively (Ngobeni *et al.,* 2024).

In addition to antimicrobial properties, *H. depressa* exhibits significant anti-inflammatory and antioxidant potential. Dichloromethane extracts demonstrated up to 81% inhibition of Cyclooxygenase 1 (COX-1), outperforming several other Sterculiaceae species in comparative studies (Al Muqarrabun and Ahmat, 2015; Reid *et al.,* 2005). Acetone and methanol extracts effectively reduced secretion of nitric oxide in lipopolysaccharide-activated RAW 264.7 macrophages, indicating strong anti-inflammatory activity, with acetone extracts showing slightly higher efficacy (Ngobeni *et al.,* 2024). Concurrently,

aqueous, methanol, and acetone extracts exhibited substantial antioxidant activity, in some cases surpassing standard antioxidants such as ascorbic acid and Trolox, highlighting their potential for mitigating oxidative stress-related damage (Ngobeni *et al.,* 2024; Xaba, 2016). The evaluation of cytotoxicity and biosafety of *H. depressa* has produced significant insights into its safety profile. *In vitro* MTT assays using African green monkey kidney cells indicated that acetone and methanol extracts caused moderate reductions in cell viability, whereas aqueous extracts exhibited no detectable toxicity (Ngobeni *et al.,* 2024). Complementary studies using Madin-Darby bovine kidney (MDBK) cells, lactate dehydrogenase (LDH), and brine shrimp lethality assays (BLSA) corroborated low cytotoxic effects, with acetone extracts even promoting cell growth in some cases (Molefe, 2013). However, in vivo BLSA tests revealed significant toxicity at higher concentrations of water and acetone extracts, suggesting the presence of potentially toxic constituents that warrant further investigation. Collectively, these findings underscore the need for additional in vivo studies to confirm safety and determine appropriate therapeutic doses (Ngobeni *et al.,* 2024).

2.7. Conclusion

The interconnected issues of microbial infections, chronic inflammation, and oxidative stress pose a major global health problem, resulting in high mortality rates and a cycle of illness and poverty, especially in developing countries. Conventional synthetic drugs have critical limitations due to antimicrobial drug resistance, adverse side effects, high costs, and inaccessibility to remote areas. This highlights the urgent need for alternative, multi-targeted therapies. Medicinal plants are a promising option because they contain numerous bioactive compounds that can simultaneously treat infection, inflammation, and oxidative damage. However, their use must be backed by rigorous scientific research to confirm their safety and effectiveness. *H. depressa* has a strong ethnobotanical history for treating many conditions. Previous scientific studies on *H. depressa* have primarily

focused on root extracts, which showed notable antimicrobial, anti-inflammatory, and antioxidant properties *in vitro*. The leaf and stem extracts of the plant remain largely unexplored; therefore, it is necessary to assess the medicinal potential of the aerial parts of this plant, as this will validate the efficacy of medicinal use of *H. depressa* in ethnomedicine.

2.8. References

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CHAPTER THREE

THE *IN-VITRO* ANTIMICROBIAL ACTIVITY SCREENING AND THE ULTRASTRUCTURAL EFFECTS OF *H. DEPRESSA* EXTRACTS ON MICROBIAL CELLS

Abstract

Microbial infections continue to pose a significant challenge for healthcare worldwide. This chapter examines the antimicrobial properties of *H. depressa* leaf and stem extracts against various pathogens. The broth microdilution assay revealed that acetone and methanol extracts were highly active against *Candida albicans* (MIC = 0.31 mg/mL) and *Staphylococcus aureus* (MIC = 0.63 mg/mL). Acetone extracts showed good selectivity (SI = 2.1 against *C. albicans*, SI = 1.0 against *S. aureus*) relative to Vero cells, while methanol extracts displayed similar activity (SI = 2.2 against *C. albicans*, SI = 1.1 against *S. aureus*) relative to RAW 264.7 cells, suggesting good antimicrobial potential (SI > 1) relative to normal mammalian cell cytotoxicity which is a good biosafety profile. The aqueous extracts were effective against *S. aureus* and *Streptococcus pyogenes*, with an MIC of 2.5 mg/mL. Selectivity Index (SI) assessments revealed strong selectivity, especially for the aqueous extract (SI=37.2), suggesting exceptional biosafety to normal host cells while exhibiting antimicrobial properties. Electron microscopy studies (SEM and TEM) demonstrated that the acetone extract caused significant ultrastructural damage to *C. albicans*, including cell wall disruption, cytoplasmic leakage, and inhibited budding. These antimicrobial effects are attributable to bioactive phytochemicals present in the extracts of this plant. The findings support the traditional use of *H. depressa* for infections and highlight its leaves and stems as a promising source of safe antimicrobial agents.

3.1. Introduction

Millions of mortalities worldwide are reportedly caused by microbial infections. Notably, a WHO study approximates 3.83 million deaths resulting from infections in Africa alone (Khameneh et al., 2019; Sartorius et al., 2024). Moreover, the high incidence of immunosuppressed individuals due to HIV / AIDS and chemotherapy as cancer treatment has exacerbated the situation by increasing the risk of microbial opportunistic infections, and the insidious upsurge in drug-resistant pathogens has also exacerbated the risk of mortality through microbial infections (Sartorius et al., 2024). The need for novel antimicrobial agents cannot be overstated, as the treatment of microbial infections is the cornerstone of healthcare systems. Even though synthetic drugs have contributed significantly to the combat against microbial infections, yet, they present notable challenges, such as microbial drug resistance, increased likelihood of adverse reactions, inaccessibility of healthcare facilities to rural areas, as well as inflated costs (Lewis, 2013; Workowski *et al.*, 2021). To tackle these challenges, research on traditional medicines as alternative treatments, particularly those with novel mechanisms of action against pathogens, is exceptionally crucial in curbing microbial infections.

Plant medicines have been used for ages by Indigenous populations, and many developing countries still rely on them for healthcare, especially in rural parts. A worldwide population of about 65% to 80% still use traditional medicines, while South Africa has about 72% among black South Africans using them for basic healthcare needs (Oyebode et al., 2016; WHO, 2013). Phytochemicals like phenolics, flavonoids, alkaloids, saponins, and terpenoids exhibit antimicrobial activity against bacteria, fungi, and viruses by disrupting cell membranes, inhibiting vital enzymes, or interfering with microbial growth (Satoh, 2014; Kumar *et al.*, 2023). The search for plants with anti-microbial activity is critical in research because this will lead to the discovery and production of novel medications for disease treatment (Mwitari *et al.*, 2013; Ngobeni, 2016).

Traditionally, *H. depressa* is utilised *to* treat STIs, respiratory diseases, heart disease, diarrhoea, and heartburn, soothe wounds, as well as support pregnant women in alleviating abdominal discomfort and nausea (Reid *et al.*, 2005; Sobiecki, 2008; Ngobeni *et al.*, 2023). Moreover, different studies agree on the significant antimicrobial potential of *H. depressa* extracts against microbes like *Candida albicans, Bacillus cereus* and even *Mycobacterium* (Hlongwane 2008; Molefe 2013; Ngobeni et al. 2024). Studies on this plant utilised root extracts, and the antimicrobial potential of leaves and stems remains unexplored. Additionally, the mechanism of action of plant medicines is a highly understudied area, presenting a significant gap in research. As a result, this chapter aims to contribute to existing literature by studying the antimicrobial activity of *H. depressa* leaves and stems extracts using the broth microdilution method and the mechanism of action using electron microscopy.

3.2. Methods

3.2.1. Plant identification and collection

H. depressa stems and leaves were collected in Thaba-Nchu (-29.197955, 26.766118) in the Free State Province, South Africa, in April and May 2024. The plant was identified and verified by an accredited botanist, and a voucher specimen was deposited at the National Museum in Bloemfontein, Free State Province, and allocated an accession number NMB 28716. The leaves and stems were washed with running water to remove all debris and then rinsed with distilled water. They were finally dried at room temperature until they were completely dry. The dried plant material was cut into smaller pieces and then ground into powder using the POLYMIX (PX-MFC 90 D) laboratory blender.

3.2.2. Extraction

Extraction was conducted using three solvents: acetone, methanol and hot water (80 °C), where 50g of each plant material powder was extracted in 800 mL of each solvent. The mixture was then shaken for 76 hours on a rotary laboratory shaker at 120 rpm and subsequently filtered using filter paper. Moreover, 800 mL of each solvent was added to the remaining plant material sediment for further extraction, and the process was repeated. Acetone and methanol extracts were evaporated to remove excess solvent using a rotary laboratory evaporator at 50 °C, while the aqueous extracts were dried/lyophilised using a freeze dryer. The extracts were stored in McCartney bottles and then further stored in a refrigerator set at 4°C to prevent spoilage.

3.2.3. Microbial preparations

H. depressa extracts were investigated for antimicrobial effects against: *Candida tropicalis* (ATCC 750), *Candida albicans* (ATCC 1231), *Escherichia coli (*ATCC 25922),

Staphylococcus aureus (ATCC 25923), *Streptococcus agalactiae* (ATCC 13813) and *Streptococcus pyogenes* (ATCC 19615). This panel represents high-priority gram-positive, gram-negative and fungal pathogens related to WHO infection control and the antimicrobial resistance concerns. All the microorganisms were grown and sub-cultured on Mueller-Hinton agar (MHA) before testing. Then, for the broth micro-dilution assay, the 24-hour microorganism cultures were inoculated in sterile Mueller-Hinton broth (MHB) and allowed to grow for 18–24 hours at 37 °C. Thereafter, the microorganism cultures were prepared to a density of 1.5 × 10^8 colony-forming units per millilitre (CFU/mL) for bacteria and 1-5 × 10^6 CFU/mL for fungi, which is equivalent to 0.5 McFarland Standard in sterile MHB.

3.2.4. Minimum inhibitory concentration determination: Broth microdilution assay method

H. depressa extracts' minimum inhibitory concentration (MIC) was determined employing a 96-well microtiter plate-based broth microdilution assay, as described by Eloff (1998) with minor changes. Each *H. depressa* extract, weighing 100 mg, was dissolved in 10 mL of 2% DMSO to prepare a stock solution with a concentration of 10 mg/mL. The stock solutions were further diluted serially in sterile distilled water to achieve five dilutions, each at concentrations of 5 mg/mL, 2.5 mg/mL, 1.25 mg/mL, 0.625 mg/mL, and 0.3125 mg/mL. Serial dilutions of each *H. depressa* extract were added to the wells in triplicates. The 24-hour microorganism cultures were then inoculated into sterile broth and adjusted to a density of 1.5 x 10^8 CFU/mL, matching the 0.5 McFarland Standard. Each well received 80 µL of this inoculated broth. The plates were sealed and incubated at 37 °C for a duration of 24 hours. Microbial growth was assessed visually by observing colour changes in the wells after adding 40 µl of Iodonitrotetrazolium chloride (INT) dye; a shift from pink to maroon indicated growth. The MIC was established as the minimum concentration that prevents growth, as indicated by no colour change of INT in the well.

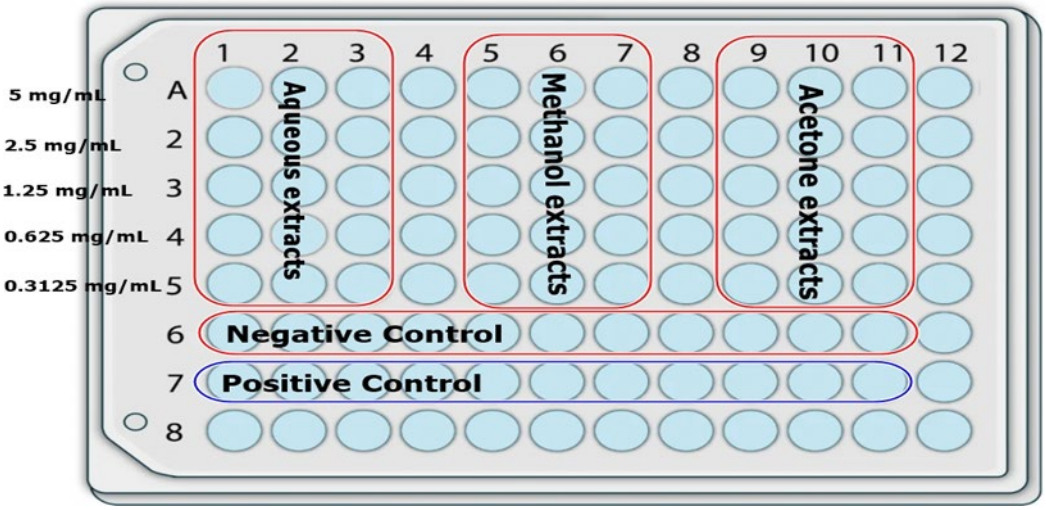

Figure 3. 1: The 96-well microplate set up

3.2.5. Selectivity Index (SI)

SI was employed to determine the relative safety of the antimicrobial extracts in medicinal applications, by determining whether *H. depressa* extracts can specifically target and inhibit microbial cells without adverse effects on normal mammalian cells. SI was calculated by dividing the cytotoxic half-maximal inhibitory concentration (CTC_{50}) by the antimicrobial MIC of the corresponding extracts according to the formula:

$$SI = \frac{CTC_{50}}{MIC}.$$

SI value greater than 1 proves that the *H. depressa* extracts can target microbial cells without affecting normal cells, suggesting good medicinal potential. Whereas SI less than 1 shows that the extracts exhibit elevated toxicity towards normal cells, suggesting poor selectivity between microbial and host cells, which compromises its suitability for medicinal applications (Martins *et al.*, 2015; Nunes *et al.*, 2016; Njeru and Muema, 2021).

3.2.6. The effect of the extracts on the morphology of microbial cells using electron microscopy

5.2.6.1. Preparation of samples

The extract concentration with the strongest antimicrobial effect in the broth microdilution assay was selected for analysis of its mechanism of action. Furthermore, 100µL of a 24-hour culture in MHB of the most inhibited microorganism was treated with 100µL of the corresponding extract concentration and incubated at 37 ^{0}C for 24 hours. The untreated microorganism served as the control.

3.2.6.2. SEM & TEM analysis

For electron microscopy, the samples were first stabilised through chemical fixation using 3% glutaraldehyde and 1% osmium tetroxide, then maintained at -4 °C for 2 hours to preserve ultrastructure, prevent degradation, and make the samples vacuum-compatible. Following fixation, the samples were dehydrated through a series of increasing ethanol concentrations, progressing from 50% to 70%, 95%, and finally to 100%. Thereafter, the samples were dried using the critical point drying technique, utilising carbon dioxide (CO_2). For the scanning electron microscopic (SEM) analysis, the samples were mounted on a specimen stub, then coated with a thin metal layer of gold-palladium alloy. The samples were then observed employing SEM, and micrographs of the control and treated samples were captured. On the other hand, for the transmission of electron microscopy (TEM), the ethanol-dehydrated samples were processed by replacing ethanol with propylene oxide, followed by embedding them in epoxy resin. Finally, the resin-infiltrated samples were sliced into ultrathin sections (90 nm) using an ultramicrotome to allow electrons to pass through. Thereafter, the sections were mounted on metal grids and

stained with 6% aqueous uranyl acetate, followed by lead citrate to improve contrast (Ellis, 2014; Pavathuparambil Abdul Manaph *et al.*, 2023). Finally, the samples were then observed under the Transmission electron microscope, with images of both the control and treated cells captured.

3.3. Results

3.3.1. The antimicrobial potential of *H. depressa* leaves and stems extracts

H. depressa aqueous, methanol and acetone extracts portrayed antimicrobial activity against *C. albicans, S. pyogenes* and *S. aureus* at differing degrees, with MIC values ranging from 0.3125 to 2.5 mg/mL. However, the most potent activity was demonstrated against *C. albicans* by the acetone and methanol extracts of *H. depressa* (MIC = 0.31 mg/mL), as depicted in Table 5.1. The tested *H. depressa* extracts had no antimicrobial activity against C. *tropicalis, E. coli* and *S. agalactiae*.

Table 3. 1: The antimicrobial potential of *H. depressa* extracts presented as MIC values

***H. depressa* extracts**	**MIC (mg/mL) against selected microorganisms**					
	C. tropicalis	*C. albicans*	*E. coli*	*S. aureus*	*S. agalactiae*	*S. pyogenes*
Acetone extract	-	0.31	-	0.63	-	-
Methanol extract	-	0.31	-	0.63	-	-
Aqueous extract	-	-	-	2.5	-	2.5

(-) = No activity

3.3.2. Selectivity index of the antimicrobial activity of *H. depressa* extracts

Table 5.2 shows the selectivity index of *H. depressa* extracts, indicating that both the acetone and methanol extracts demonstrated good selectivity (SI > 1) against *Candida albicans*, exhibiting minimal toxicity to Vero cells. This suggests that these extracts possess selective antimicrobial efficacy with low host cell toxicity. Additionally, the methanol extract also showed promising selectivity against *C. albicans* and *Staphylococcus aureus*, with low toxicity towards RAW 264.7 cells. Notably, the aqueous extract displayed outstanding selectivity (SI = 37. 2) against *S. aureus* and *Streptococcus pyogenes*, highlighting its strong potential for therapeutic application against these pathogens.

Table 3. 2: Antimicrobial activity selectivity index of *H. depressa* extracts

SI	Vero cells		RAW 264.7 macrophage cells		
	C. albicans	*S. aureus*	*C. albicans*	*S. aureus*	*S. pyogenes*
Acetone extract	2.1	1.0	0.7	0.3	n/a
Methanol extract	1.2	0.6	2.2	1.1	n/a
Aqueous extract	n/a	n/a	n/a	37.2	37.2

3.3.3. The assessment of the ultrastructural effects of *H. depressa* acetone extracts on *C. albicans* using SEM

H. depressa acetone extracts exhibited the strongest antimicrobial activity against *C. albicans* (MIC = 0.31 mg/mL). They were thus selected for evaluation of the effects on microbial cell ultrastructure after treatment using electron microscopy. SEM was employed to analyse the morphological alterations of *C. albicans* cells treated with *H.*

depressa extracts after 24 hours at 37 °C, in comparison to the normal, untreated cells, at magnifications ranging between ×1000 and ×14000 (Figure 5.2). Normal *C. albicans* cells exhibited a typical pleomorphic morphology, displaying both yeast and hyphal forms, as demonstrated in Figure 5.2. The yeast forms of *C. albicans* were observed as spherical to ovoid blastoconidial cells with a smooth and homogeneous outer surface. The hyphae are smooth, extended tubular structures, without narrowing at septal junctions and possess solid septa (Figure 5.2(B)). Budding in these cells primarily occurs at the apical tips in a spontaneous or unipolar manner, leaving visible bud scars that appear as rings or indentations (Figure 5.2(B)). Whereas pseudo-hyphae were also observed as elongated chains of blastoconidial cells, especially during intermediate phases of growth. Pseudo-hyphal cells were generally wider and shorter than true hyphae. The distinct septa in these forms appear fragile, and sporadic branching was observed at sites of septal constriction (Figure 5.2(C)). The surface appearance showed a smooth and uniform surface (Figure 5.2(D)).

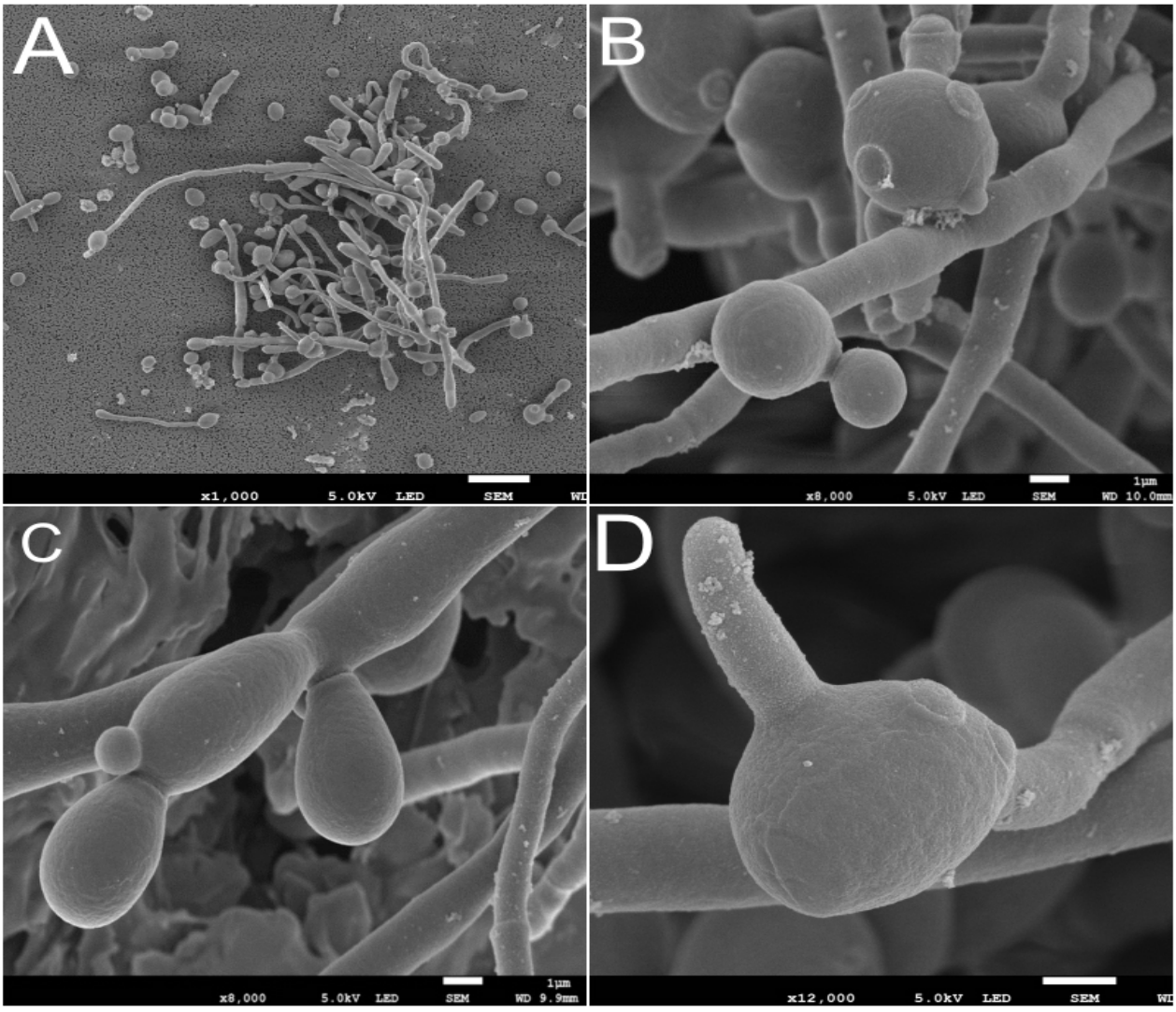

Figure 3. 2: SEM images of untreated *C. albicans* showing pleomorphic morphology. (A) Yeast and hyphal forms. (B) True hyphae with smooth tubular structures, solid septa, and apical budding scars. (C) Pseudo-hyphae are chains of blastoconidia with fragile septa and occasional branching. (D) Yeast cells with spherical to ovoid blastoconidia and smooth surfaces.

C. albicans cells treated with the *H. depressa* extract exhibited notable morphological aberrations, as shown in Figure 5.3. Treated blastoconidial cells appeared to maintain their normal ovoid or spherical (OVS), but as they matured, they became irregular and misshapen (IRM). Whereas hyphal structures lacked apical budding bulbs and appeared to form bud scars without apical swelling (NAS). Additionally, the cells exhibited decreased budding and showed disrupted or deformed bud scars, suggesting disruption of normal growth and reproductive mechanisms (DBS). Moreover, some cells display a deflated appearance due to cytoplasmic leakage, and this is linked to cell membrane damage (DCL). The surface topology of treated *C. albicans* showed significant abnormalities, including a granular or gravel-like surface appearance (GGL), with notable wrinkling and folding, as well as the development of holes or punctures on the surface of cells (PAH). Lastly, the residual particles of the plant extract were observed in the extracellular environment as a dense, irregular, amorphous or sponge-like material that adhered to the blastoconidia and hyphae. The observed abnormalities suggest that *C. albicans* is susceptible to *H. depressa* acetone extracts through suppression of reproductive pathways, pronounced cell envelope damage, and leakage of cytoplasmic content. As a result, the *H. depressa* extracts have potent antifungal activity.

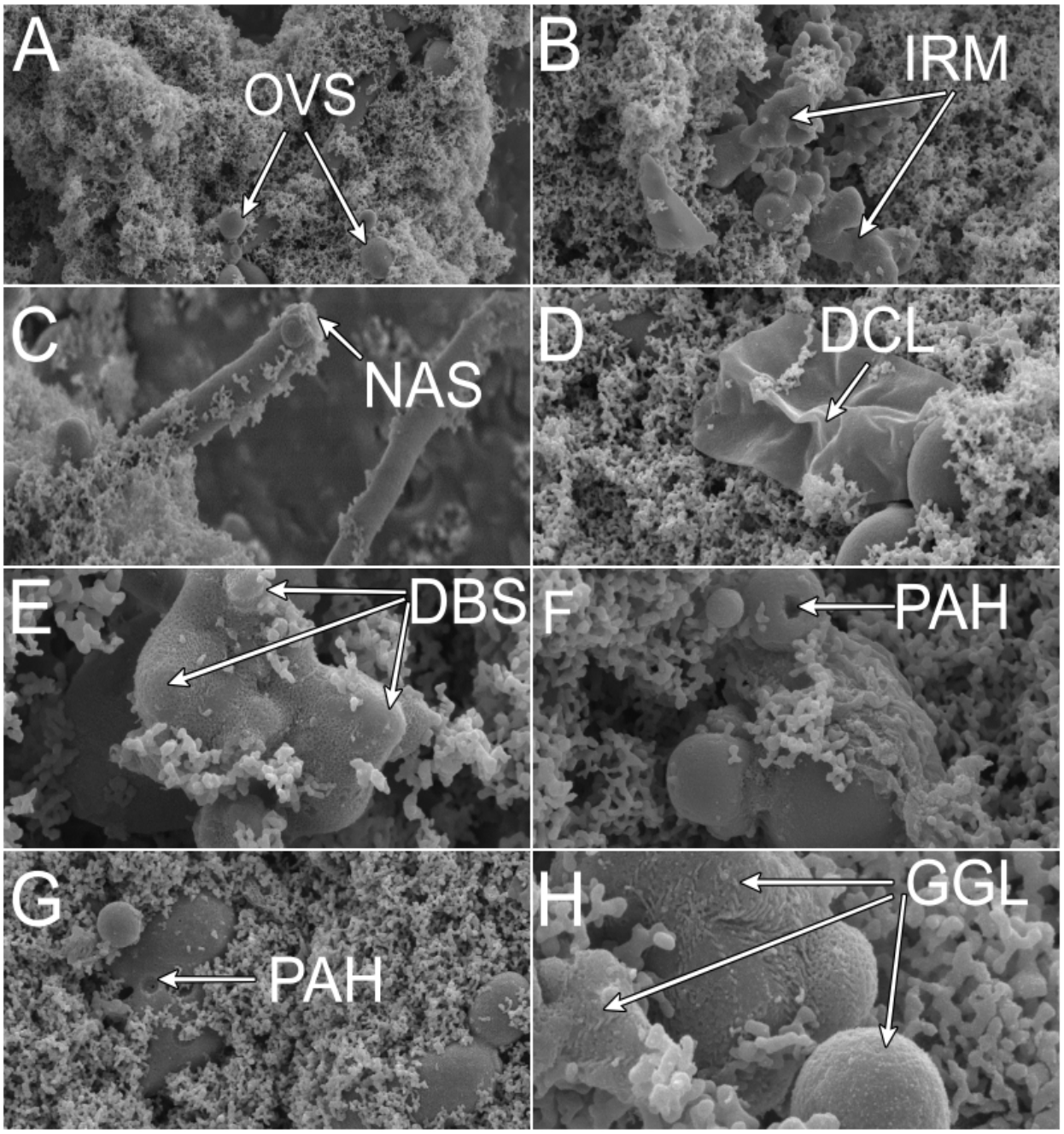

Figure 3.3: SEM micrographs of *C. albicans* after 24 hours of treatment with *H. depressa* showing diverse morphological aberrations. Ovoid spherical cells (OVS); hyphae without apical swelling (NAS); deformed bud scars (DBS); deflated cells with cytoplasmic leakage (DCL); cells with punctures and holes (PAH); granular gravel-like cell surface (GGL)

3.3.4. The TEM assessment of the ultrastructural effects of *H. depressa* acetone extracts on C. albicans

Morphological assessment of untreated *C. albicans* cells using TEM revealed characteristic pleomorphic morphology, including blastoconidial (Figure 5.4A), hyphal (Figure 5.4D), and pseudo-hyphal forms (Figure 5.4C). The cells exhibited a uniform central electron density with a structured nucleus (N) and homogenous cytoplasm containing several electron-dense elements (CT). The cell wall (CW) appeared smooth and continuous, while the cell membrane (CM) was clearly defined with uniform thickness. Budding was observed at apical tips (AT), and bud scars were visible as localised thickenings at the polar ends of the cells (BSC).

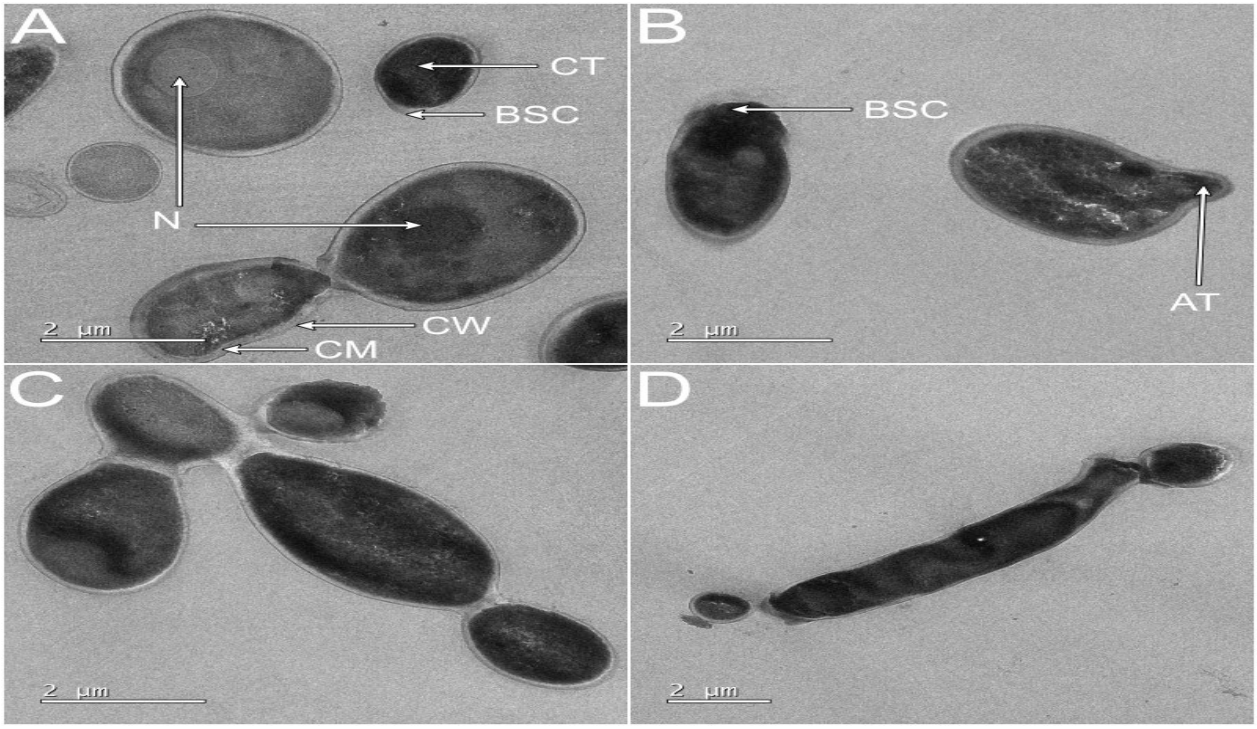

Figure 3.4: TEM micrographs of normal *C. albicans* cells. (A and B) normal blastoconidial cells; (C) pseudohyphae and (D) hyphae. Nucleus (N); Cytoplasm (CT); Cell membrane (CM), Cell wall (CW), Bud scar (BSC) and budding at apical tips (AT)

C. albicans cells treated with *H. depressa* acetone extracts exhibit structural abnormalities as shown in Figure 5.5. The cell wall appears unevenly thickened and mantle-like outer covering (CWD), while the cell membrane shows significant shrinkage(S), detaching from the cell wall in some regions (CWD). The nucleus is not as defined. The cytoplasm shows heterogeneous electron density, with areas of degeneration (F) and vacuolar enlargement (V). Overall, Figure 5.5 shows that the cells lose their typical oval shape, appearing collapsed or deformed, with membrane blebbing and occasional cytoplasmic leakage (CL).

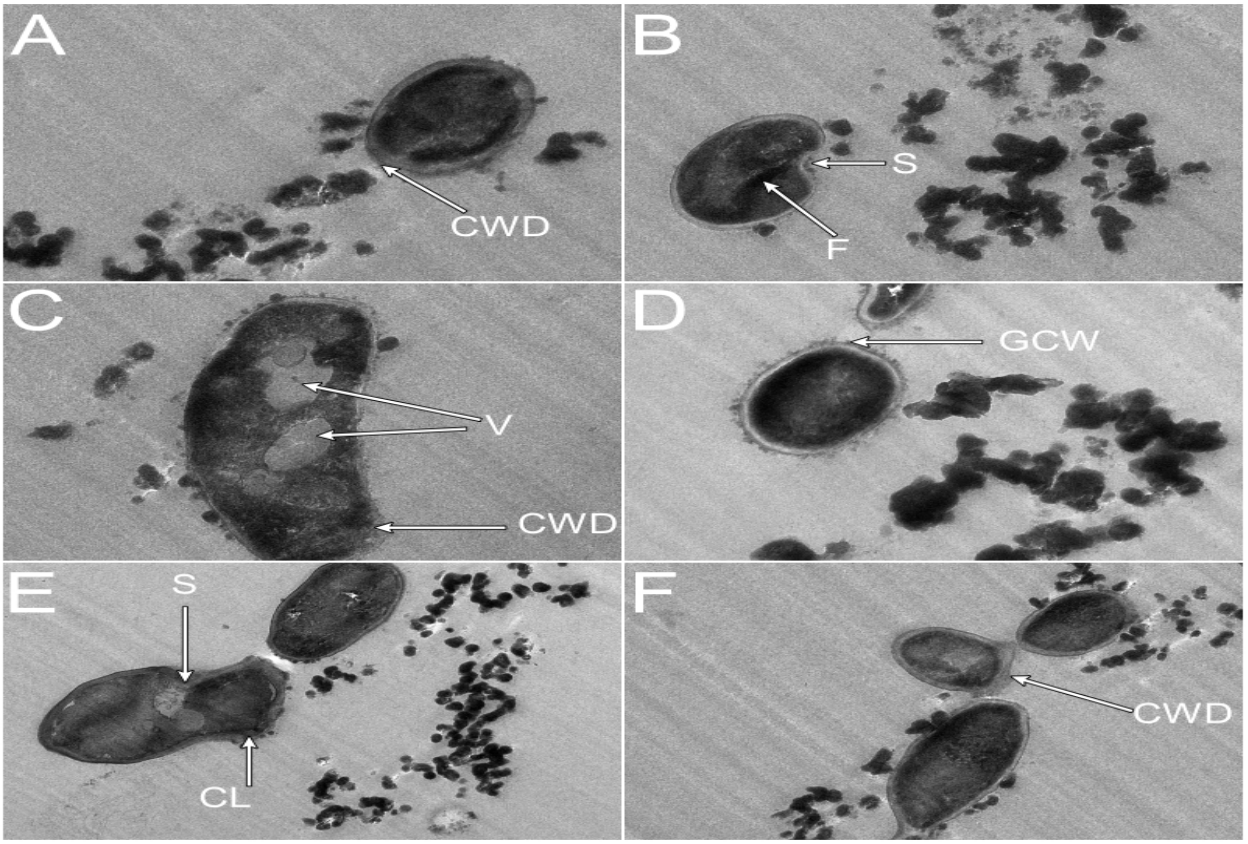

Figure 3.5: TEM micrographs of *C. albicans* after 24 hours of treatment with *H. depressa* showing: thickened and detached cell membrane (CWD) with mantle-like outer covering on the cell wall (GCW); cell membrane shrinkage (S); cytoplasm with heterogeneous electron density (F); vacuolar enlargement (V) and cytoplasmic leakage (CL).

3.4. Discussion

H. depressa leaf and stem extracts showed inhibitory activity against *C. albicans*, *S. aureus*, and *S. pyogenes*, as reflected by the MIC values in Table 5.1. The acetone and methanol extracts exhibited comparatively lower MIC values, suggesting microbial inhibition. These findings suggest that the aerial parts of *H. depressa* contain compounds that inhibit pathogens associated with candidiasis, endocarditis, and pharyngitis; however, further studies are required to confirm their therapeutic relevance. Moreover, the good SI noted for the *H. depressa* extracts indicates a promising biosafety profile, suggesting that leaves and stems of the plant are favourable for medicinal applications. These results are consistent with previous studies, including those of Reid et al. (2005), which documented the antibacterial activity of *H. depressa* ethanolic and ethyl acetate extracts from roots, leaves, and stems against *Bacillus subtilis*, *Klebsiella pneumoniae*, and *Escherichia coli*. Similarly, Hlongwane (2008) reported activity against *Mycobacterium tuberculosis*, while Ngobeni *et al.* (2024) showed that methanol and acetone extracts inhibited 13 microorganisms, including *Candida* spp., *S. aureus*, and *S. pyogenes*, with the most pronounced effects observed against *C. albicans* (MIC = 0.1 mg/mL) and *B. cereus* (MIC = 0.3 mg/mL). This further supports the use of *H. depressa* in ethnomedicine for the treatment of microbial infections. Literature suggests that plant-derived secondary metabolites such as flavonoids, terpenoids, and phenolics commonly exert broad-spectrum antimicrobial effects (Tariq *et al.,* 2019; Yadav *et al.,* 2023; Patra *et al.,* 2017; Rajeswaran and Rajan, 2025). Moreover, several flavonoids, tannins, alkaloids and phenolic compounds identified in *H. depressa* extracts have been widely associated with antibacterial and antifungal activities, which likely underpin the inhibitory effects observed against *C. albicans* and *S. aureus* (Reid et al., 2005; Hlongwane, 2016; Ngobeni et al., 2024; Nhlapo et al.,2025). The greater effectiveness of acetone and methanol extracts compared to the aqueous extract aligns with the increased solubility and extraction efficiency of the non-polar to semi-polar compounds in organic solvents (Borges *et al.,* 2020). The scanning and transmission electron microscopy findings

provide a mechanistic basis for the antimicrobial activity, showing evident ultrastructural damage to *C. albicans* cells caused by the acetone extracts, noted by overall cell distortion, membrane disruption, cytoplasmic leakage, and inhibition of budding. These alterations may be attributable to the presence of phytochemicals, which have been shown in previous research to similarly portray antimicrobial activity by disrupting membranes and walls, damaging cell organelles and essential structures and inhibiting spore germination, hyphal growth, and biofilm formation (Huang *et al.*, 2023; Lee *et al.*, 2016; Lemos *et al.*, 2020; Muniyappan *et al.*, 2023; Ngobeni *et al.*, 2020; Sasidharan *et al.*, 2011; Sun *et al.*, 2024; Yang *et al.*, 2024). Furthermore, *C. albicans* treatment with conventional antifungal drugs, like amphotericin B and flucytosine, exhibits comparable ultrastructural morphological abnormalities, including distortion, shrinkage, and increased cell surface irregularity (Kim *et al.,* 2011), suggesting that *H. depressa* aerial part could be a cheaper, accessible and potentially safer alternative antifungal agent as compared to conventional synthetic antifungal drugs.

3.5. Conclusion

The findings of this chapter further validate the immense potential of *H. depressa* aerial parts as an effective antimicrobial agent that could be harnessed in the treatment of various microbial infections. The broad-spectrum antimicrobial activity demonstrated across various microbial strains highlights the plant's pharmacological relevance and supports its traditional use in ethnomedicine. However, the relatively low selectivity index (SI) values observed for acetone extracts against *C. albicans* and *S. pyogenes*, as well as for methanol extracts against *S. aureus*, suggest possible cytotoxicity at higher concentrations and therefore emphasise the need for further in-depth investigations. Future studies should prioritise bioassay-guided fractionation and isolation of the active phytochemicals responsible for antimicrobial effects, potentially facilitating the discovery of compounds with greater specificity and safety profiles. Additionally, the observed

mechanistic effects of *H. depressa* extracts on microbial cell morphology, including disruption of cell walls and cytoplasmic disorganisation, provide valuable insights into the possible modes of action. These findings open new avenues for research into plant-derived antimicrobial agents, particularly as resistance to conventional antibiotics continues to rise. Cumulatively, the results of this study demonstrate that *H. depressa* holds promise as a reservoir of new antimicrobial compounds. Still, further pharmacological, toxicological, and in vivo evaluations are essential before clinical application can be realised.

3.6. References

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CHAPTER FOUR

THE *IN-VITRO* ANTI-INFLAMMATORY AND ANTIOXIDANT ACTIVITIES OF *H. DEPRESSA* LEAVES AND STEMS EXTRACTS

Abstract

Chronic inflammation and oxidative stress are pathological drivers of numerous life-threatening ailments. This chapter evaluates the *in vitro* anti-inflammatory and antioxidant potential of *Hermannia depressa* leaf and stem extracts. The anti-inflammatory activity was assessed by measuring the inhibition of nitric oxide (NO) production in LPS-induced RAW 264.7 macrophages. Antioxidant activity was determined using the FRAP and DPPH free radical scavenging assays. The acetone extract demonstrated potent anti-inflammatory activity (89% NO inhibition at 250 µg/mL; IC_{50} = 130.86 µg/mL). All extracts showed significant antioxidant activity, with the aqueous and methanol extracts being the most potent in the FRAP and DPPH assays, respectively. The anti-inflammatory capacity of the acetone extract was determined to be specific for NO scavenging (Selectivity Index > 1) and not a result of cytotoxicity. These findings suggest that *H. depressa* extracts possess strong, specific anti-inflammatory and antioxidant properties, providing a scientific premise for their ethnomedicinal use and highlighting their potential as an inexhaustible source of novel therapeutic compounds.

4.1. Introduction

Microbial infections of acute and chronic nature, in addition to degenerative conditions such as cancer, are major causes of prolonged inflammation and oxidative stress (Parajuli Baral *et al.*, 2015). Inflammation is the immune reaction to harmful stimuli, such as pathogens or tissue damage, to restore homeostasis, and it encompasses the secretion of proinflammatory mediators, such as nitric oxide, cytokines, and free radicals, by activated neutrophils and macrophages (Kulkarni, Virkar and D'mello, 2008; Coico and Sunshine, 2015; Rahim *et al.*, 2021). Furthermore, prolonged inflammation leads to oxidative stress, where the body's physiological antioxidants are unable to neutralise free radicals, which are byproducts of inflammation (Pizzino *et al.*, 2017). Free radicals such as superoxide (O_2^-) and hydroxyl (OH^-) cause tissue damage directly through oxidative stress or indirectly by generating detrimental molecules such as hydrogen peroxide (H_2O_2), leading to lipid peroxidation and activation of matrix metalloproteases that degrade proteins (Kulkarni, Virkar and D'mello, 2008). The resulting tissue damage triggers further inflammation through the release of more proinflammatory and chemotactic mediators, creating a continuous pathological loop of inflammation and oxidative stress (Schinella *et al.*, 2002; Iwalewa *et al.*, 2007). Alleviation of inflammation and antioxidant stress is fundamental in the treatment and management of any acute or chronic degenerative conditions and metabolic diseases.

The hallmark signs of inflammation and oxidative stress include localised erythema, pain, tissue swelling and loss of physiological function (Coico and Sunshine 2015; Kumar et al. 2013). Inflammatory conditions are conventionally treated with non-steroidal anti-inflammatory drugs (NSAIDs) that reduce pain and minimise pro-inflammatory mediators. Treatment of oxidative stress is usually with synthetic antioxidants such as N-acetylcysteine (NAC), superoxide dismutase (SOD), catalase mimetics, and nuclear factor erythroid 2-related factor 2 (Nrf2) activators that scavenge ROS, restore redox balance, and protect tissues from oxidative damage *(*Forman and Zhang, 2021; Pooja *et*

al., 2025). However, the use of synthetic drugs has limiting challenges such as adverse effects, including gastrointestinal bleeding and immunosuppression (Rahim *et al*., 2021). As a result, the quest for safer anti-inflammatory and antioxidant alternatives is critical for treating such conditions, and medicinal plants have immense potential as candidates.

Herbal medicines are an abundant reservoir of natural anti-inflammatory and antioxidant compounds. In South Africa, over 115 plants are utilised traditionally to treat various inflammatory conditions associated with pain (Iwalewa *et al*., 2007). The antioxidant and anti-inflammatory properties of medicinal plants are mainly attributed to the phytochemical content that comprises flavonoids, tannins, curcumins, saponins, terpenoids, and alkaloids (Adebayo *et al*., 2015). Furthermore, the phytochemicals' modes of action to curb inflammation and oxidative stress include free radical scavenging and inhibition of proinflammatory enzymes, including cyclo-oxygenases (COX) and lipoxygenases (LOX), in the key inflammatory pathways (Lee *et al*., 2003; Sadik *et al*., 2003). Additionally, flavonoids have been proven to inhibit the biosynthesis of prostaglandins, which are end-products of the COX and LOX pathways involved in immunologic responses. (Adebayo *et al*,. 2015)

The plant *H. depressa* has been demonstrated to possess phytochemical compounds like alkaloids, flavonoids, polyphenols and phenolic acids through advanced chromatographic and spectroscopic techniques (Molefe 2013; Ngobeni *et al*., 2024; Reid *et al*., 2005). Furthermore, in several studies, *H. depressa* extracts have exhibited impressive anti-inflammatory and antioxidant potential. For instance, the dichloromethane extract showed COX-1 inhibition of 81% (Reid *et al*., 2005), and acetone and methanol extracts also significantly reduced nitric oxide production in LPS-stimulated macrophages (Ngobeni et al. 2024). The DPPH, ABTS, and FRAP assays were employed to confirm potent Antioxidant activity by free radical scavenging by *H. depressa* extracts (Ngobeni *et al*., 2024; Xaba 2016) Additionally, in the study by Ngobeni (2024), methanol and acetone

extracts outperformed standard antioxidants such as ascorbic acid and Trolox. Therefore, these findings validate *H. depressa* as a valuable candidate for developing effective anti-inflammatory and antioxidant therapies, contributing to its pharmacological relevance.

4.2. Methods

4.2.1. Extract preparation

The extract preparation was as described in Chapter 3. For the anti-inflammatory and antioxidant assays, 150 mg of each extract was solubilised in DMSO to yield a stock solution concentrated at 100 mg/mL and stored at 4°C until required.

4.2.2. Determination of the anti-inflammatory potential of *H. depressa* extracts using nitric oxide inhibition assay

NO inhibition assay was conducted using the Griess assay with minor alterations (Tsikas 2007). RAW 264.7 cells were seeded at 1×10^5 cells/well in 96-well plates using RPMI 1640 with 10% foetal bovine serum (FBS) and incubated overnight. After removing the spent medium, 50 µL of test samples (diluted in complete medium) were added at final concentrations of 250, 125, 62.5, 31.25, and 15.63 µg/mL. Inflammation was induced by adding lipopolysaccharide (LPS, 500 ng/mL in 50 µL) to RAW 264.7 macrophages and incubating for 24 hours. Aminoguanidine (positive control) was tested at concentrations of 3.7, 7.4, and 14.8 µg/mL. Then 50 µL of spent medium was pipetted into a clean 96-well plate and combined with 50 µL of 1% sulphanilamide prepared in 5% phosphoric acid. The mixture was incubated for 10 minutes at room temperature in the dark, after which 50 µL of 0.1% N-(1-naphthyl)ethylenediamine dihydrochloride (NED) was added. Absorbance was read at 540 nm using a BioTek® PowerWave XS spectrophotometer.

4.2.2.1. Statistical data analysis

A standard curve using sodium nitrite (100µM in complete medium) was used to calculate the concentration of NO (µM) in each. The percentage NO inhibition by *H. depressa* extracts was calculated according to the formula.

$$\% \text{ NO inhibition} = \frac{A_{LPS\ treated\ cells} - A_{sample}}{A_{LPS\ treated\ cells} - A_{untreated\ cells}} \times 100$$

Where $A_{LPS\ treated\ cells}$ is the absorbance in LPS-activated RAW 264.7 macrophages, $A_{untreated\ cells}$ is the absorbance in untreated RAW 264.7 macrophages, and A_{sample} is the absorbance in LPS-stimulated macrophages treated with *H. depressa* extracts. The IC_{50} was calculated by linear interpolation.

4.2.3. Selectivity Index (SI) of the anti-inflammatory potential of *H. depressa* extracts

The anti-inflammatory SI was determined to establish whether the perceived inflammatory activity is due to specific proinflammatory marker scavenging or cytotoxicity against normal mammalian cells (LPS-activated RAW 264.7 macrophages). Furthermore, a sensitivity index greater than one indicates that *H. depressa* extracts can scavenge proinflammatory markers while maintaining the viability of normal RAW 264.7 cells, suggesting good medicinal potential. Whereas a sensitivity index less than one shows that the extracts exhibit elevated toxicity towards normal cells; therefore, suggesting low therapeutic value due to a lack of selectivity (Adebayo et al. 2017; Nunes et al. 2016). The anti-inflammatory activity index was determined as the ratio of the cytotoxic CTC_{50} to the anti-inflammatory IC_{50} of the respective extract.

$$SI = \frac{CTC_{50}}{IC_{50}}$$

4.2.4. Assessing the antioxidant potential of *H. depressa* extracts

4.2.4.1. Ferric Reducing Antioxidant Power

The *H. depressa* extracts were prepared as stipulated in Chapter 3. A range of concentrations for each extract (3.125–200 µg/mL) was prepared using the respective assay buffer. Trolox (6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was utilised as a control. The FRAP assay was conducted according to the method formulated by Pringle *et al*. (2021), with minor changes. The FRAP reagent was freshly prepared by combining 20 mL of sodium acetate buffer (300 mM, pH 3.6), 2 mL of TPTZ solution (10 mM), 2 mL of ferric chloride solution (20 mM, in distilled water), and 2.4 mL of distilled water. The assay was performed in 96-well microplates in quadruplicate, with 50 µL of each sample added per well, followed by 200 µL of FRAP reagent. The reaction mixtures were incubated at 37 °C for 5 minutes to facilitate the reduction of the ferric–TPTZ complex to its ferrous form, resulting in a blue colouration. The antioxidant-reducing ability is directly proportional to the intensity of the colour change. Absorbance was measured at a wavelength of 593 nm using a BioTek PowerWave XS spectrophotometer.

4.2.4.2. The DPPH (2,2-Diphenyl-1-Picrylhydrazyl) free radical scavenging assay

The DPPH assay was conducted employing the method formulated by Pringle *et al*., (2021) with minimal alterations. The assay was conducted in 96-well plates in quadruplicate to ensure statistical reliability. The preparation of *H. depressa* extracts was as stated in 6.2.1. A sample volume of 5 µL at varying concentrations (3.125 – 200 µg/mL) was mixed with 120 µL of Tris-HCl buffer (50 mM, pH 7.4), followed by the addition of 120

µL of freshly prepared DPPH solution (0.8 mM in ethanol). The prepared treatments were incubated at room temperature in the dark for 20 minutes to prevent photodegradation of the DPPH radical. Finally, absorbance was recorded at 510 nm wavelength using the BioTek® PowerWave XS spectrophotometer.

4.2.4.3. Statistical data analysis

Radical scavenging activity calculation was: absorbance of the control ($A_{control}$) minus the absorbance of the sample (A_{sample}), divided by the absorbance of the control, multiplied by 100. IC_{50} values were extrapolated through linear interpolation, and the same calculation was applied for the control (Trolox). Trolox equivalents were then calculated by dividing the antioxidant activity IC_{50} of the extracts by the IC_{50} of Trolox. The following formula expresses the radical scavenging activity:

$$\% \text{ Free radical scavenging} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

4.3. Results

The anti-inflammatory activity was evaluated employing the NO inhibition assay on LPS-activated macrophages, whereas the FRAP and DPPH scavenging assays were conducted to assess the antioxidant activity. The results were interpreted according to four levels. Percentage activities below 20% were considered poor; those between 20% and 50% were classified as insignificant; activities ranging from 50% to 70% were regarded as moderate; and any activity falling within the range of 70% to 100% was considered to demonstrate significant or strong activity (Mulaudzi *et al*., 2013).

4.3.1. NO inhibition assay

All three *H. depressa* extracts demonstrated a dose-dependent NO inhibition in LPS-activated RAW 264.7 macrophages. The acetone extract exhibited the most significant anti-inflammatory activity, achieving 89% NO inhibition at 250 µg/mL, which closely matched the effect of the positive control, aminoguanidine (Figure 6.1). The methanol extract showed low inhibition (48% at 250 µg/mL), while the aqueous extract was least active (37% at 250 µg/mL).

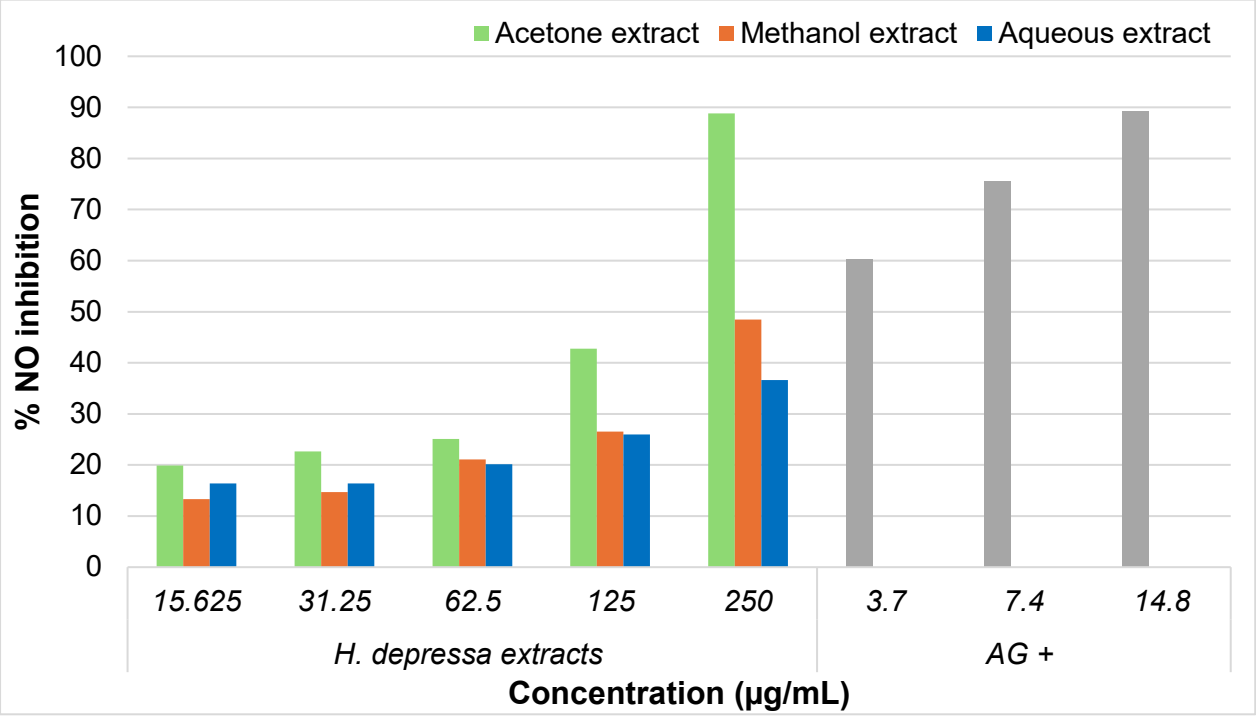

Figure 4. 1: Percentage NO inhibition in LPS-activated macrophages.

Table 4. 1: The nitric oxide half maximal inhibition (IC_{50})

H. depressa extracts	IC_{50} (µg/mL)
Acetone extract	130.86
Methanol extract	266.37
Aqueous extract	398.54
AG+ Positive Control	196.34

4.3.2. Anti-inflammatory activity Selectivity Index

The acetone extract showed the strongest anti-inflammatory effects, achieving a selectivity index above one, indicating good selectivity in inhibiting the NO pro-inflammatory marker while causing minimal cytotoxic effects on activated RAW 264.7 cells (Table 6.2). The aqueous and methanol extracts also showed promising SIs, even though the anti-inflammatory activity was weak. The highest selectivity index was observed with the aqueous extract, suggesting a strong safety profile in relation to its anti-inflammatory activity. The aqueous extract exhibited the most notable SI, which was a key factor contributing to its exceptional biosafety profile.

Table 4. 2: Anti-inflammatory activity Selectivity Index

H. depressa extracts	CTC_{50} (µg/mL)	IC_{50} (µg/mL)	Selectivity index
Acetone extract	207.21	130.86	1.6
Methanol extract	695.20	266.37	2.6
Aqueous extract	93028	398.54	233

4.3.3. FRAP and DPPH assays

The antioxidant potential of the *H. depressa* extracts was investigated employing the FRAP and DPPH assays. In the FRAP assay, all three extracts exhibited significantly lower ferric reducing capacity than the standard Trolox, as shown in Table 6.3, with Trolox equivalents manifold less than 1 across the board. This suggests that leaves and stems have a weak antioxidant capacity, as indicated by their weak ferric reducing power compared to the Trolox standard. Furthermore, all extracts demonstrated strong results in the DPPH assay, with DPPH free radical scavenging capacity ranging from 78% to 100% across all extracts (Figure 6.3). This shows that *H. depressa* aerial parts have more potent radical scavenging through hydrogen-donating capacity, which suggests strong antioxidant activity.

Table 4. 3: FRAP activity represented as Trolox equivalents

Sample	IC_{50} (µg/mL)	Trolox IC_{50} (µg/mL)	Trolox equivalent *
Methanol extracts	189	14.0	0.07
Acetone extracts	301		0.05
Aqueous extracts	109		0.13

**The closer the value is to 1, the better the antioxidant activity.*

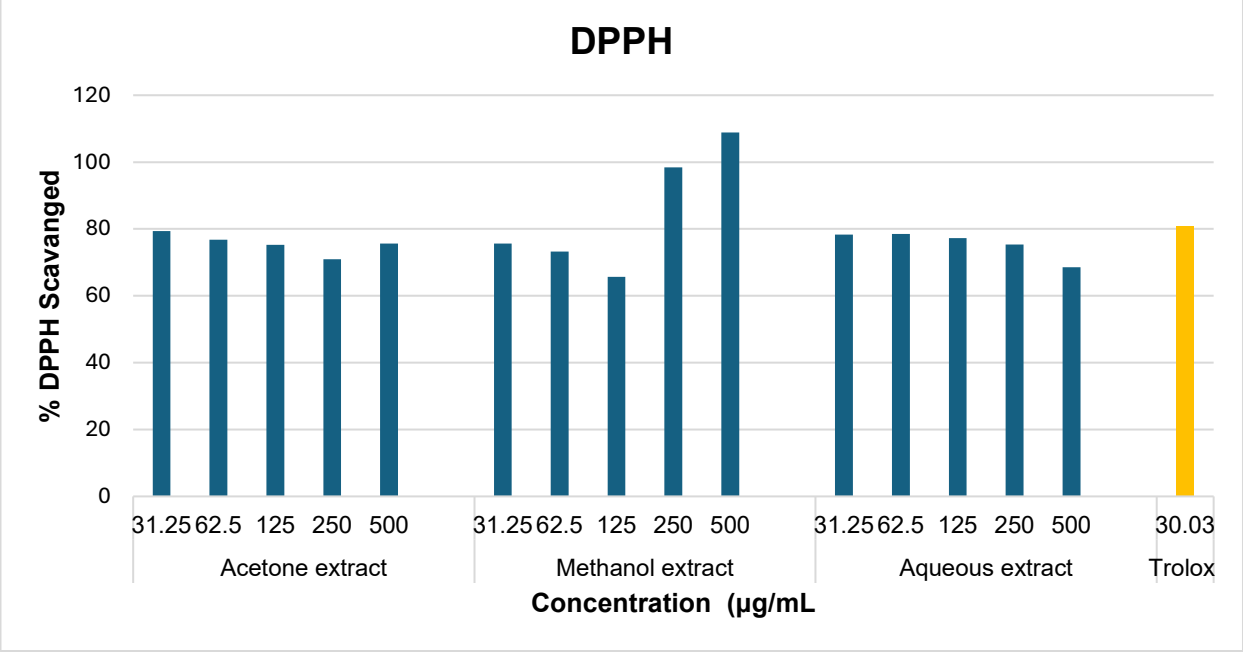

Figure 4. 2: Bar chart showing percentage DPPH free radical scavenging activity of *H. depressa* extracts**.**

4.4. Discussion

The anti-inflammatory assessment was conducted through the NO inhibition assay employing LPS-activated RAW 264.7 macrophages. Acetone extracts demonstrated the most significant anti-inflammatory activity, thereby validating the ethnomedicinal use of the plant in treating inflammation-related disorders, such as infections, pain, headaches, fever, and burns (Chapter 2; Reid *et al.,* 2005; Molefe, 2013; Seleteng Kose *et al.,* 2015). Methanol and aqueous extracts showed insignificant NO inhibition. Moreover, acetone extracts showed good specificity (SI > 1), which may indicate that the extracts can specifically target and scavenge pro-inflammatory markers while being relatively safe to normal host cells. The higher NO inhibition observed with the acetone extract may be attributable to abundant anti-inflammatory phytochemicals; these may include phenolics, flavonoids, saponins or terpenes, which have been shown to inhibit pro-inflammatory

mediators, including NO (Desai *et al.,* 2009; Pereira *et al.,* 2009; Kumar and Pandey, 2013; Ninkuu *et al.,* 2021). The antioxidant properties of the *H. depressa* leaves and stems extracts were assessed through the FRAP and DPPH assays. In the FRAP assay, leaf and stem extracts of *H. depressa* showed weak antioxidant activity in the ferric reducing capacity pathway. However, the DPPH assay showed a potent radical scavenging capacity in all three *H. depressa* leaf and stem extracts, suggesting that *H. depressa* aerial parts may be rich in hydrogen-donating antioxidants, as per the principle of the DPPH method (Pringle *et al.*,2021). The DPPH findings in this research are consistent with those of Ngobeni *et al.* (2024), who also reported strong antioxidant properties from *H. depressa* roots using the same assay; moreover, this potent antioxidant activity was linked to the LC-MS identified phytochemicals such as tannins, phenolics, glycosides, flavonoids and fatty acids in *H. depressa* roots (Ngobeni et al., 2024; Vignesh et al., 2022). Similarly, in this investigation, the antioxidant activity of *H. depressa* leaf and stem extracts may be attributed to bioactive phytochemicals such as phenolics, terpenoids, tannins, and flavonoids, as detailed in Chapter 2 (Pereira *et al.,* 2009; Hussein and El-Anssary, 2019; Rajeswaran and Rajan, 2025). The findings suggest that *H. depressa* is a particularly promising candidate for treating conditions associated with prolonged inflammation and oxidant stress, highlighting the plant's exceptional medicinal potential.

4.5. Conclusion

Anti-inflammatory findings in this chapter show that acetone extracts of *H. depressa* leaves and stems are potent inhibitors of NO related to inflammation, suggesting good potential for the medicinal applications of these extracts in treating ailments associated with prolonged inflammation and validating the use of *H. depressa* aerial parts in ethnomedicine to treat infections, pain, headaches, fever or burns (Chapter 2). Moreover, the anti-inflammatory selectivity index was greater than 1, indicating good selectivity in

inhibiting the production of the NO pro-inflammatory marker with minimal cytotoxic effects on activated RAW 264.7 cells, which is a promising indicator of the biosafety of the plant in medicinal applications. Further in vivo experiments are essential to confirm safety, understand pharmacokinetics, and evaluate the effects after prolonged use. In the FRAP assay, the extracts showed relatively weak antioxidant activity compared to Trolox, indicating low antioxidant activity in the ferric ion reduction pathway. In contrast, the DPPH assay demonstrated strong radical-scavenging capacity across all three extracts, suggesting that the aerial parts of *H. depressa* are rich in hydrogen-donating antioxidants and therefore have promising oxidative stress-reducing properties. This chapter presents scientific evidence that backs-up the ethnomedicinal utility of *H. depressa* leaves and stems in alleviating inflammation and oxidative stress, and highlights the aerial parts as an abundant reservoir of medicinal compounds for the formulation of novel treatments for chronic inflammation, metabolic diseases, and degenerative conditions.

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CHAPTER FIVE

THE *IN VITRO* ANALYSIS OF THE BIOTOXICITY OF *H. DEPRESSA* LEAVES AND STEMS EXTRACTS

Abstract

The evaluation of the medicinal potential of medicinal plants requires rigorous biosafety testing. Ethnobotanical surveys have reported *Hermannia depressa* to be a traditionally important plant for treating a diverse range of illnesses in Southern Africa. This chapter evaluates the *in vitro* cytotoxicity of *H. depressa* leaf and stem extracts (acetone, methanol, aqueous) on normal Vero and RAW 264.7 macrophage cells employing the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. All extracts were non-toxic to Vero cells at concentrations up to 250 µg/mL. In contrast, the acetone extract demonstrated significant toxicity to RAW 264.7 cells (36.95% viability at 250 µg/mL), whereas the methanol and aqueous extracts showed low toxicity. This differential cytotoxicity is related to specific extract-dependent phytochemical profiles. The toxicity of acetone extracts at high concentrations may be due to the presence of a wider range of bioactive compounds, some of which may have toxic effects. The findings suggest that *H. depressa* extracts may be viable for medicinal applications, given their low toxicity, especially at low concentrations. Moreover, the results highlight the need to isolate compounds to distinguish therapeutic from cytotoxic agents, ensuring the safe medicinal use of *H. depressa*.

5.1. Introduction

In developing countries such as South Africa, a notable fraction of the population, estimated at 80%, uses traditional medicine for their primary healthcare (Ouoba et al., 2022; Oyebode et al., 2016; Tesfahuneygn and Gebreegziabher, 2019). The production and sales of plant medicine still have no official regulation in most parts of the world, and information about their correct use is immensely lacking more especially in developing countries (Nworu *et al.*, 2014). Nonetheless, there is a general belief that because plant medicines are natural, they are safe and have minimal side effects (Philomena, 2011). This can be a misleading notion because there are safety concerns regarding the medicinal use of plants, which may arise from misidentification, improper preparation, or incorrect dosage administration by untrained personnel (Botha and Penrith, 2008; Nasri and Shirzad, 2013; Van Wyk, 2002). Biosafety of medicinal plants is a matter of grave importance; therefore, research must prioritise finding ways in which plant-based medicines can be prepared and used in a safe, regulated manner.

The prevalence of traditional medicine poisoning is high in places where herbal remedies are the main form of healthcare and are a deeply rooted practice, affecting young children and pregnant women mostly (Botha and Penrith, 2008; Nasri and Shirzad, 2013). In South Africa, traditional plant-based remedies have been associated with acute poisoning, leading to significant morbidity and mortality, with annual deaths estimated between 10,000 and 20,000 (Popat, *et al.,* 2001). A study at a hospital in South Africa (1987–1992) discovered that traditional medicines caused 7.8% of poisoning cases, and traditional medicines were associated with the highest mortality among poisoning cases. Children under the age of five years constituted 79% of the mortalities caused by traditional medicines (Popat *et al.*, 2001; Scott, 2003). The dangers of medicinal plant use are documented despite their therapeutic advantages, and measures to reduce poisoning from medicinal plants must be taken.

Toxicity in plants has been recognised for ages; plants with cardiac glycosides have been utilised both as arrows and as heart tonics (Botha and Penrith, 2008). Adverse health effects of plant medicines result from toxic compounds contained in plants; plants synthesise these compounds as a natural defence mechanism against microorganisms, insects, herbivores and environmental stress. Medicinal plant toxicity can affect various organ systems depending on the toxic compounds. It can manifest through neurotoxicity, gastrointestinal toxicity, genotoxicity, mutagenic effects, cytotoxicity and metabolic toxicity - damaging vital organs such as the liver, kidneys, heart, and lungs. Extremely toxic plants can lead to systemic collapse, including multiple organ failure, seizures, coma, and death (Ndhlala *et al.,* 2013). The degree of toxic effects is dependent on factors such as the plant species, the part of the plant used, dosage, frequency of exposure, and individual susceptibility (Philomena, 2011). Efforts to determine the safe administration of plant medicines must include the isolation and characterisation of pure compounds with medicinal effects, as this will allow them to be distinguished from toxic ones.

H. depressa also has important medicinal uses as outlined in Chapter 5; however, toxicity studies on this plant showed low to moderate cytotoxicity, depending on the various extraction solvents. *In vitro* tests on kidney cells showed that acetone and methanol extracts slightly reduced viability, while aqueous extracts were non-toxic (Ngobeni *et al.,* 2024). Additionally, in vivo brine shrimp lethality assays showed significant toxicity of acetone and aqueous extracts, especially at higher concentrations, indicating potential risks in living organisms (Molefe, 2013). Previous cytotoxicity studies on *H. depressa* mainly focused on the root extracts of this plant; hence, this chapter will focus on the toxicity assessment of *H. depressa* leaves and stems extracts.

5.2. Methods

5.2.1. Preparation of *H. depressa* extracts

The extract preparation was as outlined in Chapter 3; however, for cytotoxicity analyses, the extracts were solubilised using dimethyl sulfoxide (DMSO) to yield stock solutions concentrated at 100 mg/mL and stored at 4°C until use.

5.2.2. MTT assay toxicity analysis of *H. depressa* extracts

The toxic effects of *H. depressa* extracts were evaluated employing the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) MTT assay formulated by Mosmann (1983) with slight changes. The assay was conducted as follows: cells were seeded into 96-well microtiter plates at a concentration of 3×10^4 cells/mL using a volume of 100 µL in each well for each cell line. The incubation microtiter plates was at 37°C, 5% CO_2, and 100% humidity for 24 hours before adding the test extracts to allow cell attachment. Cells were treated using a 5-point serial dilution range from 15.6-250 µg/mL of each extract. The positive control was 25 µM of Melphalan. The treated cells were incubated for 48 hours, after which the treatments were aspirated. Then, 100 µL of MTT (0.5 mg/mL) in complete medium was added to every well and incubated for an additional 3 hours. MTT was aspirated, and 100 µL DMSO was added to each well. Finally, the wavelength at which absorbance was measured was 540 nm using a BioTek® PowerWave XS spectrophotometer (Winooski, VT, USA).

5.2.3. Statistical data analysis

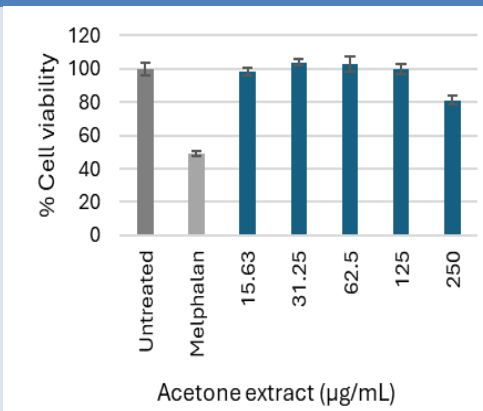
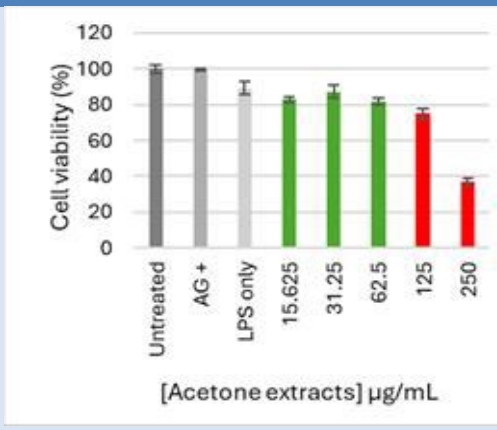
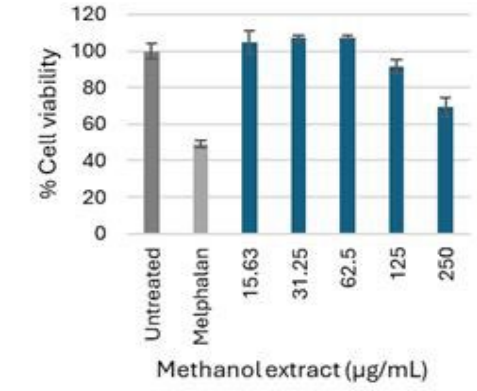
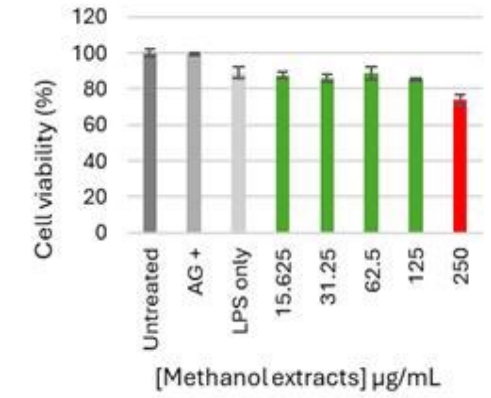
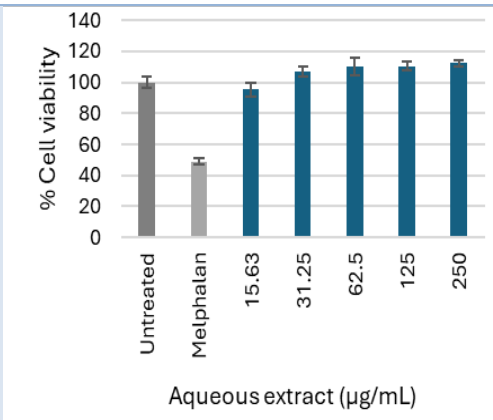
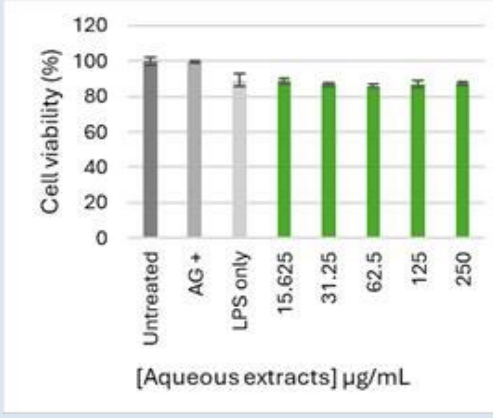
The data was analysed using Microsoft Excel, and % cell viability after treatment was calculated using the formula: $(A_{treated} / A_{untreated}) \times 100$, where $A_{treated}$ is the absorbance of

the cell line treated with *H. depressa* extracts and $A_{untreated}$ is the absorbance of untreated cells.

5.3. Results

The three (3) *H. depressa* extracts (acetone, methanol and aqueous) were evaluated for cytotoxicity using the normal Vero cells and RAW 264.7 macrophage cells using the MTT assay; the results are presented in Table 5.1. Cell viability percentages above 80% after treatment with the extracts were considered non-cytotoxic, those between 80% and 60% as weak cytotoxicity, 60% to 40% as moderate cytotoxicity, and values below 40% as strongly cytotoxic (López-García *et al.*, 2014). All three *H. depressa* extracts demonstrated no cytotoxic effects on Vero cells, as cell viability remained above 80% at a maximum concentration (250 µg/mL). The cytotoxicity results on RAW 264.7 macrophages showed that acetone extracts exhibit significant cytotoxicity at a maximum treatment concentration of 250 µg/mL, resulting in a decline in per cent cell viability of 36.95%. Additionally, methanol extracts also exhibited weak cytotoxicity at 250 µg/mL concentration, where viability decreased to 73.90% and aqueous *H. depressa* extracts showed non-significant cytotoxicity.

Table 5. 1: The cell viability percentage of Vero cells and RAW 264.7 macrophages after being treated with *H. depressa* extracts**.**

H. depressa	Vero cells	RAW 264.7 macrophages
Acetone extract		
Methanol extract		
Aqueous extract		

5.4. Discussion

In this chapter, the cytotoxicity of *H. depressa* extracts on Vero and RAW 264.7 cells were assessed using the MTT assay, and the results revealed solvent-dependent and concentration-dependent cytotoxicity profiles. The aqueous and methanol extracts showed no significant toxicity against Vero and RAW 264.7 cell lines, whereas the acetone extracts exhibited notable cytotoxicity against RAW 264.7 cells at the highest concentration, but no significant toxicity against Vero cells. This indicates potential safety concerns for acetone extracts. The differential cytotoxicity may be related to the differing inherent susceptibility profiles between Vero cells and RAW 264.7, as well as to distinct phytochemical compositions, as revealed by GC-MS analysis (Chapter 3). Mouse-derived RAW 264.7 macrophages are likely more susceptible to the extracts' effects due to their phagocytic nature, which increases intracellular accumulation and enhances their vulnerability to cytotoxic effects. In contrast, the resilience of Vero cells to acetone-extract-induced damage may be attributed to their lower propensity for uptake, reducing the likelihood of intracellular exposure. Vero cells, derived from monkey kidney epithelium, are widely used as a human-relevant model of mammalian cells. Their resilience to acetone-extract-induced toxicity is encouraging and suggests that *H. depressa* extracts may be safe at specific dosages; however, further validation in human cell models is required before definitive safety conclusions can be drawn (Ammerman, Beier-Sexton and Azad, 2008; Stanca *et al.*, 2023). The higher cytotoxicity observed in the acetone extract, especially against RAW 264.7 macrophages, is also likely due to its more diverse phytochemical content. The intermediate polarity of the acetone solvent effectively extracted a broader spectrum of compounds, including those that may be cytotoxic (Borges *et al.,* 2020). The presence of alkaloids, flavonoids, phenolics and steroidal compounds in *H. depressa* extracts may be associated with cytotoxic effects, as these phytochemical classes are known to disrupt cellular metabolism and membrane integrity, thereby accounting for the biotoxicity observed *in vitro* (Molefe, 2013; Ngobeni et al., 2024; Nhlapo et al., 2025; Reid et al., 2005). Moreover, the synergistic interactions among

the present compounds are likely to contribute to the observed higher cytotoxicity as noted in the acetone extract. The biosafety of medicinal plants is essential for their therapeutic use (Ndhlala *et al.*, 2013). However, cytotoxicity observed in RAW 264.7 macrophage cells should not be viewed solely as a negative property but also as an indicator of potent bioactivity, such as anticancer properties, and warrants further scientific investigation (Bunel *et al.,* 2014; George *et al.,* 2010). Future studies should prioritise the challenge of distinguishing therapeutic compounds from cytotoxic ones through fractionation, isolation, and elucidation of pure compounds from *H. depressa* extracts. This can separate compounds with beneficial pharmacological effects, such as antimicrobial or anti-inflammatory properties, from those that are toxic.

5.5. Conclusion

The cytotoxicity results of *H. depressa* leaf and stem extracts indicate that the plant is safe for Vero cells, which are known to have a close resemblance to human cells, suggesting that *H. depressa* aerial parts may be safe for human medicinal use. The cytotoxicity observed in RAW 264.7 macrophages indicates that the extracts should be used with caution, considering safe dosages. However, cytotoxicity is not merely a negative property, but rather an indicator of potent pharmacological activities, which may also include anticancer properties. Therefore, further in vivo biosafety studies are necessary to establish a more comprehensive biosafety profile.

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CHAPTER SIX

THE PHYTOCHEMICAL PROFILING OF *H. DEPRESSA* EXTRACTS

Abstract

This chapter aimed to comprehensively profile the phytochemical constituents of *Hermannia depressa* N.E.Br. leaf and stem extracts to identify the bioactive compounds responsible for its documented medicinal uses. Crude extracts were prepared using solvents of varying polarity: acetone, methanol, and hot water (80 °C). The chemical composition of the active extracts was determined using Gas Chromatography-Mass Spectrometry (GC-MS). The identified compounds were confirmed through comparison of their mass spectra and retention indices with those available in Wiley and NIST standard databases. The percentage yield of extraction was highest for methanol (29%), followed by acetone (26%) and aqueous (6.3%) solvents. GC-MS analysis revealed a diverse array of pharmacologically active phytochemicals. The acetone and methanol extracts were particularly rich in terpenes (e.g., neophytadiene, phytol, trans-β-ionone, β-myrcene, limonene), phenolic compounds (e.g., scopoletin, 2,4-di-tert-butylphenol, 3-methoxybenzoic acid), aldehydes (p-anisaldehyde), and nitrogen-containing compounds (benzothiazole). Notable high-abundance compounds included Vitamin E and neophytadiene. These compounds are associated with a broad spectrum of bioactivities, such as antimicrobial, anti-inflammatory, antioxidant, and anticancer properties. The prevalence of terpenes and phenolics provides a concrete chemical basis for the plant's

traditional use in treating headaches, respiratory conditions, gastrointestinal ailments, and various microbial infections.

6.1. Introduction

The use of plants as medicine dates back 6,000 years, where diverse cultures around the globe have utilised herbs to treat different diseases (Khan, 2014; Jamshidi-Kia, Lorigooini and Amini-Khoei, 2018) Globally, medicinal plants used to treat diverse diseases are estimated to be around 35,000. Traditional plant-based medicine was developed through the extensive empirical expertise of many healers over centuries and was passed down from ancestors to younger generations (Jamshidi-Kia, Lorigooini and Amini-Khoei, 2018). As civilisations thrived, they created their own indigenous “Materia medica”, where information about the diverse plant species and their medicinal use was documented (Khan, 2014). This consequently led to the emergence of modern medicine, which evolved into the current functioning healthcare system.

“*Phyto*” is a Greek-derived word meaning plant; therefore, in the literal sense, phytochemicals are plant chemicals (Velavan, 2015). The main categories of phytochemicals are primary and secondary metabolites, and this classification is based on their function in plant metabolism (Kalimuthu and Prabakaran, 2013). Primary metabolites are essential compounds linked to vital cellular functions, including growth and energy metabolism. Primary metabolites are vital for essential cell functions, especially those associated with growth and energy metabolism, and they include carbohydrates, proteins, amino acids, nucleosides, and chlorophylls. Secondary metabolites perform specific roles in plants, supporting development through hormonal activity and photosynthesis, aiding reproduction and survival in nature, as well as actively

contributing to their medicinal properties (Kalimuthu, Kalimuthu and Prabakaran, 2013; Böttger *et al.*, 2018).

Approximately 139,000 secondary metabolites have been discovered so far, with the primary categories comprising phenolics, alkaloids, saponins, terpenes, lipids, steroids, flavonoids, tannins and glycosides (Kalimuthu and Prabakaran, 2013; Hussein and El-Anssary, 2019). Specific classes of phytochemicals have diverse therapeutic profiles: antimicrobial, analgesic, anti-inflammatory, antioxidant, antidiabetic, anticancer, fertility-enhancing functions, cardiotonic effects and immunomodulatory properties (Ghanghro *et al.*, 2015; Mera, Falconí and Córdova, 2019; Kumar *et al.*, 2023). Approximately 7,000 compounds with pharmacological properties have been identified from medicinal plants, leading to the derivation of numerous groundbreaking novel drugs, such as quinine, Taxol, and aspirin. (Tshibangu *et al.*, 2002). This demonstrates that medicinal plants are an immense and vast source of pharmacologically active agents necessary to combat diverse medical conditions.

H. depressa root extracts have previously been analysed for their photochemical constituents utilising techniques such as thin layer chromatography (TLC), high-performance liquid chromatography (HPLC), vacuum liquid chromatography (VLC) and liquid chromatography tandem mass spectrometry (LC-MS/MS). Compounds such as tannins, phenols, saponins, terpenoids, and cardiac glycosides were identified using preliminary phytochemical screening methods (Molefe, 2013; Ngobeni *et al.,* 2024; Reid *et al.,* 2005). LC-MS/MS detected compounds classified as alkaloids, flavones (e.g., Waltherione D, quercetin, tricin), steroids, lignans, and fatty acids in different extracts. However, the *H. depressa* stems and leaves remain unexplored; hence, this shows a substantial gap in research on the phytochemical characterisation of this plant. Isolating and elucidating pure bioactive compounds is also critically needed. Consequently, this

chapter aims to identify bioactive compounds from crude acetone, methanol, and aqueous extracts of *H. depressa* leaves and stems.

6.2. Methods

6.2.1. Extract preparation

The extract preparation was as described in Chapter 3. For phytochemical analyses, 150 mg of each extract was solubilised in DMSO to obtain a stock solution at 100 mg/mL, which was stored at 4°C until required.

6.2.2. Determination of Percentage Yield

The percentage yield of the extracts was determined using the formula $(M_1/M_0) \times 100$, where M_1 = mass of final extract and M_0 = mass of initial plant material.

6.2.3. Detection of bioactive compounds using GC-MS

H. depressa extracts were prepared by adding 0.5 mL phosphate buffer to 10 mL of the sample in polytetrafluoroethylene (PTFE) lined screw-cap tubes, followed by phase-transfer catalyst, 1 mL extraction solvent, 10 µL internal standard, and 180 mg methyl iodide. Tubes were sealed and stirred at 70 °C for 90 minutes, cooled, saturated with sodium chloride (NaCl), and the organic layer was separated and dried over anhydrous sodium sulphate (Na_2SO_4). Supernatants were transferred into clean vials for analysis. GC–MS was performed on an HP 6890 Series gas chromatograph with split / splitless injectors, an autosampler and a Supelco SPB-M-5 capillary column (30 m × 0.32 mm, 0.25 µm film) using helium (99.9%) as carrier gas. Injection volume was 1 µL (splitless,

50:1 split after 1 min), injector temperature 260 °C, detector/transfer line temperature 280 °C, with oven programming: 50 °C (5 min), ramp 5 °C/min to 150 °C, ramp 10 °C/min to 210 °C (11 min hold), total run time 45 min. The mass spectrometer (HP 5973) operated in EI mode at 70 eV, scanning m/z 50–500 mode, calibrated with perfluorotributylamine (PFTBA) (m/z 69, 219, 502). Peaks were identified by comparison with authentic standards, relative retention indices, and spectral libraries, as per the WILEY online library and the National Institute of Standards and Technology (NIST) system (Sujatha et al., 2013).

6.3. Results

6.3.1. Percentage Yield

The three solvents used in this study yielded different amounts of crude extract mass, as demonstrated in Figure 3.1. Methanol extraction produced the highest yield (29%), followed by acetone (26%), and lastly, hot water (6.3%).

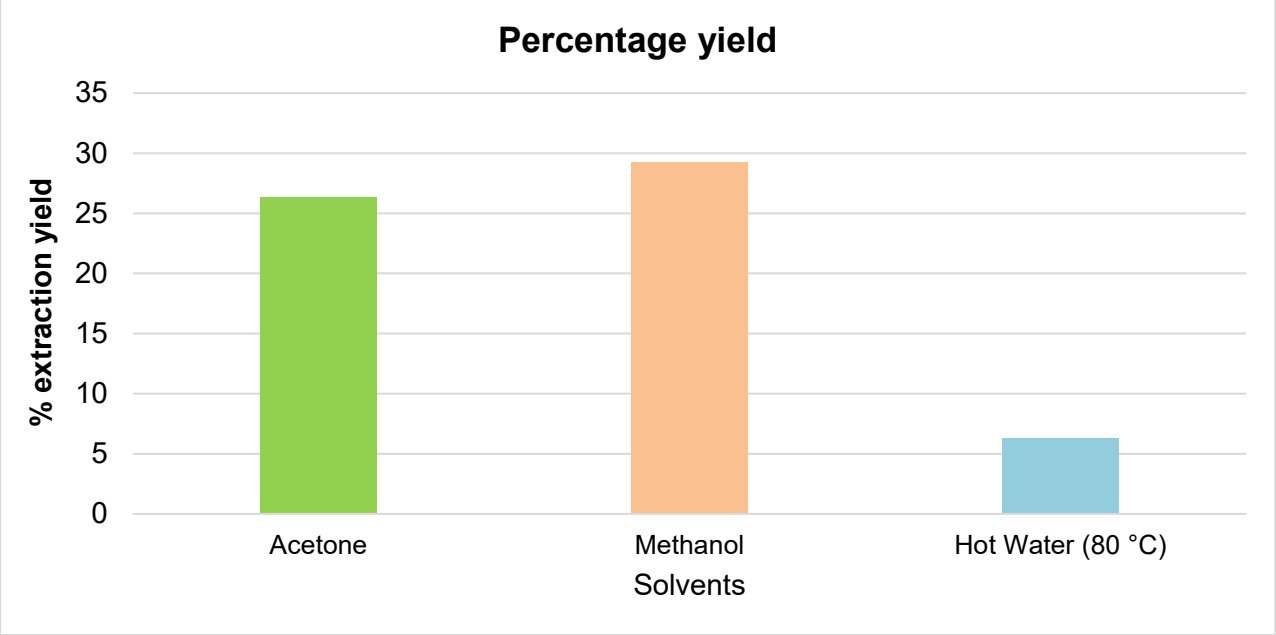

Figure 6. 1: Different percentage yields of *H. depressa* extracts using acetone, methanol, and hot water (80 °C)

6.3.2. Detection of bioactive compounds using GC-MS

The phytochemical analysis of *H. depressa* acetone and methanol extracts was conducted using GC-MS, and the detected compounds are presented in Tables 6.1 and 6.2, respectively. Moreover, GC-MS identified phytochemical classes with important pharmacological properties, including terpenes, phenolic acids, phenols, phenylpropanoids, flavonoids, and coumarins. The documented pharmacological properties of the prominent compounds identified include antimicrobial, anticancer, antioxidant, anti-inflammatory, and antidiabetic effects, among others, as demonstrated in Table 6.3. These findings support the plant's ethnomedicinal use and make it a promising candidate for the discovery of new drugs.

Table 6. 1: Phytochemicals identified in *H. depressa* leaf and stem acetone extract

No.	RT*	Compound	Molecular formula	Molecular weight (g/mol)	% Peak area
1	11.1163	beta-Myrcene	$C_{10}H_{16}$	136.2	0.09
2	11.8688	Limonene	$C_{10}H_{16}$	136.2	0.18
3	12.3408	trans-Carane	$C_{10}H_{18}$	138.3	0.25
4	12.6526	E-Citral	$C_{10}H_{16}O$	152.2	0.14
4	13.4986	Dibutyl disulfide	$C_8H_{18}S_2$	178.4	0.11
5	14.1082	Menthone	$C_{10}H_{18}O$	154.3	0.21
6	14.2737	iso-Menthone	$C_{10}H_{18}O$	154.3	0.11
7	14.3199	3-Methoxybenzoic acid	$C_8H_8O_3$	152.2	0.36
8	15.315	Benzothiazole	C_7H_5NS	135.2	0.69
9	15.4349	5-tert-butyl-m-Cymene	$C_{14}H_{22}$	190.3	1.04
10	15.7851	Hydroxy-3-isopropyl-6-methyl-2-cyclohexen-1-one	$C_{10}H_{16}O_2$	168.2	0.11
11	16.1167	3-methyl-3-Decen-2-one	$C_{11}H_{20}O$	168.3	0.12
12	16.3573	3-ethyl-4-hydroxy-6-methyl-2H-Pyran-2-one	$C_8H_{10}O_3$	154.2	0.13
13	17.043	1,1,6-trimethyl-1,2-dihydronaphthalene	$C_{13}H_{16}$	172.3	0.16
14	17.6051	Methyl eugenol	$C_{11}H_{14}O_2$	178.2	0.17
15	17.7264	2-methoxy-1,4-Benzenediol	$C_{14}H_{14}O_6$	278.3	0.34
16	18.172	Geranylacetone	$C_{13}H_{22}O$	194.3	0.25
17	18.3168	p-Anisaldehyde	$C_8H_8O_2$	136.2	1.47
18	18.3963	2,6-di-tert-Butylquinone	$C_{14}H_{20}O_2$	220.3	0.28

19	18.981	2,4-Di-tert-butylphenol	$C_{14}H_{22}O$	206.3	0.84
20	19.1191	Geranyl Linalool Isomer	$C_{20}H_{34}O$	290.5	0.32
21	19.2782	(Z)- trimethyl-1-propenyl-Pyrazine	$C_{10}H_{14}N_2$	162.2	0.35
22	19.4124	5,6,7,7a-tetrahydro-4,4,7a-trimethyl-2(4H)-Benzofuranone	$C_{11}H_{16}O$	180.0	0.28
23	19.9877	1,4a.beta.-dimethyl-1.alpha.-hydroxy-3,4,4a,5,6,7-hexahydronaphthalen-2(1H)-one	$C_{12}H_{20}O_2$	196.3	0.24
24	21.9259	Iso Jasmone	$C_{11}H_{16}O$	164.2	0.19
25	22.0234	4-hydroxydibenzothiophene Dibenzothiophene-4-ol	$C_{12}H_8OS$	200.2	0.29
26	22.4181	m-Phenoxybenzyl Methyl Ether	$C_{14}H_{14}O_2$	214.5	0.42
27	22.5297	Neophytadiene	$C_{20}H_{38}$	278.5	2.29
28	22.6113	7,7-Dimethyl-6-methylidene-5-(2'-oxo-1'-propyl)-1-oxaspiro[2.6]nonan-4-one	$C_{14}H_{20}O_2$	220.3	0.47
29	22.7824	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296.5	0.40
30	22.9733	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296.5	0.71
31	23.0996	cis-4,5-Dimethoxy-2-ethoxy-.beta.-methylstyrene	$C_{13}H_{18}O_3$	222.3	0.13
32	23.3748	trans-beta-Ionone	$C_{13}H_{20}O$	192.3	0.68
33	23.9823	Naphthalene, decahydro-1,1,4a-trimethyl-6-methylene-5-(3-methyl-2,4-pentadienyl)-, [4aS-(4a.alpha.,5.alpha.,8a.beta.)]-	$C_{20}H_{32}$	272.5	10.03
34	24.1761	Scopoletin	$C_{10}H_8O_4$	192.2	1.97

35	24.2799	2(3H)-Benzothiazolethione	$C_7H_4N_2O_2S_2$	212.3	0.24
36	24.34	Methyl trimethylpyrrole-2-carboxylate	$C_9H_{13}NO_2$	167.2	0.20
37	26.5547	6,7,8,9-Tetrahydro-1,2,3-trimethoxy-9-methyl-5H-benzocycloheptene	$C_{11}H_{14}O$	162.2	3.57
38	26.9623	9(11)-Dehydrotestosterone	$C_{19}H_{30}O_2$	290.4	3.79
39	27.157	3,3'-dimethyl-2,2'-biquinoline	$C_{20}H_{16}N_2$	284.4	0.43
40	27.189	4',6-Dimethoxyaurone	$C_{17}H_{14}O_4$	282.3	0.21
41	27.3873	(cis)-2,5-dihydro-2-methoxy-3,5-diphenyl-1,2-oxaphosphole-2-oxide	$C_{15}H_{13}O_3P$	260.2	0.81
42	27.4364	8.alpha.,13-epoxylabdano-20,2.beta.-lactone	$C_{20}H_{30}O_3$	318.5	0.41
43	29.3341	2-(2h-benzotriazol-2-yl)-4-(1,1,3,3-tetramethyl butyl)phenol	$C_{20}H_{25}N_3O$	323.4	0.73
44	29.3684	2,7-dihydroxy-3,4,6-trimethoxy-9,10-dihydrophenantrene	$C_{17}H_{18}O_5$	302.3	0.71
45	29.3787	1,4-Dihydro-9-isopropylidene-5,6,7,8-tetramethoxy-1,4-methanonaphthalene	$C_{14}H_{14}$	182.3	1.53
46	33.5488	Vitamin E	$C_{29}H_{50}O_2$	430.7	27.58

*Retention time

Table 6. 2: Phytochemicals identified in *H. depressa* leaf and stem methanolic extract

No.	RT*	Compound	Molecular formula	Molecular weight (g/mol)	% Peak area
1	14.0522	2,3-dihydro-3,5-dihydroxy-6-methyl-4h-pyran-4-one	$C_6H_8O_4$	144.1	0.30
2	15.3194	Benzothiazole	C_7H_5NS	135.2	1.06
3	15.4425	5-tert-butyl-m-Cymene	$C_{14}H_{22}$	190.3	0.63
4	18.3342	p-Anisaldehyde	$C_8H_8O_2$	136.2	0.29
4	18.9891	2,4-Di-tert-butylphenol	$C_{14}H_{22}O$	206.3	0.93
5	19.1289	Geranyl linalool isomer	C20H34O	290.5	0.27
6	22.5339	Neophytadiene	$C_{20}H_{38}$	278.5	0.99
7	22.6164	7,7-Dimethyl-6-methylidene-5-(2'-oxo-1'-propyl)-1-oxaspiro[2.6]nonan-4-one	$C_{14}H_{20}O_2$	220.3	0.16
8	22.7875	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296.5	0.22
9	23.3529	Methyl palmitoleate	$C_{17}H_{32}O_2$	268.4	0.20
10	23.3778	trans-beta-Ionone	$C_{13}H_{20}O$	192.3	0.41
11	23.449	Methyl palmitate	$C_{17}H_{34}O_2$	270.5	1.92
12	23.9835	Naphthalene, decahydro-1,1,4a-trimethyl-6-methylene-5-(3-methyl-2,4-pentadienyl)-, [4aS-(4a.alpha.,5.alpha.,8a.beta.)]-	$C_{20}H_{32}$	272.5	7.07
13	24.1784	Scopoletin	$C_{10}H_8O_4$	192.2	0.53
14	24.2736	2(3H)-Benzothiazolethione	$C_7H_4N_2O_2S_2$	212.3	0.62
15	24.4181	Methyl margarate	$C_{18}H_{36}O_2$	284.5	0.15
16	24.9556	(2-Methoxyphenyl) trimethylstannane	$C_{10}H_{16}OSn$	270.9	0.16
17	25.0819	Methyl linoleate	$C_{19}H_{34}O_2$	294.5	1.51

18	25.1463	Methyl linolenate	$C_{19}H_{32}O_2$	292.5	1.75
19	25.2519	Phytol	$C_{20}H_{40}O$	296.5	0.36
20	25.2819	(8.beta.,13.beta.)-Kaur-16-ene,	$C_{20}H_{32}$	272.5	0.72
21	25.354	Methyl stearate	$C_{19}H_{38}O_2$	298.5	0.37
22	26.5454	6,7,8,9-Tetrahydro-1,2,3-trimethoxy-9-methyl-5H-benzocycloheptene	$C_{11}H_{14}O$	162.2	0.41
23	26.833	NORUNS-12-ENE	$C_{30}H_{50}$	189.3	0.29
24	26.8753	2-(Methylthio)-8-phenyl-4,5,6,7-tetrahydro-3Hcyclohepta[c]pyrrole	$C_{18}H_{19}NS$	281.4	0.66
25	26.9629	9(11)-Dehydrotestosterone	$C_{19}H_{30}O_2$	290.4	1.33
26	28.2496	(+-)-scleroderodione			0.40
27	28.3386	Silicone grease, Siliconfett			0.14
28	28.3591	2,3-dimethyl-4-azaphenanthrene	$C_{15}H_{13}N$	207.3	0.05
29	28.4502	Benzene, 1-phenyl-4-(2-cyano-2-phenylethenyl)	$C_{21}H_{15}N$	281.3	0.08
30	28.5749	Desacyl-Kondurangogenins A	$C_{30}H_{48}O_6$	504.7	0.20
31	28.6565	2-Propen-1-one, 3-[4-(1-methylethyl)phenyl]-1-phenyl- (CAS)	$C_{16}H_{14}O_2$	238.3	0.16
32	28.8851	Taraxasterol	$C_{30}H_{50}O$	426.7	0.43
33	30.8382	Squalene	$C_{30}H_{50}$	410.7	4.91
34	33.5639	Vitamin E	$C_{29}H_{50}O_2$	430.7	34.92

Table 6. 3: Pharmacologically significant phytochemicals identified from *H. depressa* leaf and stem extracts

Compound	Solvent	Class	Pharmacological Activities	Toxicity Profile	Reference
(8.beta.,13.beta.)-Kaur-16-ene	Methanol	Diterpenes	Anticancer	n. d	(Rocha *et al.*, 2019)
2,3-Dihydro-3,5-Dihydroxy-6-Methyl-4H-Pyran-4-One	Methanol	Diterpenes	Antioxidant, Anti-inflammatory, Anti-ageing (skin protection)	Oral toxicity, DNA damage, mutagenicity	(Sato *et al.*, 2025)
2,4-Di-tert-butylphenol	Acetone, Methanol	Phenol	Anticancer, Antioxidant, Antibacterial, Antiviral, Antifungal, Anti-inflammatory	DNA damage, Neurotoxicity, Alimentary canal damage	(Varsha *et al.*, 2015; Zhao *et al.*, 2020; Aravinth *et al.*, 2023; Ren *et al.*, 2023)
2-Methoxy-1,4-Benzenediol	Acetone	Phenol	Antimicrobial	Acute oral toxicity, Respiratory irritation, DNA damage	(Sujatha et al. 2013)
3,7,11,15-Tetramethyl-2-hexadecen-1-ol (phytol)	Acetone, Methanol	Diterpene	Antimicrobial, Antioxidant, Anti-inflammatory, Analgesic	Cytotoxic and Genotoxic at high concentrations	(Islam *et al.*, 2018; Madhankumar and Murugesan, 2019; Mgbeje *et al.*, 2020)
3-Methoxybenzoic acid	Acetone	Phenolic acid	Antioxidant, Antimicrobial, Anti-inflammatory, Anticancer, Antidiabetic, Neuroprotective, Cardioprotective	n. d	(Czarnecka *et al.*, 2018)
4',6-Dimethoxyaurone	Acetone	Flavonoid	Anticancer	n. d	(Yang et al., 2024)
Benzothiazole	Acetone, Methanol	Phenazine alkaloids	Anti-inflammatory, Antidiabetic, Analgesic, Antimicrobial, Anticancer, Antitumor, Antibiotic, Antifungal, Antiviral, Antimalarial, Anticonvulsant	Acute toxicity, Dermatitis, Skin allergy, Hepatotoxicity	(Tariq, Kamboj and Amir, 2019; Yadav *et al.*, 2023)
E-Citral	Acetone	Monoterpene	Anticancer, Hepatoprotective, Anti-inflammatory	No toxic effects on humans	(Thomas *et al.*, 2016; Kiyama, 2020)
Geranylacetone	Acetone	Terpene	Antimicrobial, Germicidal	Skin irritation, Respiratory irritation	(Miyazawa, Nankai and Kameoka, 1996; Romankiewicz *et al.*, 2007; Stobiecka, 2015; Saad *et al.*, 2019; Wróblewska-Kurdyk *et al.*, 2022)
Limonene	Acetone	Monoterpene	Anticancer, Antiviral, Anti-inflammatory, Antibacterial	Low toxicity, Skin irritation,	

					(Gad and Hakkinen, 2005; Isac-García *et al.*, 2016)
Menthone and iso-Menthone	Acetone	Monoterpene	Antioxidant, Anti-inflammatory, Antibacterial, Antiviral, Neuroprotective	Low oral toxicity, Low skin irritation. Hepatotoxicity	(Zhao *et al.*, 2022; Jasemi *et al.*, 2024)
Methyl eugenol	Acetone	Phenylpropanoids / Tetrahydroisoquinoline alkaloids	Antioxidant, Anti-inflammatory, Antimicrobial, Anticancer, Neuroprotective, Hepatoprotective, anxiolytic, antidepressant, anaesthetic	Genotoxic, Acute toxicity, Carcinogenicity	(Hong Tan *et al.*, 2012; Buchbauer and Wallner, 2015; de Oliveira *et al.*, 2024)
Methyl linoleate	Methanol	Phenolic acids	Antioxidant	lung toxicity after intravenous administration	(Hempenius *et al.*, 1992; Pekkarinen *et al.*, 1999)
Neophytadiene	Acetone, Methanol	Diterpene	Anti-inflammatory, Neuroprotective, Anticancer, Antidiabetic, Analgesic, Hepatoprotective, Antioxidant, Cardioprotective, Antipyretic, Wound healing properties, Antimalarial properties	Non-toxic in animal models	(Rajeswaran and Rajan, 2025)
Scopoletin	Acetone, Methanol	Phenolic coumarin	Antidiabetic, Anti-inflammatory, Antioxidant, Antimicrobial, Hepatoprotective, Anticancer, Anti-angiogenesis, Anti-gout, Antiaging, Anticonvulsant, Immunomodulatory, Analgesic, Anti-atherosclerotic, Cardioprotective, Neuroprotective, Anti-depressant	No acute toxicity, no weight gain or loss, or gross behavioural variation	(Firmansyah, Winingsih and Manobi, 2021; Gao *et al.*, 2024)
Squalene	Methanol	Triterpene	Antioxidant, Anti-inflammatory, Anticancer, Drug carrier, Detoxifier, Skin hydrating	Non-toxic in in-vitro and in-vivo models	(Kim and Karadeniz, 2012; Lou-Bonafonte *et al.*, 2018)
Taraxasterol	Methanol	Triterpenoids	Anti-inflammatory, Antioxidant, Anticancer, Antidiabetic, chemoprotective, Anti-viral, Anti-allergic	Non-toxic in in-vitro and in-vivo models	(Jiao *et al.*, 2022; Obafemi *et al.*, 2024)
Vitamin E	Acetone, Methanol	Vitamin	Antioxidant	High doses are associated with the risk of bleeding, Low risk for kidney toxicity in humans	(Baltusnikiene, Staneviciene and Jansen, 2023)

p-Anisaldehyde	Acetone, Methanol	Phenolic aldehyde	Antimicrobial, Antioxidant, Antifungal	Low Cytotoxicity against H9c2 rat cardiac myoblasts	(Clarke, 2008; Lin *et al.*, 2022)
trans-β-Ionone	Acetone, Methanol	Terpenes	Antioxidant, Anticancer	low to moderate oral toxicity, No systemic toxicity	(Lalko *et al.*, 2007; Patra *et al.*, 2017; Paparella, Shaltiel-harpaza and Ibdah, 2021)
β-Myrcene	Acetone	Monoterpene	Antioxidant, Anti-inflammatory, Analgesic, Anticancer, Anxiolytic, Anti-ageing	Non-toxic in in-vitro and in-vivo models	(Surendran *et al.*, 2021)

n. d = not documented

6.4. Discussion

In this chapter, GC–MS analysis of *H. depressa* leaf and stem extracts revealed a variety of bioactive compounds, including monoterpenes, diterpenes, triterpenes, phenolic acids, phenols, aldehydes, flavonoids, and coumarins across both acetone and methanol extracts, as shown in Table 6.3. Compounds found in acetone and methanol extracts possess multiple documented biological properties. Notable antimicrobial, anti-inflammatory and antioxidant compounds included methyl eugenol, benzothiazole, phytol, neophytadiene, scopoletin, 3-Methoxybenzoic acid, and 2,4-Di-tert-butylphenol. Additionally, other pharmacological properties of the compounds include analgesic, anticancer and antidiabetic, hepatoprotective and cytotoxic properties, among others (de Oliveira *et al.,* 2024; Tariq *et al.,* 2019; Rajeswaran and Rajan, 2025; Firmansyah *et al.,* 2021; Zhao *et al.,* 2020; Yadav *et al.,* 2023). This demonstrates that *H. depressa* leaves and stems possess a multifaceted pharmacological profile, with many compounds contributing to multiple therapeutic categories. For instance, the inhibitory effects observed against *C. albicans*, *S. aureus*, and *S. pyogenes* may be associated with the presence of terpenoids, phenolic compounds, and aldehydes identified in the acetone and methanol extracts, as shown in Table 6.3. These classes are widely reported to disrupt microbial membranes, interfere with enzymatic systems, and inhibit biofilm formation, and may therefore account for the antimicrobial activity observed in Chapter 3. The anti-inflammatory and antioxidant activities noted in Chapter 4 may be linked to the occurrence of phenolic compounds, flavonoids, coumarins, and terpenoids in the extracts (Table 6.3). Their combined presence may explain the strong radical-scavenging capacity and nitric-oxide inhibition observed, particularly in the acetone extracts. In chapter 5, *H. depressa* extracts exhibited cytotoxic activity, especially in acetone extracts, which may be attributed to phenolic compounds, terpenoids, and aromatic derivatives, which are reported to damage normal cells at higher concentrations and may therefore account for the higher cytotoxic activity recorded against RAW 264.7 macrophages. Furthermore, these findings correlate with those of Ngobeni *et al.,* (2024), who used LC-MS to analyse

the phytochemicals in the roots of *H. depressa* and identified similar phytochemical classes such as phenolics, flavonoids, and alkaloids, which attributed *H. depressa*'s antimicrobial, anti-inflammatory, cytotoxic and antioxidant activities. Together, these compounds substantiated the plant's traditional use for treating respiratory disorders, diarrhoea, stomach-aches, cancer, gonorrhoea, and other sexually transmitted infections (Hlongwane, 2008; Molefe, 2013; Nhlapo et al., 2025; Reid et al., 2005). Moreover, the combination of multiple bioactive compounds gives *H. depressa* high potential for medicinal applications and the discovery of new drugs (Cragg and Newman, 2013). Identified compounds with multiple pharmacological properties should be isolated and evaluated as foundational molecules for developing new, more effective medicines. Moreover, future research should incorporate isolation and testing for the biological activity of the unexplored *H. depressa* phytochemicals. Safety studies, including toxicity tests, are also needed to ensure they can be used without harmful effects.

6.5. Conclusion

GC-MS analysis of *H. depressa* leaf and stem extracts demonstrated that the plant is rich in pharmacologically active phytochemicals comprising multiple chemical classes such as terpenes, phenolic acids, phenols, aldehydes, flavonoids, and coumarins. Terpenes in their diverse isotopes were the most prevalent phytochemicals. The presence of these bioactive compounds provides a scientific basis for the ethnomedicinal uses of *H. depressa* in treating various diseases. Compounds such as; 2,4-Di-tert-butylphenol, 3-Methoxybenzoic acid, Benzothiazole, Methyl eugenol, Neophytadiene, Scopoletin, Squalene, Taraxasterol and β-Myrcene; with multiple pharmacological effects, particularly those that combine antimicrobial, antioxidant and anti-inflammatory properties, stand out as promising candidates for the prevention and management of complex diseases such as communicable diseases, cancer, and metabolic disorders. However, some of the compounds are known to have considerable toxicity profiles; as a result, biosafety

concerns must also be addressed to establish safe dosage concentrations and assess potential cytotoxicity. These findings highlight *H. depressa* as a promising source of bioactive molecules that can be used in the discovery and development of novel drugs for the management of infections, chronic inflammation and oxidative stress.

6.6. References

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CHAPTER SEVEN

KEY FINDINGS, INTEGRATED DISCUSSIONS AND FUTURE PROSPECTS

7.1. Key Findings and Integrated Discussions

The outcomes of this study deliver a key understanding of the medicinal potential of *H. depressa* leaves and stems, validating its ethnomedicinal use in Southern African traditional medicine, particularly in treating ailments associated with infection, prolonged inflammation, and oxidative stress. Table 7.1 summarises the key findings of this investigation. The antimicrobial results obtained in Chapter 3 validate the traditional use of *H. depressa* for treating infections, with notable efficacy of acetone and methanol extracts against *C albicans* and *S. aureus*. This potent antimicrobial activity may be attributable to the diverse phytochemical profile, which includes notable compounds such as Benzothiazole, Neophytadiene, Phytol, trans-β-Ionone and others. SEM and TEM analyses revealed that *H. depressa* extracts induce ultrastructural damage in *C. albicans* cells, supporting a mechanism of action attributed to phytochemicals that disrupt cell membranes and compromise overall cellular structures (Clarke, 2008; Patra, 2012; Firmansyah, Winingsih and Manobi, 2021; Rajeswaran and Rajan, 2025). This action pathway parallels that of conventional synthetic antifungal drugs (Kim *et al.,* 2011); therefore, *H. depressa* leaves and stems could be a potentially safer, more accessible, and cheaper alternative, especially in rural areas. Moreover, the acetone extract demonstrated the most potent anti-inflammatory properties, validating the ethnomedicinal application of the plant in managing inflammation-related disorders, such as infections, pain, headaches, and fever. The DPPH assay demonstrated a potent radical scavenging capacity in all three *H. depressa* extracts, suggesting that *H. depressa* aerial parts may be rich in hydrogen-donating antioxidants, thereby exhibiting potent antioxidant stress-alleviating properties. The simultaneous antimicrobial, anti-inflammatory and antioxidant activities further boost *H. depressa* leaf and stem extract's therapeutic potential,

especially for life-threatening chronic diseases associated with infection, inflammation, and oxidative stress. Although all extracts showed promising bioactivities, acetone extracts are regarded as possessing the most potential for effective medicinal application. Furthermore, biosafety is crucial in evaluating the therapeutic value of medicinal plants. In Chapter 5, the cytotoxicity of *H. depressa* extracts was assessed against Vero cells and RAW 264.7 cells, showing that both the methanol and aqueous extracts possess no significant toxicity. However, acetone extracts, while demonstrating greater potency in most pharmacological assays, exhibited concerning cytotoxic levels against RAW 264.7 cells. The susceptibility of these cells was likely due to their phagocytic nature, which exposed intracellular organelles to high concentrations of toxic phytochemicals. This presents both a challenge and an opportunity, as although these extracts have remarkable therapeutic potential, their clinical use would require careful consideration of dosage, to minimise potential adverse effects, as well as isolation and distinguishing potentially toxic phytochemicals from medicinal ones (Robison and Barr, 2006; Borges *et al.*, 2020). The phytochemical analysis reveals a complex profile of medicinal compounds that can be attributed to its medicinal efficacy. GC-MS phytochemical analysis uncovered the presence of important pharmacological compounds, including terpenes, phenolics, phenylpropanoids, and flavonoids. Specific compounds such as neophytadiene, phytol, scopoletin, 2,4-di-tert-butylphenol, Methyl eugenol, etc., were notable due to their numerous documented bioactivities. The pharmacological properties of these compounds include antimicrobial, cardioprotective, anti-inflammatory, antidiabetic, antioxidant, anticancer, hepatoprotective antipyretic effects and analgesic properties, as reported in Chapter 6; and they are believed to have synergies that result in the multifaceted therapeutic effects traditionally attributed to this plant (Newman and Cragg, 2007; Hussein and El-Anssary, 2019; Tariq, Kamboj and Amir, 2019; Zhao *et al.*, 2020; Rajeswaran and Rajan, 2025). Nonetheless, acetone extracts particularly stood out for their high concentration of bioactive compounds; this is likely due to the intermediate polarity of the solvent, which allows it to dissolve a broader range of compounds with varying polarities. However, several vital considerations emerge from this discovery. The

cytotoxicity associated with the pharmacologically active acetone extracts indicates that further biosafety studies are necessary to optimise preparation methods and dosages, thereby maximising benefits while minimising adverse effects. The outstanding safety profile of the aqueous extract provides scientific validation for traditional water-based preparation methods while suggesting potential pathways for developing standardised herbal medicines. This research provides robust scientific validation for the traditional use of *H. depressa* leaves and stems, particularly in treating infections, chronic inflammation, and oxidative stress.

Table 7.1: Comparative analysis of acetone, methanol, and aqueous extracts of *H. depressa* leaves and stems

Parameter	Acetone Extract	Methanol Extract	Aqueous Extract	Key Findings
Extraction Yield	26%	29%	6.28%	Methanol extracted the highest mass of crude material.
Antimicrobial Activity (MIC)	*C. albicans*: 0.31 mg/mL *S. aureus* : 0.63 mg/mL	*C. albicans* : 0.31 mg/mL *S. aureus*: 0.63 mg/mL	*S. aureus*: 2.5 mg/mL *S. pyogenes*: 2.5 mg/mL	Acetone and methanol have more potent antimicrobial activity than aqueous extract
Antimicrobial Selectivity Index (SI)	Selective	Slightly Selective	Selective (most significant SI)	Aqueous showed the most significant selectivity due to a good safety profile.
Anti-Inflammatory Activity (NO Inhibition)	Strong	Weak	Weak	Acetone extract has potent and specific anti-inflammatory activity.
Antioxidant Activity (FRAP)	Weak	Weak	Weak	All extracts exhibit weak ferric reducing power compared to Trolox.
Antioxidant Activity (DPPH)	Strong	Strong	Strong	All extracts showed potent DPPH free radical scavenging.
Cytotoxicity				Acetone extract showed concerning toxicity against RAW 264.6 cells at high concentrations.
Vero cell line	Non- toxic	Non-toxic	Non-toxic	
RAW 264.7 cells	Toxic	Non-toxic	Non-toxic	
Phytochemical analysis	16 bioactive compounds across 9 phytochemical classes,	15 bioactive compounds across 8 classes, contributing	Not profiled by GC-MS	Acetone extracted the most diverse range of compounds. Both organic

	contributing about 62 pharmacological activities	about 45 pharmacological activities		extracts are rich in terpenes and phenolics.

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7.2. General Conclusions

Infection, inflammation, and oxidative stress form a self-perpetuating pathological loop that persists for years and worsens progressively, even when the original infection is controlled or resolved. This triad is challenging to treat effectively, causing millions of morbidities and mortalities globally and burdening healthcare systems; therefore, effective treatment requires an agent with a broader scope of therapeutic activities. This research provides comprehensive scientific validation for the traditional use of *H. depressa* leaves and stems, focusing on microbial infections, chronic inflammation and oxidant stress. The phytochemical screening identified bioactive compounds, including terpenes, phenolic aldehydes, flavonoids, and coumarins, which cement the basis for the ethnomedicinal use of the plant. Moreover, this phytochemical characterisation changes traditional knowledge about *H. depressa's* therapeutic value into an evidence-based understanding of the plant's medicinal properties. Acetone extract demonstrated potent and selective anti-inflammatory activity, and all three extracts exhibited potent DPPH free radical scavenging antioxidant activity, suggesting the promising potential of *H. depressa* leaves and stems in the treatment of life-threatening pathological cycles propagated by microbial infections, inflammation, and oxidative stress. Additionally, the relative safety of the *H. depressa* extracts on Vero cells shows good potential for medicinal use. However, the cytotoxicity finding, particularly of the acetone extracts against RAW 264.7 cells, necessitates further in vivo biosafety studies. Although this research represents the initial phase of medicinal evaluation and potential novel therapeutic agent development from *H. depressa* leaves and stems, this study has scientifically validated the therapeutic significance of *H. depressa* in Southern African traditional medicine, highlighting the aerial parts of this plant to have potent medicinal value and numerous pharmacologically active compounds.

7.3. Limitations of the Study

- The use of only three solvents may have missed important bioactive compounds; sequential extraction with more solvents with a broader polarity spectrum could yield a more comprehensive phytochemical analysis.
- GC-MS analysis did not include aqueous due to lack of solubility; the assay could not be repeated due to limited resources.
- All tests were performed *in vitro*, which do not fully represent in vivo interactions such as metabolism, absorption, and immune responses.
- Antimicrobial testing was limited to a small pathogen panel, excluding many resistant and clinically relevant strains that could not be attained due to insufficient resources and the absence of authorisation to work with pathogens that require higher biosafety levels.
- This study did not include a direct comparative analysis of the biological activity or toxicity between leaf, stem, and root extracts; therefore, conclusions regarding relative potency or safety among different plant parts cannot be drawn.

7.4. Recommendations for future research

- Conducting bioassay-guided fractionation to isolate, characterise and elucidate bioactive compounds.
- Evaluation of synergistic bioactivities between isolated compounds.
- Investigation of specific molecular and enzymatic pathways and mechanisms of action studies for antimicrobial, anti-inflammatory and antioxidant activities.
- Validation of efficacy and toxicity findings through in vivo studies to confirm safety and therapeutic potential relevance.
- Explore development of *H. depressa*-based therapeutic formulations (e.g., gels, creams, capsules).

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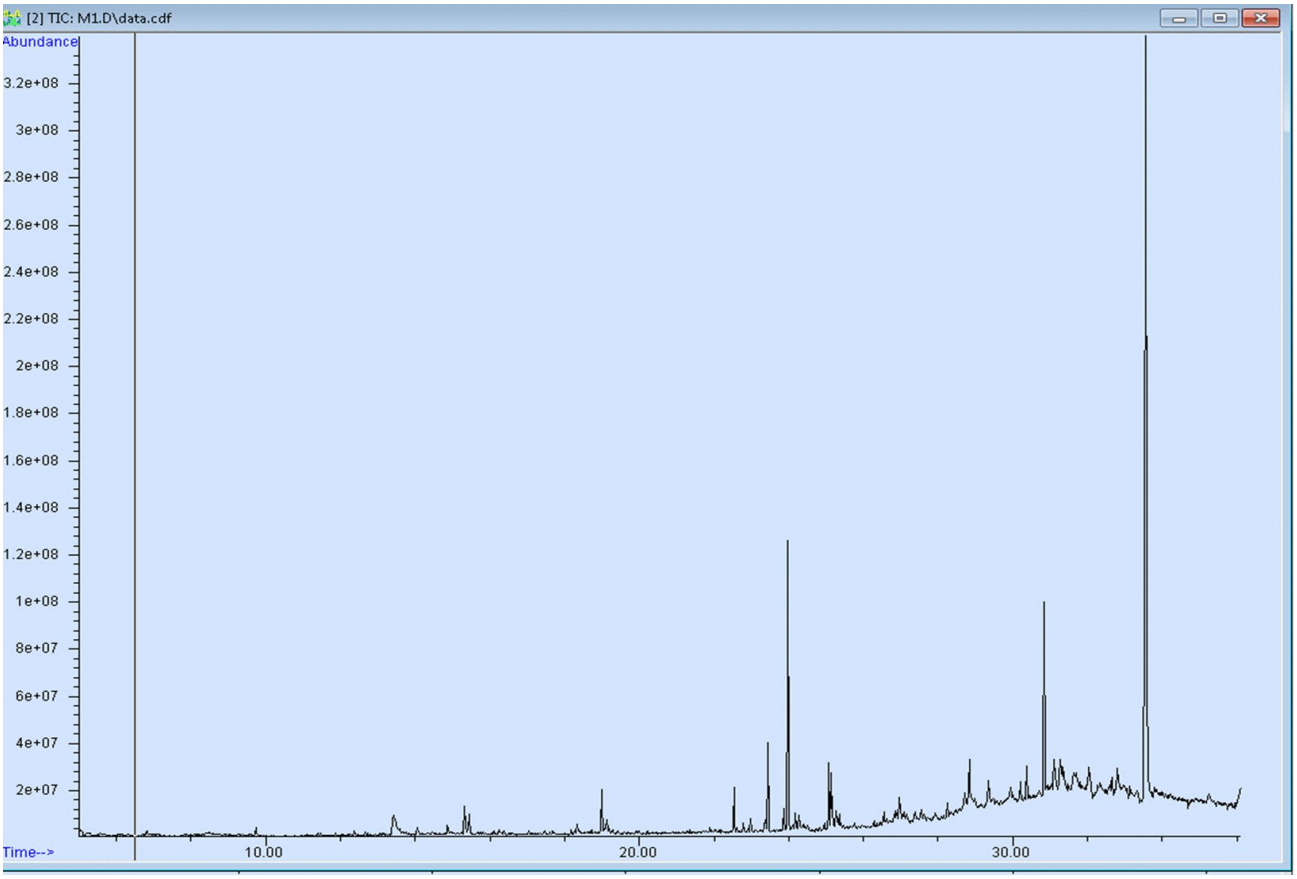
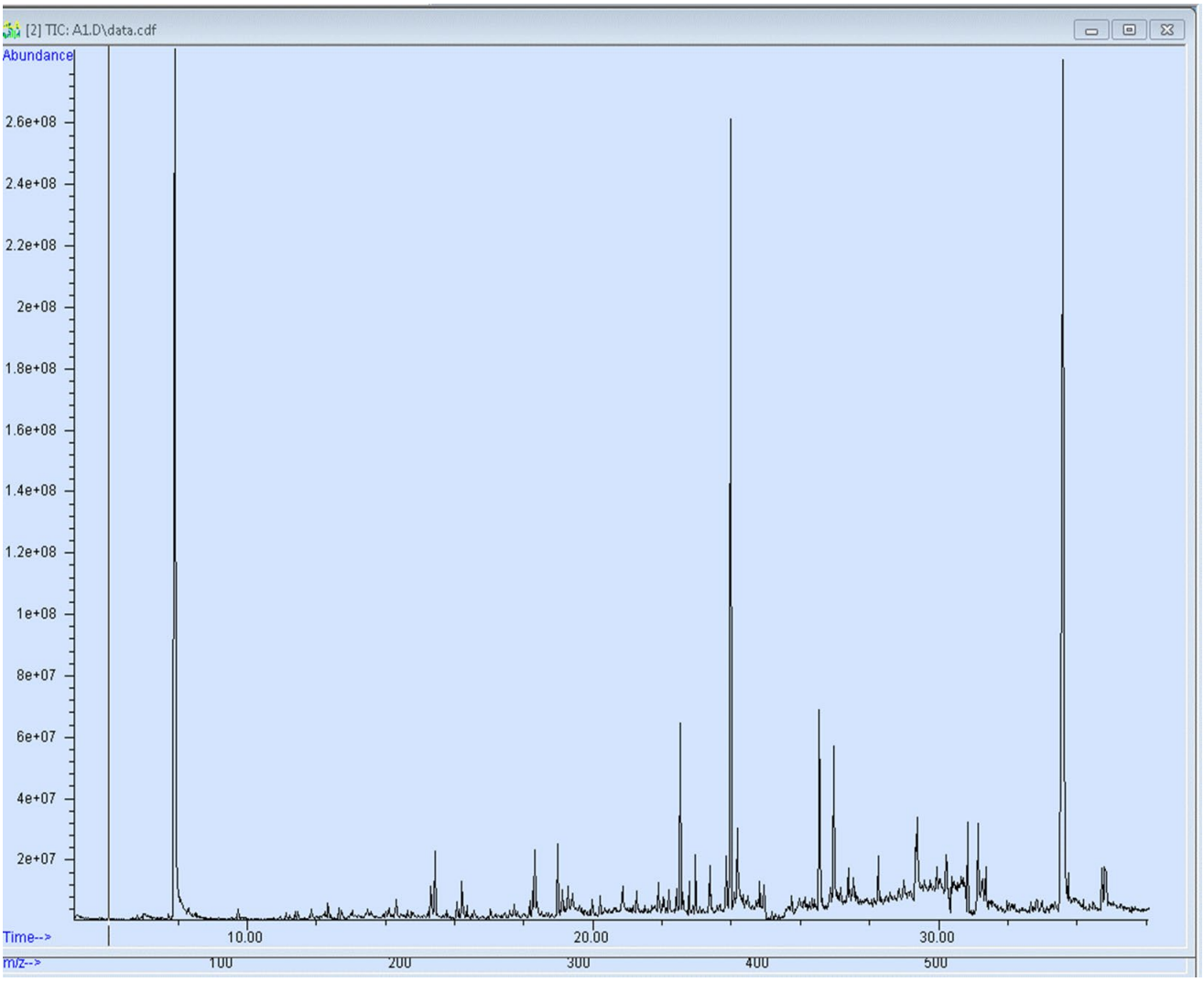
7.6. APPENDICES

1. GC-MS Chromatograms of *H. depressa* extracts



Appendix 1.1: GC-MS chromatogram of *H. depressa* methanol extracts



Appendix 1.2: GC-MS chromatogram of *H. depressa* acetone extracts

2. Published articles from this research.

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Review Article

A Review: Medicinal Uses, Phytochemistry and Pharmacological Properties of Plants from the *Hermannia Genus*

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ABSTRACT

Introduction: Medicinal plants play a pivotal role in treating illnesses and modern medicines are still being derived from plants. Hermannia genus is a significant traditional herbal medicine. This review evaluates the medicinal uses, phytochemistry and pharmacological properties of plants from the genus Hermannia genus based on available research. **Methods**: Studies accessed from online research databases were systematically selected and analysed to construct a comprehensive review of the medicinal uses, phytochemistry and pharmacological properties of plants from the genus. **Results**: Hermannia species are used in traditional medicine to treat or manage; respiratory conditions, gastrointestinal issues, skin conditions, sexually transmitted infections, and diabetes. Scientific findings also discovered promising pharmacological activities within members of the genus such as antimicrobial, anti-inflammatory, antioxidant, antidiabetic and anticancer activities. To date, over 30 types of secondary metabolites have been identified from the genus, including the 2 pure compounds that were isolated and tested for pharmacological activities. Further research must prioritize other unexplored species of the genus and efficacy and mechanism of action studies on isolated compounds. **Conclusion**: The genus Hermannia is important in the treatment of diseases of high public health concern. The pharmacological studies and presence of secondary metabolites and bioactive compounds further validates the traditional uses of the genus. Therefore, the findings suggest that the genus has species that may serve as candidates for novel drug discovery for the treatment of various illnesses. Efficacy and mechanism of action studies still need to be conducted on isolated compounds and other unexplored species of the genus.

Keywords: Hermannia, traditional medicine, phytochemistry, pharmacological activities, secondary metabolites, bioactive compounds, drug development.

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INTRODUCTION

The use of plants for medicinal purposes is a practice as old as humanity itself.[1] Evidence suggests that even in prehistoric times, humans understood the medicinal benefits of plants, learning through observation, experimentation, and self-medication. As civilisations developed, so did their understanding of plants as medicines.[2] The dawn of the early modern period introduced iatrochemistry, which developed an understanding of the chemical basis of medicinal properties in plants. This period was characterised by increased global exploration and trade of herbal medications, leading to the discovery and introduction of new medicinal plants from all around the globe, further enhancing the pharmacopoeia of the time.[1]

The 19th century was a turning point which introduced the identification and isolation of active compounds from plants, such as morphine from *Opium poppy* and digitoxin from *Digitalis purpurea*. This advancement paved the way for the discovery and development of synthetic drugs in the 20th century, resulting in the reclined use of plant-based medicines.[3] However, traditional medicine systems, deeply rooted in many cultures, continued to thrive, to date approximately 80% of the African population relies on them for primary healthcare.[3, 4] In recent years, there has been a resurgence of interest in plant-based medicines. Factors increasing medicinal plant use include cultural beliefs in traditional practices, perceptions of plant-based remedies as natural and therefore safe, cost-effectiveness, perceived efficacy, self-medication tendencies, distrust towards modern medicine, extended wait times in hospitals, and widespread promotion.[4,5] Additionally, there is a growing awareness of the potential side effects of synthetic drugs, increasing antibiotic resistance, and the recognition of plants as a valuable source of novel drug leads.[6]

The genus Hermannia, also known as "doll's roses" or "*poproste*" in Afrikaans, holds profound importance in traditional herbal medicine.[7] This genus represents a diverse group of plants classified within the Kingdom Plantae, Phylum Magnoliophyta, Class Magnoliopsida, Order Malvales, and Family Malvaceae. With an estimated 180 species globally Hermannia can be found in the United States, Mexico, Australia, Arabia tropical, East Africa, Northeast Africa and Madagascar. Nevertheless, in Africa, its predominant habitation is in in Southern African countries such as South Africa, Zimbabwe, Namibia, and Lesotho.[8,9] Hermannia species grow in diverse habitats, from the arid areas of the Karoo and Namibian deserts to the humid, summer-rainfall mountains of the Drakensberg and the sea-spray zones along Southern African coasts. This ecological versatility aligns with its extensive morphological diversity within the genus—the morphological variations from creeping herbs to thick-stemmed bushes and annuals.[9]

The Hermannia genus has diverse traditional medicinal uses that highlight its significance. Indigenous Southern African groups, including the

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Batswana, Bakwena, Basotho, Xhosa, and Zulus, have used the genus to treat many illnesses, such as respiratory diseases, heart conditions, gastrointestinal issues, skin conditions, sexually transmitted infections as well as epilepsy.[9, 10, 11, 12, 7] Furthermore, species of this genus have been scientifically investigated for their pharmacological properties such as antimicrobial, anti-inflammatory, antioxidant, anticancer and antidiabetic activities. The genus species that were studied and noted to possess significant therapeutic effects include *Hermannia depressa*, *Hermannia geniculata*, *Hermannia cuneifolia* and *Hermannia incana*.[13, 14, 15, 16, 17, 18, 19, 20]

This genus has a rich ethnobotanical history and significance in traditional medicine, especially within Southern Africa, underscoring the necessity for scientific phytochemical and pharmacological research because the genus could be a source of bioactive compounds for novel drug development. This review aims to determine the extent to which the genus has been studied focusing on phytochemistry, pharmacological activities, identified and isolated bioactive compounds and also to determine the existing gaps in literature.

REVIEW METHODOLOGY

This review seeks to comprehensively evaluate and critically analyse the existing research on the genus Hermannia, focusing mainly on its phytochemistry, pharmacological potential and bioactive compounds. The literature search on the genus Hermannia was conducted through multiple electronic databases including Google Scholar, PubMed, Elsevier Science, Semantic Scholar,Taylor and Francis Online, Wiley Online Library, and Science Direct, using keywords such as "Hermannia", "traditional uses", "medicinal uses", "phytochemistry", "bioactive compounds", "biological activities" and "pharmacological activities ". As shown in Figure 1, a total of 26 studies that comprised 20 full text articles and 6 unpublished dissertations available online that report on the ethnobotanical uses, biological activities, and the screening, isolation and identification of bioactive compounds within the genus Hermannia were consulted to provide an up-to-date review of literature. The review includes studies conducted from 2005 to 2024. The studies that are accessible online and specifically mention the search terms in their content were incorporated. Excluded studies are those that did not meet the criteria of the search terms and that do not report the medicinal use of Hermannia human diseases and illnesses.

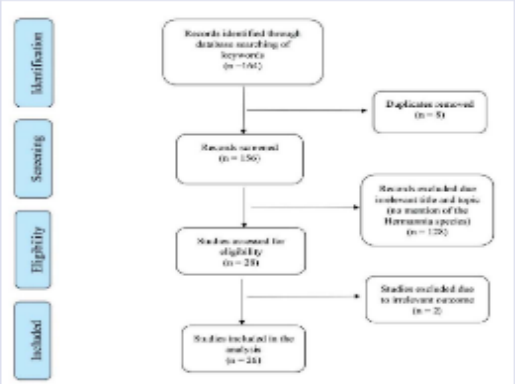

Figure 1: PRISMA flowchart of selected studies for the review

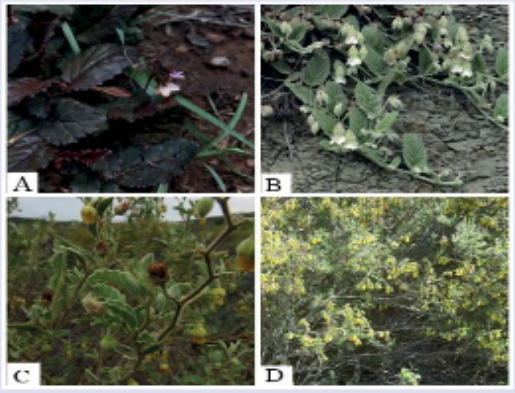

Figure 2: Hermannia species with their different morphological variations. Figure 2A – *H.depressa*, Figure 2B – *H.geniculata*, Figure 2C – *H. incana*, Figure 2D – *H. cuneifolia*.[21]

Botanical Characteristics o Hermannia

The genus Hermannia is capable of growing in a wide range of varied habitats and that explains its extensive morphological diversity. The growth forms range from low-growing, ground-hugging types to more erect and bushy species which may have woody or herbaceous stems. Low-growing and ground-hugging species such as *Hermannia depressa* and *Hermannia geniculate* (Figure 2A and Figure 2B) are widespread throughout the genus. The low-growing types are found in both summer- and winter-rainfall areas. The ground-hugging species can spread without rooting at nodes (procumbent), spread with raised terminal parts (decumbent) or have slight rooting at nodes (repent).[9]

Ascending growth forms include both sub-herbaceous and woody plants. Moreover, there are fewer erect single-stemmed species with branches, and they often exhibit a reseeding life strategy, typically in fire-prone areas, and are relatively short-lived.[9] Root types in Hermannia comprise three forms: a woody rootstock, a branched rootstock, and an erect rootstock; nevertheless, most species have a primary root with secondary adventitious roots radiating from it. Stems can range from thin and branch-like to robust and trunk-like forms, occasionally with a silvery waxy coating or resinous appearance as an anti-herbivory characteristic. Branching patterns are mostly alternate, though some species show unique forms like dichotomous branching.[9] Leaves of this genus exhibit substantial diversity in shape, size, margin, and indumentum. Most species have flattened leaves, with shapes ranging from narrow linear, lanceolate, and oblanceolate to broader elliptic, ovate, oblong, cuneate, and orbicular forms. Orbicular and cordate leaves are rare, appearing only in a few species from summer rainfall regions. Hermannia are flowering plants with inflorescences that consist of peduncles and pedicels with bracts and vary in calyx shape and lobe formation, adapting to different pollination strategies.[9]

Uses of Hermannia in Traditional Medicine

Hermannia has a long-standing history of medicinal use among various cultural groups in Southern Africa and Europe. *H. depressa* also known as "*Seletjane*" in Sesotho[22] and "*Root-opslag*" in Afrikaans, is utilised as a protective charm to ward off relationship conflicts, applying it as an ointment on the body or placing it around their homes by the Zulu people.[7] As outlined in Table 1 decoctions of *H. depressa* are also utilised to relieve coughs, and the plant is combined with others to address diarrhoea; additionally, it serves as an emetic, and the leaf sap mixed with water is used to treat stomach aches due to its purgative and diaphoretic properties.[23-24] Moreover, crushed leaves are applied

in cancer treatment, while decoctions of its roots are used to treat gonorrhoea and other sexually transmitted infections.[25-10]

H. geniculata, referred to by the Basotho as "*kgwakgwa*," is a staple in traditional Basotho medicine, particularly for managing blood sugar disorders, where dried roots, when boiled in water and taken three times daily, help to control diabetes symptoms, treat colic, and alleviate heartburn and stomach disorders, including flatulence in pregnant women. [26-11] The root extract is also used for treating ulcers and skin conditions, showcasing its wide range of medicinal applications. Other Hermannia species, such as *Hermannia incana*, are also used medicinally. *H. incana* serves as an emetic, and its leaf sap is employed to treat stomach aches and diarrhoea. Decoctions of the entire plant are used to soothe coughs; among the Xhosa, the roots treat dysuria. Traditional ointments combining *H. incana* with *Lobostemon fruticosus* and *Psoralea decumbens* are used for erysipelas or eczema.[20]

Additionally, *Hermannia cuneifolia*, known as "*pietsterbos*" in Afrikaans has leaves used as plasters. Its leaves are also infused in tea to cleanse the blood, and a root infusion was historically used by European settlers for epilepsy. Additionally, a leaf lotion was applied to eczema and shingles.[7] Notably, in parts of Europe, *Hermannia althaeifolia* was cultivated and applied medicinally as a fragrant tea for treating syphilis.[11] Table 1 shows the documented medicinal uses and plant parts utilised. *Hermannia depressa* stands out as the most prominent for its use in traditional medicine, followed by *Hermannia geniculate*[9] as shown in Figure 3. Leaves, followed by roots, are the most frequently utilized plant parts of the genus as illustrated in Figure 4. While there are references to the use of the whole plant, existing literature does not document the medicinal utilisation of flowers. The diverse traditional uses of Hermannia species underscore their importance in ethnomedicine and emphasise the need for ongoing scientific investigation into their therapeutic properties.

PHARMACOLOGICAL ACTIVITIES OF GENUS HERMANNIA

Antimicrobial activity of the genus Hermannia

Antimicrobial testing evaluates the potential of plant extracts to inhibit the growth of pathogens. Notably, bacterial and fungal pathogens have been examined for susceptibility to Hermannia extracts far more extensively in comparison to viral infections. A conducted study where twelve Hermannia species were investigated for antibacterial; the species comprised *Hermannia althaeifolia, Hermannia cuneifolia, Hermannia flammula, Hermannia holosericea, Hermannia incana, Hermannia involucrate, Hermannia lavandufolia, Hermannia muricata, Hermannia saccifera, Hermannia salviifolia, Hermannia scabra* as well as *Hermannia trifurca*. The study found *all* 12 species possessing promising antimicrobial activity at varying degrees, strong inhibitors have minimum inhibitory concentrations (MIC) ranging from 0.5 to 0.0195 mg/mL and *H. saccifera* showed the most potent bactericidal activity, particularly against *Staphylococcus aureus, Bacillus cereus*, and *Enterococcus faecalis, Table 1 shows the specific antibacterial activities of the species.* [11]

Reid et al. (2005) document the antibacterial activity of *H. depressa* where ethanolic and ethyl acetate extracts from its roots, leaves and stems showed efficacy against pathogens such as *Bacillus subtilis,*

Table 1: Documented traditional uses of Hermannia species.

Species	Plant part used	Uses	Number of uses
	Not specified	protective charm[22]	
	leaves	emetic[23]	
	leaves	stomach-ache[23]	
	leaves	purgative[23]	
H. depressa	leaves	diaphoretic[23]	9
	leaves	soothe coughs[23]	
	leaves	cancer[25]	
	roots	Gonorrhoea[10]	
	roots	Unspecified STIs[10]	
	roots	blood sugar disorders[26 17]	
	roots	Diarrhoea[26 17]	
	roots	Heartburn[26 17]	
	roots	stomach disorder[26 17]	
H. geniculata	roots	flatulency in pregnant women[26]	8
	roots	colic[17]	
	roots	ulcer[17]	
	roots	skin diseases[26]	
	leaves	stomachache[20]	
	leaves	diarrhoea[20]	
	leaves	purgative[20]	
H. incana	leaves	diaphoretic effects[20]	6
	Whole plant	soothe coughs[20]	
	roots	Dysuria[11]	
	leaves	sores[11]	
	leaves	used as plasters[7]	
H. cuneifolia	leaves	Blood cleansing[7]	5
	leaves	eczema[7]	
	leaves	shingles[7]	
H. althaeifolia	Not specified	syphilis[11]	1
H. pinnata	roots	Diabetes mellitus[27]	1
H. salviifolia	roots	Convulsions[11]	1

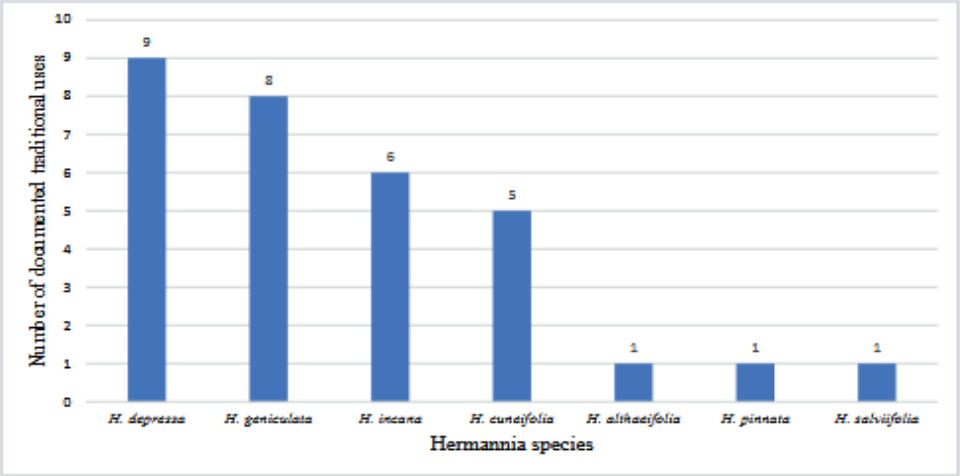

Figure 3: Comparison of Hermannia species based on documented traditional uses

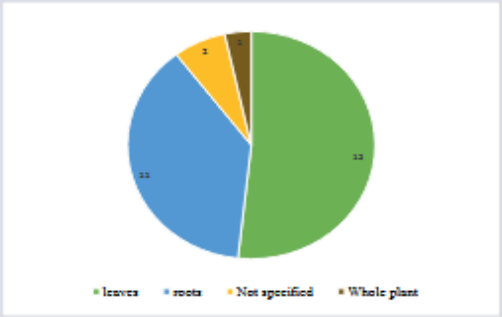

Figure 4: Comparison of frequently used plant parts from Hermannia genus

Escherichia coli, and Klebsiella pneumoniae; demonstrating potent antibacterial activity against *Bacillus subtilis*. Moreover, Hlongwane noted good antimicrobial activity of *H. depressa* extracts against *Mycobacterium tuberculosis*. [13] *H. depressa* methanol and acetone extracts showed antimicrobial activity against 13 microorganism including *Candida albicans, Candida krusei, Candida parapsilosis, Bacillus cereus, Clostridium perfringens, Enterococcus faecalis, Escherichia coli, Neisseria gonorrhoeae, Pseudomonas aeruginosa, Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus pneumoniae* and *Streptococcus pyogenes*, nonetheless the highest inhibitions were observed in *Candida albicans* and *Bacillus cereus* with MICs as low as 0.1 and 0.3 mg/mL respectively.[19] *H. geniculata* has been minimally screened for antimicrobial potential as compared to *H. depressa* however isolates from *H. geniculata* have shown antimycotic against *Candida albicans*.[14] Another species of this genus that has been studied is *H. cuneifolia*, and the results showed inhibition of strains of *Bacillus cereus, Staphylococcus aureus, Klebsiella oxytoca* and *Acinetobacter species*.[28] Plants from the Hermannia genus have great potential agents in the treatment of infectious diseases mainly caused by various bacteria and fungi. Therefore, more members of the genus need to be screened for antimicrobial activity extensively.

Anti-inflammatory activity of genus Hermannia

Hermannia extracts demonstrated consistent anti-inflammatory activities across different in vitro studies where techniques such as Cyclooxygenase-1 (COX-1) inhibition, 5-lipoxygenase inhibition and nitric oxide (NO) inhibition assays were employed as shown in Table 1. *H. depressa* dichloromethane extracts portrayed 81% COX-1 inhibition, the highest compared to the five other Sterculiaceae species screened in a study by Reid et al., .[16,23] Ngobeni et al. assessed the production of NO of lipopolysaccharide-stimulated RAW 264.7 macrophages treated with *H. depressa* acetone and methanol extracts and both extracts showed strong anti-inflammatory activity, in addition, their findings suggest that acetone extract is slightly more effective than the methanol.[29]

H. geniculata also demonstrated significant anti-inflammatory properties through various pharmacological studies. Hermannol a compound isolated from *H. geniculata* showed strong anti-inflammatory properties through potent inhibition of 5-lipoxygenase.[17] Another study also found flavonoid and phenol extract of *H. geniculata* with potent inhibition of 5-lipoxygenase.[29] Furthermore, another study was also in agreement with the findings where flavonoid extract exhibited significant inhibition of the 5-LOX enzyme, even better than the standard drug indomethacin.[30]

Furthermore, in a study that screened 12 Hermannia species for anti-inflammatory activity, eleven of the twelve species portrayed moderate activity against the 5-lipoxygenase enzyme, and *H. cuneifolia* showed a more potent anti-inflammatory activity.[31] The potential of the genus in the development of new, safe, and effective treatments for inflammatory diseases is therefore undeniable and further research is necessary, with a higher focus on the less explored in-vivo experimentations

Antioxidant activity of genus Hermannia

Hermannia species possess notable antioxidant activity. Most studies utilised the DPPH (2,2-diphenyl-1-picrylhydrazyl), the ABTS

(2,2-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid) radical scavenging assays, metal ion chelation assays and the ferric reducing antioxidant power (FRAP) to evaluate their antioxidant potential. [15, 11, 19] In a study by Essop et al. (2008)[11], ten of twelve Hermannia species, including *H. althaetfolia, H. cunetfolia, H. flammula, H. holosericea, H. incana, H. involucrate, H. lavandufolia, H. muricata, H. saccifera and H. scabra* portrayed good free radical scavenging activity and *H. cunetfolia* exhibited the strongest antioxidant activity.[11] Moreover, several studies document that aqueous, methanol and acetone extracts of *H. depressa* exhibit significant antioxidant activity.[19 15] Ngobeni et al. (2024) also notably added that methanol and acetone extracts of *H. depressa* exhibited superior antioxidant capacity compared to standards like ascorbic acid and Trolox.

Furthermore, ethyl acetate extract leaves and flavonoids isolated from the roots of *H. geniculata* have demonstrated significant antioxidant properties in vitro. For instance, ethanolic and hydro-ethanolic extracts of the roots showed remarkable free radical scavenging abilities across different assays, including DPPH, ABTS, hydroxyl radicals, and superoxide anions, in some cases outperforming standard antioxidants like silymarin.[29 14] The flavonoid and phenolic compounds also displayed significant antioxidant activity, underscoring the potential of *H. geniculata* as a rich source of natural antioxidants. Similarly, Hermannol, a xanthene derivative isolated from the roots, was discovered to possess strong antioxidant properties, potentially through both radical scavenging and metal-chelating mechanisms.[17] These findings suggest that the Hermannia genus is a valuable candidate for developing effective natural antioxidant therapies, contributing to its pharmacological relevance.

Toxicity studies on genus Hermannia

The toxicity studies on *H. geniculata* roots have provided insights into its safety and pharmacological properties. In an evaluation involving Wistar rats, the administration of an aqueous root extract at doses showed no significant toxic effects on vital organs like the liver, kidneys, lungs, and heart over however, a reduction in white blood cell count was observed, hinting at potential long-term impacts on immunity.[26] Cytotoxicity studies using *H. geniculata* roots and flavonoid extraction demonstrated an agreement in findings that this plant has low toxicity towards Vero cells and RAW 264.7 macrophages, indicating it is not harmful to normal cells. However, it demonstrated high toxicity towards HepG2 cancer cells, suggesting potential anti-cancer properties.[30] These findings are promising and can drive research on *H. geniculata* in developing safe treatments, although further detailed studies are necessary to understand its therapeutic applications and biosafety profile extensively.

Studies by Molefe and Ngobeni et al have a consensus indicating that *H. depressa* has no significant toxicity to normal cells in their research. Ngobeni et al utilised the 3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide (MTT) assay, treating the African green monkey kidney cells with *H. depressa* acetone, methanol and aqueous extracts. Acetone and methanol showed some moderate decrease in cell viability; in contrast, the aqueous extracts showed no toxicity. [19] Molefe conducted the MTT assay using the Madin-Darby bovine kidney cell (MDBK) lines, lactate dehydrogenase (LDH) and the brine shrimp lethality assay (BLSA) assays. *H. depressa* extracts exhibited low in-vitro cytotoxic effects on MDBK cells, especially for the acetone extract, which even stimulated cell growth. Nonetheless, the in vivo BLSA showed significant toxicity, especially for higher concentrations of water and acetone extracts, indicating potential toxic constituents requiring further investigation.[32] *H. depressa* has limited cytotoxicity studies, and the recommendation is that further in vivo studies are crucial to confirm these findings and determine safe doses for potential therapeutic applications.[32 19]

Antidiabetic Activity of genus Hermannia

H. geniculata is the most studied member of the genus on antidiabetic properties and these findings led to promising but varied potential in managing hyperglycaemia. The ethanolic root extract showed potent inhibition of α-glucosidase, while α-amylase inhibition was milder.[18] Meanwhile, Hermannol, a xanthene derivative isolated from the roots, demonstrated moderate inhibitory activity against α-amylase.[30] Furthermore, the flavonoid and phenol extracts exhibited significant α-glucosidase inhibition but were less effective against α-amylase. Notably, the ethyl acetate extract, particularly the isolated compound 1,3-dibutyl-2,8-dihydroxy-9H-xanthen-9-one, showed strong α-amylase inhibitory activity and significant α-glucosidase inhibition, surpassing acarbose in effectiveness.[14] These findings suggest that various extracts and isolated compounds from *H. geniculata* possess anti-diabetic properties, particularly through the inhibition of key carbohydrate-catabolizing enzymes, highlighting its potential for managing diabetes; however, there is a clear gap in the literature on the antidiabetic effects of the other members of the genus Hermannia.

PHYTOCHEMISTRY OF GENUS HERMANNIA

Plants produce secondary metabolites, which are organic compounds resulting from secondary metabolic processes. Secondary metabolites are categorised based on their structural diversity, biosynthesis, and functions, resulting in the identification of over 214,000 secondary metabolites in the scientific literature.[33] These compounds are classified into various groups, including alkaloids, terpenoids, steroids, polyphenols, fatty-acid-derived compounds, non-ribosomal polypeptides, and enzyme cofactors. The plants' phytochemicals have notable pharmacological applications because of their antimicrobial, antiviral, antioxidant, anti-inflammatory, anticancer, and cardioprotective properties.[34, 35, 33, 36] The genus Hermannia has been used for many traditional medicine purposes, which may be attributed to the present bio-active compounds.

H. depressa is utilised in various traditional medicinal practices and has demonstrated significant pharmacological potential. Techniques utilised for phytochemical screening range from older standard qualitative methods such as the froth test and the ferric chloride test to the advanced modern techniques that can be both qualitative and quantitative such as vacuum liquid chromatography (VLC), thin layer chromatography (TLC), high-performance liquid chromatography (HPLC) and Liquid Chromatography with tandem mass spectrometry (LC-MS/MS).[32 19] Phytochemical screening has identified the presence of tannins, saponins, phenols, terpenoids and cardiac glycosides in extracts of *H. depressa* and these metabolites contribute to its therapeutic potential.[23, 37, 12, 32] Utilising LC-MS/MS analysis, Ngobeni et al. (2024) identified alkaloids and flavones, such as Waltherione D, quercetin, and tricin, in aqueous and acetone extracts. Methanol extracts showed the presence of steroids, fatty acids, and lignans. Identified compounds are outlined in Table 2 [19] The quantitative phytochemical analysis of *H. depressa* was conducted utilising the Folin-Ciocalteu method for phenolic content and Aluminium colourimetric for flavonoid content. The highest phenolic content was observed in acetone extracts at 8.45 mg gallic acid equivalent per gram (GAE/g), while flavonoid content was notably high in aqueous extracts, recorded at 0.97 mg quercetin equivalent per gram (QE/g).[19]

Meanwhile, qualitative and semi-quantitative screening of phytochemicals in *H. geniculata* root extracts using standard methods showed the presence of saponins, phenols, flavonoids, anthraquinones, alkaloids, tannins, triterpenes and phytosterols as well as traces of while anthraquinones and phytosterols.[38 14] Moreover, Mojau isolated a pure bioactive compound utilising analytical techniques TLC and column chromatography (CC) and characterised it using Nuclear Magnetic

Table 2: Hermannia species with noteworthy biological activities.

Biological activity	Species	Extract/ Compound	Description	Reference
Antimicrobial activity.	*H. depressa*	Ethanolic extract	0.195 mg/ml MIC against *B. subtilis*	23
		methanolic extract	1.25 mg/mL MIC against *B. cereus* and *C. albicans* being the most inhibited.	19
		acetone extract	1.25 mg/mL MIC against *C. albicans* 0.5 mg/ml MIC against *E. faecalis*	19 24
	H. geniculata	1,3-dibutyl-2,8-dihydroxy-9H-xanthene-9-one compound (isolated from ethyl acetate extracts)	3.25 mg/mL MIC against Candida albicans (HO321 and HO325 strains) 6.5 mg/mL MPC MIC against Candida albicans (HO321 and HO325 strains)	14
	H. cuneifolia	acetone extract	0.5 mg/mL MIC against *C. neoformans*	11
	H. involucrata	acetone extract	0.5 mg/mL MIC against *C. neoformans*	11
	H. muricata	acetone extract	0.5 mg/mL MIC against *C. neoformans*	11
	H. saccifera	acetone extract	0.0195 mg/ml MIC against both *S. aureus and B. cereus and* 0.125 mg/ml MIC against *E. faecalis.*	11
	H. salviifolia	acetone extract	0.5 mg/mL MIC against *P. aeruginosa*	11
	H. scabra	acetone extract	0.5 mg/ml MIC against *P. aeruginosa* and *C. neoformans*	11
	H. cuneifolia	Methanol extract	0.05 mg/ml MIC against *B. cereus* strains 0.19 mg/ml MIC against both *S. aureus* (ATCC 4330) 0.09 mg/ml MIC against both *S. aureus* (ATCC 867716) 0.19 mg/ml MIC against MRSA	28
		Acetone extract	0.05 mg/ml MIC against *B. cereus* strains 0.19 mg/ml MIC against *S. aureus* 0.09 mg/ml MIC against MRSA 0.78 mg/ml MIC against *K. oxytoca*	
Anti-inflammatory activity	*H. depressa*	acetone extracts	77.5% NO production inhibition from oligosaccharide (LPS)-activated malignant macrophage cell line RAW264.7	19
		dichloromethane extracts	78% (stem) and 81% (root) COX-1 inhibition	23
	H. geniculata	Ethanol (Hermannol)	3.64 ± 0.123 mg/mL. IC_{50} value for inhibition of NO production	30
		Ethanol	0.67 ± 0.042 mg/mL. IC_{50} 5-lipoxygenase enzyme inhibition.	17
		acetone extracts	0.14±0.06 mg/mL lowest IC_{50} value for 5-LOX enzyme inhibition	30
		aqueous	56.53%. 5-lipoxygenase enzyme inhibition.	11
	H. depressa	methanol	0.24±0.691 µg/ml IC50 value DPPH inhibition	15
		Acetone	0.23±0.37 µg/ml IC50 value DPPH inhibition	15
		ethyl acetate	0.003576 ± 0.00044 mg/mL lowest DPPH inhibition and IC_{50} values	19
Antioxidant activity		1,3-dibutyl-2,8-dihydroxy-9H-xanthen-9-one compound (isolated from ethyl acetate extracts)	0.199 µg / mL IC50 value for DPPH inhibition IC50 of 0.077 µg/mL IC50 value for ABTS inhibition	14
		Ethanol	0.474 µg/mL IC50 value for Hydroxyl radical inhibition	14
	H. geniculata	hexane	0.111 µg/mL IC50 value for ABTS inhibition	14
		acetone	IC50 of 0.021 µg/mL IC50 value in hydroxyl radical inhibition	14
		Ethanol (Hermannol)	0.056 µg/mL. IC_{50} value in hydroxyl radical inhibition	14
			0.29± 0.011 mg/mL DPPH inhibition IC_{50} value 0.28 ±0.07 mg/mL metal chelation IC50 value	17
			10.26 ± 0.29 µg /ml IC50 in DPPH inhibition	11
			10.32 ± 0.34 µg /ml IC50 value for ABTS+ inhibition.	11
Antidiabetic activity	*H. geniculata*	Ethanol (Hermannol)	0.59 ± 0.086 IC50 for α-amylase inhibition. 0.04 ± 0.002 IC50 for α-glucosidase inhibition.	17

Table 3: Compounds identified from the genus.

Hermannia species	Source (Plant part)	Extract	Type	Compound	Molecular formula	References
H. depressa	leaves	Ethanol	Fatty Acid	Lauric acid	$C_{12}H_{24}O_2$	25 39
			Fatty Acid	Myristic acid	$C_{14}H_{28}O_2$	25 40
			Fatty Acid	Palmitic acid	$C_{16}H_{32}O_2$	25 41
			Fatty Alcohol	Stearyl alcohol	$C_{18}H_{38}O$	25 42
			Alkaloid	Waltherione D	$C_{22}H_{22}NO$	
			Alkaloid	Isomer of Waltherione D	$C_{22}H_{22}NO_4$	
			Alkaloid	Waltherione C	$C_{22}H_{21}NO_3$	
		Aqueous	Accridone alkaloid	Buxifoliadine D	$C_{23}H_{23}NO_3$	19
			Flavonoid	Quercetin	$C_{15}H_{10}O_7$	
			Flavone	Tricin	$C_{17}H_{14}O_7$	
			Flavone	Gramrione	$C_{17}H_{14}O_7$	
			Alkaloid	Waltherione D	$C_{22}H_{22}NO_4$	
				8-Dihydroantidesmone	$C_{19}H_{31}NO_3Na$	
				Hibtherin A	$C_{23}H_{36}O_3$	
				2-Methoxy-5-octylaniline	$C_{15}H_{25}NO$	
			Steroid	2-Methoxyestradiol	$C_{19}H_{26}O_3$	
		Acetone	Flavonoid	4'-Methoxynaringenin	$C_{15}H_{10}O_6$	
			Carbohydrate	Hexose or glucose	$C_6H_{12}O_6$	
			Flavonoid	Jaceosidin	$C_{17}H_{14}O_7$	
			Fatty acid	Docosahexanoic acid	$C_{22}H_{32}O_2$	19
			Fatty acid	Vernolic acid	$C_{18}H_{32}O_3$	
			Phenolic	6-Gingerol	$C_{17}H_{26}O_4$	
			Polyphenolic	4-O-Caffeoylquinic acid	$C_{16}H_{18}O_9$	
			Lignans/Polyphenol	Matairesinol	$C_{20}H_{22}O_6$	
			Lignan/Polyphenol	Pinoresinol	$C_{20}H_{22}O_6$	
		Methanol	Lignan/ Polyphenol	Medioresinol	$C_{21}H_{24}O_7$	
				7-Methoytaxifolin-3-glucoside	$C_{21}H_{22}O_{12}$	
			Caffeic Acid [43]	Yunnaneic acid F	$C_{29}H_{26}O_{14}$	
			flavonoid [44]	Salvigenin	$C_{20}H_{20}O_6$	
H. geniculata	roots	ethanolic	keto acids	2-keto-butyric-acid	$C_4H_6O_3$	30 45
				2,2-Bis(4-nitrobenzyl)-1- phenylbutane-1,3-dione	$C_{24}H_{22}N_2O_6$	30
			Alkane	n-Undecane	$C_{11}H_{24}$	30 46
				1,4,5,8-tetrathiadelin	$C_6H_{10}S_4$	30
				Imidazo (1,5-a) pyrimidine	$C_6H_5N_3$	30
	leaves	ethyl acetate		9-(7 methyl octyl)- 9H-xanthene-2,3-diol	$C_{22}H_{27}O_3$	17
				1,3-dibutyl1-2,8-dihydroxy-9H-xanthene-9-one	$C_{21}H_{24}O_4$	14

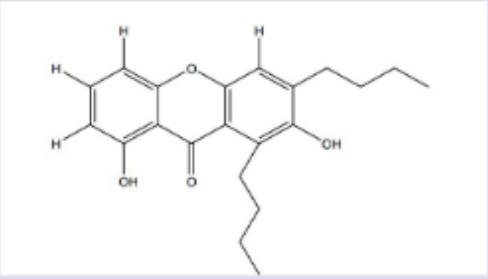

Figure 6: 9-(7-methyl octyl)-9H- xanthene2, 3-diol (hermannol).[17]

Resonance (NMR) and then confirmed NMR results using Fourier Transform Spectroscopy (FTIR) from the ethyl acetate extract of *H. geniculata*. The isolated compound was determined as 1,3-dibutyl1-2,8-dihydroxy-9H-xanthen-9-one (Figure 5).[14] Further analysis was conducted to examine its pharmacological properties, and it was found to possess strong antidiabetic and antioxidant activities and moderate antifungal effects against *C. albicans*.[14]

Hermannol (7-methyl octyl)-9H-xanthene2, 3-diol) (Figure 6) was also isolated from ethanol extracts of *H. geniculata* roots using column chromatography, preparative TLC, and characterised by NMR, Mass Spectrometry, Infrared Spectroscopy, and Ultraviolet spectroscopy. Furthermore, the pharmacological properties identified for the compound included significant antioxidant, antidiabetic, and anti-inflammatory activities, with inhibitory effects on α-glucosidase and 5-lipoxygenase enzymes. In addition, cytotoxicity assays demonstrated selective antiproliferative effects on HepG2 liver cancer cells.[17]

The conducted research has unravelled the extraordinary medicinal potential of the Hermannia genus, however, a notable gap in the phytochemical analysis of other members of Hermannia was observed, especially relating to the isolation, elucidation and determination of biological activities of pure bioactive compounds from the genus.

CONCLUSION

The genus Hermannia has over 30 documented traditional medicinal uses. The medicinal uses of the species from the genus are validated by the discovered biological activities in scientific research, which include antimicrobial, anti-inflammatory, antioxidant, antidiabetic anticancer and toxicity activities. This genus is a rich source of bioactive compounds with over 30 identified from different classes, which include alkaloids, flavonoids, phenolics, terpenoids, fatty acids and many more. These compounds are the foundation of the potent biological activities of the genus. 1,3-dibutyl1-2,8-dihydroxy-9H-xanthene-9-one and hermannol (9-(7-methyl octyl)-9H- xanthene2, 3-diol) are 2 pure compounds that were isolated, elucidated and discovered to possess potent pharmacological properties.

Despite the increasing number of studies of the genus, *H. depressa* and *H. geniculate* remain the most studied species by far and the phytochemical and pharmacological statuses of the other species remain under-explored. The most used parts of the plants in this genus are the roots followed by the leaves and stems, consequently leaving the phytochemical profiling and pharmacological characterisation of the flowers under-explored, this may be because flowers are seasonal and short-lived due to grazing of animals. Moreover, the potential of the genus in terms of isolation and characterisation of pure compounds also remains substantially untapped.

Hermannia genus is a promising source of bioactive compounds for novel drug discovery, particularly in the treatment of bacterial infections, inflammatory conditions, oxidative stress, cancer and management of diabetes. Therefore, rigorous research on the genus is imperative, and future research should prioritise phytochemical screening and pharmacological evaluations screening of a more under explored plant species, isolation and elucidation of more pure bioactive compounds, their mechanisms of action and biosafety evaluation. This will fuel therapeutic products development from the genus, thus contributing to health and wellbeing of communities.

AUTHOR CONTRIBUTION

Mfundisi Nhlapo searched the literature, collected the data, and drafted the manuscript; Brian Ngobeni contributed to the data analysis, drafting and reviewing the manuscript. Idah Manduna contributed to the literature search and comments and corrections to the final version of the manuscript

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CONFLICT OF INTEREST

The authors declare no conflict of interest

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