ANTIFUNGAL ACTIVITY AND TOXICITY ASSESSMENT OF TRADITIONAL MEDICINAL PLANTS COMMONLY USED IN THE TREATMENT OF RESPIRATORY DISEASES IN SOUTH AFRICA

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BLOEMFONTEIN 30 SEPTEMBER 2025

DECLARATION OF INDEPENDENT WORK

DECLARATION WITH REGARD TO

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I, MOLEBOHENG EMILY BINYANE-MOTSEKI, identity number _____ and student number _____, do hereby declare that this research project submitted to the Central University of Technology, Free State for the Doctor of Health Sciences in Biomedical Technology, is my own independent work; and complies with the Code of Academic Integrity, as well as other relevant policies, procedures, rules and regulations of the Central University of Technology, Free State; 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.

_____

30 SEPTEMBER 2025

SIGNATURE OF STUDENT DATE

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SUMMARY

Cryptococcus neoformans, *Cryprococcus gattii*, *Aspergillus fumigatus* and *Candida albicans* cause fungal respiratory coinfections in Human immunodeficiency virus (HIV) and Coronavirus disease 2019 (COVID-19) patients. Medicinal plants have been reported to have antifungal activities and other pharmacological properties against these fungal species. Defender is the indigenous herbal medicinal supplement prepared from medicinal plants, including *Zingiber officinale*, *Salvia rosmarinus*, *Petroselium crispum*, *Allium sativum*, *Capsicum annuum*, and *Cannabis sativa,* which have been used traditionally to treat various respiratory conditions and diseases. *Felicia* and *Searsia* species have been used traditionally to treat respiratory diseases and conditions. The aim of the study was to assess the antifungal activity and toxicity of traditional medicinal plants commonly used in the treatment of respiratory diseases in South Africa. This study also determined other pharmacological activities of these medicinal plants.

Qualitative phytochemical analysis included tests for flavonoids, anthraquinones, tannins, steroids, and terpenoids. Quantitative phytochemical analysis included tests for total flavonoid and total phenolic contents and liquid chromatography coupled with mass spectrometry (LCMS). Total flavonoid and phenolic contents were tested using the Folin-Ciocalteu reagent and the aluminium chloride colorimetric method and were measured spectrophotometrically. The antioxidant activity was analysed using the diphenyl picrylhydrazyl (DPPH) radical scavenging method. The anti-inflammatory activities of the medical plant extract and Defender were determined by measuring their ability to inhibit nitric oxide production in RAW 264.7 macrophages activated by Lipopolysaccharide (LPS). Cytotoxicity of plant extracts and Defender was performed using the Hoechst 33342/Propidium iodide (PI) dual staining method and 3-(4,5-dimethylthiazol-2yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. Antifungal activity of plant extracts and

Defender was determined against *Cryptococcus neoformans*, *Cryptococcus gattii*, *Candida albicans* and *Aspergillus fumigatus* using the serial dilution assay. The potential inhibition of plant extracts on human P450 3A4 was determined using a Vivid® CYP450 Screening Kit. Plant extracts and Defender were tested against *Cryptococcus gattii* and *Cryptococcus neoformans* in the absence and presence (100-800 µg/ml) of exogenous ergosterol, and their Minimum Inhibitory Concentrations (MICs) were determined after 24 h of incubation at 35°C.

Phytochemical analysis revealed the presence of flavonoids, tannins, anthraquinones, and steroids in the aqueous and methanol extracts of *Searsia erosa* and *Felicia filifolia*, and steroids and flavonoids in Defender. The highest phenolic contents were found in *Searsia erosa* methanol extract (426±0.1 mg GAE/g sample) and Defender (236±0.0 mg GAE/g sample), while *Felicia filifolia* aqueous extract had a phenolic amount of 127±0.0 mg GAE/g sample. The highest flavonoid amounts were observed in *Searsia erosa* methanol extract (257±3.3 mg QE/g extract) and Defender (86±1.4 mg QE/g extract), and *Felicia filifolia* methanol extract had a flavonoid content of 83±1.1 mg QE/g extract. LCMS analysis of *Felicia filifolia* methanol extract revealed the presence of Acerosin, Aphidicolin, Dictamnoside B, Lamiide, and Prostaglandin E2, 5,7,4'-Trihydroxy-3,6,8,3',5'-pentamethoxyflavone, Ranupenin 3-rutinoside, Rutin, Scandoside, and Loniphenyruviridoside D. LCMS analysis of *Searsia erosa* confirmed the presence of phytochemicals, including chlorogenic acid, caffeoyl quinic acid, 3,4-Dicaffeoyl quinic acid, flavonoid-7-O-glycoside, pentacarboxylic acids, quinic acids and derivatives, saccharolipids, and unspecified terpene glycosides.

The antioxidant activities of Defender (74%), *Searsia erosa* methanol extract (74%), and *Felicia filifolia* aqueous extract (68%) were higher than those of the positive controls, trolox (64%) and ascorbic acid (53%), at a concentration of 250 µg/mL. The *Felicia filifolia* methanol extract at concentrations of 50 and 200 µg/ml, the *Searsia erosa* methanol extract at 7.5 µg/mL, the *Searsia erosa* aqueous extracts at 50 and 200 µg/mL, and Defender at 500 µg/mL demonstrated potential anti-inflammatory activities. *Felicia filifolia* methanol extract exhibited the most significant cytotoxicity at a minimum concentration

(MIC) of 62.5 µg/mL on Vero cells. *Searsia erosa* aqueous extract (MIC=1 µg/mL), Defender (MIC=1, 10, 50 µg/mL), and *Felicia filifolia* methanol extract (MIC=1 µg/mL) were effective against *C. neoformans*, *C. gattii*, *C. albicans*, and *A. fumigatus*. *Searsia erosa* methanol extract (MIC=1 µg/mL) only inhibited the growth of *C. albicans* and *A. fumigatus*. MICs of all tested extracts and Defender increased in the presence of ergosterol, and the increase was concentration dependent. Results of the CYP3A4 activity assay revealed a concentration-dependent decrease in substrate production for tested extracts.

Searsia erosa and *Felicia filifolia* methanol extracts are potential anti-inflammatory, antioxidant, and cytotoxic (anticancer) agents. They are also potential antifungal treatments against *C. albicans*, *C. neoformans*, *C. gattii*, and *A. fumigatus*. Defender is a potential antioxidant, anti-inflammatory agent, and antifungal treatment against *C. albicans*, *A. fumigatus, C. neoformans*, and *C. gattii*. *Searsia erosa* and *Felicia filifolia* are possible inhibitors of CYP3A4, and this finding has dose implications. Tested plant extracts and Defender are possible antifungal treatments for infections caused by *C. neoformans and C. gattii,* working by binding to ergosterol. Therefore, these medicinal plants and Defender can be used to treat fungal respiratory infections caused by *C. albicans*, *C. neoformans*, *C. gattii*, and *A. fumigatus* in COVID-19 and HIV patients.

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LIST OF ABBREVIATIONS

ABPA:	Allergic bronchopulmonary aspergillosis
AIDS:	Acquired immunodeficiency syndrome
CAC	COVID-19 associated candidiasis
CAPA	COVID-19-associated pulmonary aspergillosis
CFU	Colony forming unit
COVID-19:	Coronavirus disease 2019
CPA	Chronic pulmonary aspergillosis
CYP3A4	Cytochrome P450 3A4
DCM	Dichloromethane
GAE	Gallic acid equivalents
HIV:	Human immunodeficiency virus
ICU:	Intensive care unit

INT:	Iodonitrotetrazolium
IPA:	Invasive pulmonary aspergillosis
LCMS:	Liquid chromatography coupled with mass spectrometry
MeOH	Methanol
MIC:	Minimum Inhibitory Concentration
MPs	Medicinal plants
MS	Mass spectrometry
MTT:	3-(4, 5-dimethyl thiazol-2yl)-2, 5-diphenyl tetrazolium bromide
PDA	Photodiode array
QE	Quercetin equivalents
QTOF	Quadrupole time-of-flight
RIs	Respiratory infections
SA:	South Africa
SARS-CoV-2:	Severe acute respiratory syndrome coronavirus-2
TDM	Therapeutic drug monitoring
TFP	Total flavonoid content
TMPs	Traditional medicinal plants
TPC	Total phenolic content
UPLC	Ultra-performance liquid chromatography
USA	United States of America

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PUBLICATIONS FROM THIS THESIS

- Binyane M.E and Mfengwana P.H., (2022). Traditional Medicinal Plants as the Potential Adjuvant, Prophylactic and Treatment Therapy for COVID-19 Disease: A Review. Medicinal Plants, *IntechOpen*, chapter 4, pp.1-20. DOI: 10. 57 72/ inte chopen.104491. https://www.intechopen.com/books/11299.

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CONFERENCE PRESENTATIONS

- M.E Binyane, S.S Mashele, and P.H Mfengwana. **The effect of the South African traditional medicinal plants, *Searsia* and *Felicia* species on CYP3A4 activity *in vitro***. 57th Annual South African Society for Basic and Clinical Pharmacology conference. Irene Country Lodge, Centurion, South Africa, 15-17 September 2024.

- **M.E Binyane**, S.S Mashele, and P.H Mfengwana. **Anti-inflammatory and antifungal activities and toxicity assessment of *Searsia erosa*, the South African traditional medicinal plant commonly used to treat respiratory diseases**. 19th world Congress of Basic and Clinical Pharmacology, Scottish Event Campus, Glasgow, Scotland, United Kingdom, 2-7 July 2023.

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- **M.E Binyane**, S.S Mashele, and P.H Mfengwana. **Phytochemical analysis and toxicity assessment of traditional medicinal plants commonly used in treatment of respiratory diseases in South Africa**. International Conference on Traditional Medicine and Phytochemistry, Virtual, 12-14 July 2021.

CHAPTER 1

GENERAL BACKGROUND

1.1 INTRODUCTION

Fungal respiratory infections are the significant causes of mortality and morbidity in patients with viral conditions, including Human immunodeficiency virus (HIV) and Coronavirus disease 2019 (COVID-19) [1,2]. Fungal coinfections caused by *Aspergillus* and *Candida* species are prevalent in hospitalised COVID-19 patients and among immunocompromised populations [3].

Cryptococcus neoformans and *Aspergillus* species are major pathogens known to cause pulmonary diseases in HIV infected patients [4]. Globally, the main opportunistic fungi among HIV patients are *Cryptococcus neoformans* and *Candida albicans* [5].

Triazoles, including fluconazole, isavuconazole, itraconazole, posaconazole, and voriconazole, are licensed for the systemic treatment of pulmonary fungal diseases [6]. The currently available antifungal drugs for the pharmacological management of *Aspergillus*, *Candida* and *Cryptococcus* infections normally fail due to the emergence of antifungal drug resistance, drug interactions, and adverse effects [6,7,8]. Therefore, the development of novel antifungal drugs is highly required [8].

Plant-based management of respiratory symptoms and infections has been in use in South Africa (SA) for several centuries [9]. Plants have been reported to be the source of new chemical entities, and a few clinical studies have revealed the therapeutic benefits of plant-originating molecules on the human organism [10]. This study was aimed at assessing the antifungal activity and toxicity of traditional medicinal plants (TMPs, *Felicia filifolia*, *Searsia erosa,* and Defender, containing *Zingiber officinale*, *Salvia rosmarinus*, *Petroselium crispum*, *Allium sativum*, *Capsicum annuum*, and *Cannabis sativa*) commonly used in the treatment of respiratory diseases in SA with the hope of discovering alternative antifungal drugs of medicinal plant (MPs) origin to treat respiratory fungal infections and diseases in COVID-19 and HIV patients.

1.2 LITERATURE REVIEW

Fungal pathogens and infections cause increased risks to public health globally [11]. Fungal respiratory co-infections associated with HIV and COVID-19 occur in severely ill patients, patients with underlying conditions, and immunosuppressed patients [1,12]. They are significant causes of sickness and death in HIV-positive patients [1].

COVID-19 is caused by Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-COV-2) [12]. This disease was declared a global pandemic by the World Health Organisation on 30 January 2020 [3]. Freer and Mudaly (2022) have reported that approximately 8 million of the 60 million of the South African population, and almost a fifth of the global population, are HIV positive [13]. HIV was first discovered in 1983 and in 2022 had already claimed about 40.4 million lives globally [14]. It belongs to the genus of *Lentivirus* in the Retroviridae family [14,15]. HIV mainly targets CD4+ T-lymphocyte helper cells, resulting in severe immune subversion with a continuous loss of cells [15]. This causes the immune system to weaken, resulting in many immunological abnormalities, including oncological complications and high risks of infections [16]. If it remains untreated, HIV infection progresses to acquired immunodeficiency syndrome (AIDS) [15].

The most common invasive fungal diseases are cryptococcosis, aspergillosis, and candidiasis [12]. Cryptococcosis is a contagious fungal disease caused by the *Cryptococcus* yeast species [17]. Cryptococcosis is estimated to be the cause of mortality worldwide, with 624 700 cases of deaths annually [18]. It is still a common and highly lethal disease of HIV positive individuals in the developing world, and its cases are frequently reported in COVID-19 patients [17,19].

Cryptococcosis is a life-threatening fungal disease that manifests in cutaneous, pulmonary, and meningococcal forms [20]. Pulmonary cryptococcosis caused by *the Cryptococcus neoformans-Cryptococcus gattii* complex can affect both immunocompromised and immunocompetent individuals [21]. It has symptoms similar to those of other pulmonary infections, including coughing, pleuritic chest pain, fever, dyspnoea, weight loss, malaise, and, more rarely, haemoptysis [22].

Pulmonary aspergillosis is commonly caused by *Aspergillus fumigatus* [23]. There are three main types of pulmonary aspergillosis infection, namely, allergic bronchopulmonary aspergillosis (ABPA), chronic pulmonary aspergillosis (CPA), and invasive pulmonary aspergillosis (IPA) [24]. Symptoms of pulmonary aspergillosis are nonspecific and include chronic productive cough, antibiotic-unresponsive fever, sputum production, wheezing, weight loss, dyspnoea, pleuritic chest pain and mild or severe life-threatening haemoptysis [23,24].

A weak immune system, lack of iron and zinc, and hospital-acquired and iatrogenic infections predispose COVID-19 patients to candidiasis [25]. *Candida* species are acquired in the respiratory tract through numerous risk factors, including, among others, immunosuppressive conditions such as HIV [26]. The opportunistic infection candidiasis is caused by *Candida* species [25]. Clinical signs of invasive candidiasis include fever with unspecified cause, known risk factors, and poor response to antibiotics [27].

The recommended gold standard treatment for cryptococcosis includes amphotericin B, flucytosine and fluconazole [22]. The first-line treatment of COVID-19 associated pulmonary aspergillosis (CAPA), ABPA, and IPA is the second-generation antifungal drugs, including isavuconazole, posaconazole, and voriconazole [24,28]. Triazole antifungal drugs such as liposomal amphotericin B or echinocandins, such as caspofungin, are used in case of voriconazole intolerance [28]. Amphotericin B and caspofungin are reported to be more effective against all *Candida* spp. [29]. Azoles, echinocandins, and polyenes are major antifungal classes prescribed for the treatment of candidiasis [25].

In trying to find new alternative treatments, some research studies have explored the activity of aspirin against cryptococcosis. Aspirin is the trade name for acetylsalicylic acid, and it is a member of the family of salicylates that have salicylic acid as the active agent [30]. It is derived from the medicinal plant, the willow tree [31]. Aspirin is primarily used to relieve pain, inflammation, and fever [32]. Aspirin is also used for the prevention of secondary events of thrombosis [30]. It has pharmacological activities, including antitumor, anticancer, antifungal, and antimicrobial effects [33]. The study conducted by

Ogundeji *et al*. (2016) has revealed the potential clinical application of aspirin as a candidate anti-Cryptococcus drug effective against *Cryptococcus neoformans* and *Cryptococcus gattii;* however, prolonged use of aspirin leads to gastritis and haemorrhagic complications [34,35].

Conventional antifungal drugs frequently fail due to severe adverse effects and drug resistance, and research interest has shifted to natural treatments, particularly medicinal plants, in search of novel antifungal therapies [36]. MPs have contributed significantly to the development of complementary medicines and remain the most used form of therapeutic intervention to date [37]. Herbal medicines are believed to be safe, cause fewer side effects, and are affordable and easily accessible [37]. Scientific research has taken interest in the botanical diversity of SA whereby phytopharmacological studies of South African plants are conducted with the aim of developing new allopathic medicines [38]. Plants have been reported to be the source of new chemical entities, and several clinical studies have revealed the therapeutic benefits of plant-based molecules on the human organism [10]. This study was aimed at assessing the antifungal activity and toxicity of the TMPs *Felicia filifolia* and *Searsia erosa,* commonly used in the treatment of respiratory diseases in SA and herbal medicine. Defender is known for its immune system-boosting properties.

Felicia filifolia, previously called *Aster fillifolius*, belongs to the *Asteraceae* family [39,40]. In SA, it is widely distributed in the Eastern Cape, Free State, Kwazulu-Natal, Limpopo, Northern Cape and Northwest provinces [41]. *Felicia filifolia* is known as wild aster in English, sehalahala-se-seholo in Sesotho and draaibos in Afrikaans [39,40,42]. A mixture of *Felicia filifolia* and *Mentha longifolia* is used to treat chest problems, asthma, and bronchitis [43]. Medicinal plants belonging to the genus *Felicia* have been reported to have strong antipyretic, antinociceptive, anti-inflammatory, antioxidant, anthelmintic, antibacterial, antifungal and cytotoxicity activities in animal models [39,44].

Searsia is recently called genus Rhus, and it belongs to *the Anacardiaceae family,* which contains over 250 individual species of flowering plants [45]. *Searsia* species, including *Searsia erosa*, *Searsia natalensis*, and *Searsia undulata,* are used to treat respiratory

diseases such as colds and influenza [46]. They are also used traditionally in SA to treat infections caused by microorganisms [47]. The polar fractions of *Searsia penduline* and *Searsia pentheri* are more effective against fungal pathogens, including *Aspergillus fumigatus*, *Candida albicans* and *Cryptococcus neoformans* [47].

Defender is the herbal medicine prepared from a mixture of MPs including *Zingiber officinale*, *Salvia rosmarinus*, *Petroselium crispum*, *Allium sativum*, *Capsicum annuum*, and *Cannabis sativa*. *Zingiber officinale* is used to treat coughs, colds and flu [48]. The aqueous and methanol extracts of *Zingiber officinale* are reported to serve as the most effective antimicrobial therapies to treat some respiratory tract infections [48]. Other biological activities of *Zingiber officinale* include anti-inflammatory, antioxidant, anticancer, and respiratory protective [49]. *Salvia* species are used for the treatment of colds [47]. *Salvia Rosmarinus* has biological properties, including antioxidant, anticancer and antifungal activity against *Aspergillus parasiticus* [50].

Petroselium crispum is traditionally used to manage urinary tract infections, diabetes, and fluid retention [51]. It has pharmacological activities, including antifungal, antibacterial, antioxidant, anti-ulcer, and anti-inflammatory [51,52]. *Allium sativum* is used to treat coughing, fever, tuberculosis, and lung inflammation [53]. It has medicinal properties, including antioxidant, anti-inflammatory, anticancer, and antifungal activity against *Candida albicans* [53]. *Capsicum annuum* Linn is used to treat coughs, colds and asthma [54]. It has biological properties, including antifungal, antioxidant, and analgesic [54]. *Cannabis* is used to treat viral diseases, chronic pain, cancer, inflammatory conditions and inflammatory bowel diseases [55]. It has antioxidant, anti-inflammatory, analgesic, anticancer and calming properties [55].

1.3 RESEARCH OBJECTIVES AND OUTCOMES

Problem statement: Fungal pathogens, including *Cryptococcus*, *Candida*, and *Aspergillus* species, cause pulmonary diseases in HIV-infected patients and contribute to increased mortality rates in COVID-19 patients [4,17,56]. Antifungal therapy for pulmonary fungal diseases includes extended spectrum triazoles, voriconazole, posaconazole, and

liposomal amphotericin B [6]. Fluconazolei is effective against fungal infections, including candidiasis, cryptococcosis and aspergillosis [57,58]. However, fluconazole can cause drug-induced liver injury [59], and its long-term use exerts selection pressure, thereby causing the emergence of resistant strains [60]. Voriconazole has adverse effects, including photopsia, hepatotoxicity, skin rash, and visual hallucinations [61]. Moreover, voriconazole requires therapeutic drug monitoring (TDM), especially for COVID-19 patients in the intensive care unit (ICU) [62].

Emergence of azole-resistant *C. albicans*, *A. fumigatus* and *C. neoformans* has been reported [63]. The appearance of these drug-resistant microbial strains has also increased public pressure to minimise the use of synthetic antimicrobials, and this has directed research towards finding better antimicrobial agents of natural product origin [64]. One approach is to screen local medicinal plants for possible antifungal properties to develop alternative antifungal drugs for the treatment of infectious diseases [65].

1.3.1 Aim of the study

To assess antifungal activity and toxicity of TMPs commonly used in treating respiratory diseases in SA.

1.3.2 Objectives of the study

- To perform a qualitative and quantitative phytochemical analysis of selected MPs.
- To identify the secondary metabolites which are present in selected MPs using Liquid chromatography-mass spectrometry (LC-MS)
- To determine the cytotoxicity of selected MPs using the 3-(4, 5-dimethyl thiazol-2yl)-2, 5-diphenyl tetrazolium bromide (MTT) assay and Hoechst 33342/ Propidium Iodide dual staining methods.
- To determine the antifungal activity of selected MPs against *Cryptococcus neoformans*, *Cryptococcus gattii*, *Candida albicans*, and *Aspergillus fumigatus* using the iodonitrotetrazolium (INT) assay.
- To investigate the possible effect of selected MPs on ergosterol using the Ergosterol Effect Assay.
- To investigate the selected MPs' possible herb-drug interactions with CYP3A4.

1.4 METHODOLOGY

1.4.1 Plant extraction

Fresh leaves of *Searsia erosa and Felicia Filifolia* were washed, air-dried at room temperature and then ground into fine powder using an electric blender. Powdered plant materials were soaked separately in purified water, 100% methanol (MeOH), 1:1 (v/v) absolute methanol: dichloromethane (MeOH: DCM), 100% dichloromethane (DCM) and 100% Hexane for 72 hours with occasional stirring. Following that, extracts were filtered, aqueous extracts were freeze-dried, and organic solvents were concentrated with a rocket evaporator [66]. Extraction of MPs for the preparation of Defender was performed by Cberkie Projects and Innovation (Pty) Ltd using its in-house method.

1.4.2 Qualitative and Quantitative phytochemical analysis

MPs were washed, dried, powdered and extracted, and the phytochemical composition (tannins, saponins, flavonoids, alkaloids, protein, steroids, cardiac glycosides, coumarins and anthroquinones) was determined using standard qualitative phytochemical tests [67,68,69,70,71]. Total flavonoid and phenolic contents were measured using the Folin-Giocalteu colorimetric method [67,72,73].

1.4.3 Liquid chromatography-mass spectrometry identification of secondary metabolites

Phytochemical profiles of medicinal plant extracts were determined by using Liquid chromatography-mass spectrometry (LC-MS). LC-MS Analysis of plant extracts was performed using a Shimadzu chromatograph equipped with SPD-10A UV and LC-MS 2010 detectors and an EC 150/2 NUCLEODUR C18 Gravity SB 150×2mm×5µm column with the National Institute of Standards and Technology library (NIST 11 MS library) [44].

1.4.4 Cytotoxicity assessment of selected medicinal plants

The cytotoxicity screening of MPs was determined by assessing cell viability using the MTT assay and Hoechst 33342/ propidium iodide assay [74].

1.4.5 Determination of the Minimal Inhibitory Concentration of selected medicinal plants against respiratory pathogens

The minimal inhibitory concentrations (MICs) of MPs were determined against *C. neoformans*, *C. gattii*, *C. albicans* and *A. fumigatus*. Tryptic soya broth was inoculated with diluted 1 x 10^8 colony-forming units (CFU)/ml of fungal pathogens, and then 100 µL was introduced to all wells of the 96 microtitre plate. Fluconazole and salicylic acid were used as positive controls. The colour reagent, *p*-iodonitrotetrazolium violet, was added after 24 h, and the colour changes (demonstrating microbial growth) were observed after 2-6 h. The MIC value was read as the lowest dilution having no evidence of fungal growth [75].

1.4.6 Investigate the possible effect of medicinal plants on ergosterol using Ergosterol Effect Assay

The MICs of MPs were determined against *C.neoformans* and *C. gattii* in the presence (200-800 µg/mL) and absence of exogenous ergosterol. Salicylic acid and fluconazole were used as controls. The MICs were determined after 24 h of incubation at 35°C [18].

1.4.7 CYP3A4 assay

Cytochrome P450 3A4 inhibitory effect of MPs was performed using Vivid® CYP3A4 Red Kit reagents and Vivid® BOMR Red substrate as per the manufacturer's instructions. Fluorescence intensity was measured at 550/590 (Ex/Em) nm using a BioTek® PowerWave XS spectrophotometer (Winooski, VT, USA). Results were determined using a Vivid® Fluorescent Standard curve and are expressed as [substrate produced] (nM).

1.4.8 Study layout

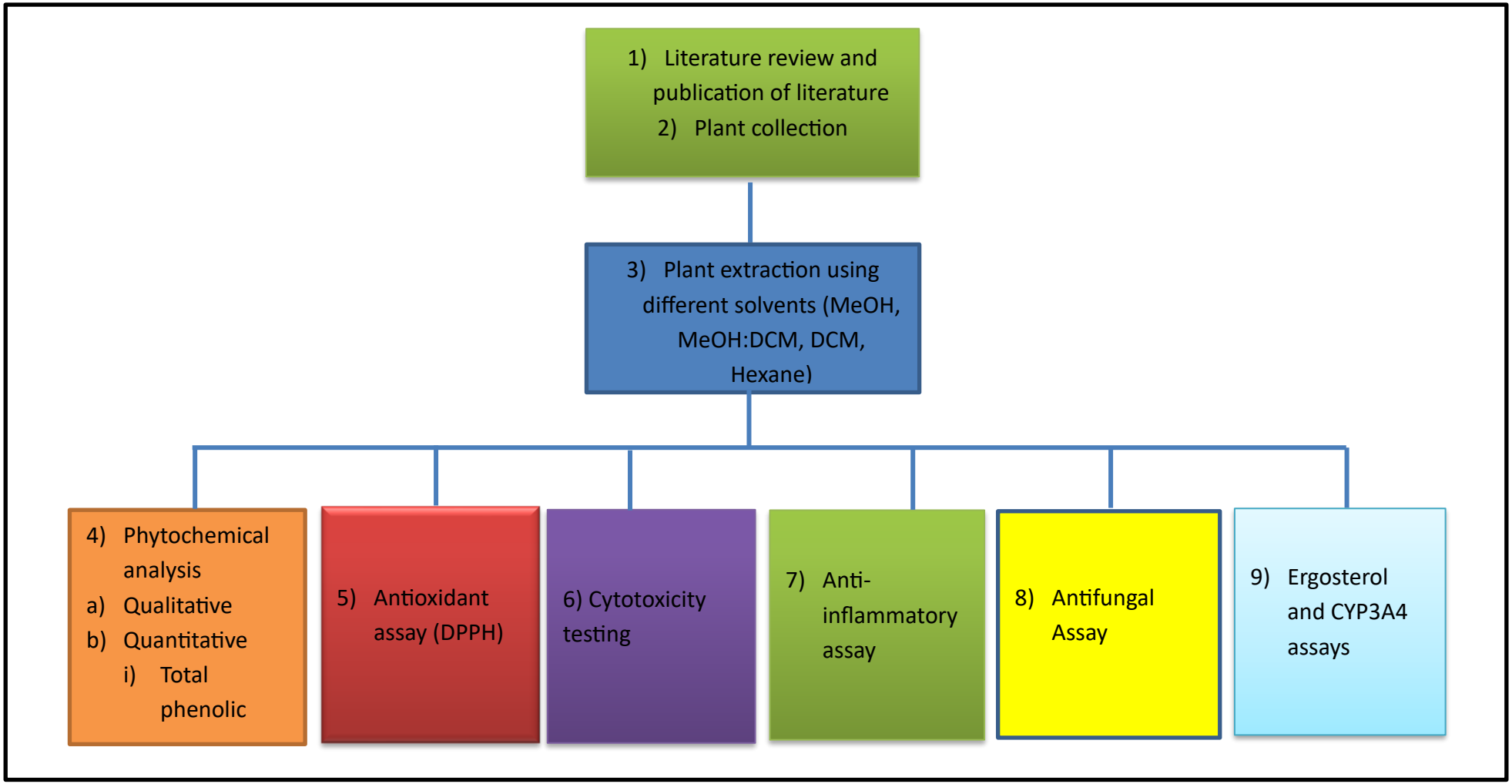

Figure 1: A diagram showing the steps followed to investigate the pharmacological activities of medicinal plants used to treat respiratory diseases in South Africa

1.5 STATISTICAL ANALYSIS

Tests were performed in triplicate. Results were expressed as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) was carried out to test any significant difference between the control and test groups and, *P*-values less than 0.05 were considered significant [80].

1.6 STUDY OVERVIEW

Chapter 1: Provides the overview of the study, i.e. the introduction, literature review, problem statement, objectives, and methodology used in the study are summarised. This chapter also provides the contents of the thesis and a brief description of what each chapter entails. The statistical analysis is also covered.

Chapter 2: This section provides published review of South African TMPs used for treating fungal coinfections in COVID-19 patients with respiratory diseases.

Chapter 3: This section provides a review of medicinal properties of TMPs used in preparation of defender, the herbal medicine

Chapter 4: This section of thesis provides phytochemical analysis of MPs. Both qualitative tests and quantitative tests are covered. Quantitative tests performed in the study included total phenolic and flavonoid contents, and LC-MS. The results of the analysis are also reported and discussed.

Chapter 5: This section covers the determination of antioxidant, anti-inflammatory, and antifungal activities and the toxicity assessment of MPs, and the results are reported and discussed in this chapter.

Chapter 6: This section covers the analysis of Ergosterol effect and the CYP3A4 inhibitory effect of MPs. Results are reported and discussed in this chapter

Chapter 7: This section covers final conclusions, study limitations and recommendations for future studies.

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CHAPTER 2

A REVIEW OF SOUTH AFRICAN TRADITIONAL MEDICINAL PLANTS USED FOR TREATING FUNGAL COINFECTIONS IN COVID-19 PATIENTS WITH RESPIRATORY DISEASES

ABSTRACT

Fungal infections are still most prevalent in the South African population. Fungal respiratory infections and diseases are the cause of severe clinical challenges and mortality in patients with compromised immune systems. Clinical signs of coronavirus disease of 2019 (COVID-19), such as lung injury, hyperglycemia due to diabetes, host iron and zinc depletion, hypoxia, immunosuppression, steroid therapy, and long-term hospitalisation, predispose patients to opportunistic fungal infections. Fungal pathogens, including *Cryptococcus*, *Aspergillus*, and *Candida* species, cause coinfections in patients infected with COVID-19, and this has a negative impact on the patients' pharmacological management goals. *Cryptococcus*, *Aspergillus*, and *Candida* species cause respiratory infections and illnesses, including pneumonia, pulmonary aspergillosis, pulmonary candidiasis, and pulmonary cryptococcosis. South African traditional medicinal plants (TMPs) have been used in the treatment of respiratory symptoms and diseases caused by these fungal pathogens. Medicinal plants (MPs) contain secondary metabolites possessing antifungal activity against *Cryptococcus*, *Aspergillus*, and *Candida* species. Moreover, MPs are cheaper and easily accessible, and they are believed to be safe. This review documents the use of South African TMPs such as *Artemisia absinthium*, *Artemisia afra*, *Dicoma anomala*, *Felicia* species, *Mentha* species, *Ruta graveolens*, and *Seasia erosa,* in the treatment of fungal infections and diseases caused by *Cryptococcus*, *Aspergillus*, and *Candida* species.

2. INTRODUCTION

COVID-19 patients with asymptomatic, mild, moderate, severe, and critical disease states are at risk of developing coinfection with pathogenic fungal species, such as *Aspergillus*, *Candida*, and *Cryptococcus* [1,2]. Research reports suggest that COVID-

19 predisposes patients to fungal and other viral coinfections, and superinfections [3]. Concurrently occurring coinfections pose a massive challenge because they complicate diagnoses and COVID-19 management [3]. COVID-19, caused by SARS-CoV-2 [1,2,3,4] causes respiratory symptoms such as shortness of breath, fever, fatigue, runny nose, headache, chest pain, congestion, anosmia, ageusia, sore throat, confusion, and vomiting [3,5,6], similar to those caused by *Aspergillus*, *Candida*, and *Cryptococcus* species infections [3].

An estimate of 15% of COVID-19 patients admitted to the hospital's ICU become co-infected by *Aspergillus* [7]. *Aspergillus* causes pulmonary aspergillosis, including ABPA, CPA, and IPA [8]. COVID-19-associated pulmonary aspergillosis (CAPA) is reported to have a 52% death rate [9]. *A. fumigatus/ A. fumigatus* and *A. flavus* are the most common *Aspergillus* species causing coinfection in COVID-19 patients [4]. Conducted cohort studies on COVID-19-associated pulmonary aspergillosis have described its incidence to be between 2 and 33% [2,10]. Aspergillosis is treated by the antifungal drug class, triazoles [1,11], with voriconazole and isavuconazole being the first-line therapies [7,9]. However, there are challenges associated with treatment therapy, including the occurrence of azole-resistant *A. fumigatus* [11] and drug-drug interactions associated with the use of voriconazole, which leads to increased cardiotoxic effects of anti-SARS-CoV-2 agents [1].

The study conducted on COVID-19 patients who were severely and critically ill has revealed that dexamethasone is associated with increased pulmonary aspergillosis risk and death [12]. COVID-19-associated candidiasis (CAC) has occurred in various hospitals across countries [3]. CAC is an opportunistic infection caused by fungal species belonging to the genus *Candida* [3,13]. Studies conducted in various countries, including the UK, Italy, Egypt, China, Iran, India, Gambia, and have revealed that *Candida* species, including *C. albicans*, *C. tropicalis*, *C. glabrata*, *C. auris*, and *C. parapsilosis,* are implicated in CAC [4,13,14]. Treatment of *Candida* infections includes azoles, echinocandin, Amphotericin B, and its liposomes [15]. However, there is an emergence of multidrug-resistant *Candida* species, such as C. *glabrata, C. auris*, inherently resistant *C. krusei*, *C auris* resistant to fluconazole, and

Amphotericin B, and fluconazole-resistant *C. parapsilosis* and *C. tropicalis* [4,15]. Moreover, COVID-19 patients receiving treatment therapy, including tocilizumab, interferon type 1β, and lopinavir-ritonavir, are at an elevated risk of developing coinfections with *Candida* spp. [16].

Chloroquine, hydroxychloroquine, azithromycin, and protease inhibitors can cause direct myocardial toxicity, arrhythmias, and death [1]. COVID-19 patients coinfected with human immunodeficiency virus (HIV) or those with compromised immune systems are at risk of developing cryptococcosis [15]. The literature reveals a growing number of cryptococcosis cases in COVID-19 patients who were receiving corticosteroids and immunomodulators [17,18,19]. Pulmonary cryptococcosis is caused by two cryptococcal pathogenic species namely, *C. neoformans* and *C. gattii* [20,21].

The recommended treatment therapy for cryptococcosis includes initial treatment with Amphotericin B in combination with flucytosine, followed by maintenance therapy with fluconazole [15,22]. However, fluconazole-resistant *Cryptococcus* has been reported, and there is also an increased risk of antifungal toxicity [19]. Phytotherapy is considered an important solution in treating respiratory infections and diseases in adults and children [23]. Research reports that medicinal plants contain a variety of active secondary metabolites, including alkaloids, saponins, and terpenoids with antifungal activity [24].

In SA, many people utilise TMPs more than Western medicines because TMPs are cheaper, widely available, and considered to be more effective [25]. South African TMPs such as *Artemisia absinthium*, *Artemisia afra*, *Dicoma anomala, Felicia* species, *Mentha* species, *Ruta graveolens*, and *Searsia erosa* have been shown to possess antifungal activity against fungal pathogens including *Cryptococcus*, *Aspergillus*, and *Candida* species [19,26,27,28,29].

Fungal species such as *Candida*, *Aspergillus*, and *Cryptococcus* cause respiratory coinfections in HIV and COVID-19 patients, and this affects the diagnosis and management of such patients. TMPs are widely available and possess antifungal

activities against these pathogens and could be the potential treatment of infections and diseases caused by these pathogens.

2.1 SOUTH AFRICAN TRADITIONAL MEDICINAL PLANTS USED IN THE TREATMENT OF RESPIRATORY DISEASES CAUSED BY FUNGAL PATHOGENS

2.1.1 *Artemisia* species

Artemisia is the most widely distributed genus belonging to the *Asteraceae* family [26,27]. It consists of over 500 plant species of small herbs and shrubs, which are classified as annual, biennial, and perennial natural plants [27,30]. These plants are used as traditional medicines [26]. Amongst all 500 *Artemisia* species, two species, *Artemisia afra* and *Artemisia absinthium,* are the most used in SA [30]. *Artemisia afra* Jacq. ex Willd. (**Figure 2),** also known as 'Wilde als' in Afrikaans, 'African wormwood' in English, 'Lengana' in Sesotho, 'Umhlonyane' in isiXhosa and 'Mhlonyane' in isiZulu is a South African medicinal plant commonly used to treat respiratory symptoms and conditions such as bronchitis, asthma, colds, coughs, fever, pneumonia, sore throat, chills, whooping cough and headache [6,19,28,30,31].

A. afra is also used in combination with other TMPs such as *E. globulus* and *Lippia asperifolia* as prophylaxis for lung inflammation and to treat influenza [28]. The crude extract of *A. afra* has shown antifungal activity against *Candida albicans*, *Cryptococcus neoformans*, and *Aspergillus* species, including *Aspergillus ochraceus*, *Aspergillus niger*, and *Aspergillus parasiticus* (**Table 1**) [19, 28,32]. The leaves of *A. afra* contain numerous phenolic compounds that possess antimicrobial activity [33]. *A. afra* methanolic crude extract contains scopoletin, betulinic acid, and acacetin with good antimicrobial activity [34]. Other secondary metabolites, including alkaloids, tannins, saponins, steroids, cardiac glycosides and anthraquinones, are found in the crude extract and essential oil of *A. afra* [35]. Toxicity testing results of *A. afra* extract on McCoy fibroblast cell lines indicated moderate toxicity [19].

A. absinthium (**Figure 3),** also known as Wormwood, Green ginger, Absinthium, or Absinthe in English, is used traditionally to treat fever [27,29]. However, when used for a long period, *A. absinthium* is reported to be responsible for central nervous system-associated adverse effects in patients, such as convulsions, hallucinations, and insomnia [27]. It contains secondary metabolites, including lactones, terpenoids,

flavonoid glycosides, organic acids, tannins, and phenols [27]. Moreover, *A. absinthium* has antifungal activity against *C. albicans*, *A. niger, and A. flavus* (**Table 1**) [29]. *A. absinthium* is reported to be nontoxic when tested on Wistar Hannover rats for thirteen weeks [77].

Artemisia species are available throughout all seasons and are used to treat respiratory symptoms. They contain secondary metabolites responsible for antifungal activities against *C. albicans*, and *Aspergillus* species, and are not toxic when tested *in vitro* and *in vivo*.

2.1.2 *Dicoma anomala*

Dicoma anomala (**Figure 4**) is the herbaceous plant belonging to the *Asteraceae* family of plants [36,37]. It is known as 'Maagbitterwortel' in Afrikaans, 'Fever bush' in English, 'Hloenya' in Sesotho, 'Inyongana' in isiXhosa, and 'Isihlabamakhondlwane' in isiZulu [36,37]. In SA, *Dicoma anomala* is distributed in various provinces, including the Free State, Limpopo, Gauteng, Northwest, Northern Cape, and Kwazulu natal [36,39,78]. Two subspecies, *Dicoma anomala* and *Dicoma gerrardii,* are found in SA [37]. *Dicoma anomala* is used traditionally to treat respiratory symptoms and diseases, including coughs, colds, and fever [36,37,38]. It has antifungal activity against *C. albicans* and *A. niger* (**Table 1**) [36,39].

Figure 2. *Artemisia afra*

Figure 3. *Artemisia absinthium*

Dicoma anomala produces bioactive compounds, including phenolic acids, flavonoids, tannins, saponins, triterpenes, phytosterols, acetylenic compounds, sesquiterpene lactones, and diterpenes [40].Results of acute and subchronic oral toxicity assessment

of aqueous root extract of *Dicoma anomala* in rats for 14 days of acute and 90 days of subchronic toxicity testing have revealed that *Dicoma anomala* is not toxic at 0.5 to 2000 mg/kg [39]. *Dicoma anomala* dichloromethane: methanol extract was found to be nontoxic at concentrations below 200 µg/ml when tested on Chang liver cells [79].

Dicoma anomala, known to be effective against respiratory conditions, has been used traditionally in various parts of SA. It contains secondary metabolites responsible for its antifungal properties, and *in vivo* tests have shown that it is nontoxic when orally administered.

Figure 4. *Dicoma anomala*

Dicoma anomala is widely distributed in SA and has been used traditionally to treat respiratory diseases. Its antifungal activity is due to the phytochemical compounds naturally present in it. More safety *in vivo* tests and clinical studies are required to confirm the safety of this *Dicoma anomala* in humans.

2.1.3 *Felicia muricata*

The genus *Felicia* consists of small shrubs of eighty-five known species of annual and perennial herbaceous plants [80]. *Felicia muricata* (**Figure 5**) is an aromatic herb belonging to the *Asteraceae* family [41,42]. It is known as 'white Felicia' in English and 'Ibhobhosi' or 'Ubosisi' in isiXhosa [41,42,43]. *Felicia muricata* is widely distributed in SA, and in the Eastern Cape province, it is used traditionally to treat respiratory symptoms, including headaches and fever [41,43,44,45]. It has antifungal activity against *Aspergillus* species, including *A. niger and A. flavus* (**Table 1**) [41]. *Felicia muricata* contains secondary metabolites, including phenols, proanthocyanidins, flavonols, sesquiterpene lactones, triterpenoids, and flavonoids [41,46]. The study conducted in Wistar rats using *Felicia muricata* aqueous leaf extract at 50, 100, and

200 mg/kg body weight for fourteen days revealed that the plant is mildly toxic and safe for oral requires further investigation [81].

Felicia species is annually available in most parts of SA and has been used traditionally to treat respiratory symptoms. There is a need to conduct more antifungal tests on the plants and their secondary metabolites. The current study focuses on *Felicia filifolia* and it is important to perform safety profiling of this medicinal plant.

Figure 5. *Felicia muricata*

2.1.4 *Searsia* species

The genus *Searsia* (formerly known as *Rhus*) belongs to the family *Anacardiaceae*. It is widely distributed in tropical and subtropical areas globally, mostly in the African continent, especially southern Africa [88,89]. Most *Searsia* species, such as *Searsia erosa*, *Searsia divaricate*, *Searsia lancea*, *Searsia natalensis*, and *Searsia undulata,* are traditionally used to treat respiratory illnesses, including colds, influenza, and microbial infections [68,90]. *Searsia* species have pharmacological activities, including anti-inflammatory, anticancer, antiviral, antimalarial, antidiarrheal, and antioxidant activities [91]. *Searsia erosa* (**Figure 6A),** also known as Broom karee, Besembos in English, and Tśilabele in Sesotho [68,72,73]. It is used traditionally to treat respiratory diseases, including colds [72,73].

It has antifungal activity against *Cryptococcus neoformans* (**Table 1**) [74]. Aqueous extracts of *Searsia erosa* were found to be nontoxic when tested using the brine shrimp lethality assay [74]. *Searsia lancea* (**Figure 6B),** also known as African sumac and Willow rhus in English, is used to treat colds and influenza [68]. It contains bioactive

compounds, including flavonoids, tannins, and phenols [75]. *Searsia lancea* has the antifungal activity against *A. flavus* (**Table 1**) [76]. *Searsia natalensis,* also known as Natal rhus in English, is used to treat influenza [76]. It possesses secondary metabolites, including epicatechin, 3β-sitosterol, glucoside stigmasterol, and lupeol [75]. *Searsia natalensis* (**Figure 6C**) has the antifungal activity against *C. albicans* and *A. Niger* [95]. There are no studies documenting the toxicity analysis of reported *Searsia* species, and further studies are warranted to determine the safety of these medicinal plants.

Figure 6. *Searsia* species, (A) *Searsia erosa*, (B) *Searsia lancea*, (C) *Searsia natalensis*

Searsia species are mostly distributed in SA and the African continent. They have used traditionally to treat microbial infections and respiratory diseases. Antifungal activities of various *Searsia* species have been reported and it is important to test these MPs for their safety *in vitro* and *in vivo*. The focus of the current work is *Searsia erosa*.

Table 1 below shows the traditional use of South African TMPs in respiratory conditions, including asthma, bronchitis, colds, coughs, sore throats, headaches, lung inflammation, influenza, chills, whooping cough, pneumonia, and fever [6,19,28,29,30,31,63,70,85]. These TMPs are also reported to possess antifungal activity against *Aspergillus*, *Candida*, and *Cryptococcus* species, which are implicated in coinfections with COVID-19.

Table 1. Traditional medicinal plants used in respiratory diseases caused by fungal pathogens causing co-infections in COVID-19 patients

South African TMPs	Venicular names	Traditional uses in in respiratory conditions	Inhibited fungal pathogens implicated in coinfections in COVID-19 patients	Secondary metabolites responsible for the antifungal activity
Artemisia afra	Wild als, African wormwood, Lengana,	Asthma, bronchitis, colds, coughs, sore throat, chills, fever	*C. albicans*, *C. neoformans* [28,30,33]	Phenolic compounds, scopoletin, betulinic acid, acacetin, alkaloids, tannins, saponins, steroids, cardiac glycosides, anthraquinones

	Umhlonyane, Mhlonyane [6,19,28,30,31]	headaches, lung, inflammation, influenza, whooping cough, pneumonia [6,19,28 ,30,31]		[33,34,35]
Artemisa absinthium	Wormwood, Green ginger, Absinthium, Absinthe [27,29]	Fever [29]	*C. albicans*, *A. niger*, *A. flavus* [27,29]	Lactones, terpenoids, flavonoids, flavonoid glycosides, organic acids, tannins, phenols [27]
Dicoma anomala	Fever bush, Hloenya, Maagbitterwortel, Inyongana,	Cold, cough, fever, Sore [36, 37, 38]	*C. albicans*, *A. niger* [36,39]	Phenolic acids, flavonoids, tannins, saponins, triterpene, phytosterols, acetylenic compounds,

	Isihlabamakho-ndlwane [36,37]			sesquiterpene, lactones, diterpene [40]
Felicia muricata	White Felicia, Ihbosisi	Headaches, fever, [41,43,44,45]	*A. niger*, *A. flavus* [41]	Phenols, proanthocyanidins, flavonols, sesquiterpene, lactones, triterpenoid [41,42,43], flavonoids [41,46]
Mentha spicata	Spearmint, brown mint, Garden mint, Lady's mint, Imboza [47,48]	Asthma, cold, fever, flu [48,49,50]	*A. niger,* *C. neoformans*, *C. albicans* [48,51,52,53]	Biopeptides, flavonoids, tannins, sterols, polyphenols, sterols, triterpenes, glycosides [53,54]
Mentha longifolia	Wild mint, Horsemint, Silver mint, Koena,	Common cold, cough, sore,	*C. albicans*, *C. glabrata*,	Flavonoids, ceramides, cinnamates, ester, ketones, monoterpenes,

	Inxina, Inzinziniba [49,55,56,57,58,59]	throat, fever, headache, flu [60,61]	*A. flavus*, *A. fumigatus*, *A. niger* [55,57,62]	phenols, polyene, sesquiterpenes [60]
Ruta graveolens	Ruta, rue, Garden rue, Herb of grace, Wynruit [63,64,65,66]	Fever, headache, colds, influenza, [64,66]	*C. albicans*, *C. tropicalis*, *C. parapsilopsis*, *C. glabrata* , *A. flavus*, *A. fumigatus*, *A. niger*, *C. neoformans* [67,68,69,70]	Coumarins, coumarin dimers, dihydrofuranocoumarins, quinolone, furoquinoline, dihydrofuroquinoline, phenolic acids, alkaloids, flavonoids [71]

Searsia erosa	Broom karee, Besembos, Tśilabele	Colds [89,90]	*C. neoformans* [91]	Alkaloids, flavonoids, terpenoids, saponins, tannins [91]
Searsia lancea	African sumuc, Willow rhus, [86]	Colds, influenza [86]	*A. flavus* [92]	Flavonoids, tannins, phenols [91]
Searcia natalensis	Natal rhus [92]	Influenza [92]	*C. albicans*, *A. Niger* [92]	epicatechin, 3β-sitosterol, 3β-sitosterol glucoside stigmasterol, lupeol [92]

[86,89,90]

2.2 CONCLUSIONS

This review summarizes TMPs commonly used in the treatment of respiratory diseases caused by fungal pathogens such as *Aspergillus*, *Candida* and *Cryptococcus* species implicated in coinfection in COVID-19 patients. *Artemisia absinthium*, *Artemisia afra*, *Dicoma anomala*, *Felicia* species, *Mentha* species, *Ruta graveolens*, and *Searsia erosa* have been used in SA for the treatment of respiratory symptoms and diseases including asthma, bronchitis, colds, coughs, sore throat, headaches, lung inflammation, influenza, chills, whooping cough, pneumonia, fever and flu. These TMPs contain secondary metabolites responsible for their antifungal activities. *In vitro* and *in vivo* toxicity studies reported within this chapter have revealed that some of these TMPs are nontoxic for oral administration. However, further testing using animal models and clinical studies is required to profile pharmacokinetics and pharmacodynamics of these TMPs before recommendations to use in coinfections in COVID-19 patients.

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CHAPTER 3

MEDICINAL PROPERTIES OF TRADITIONAL MEDICINAL PLANTS USED IN PREPARATION OF DEFENDER, THE HERBAL MEDICINE

ABSTRACT

Defender is the herbal medicine prepared from medicinal plants (MPs), *Allium sativum*, *Cannabis sativa*, *Capsicum annuum*, *Petroselium crispum*, *Zingiber officinale*, *Salvia rosmarinus*, and *Zingiber officinale*. Some of these MPs are used to treat respiratory infections and symptoms. *Allium sativa* is used to treat tuberculosis (TB), fever, lung inflammation, and coughs. *Capsicum annuum* Linn is used to treat asthma, coughs, and colds. *Salvia* species are used for the treatment of colds, and *Zingiber officinale* is used to treat infections of the respiratory tract. These MPs including *Salvia Rosmarinus*, *Petroselium crispum*, and *Cannabis sativa,* contain secondary metabolites such as alkaloids, tannins, flavonoids, saponin glycosides, terpenoids, and steroids responsible for their medicinal properties, such as anti-inflammatory, antioxidant, antifungal, and anticancer activities. This review aims to document the South African traditional medicinal plants (TMPs) used in preparing Defender, their applications for respiratory and other conditions, and the secondary metabolites responsible for their medicinal properties.

3. INTRODUCTION

Respiratory infections (RIs) and diseases are still the main global health challenge and are counted amongst the leading causes of death and sickness [1,2]. In SA, RIs and diseases are responsible for the increased numbers of all adjusted life years, disabilities, and death rates [3]. MP-based management of respiratory infections and symptoms has been used for many centuries in SA [3]. Traditional medicine is foundational to healthcare for numerous South African communities [4]. MPs possess pharmacological properties because of the phytochemical constituents they contain, such as alkaloids, flavonoids glycosides, saponins, steroids, tannins, and terpenoids [5]. These pharmacological

properties include antifungal, anti-inflammatory, antioxidant, and anticancer activities [6].

Respiratory diseases are still a health problem and because many communities in SA especially in rural areas prefer MPs over western treatment, and studies have revealed that these MPs possess pharmacological activities due to their naturally present phytochemicals, it is crucial to perform various test to further document safety and efficacy profiles of these MPs.

3.1 MEDICINAL PLANTS USED IN PREPARATION OF DEFENDER, THEIR USE IN RESPIRATORY DISEASES AND SYMPTOMS AND THEIR OTHER MEDICINAL PROPERTIES

The COVID-19 pandemic has created opportunities for the production of herbal medicines, especially those with immunomodulatory properties [2], and Defender (**Figure 10**) is one of them. Defender is the herbal medicine containing six medicinal plants used in respiratory diseases and other conditions, (1) *Allium sativum*, (2) Capsicum *annuum* Linn, (3) *Salvia Rosmarinus*, (4) *Petroselium crispum*, (5) *Cannabis sativa, and* (6) *Zingiber officinale*.

Figure 7. Defender

3.1.1 Allium sativum

Allium sativum **(Figure 8)** also known as Garlic belongs to the *Amaryllidaceae* family [6]. It is used to treat respiratory diseases and symptoms including influenza, whooping cough, cold, cough, fever, earache, lung inflammation, and tuberculosis [5, 7,8]. *Allium sativum* can also be used in the management of conditions including joint disease, seizures and disorders of the gastrointestinal tract [6]. It has biological activities such as antifungal activities against *Candida albicans*, *Cryptococcus* and *Aspergillus* species, and antioxidant, anti-inflammatory and anticancer activities [5,7,8]. *Allium sativum* also contains S-allyl-cysteine, the organosulfur compound responsible for its antioxidant, anti-inflammatory and anticancer properties [5]. Allicin, the sulfenic acid thioester found in *Allium sativum,* is responsible for its antioxidant and anticancer activities [5]. Allicin and ajoene are responsible for antiviral activity against influenza B [5].

Figure 8. *Allium sativum* [9]

Allium sativum is a very useful component of Defender that has been used traditionally for the treatment of respiratory diseases and it has been reported to be effective against all three fungal pathogens tested in this study.

3.1.2 Capsicum annuum Linn

Capsicum annuum (**Figure 9**) Linn belongs to the family *Solanaceae* and is also known as chili pepper [10]. It has other English names, such as bell pepper, cayenne pepper, pod pepper, red pepper, and sweet pepper [10]. *Capsicum annuum* Linn used traditionally for the treatment of colds, coughs, and asthma [11]. It is also used to treat cancer, rheumatism, flatulence, and dyspepsia and to improve circulation and digestion [9].

Capsicum annuum has pharmacological activities, including antifungal, antiviral, antioxidant, anti-inflammatory, and anticancer [10,11]. It has phytoconstituents, including alkaloids, flavonoids, glycosides, phenolic compounds and carotenoids, responsible for its pharmacological activities [12].

Figure 9. *Capsicum annuum* major species [12]

Capsicum annuum is used to treat respiratory symptoms and diseases and is widely available and is used in many homes for preparation of food. It is the important part of Defender due to its reported antifungal properties and effectiveness in respiratory infections.

3.1.3 Salvia Rosmarinus

Salvia Rosmarinus (**Figure 10),** also known as rosemary, belongs to the *Lamiaceae* family [13]. *Salvia* species are used for the treatment of colds [14], and *Salvia Rosmarinus* is used to treat respiratory disorders and sore throats [13]. *Salvia Rosmarinus* is also used for the treatment of other diseases, including renal colic, epilepsy, diabetes, and cardiovascular and rheumatic diseases [13]. It has pharmacological properties, including antifungal activity against *Aspergillus parasiticus and* antioxidant, anti-inflammatory, and anticancer activities [13]. *Salvia Rosmarinus* contains phytoconstituents, including terpenoids, flavonoids, and hydroxycinnamic derivatives [13].

Salvia Rosmarinus is the crucial component of Defender due to its ability to treat respiratory conditions and symptoms, and its pharmacological activities such as antioxidant, antifungal and anti-inflammatory.

Figure10. *Salvia Rosmarinus*

3.1.4 Petroselium crispum

Petroselium crispum (**Figure 11),** also known as parsley, belongs to the family *Apiaceae* [16]. It is traditionally used to manage urinary tract infections, diabetes, and fluid retention [17]. *Petroselium crispum* has a diuretic effect [18], and that is the main reason for its use in the preparation of Defender. It has pharmacological activities, including antifungal, antibacterial, antioxidant, anti-ulcer, and anti-inflammatory activities [16,17]. Phytochemical composition of *Petroselinum crispum* consists of flavonoids, phenolic compounds, coumarins, carotenoids, organic acids, essential oil, minerals, and vitamins [18].

Figure 11. *Petroselium crispum* [19]

Petroselium crispum is widely available and used in various homes in food or salads. It was selected in preparation of Defender for its diuretic, antifungal and antioxidant effects.

3.1.5 Cannabis sativa

Cannabis sativa (**Figure 12),** also known as hemp and marijuana, belongs to the *Cannabaceae* family [20]. It is used traditionally to treat respiratory diseases and several other diseases, including digestive, genital, urinary, nervous, and circulatory diseases, diabetes, inflammation, and chronic pain [20]. *Cannabis sativa* has pharmacological activities, including antioxidant, anti-inflammatory, anticancer, and analgesic activities [20]. It has phytochemical components such as cannabinoid, flavonoid, stilbenoid, carotenoid, alkaloid, and lignanamide classes [20].

Figure 12. *Cannabis sativa* [20]

Cannabis sativa, a very important component of Defender was selected for its antioxidant, and anti-inflammatory activities, and its ability to treat respiratory diseases.

3.1.6 Zingiber officinale

Zingiber officinale (**Figure 13**), also known as ginger, belongs to the *Zingiberaceae* family [21,22]. It is used for the treatment of respiratory symptoms and diseases, including common colds, coughs, asthma, influenza, headaches, sore throats, fever, and other diseases such as arthritis, rheumatism, nausea, flatulence, muscular aches, pains,

cramps, constipation, hypertension, dementia, infectious diseases, helminthiasis, colic, and diarrhoea [21,23,24,25]. It has pharmacological activities, including immunomodulatory, antitumorigenic, anti-inflammatory, antiapoptotic, antihyperglycemic, antilipidemic, antiemetic, antipyretic, antioxidant, antibacterial, and analgesic [21,24,25]. Active compounds in ginger include phenolic and terpene compounds, and phenolic compounds in ginger include gingerols, paradols and shogaols, and paradols [24]. The profile and chemistry of *Zingiber officinale* make it perfect for anti-inflammatory therapy in the context of upper respiratory affections [24].

Figure 13. *Zingiber officinale*

Zingiber officinale is the important component of Defender, selected for its ability to treat respiratory symptoms and diseases, and for its antioxidant, anti-inflammatory and antimicrobial activities.

3.2 CONCLUSION

RIs and diseases are a global problem, and MPs can be used to treat such diseases and associated symptoms. Defender is prepared from six MPs, and five (*Allium sativum*, *Salvia Rosmarinus*, *Cannabis sativa*, *Capsicum annuum*, and *Zingiber officinale*) have been used traditionally to treat RIs and diseases, and one (*Petroselium crispum*) is added for its diuretic effect. These medicinal plants have pharmacological activities such as antimicrobial (antifungal, antibacterial and antiviral), antioxidant, anti-inflammatory and anticancer activities, and their phytoconstituents, including alkaloids, coumarins, hydrocinnamic derivatives, flavonoids, phenolic compounds, glycosides, terpenoids,

carotenoids, organosulfur compounds, essential oils, minerals and vitamins, are responsible for such activities.

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CHAPTER 4

QUALITATIVE AND QUANTITATIVE PHYTOCHEMICAL ANALYSIS OF *SEARSIA EROSA*, *FELICIA FILIFOLIA* AND DEFENDER

ABSTRACT

Qualitative and quantitative phytochemical screenings of traditional medicinal plants (TMPs) of the *Felicia* and *Searsia* species and the Defender were performed. Medicinal plants (MPs) were extracted using water and methanol. Qualitative phytochemical analysis included tests for anthraquinones, flavonoids, steroids, tannins, and terpenoids. Quantitative phytochemical analysis performed was total phenolic and flavonoid contents and liquid chromatography coupled with mass spectrometry (LCMS). Total phenolic and flavonoid contents were measured spectrophotometrically using the Folin-Ciocalteu reagent and the aluminium chloride colorimetric method. The results of the phytochemical analysis revealed the presence of tannins, flavonoids, steroids, and anthraquinones in the aqueous and methanol extracts of *Felicia filifolia* and *Searsia erosa*, as well as flavonoids and steroids in Defender. The highest flavonoid amounts were observed in *Searsia erosa* methanol extract (257±3.3 mg QE/g extract) and *Felicia filifolia* methanol extract (83±1.1 mg QE/g extract), and the Defender had a flavonoid content of 86±1.4 mg QE/g extract. The highest phenolic contents were found in *Searsia erosa* methanol extract (426±0.1 mg GAE/g sample) and *Felicia filifolia* aqueous extract (127±0.0 mg GAE/g sample), and the total phenolic content of the Defender was 236±0.0 mg GAE/g sample. LCMS analysis of *Searsia erosa* revealed the presence of phytochemicals including flavonoid-7-O-glycoside, unspecified terpene glycosides, pentacarboxylic acids, quinic acids and derivatives, caffeoyl quinic acid, 3,4-Dicaffeoyl quinic acid, saccharolipids, and chlorogenic acid. An LCMS study of *Felicia filifolia* methanol extract confirmed the presence of Acerosin, Aphidicolin, 5,7,4'-Trihydroxy-3,6,8,3',5'-pentamethoxyflavone,

Ranupenin 3- rutinoside, Rutin, Scandoside, Loniphe- nyruviridoside D, Dictamnoside B,Lamiide, and Prostaglandin E2.

4.1 INTRODUCTION

Chapters 2 and 3 discussed MPs used in RIs and diseases and highlighted that pharmacological properties of such MPs are attributed to the presence of secondary plant metabolites. MPs and numerous modern medicines depend on secondary metabolites, also called phytoconstituents and phytochemical compounds, for their pharmacological actions [1]. Secondary metabolites are substances produced by plants, and their production is dependent on physiological conditions and developmental stages of plants [2]. They enable plants to cope with stress and are important for plants' survival and growth [2,3]. In humans, secondary metabolites are important to improve health and are harvested for medicinal purposes as immunomodulators, antibiotics, antitumor agents, enzyme inhibitors, and so forth [3].

Major phytochemical components found in MPs are tannins, anthroquinones, flavonoids, steroids, alkaloids, cardiac glycosides, phlobatannins, terpenoids, and reducing sugars [4]. In this chapter, qualitative phytochemical analysis screening of selected MPs was performed according to Ranjan *et al.* (2013) [5]. Total phenolic content (TPC) and total flavonoid content (TFC) were performed with slight modification from Phuyal *et al.* (2020) and Mahmood e*t al.* (2011) [6,7]. LCMS analysis was performed according to Masike and Madala. (2018) [8].

4.2 PHYTOCHEMICAL ANALYSIS

Phytochemical tests performed in this study involved the determination of tannins, flavonoids, and flavonoid glycosides, steroids, anthroquinones, and terpenoids in selected MPs.

4.2.1 Tannins

Tannins (**Figure 14**) are present in numerous plant species and are found in the plant tissues' outer layer, root, stem, and bark [9]. Tannins are polyphenolic compounds used in traditional medicines and food stuffs because they possess pharmacological properties [9]. They have antimicrobial (antibacterial, antifungal, antiviral, and antiparasitic), anticarcinogenic, anticancer, antitumor, cytostatic, antimutagenic, and hepatoprotective effects and can be used in the treatment of skin inflammation [9, 10, 11].

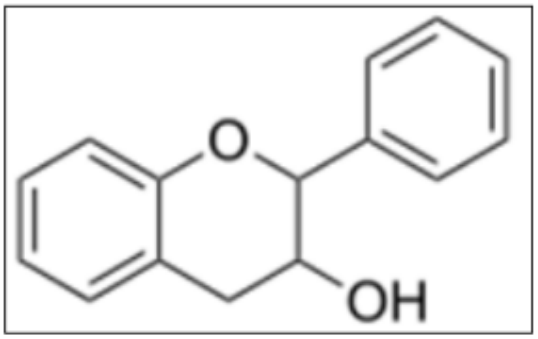

Figure 14: Structure of tannins [9]

Tannins are chemically divided into two major groups, namely hydrolysable tannins and condensed tannins [11]. Hydrolysable tannins include gallic acid esters, gallotannin and ellagitannin [9,10]. Condensed tannins include non-hydrolysable oligomeric and polymeric proanthocyanidins such as catechin and epicatechin and flavolans [9,10]. Complex tannins are those in which a catechin unit is bound to an ellagitannin or gallotannin unit glycosidically [10].

4.2.2 Flavonoids

Flavonoids **(Figure 15)** are secondary metabolites consisting of polyphenolic structures [12]. They are present in plants (flowers, stems, bark, roots, and seeds), grains, tea, wine, vegetables, and fruits [12,13] and responsible for characteristics such as flavour, fragrance, and colour [13]. Flavonoids have antimicrobial (antibacterial, antiviral, and antiparasitic), anti-inflammatory, anticancer, anti-ageing, immunomodulatory, neuro-

protective and cardioprotective properties [13]. They have antioxidant activity, and their potency depends on the number and location of free hydroxyl groups [14]. The synthesis of flavonoids in plants is triggered by microbial infection [15]. Main classes of flavonoids are flavones, isoflavones, flavonols, flavanones, flavanols, chalcones, anthocyanidins, and aurones [14].

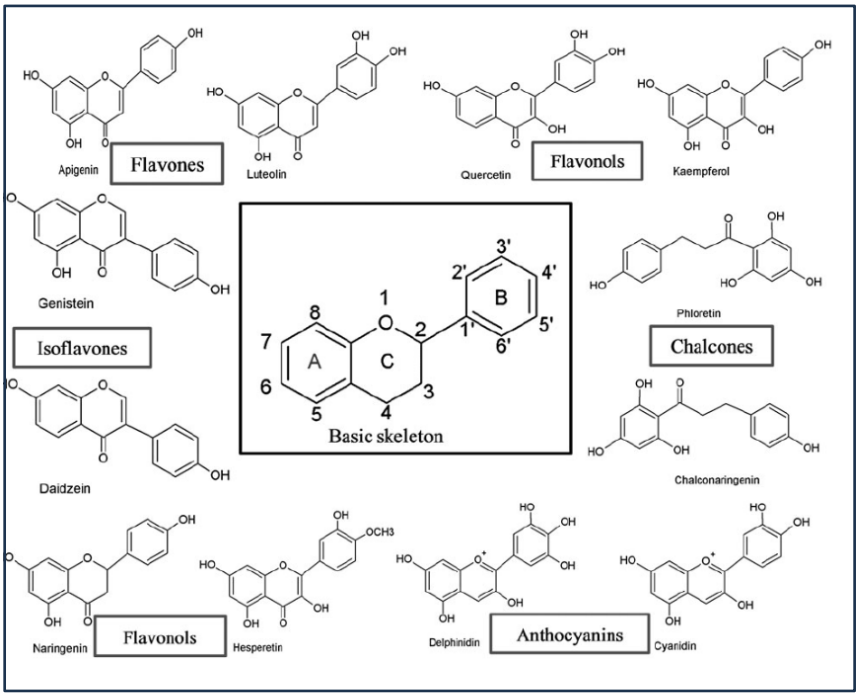

Figure 15. Structures of flavonoids [12]

4.2.3 Steroids

Phytosterols (**Figure 16**) are plant-based steroids [16]. They are used as antihormones, anticancer, antibacterial, anti-inflammatory, antioxidant, antidiabetic, anti-atherosclerotic, anaesthetic, and antiasthma agents [16,17]. There are two categories of plant steroids namely phytosterols and brassinosteroids [16,17].

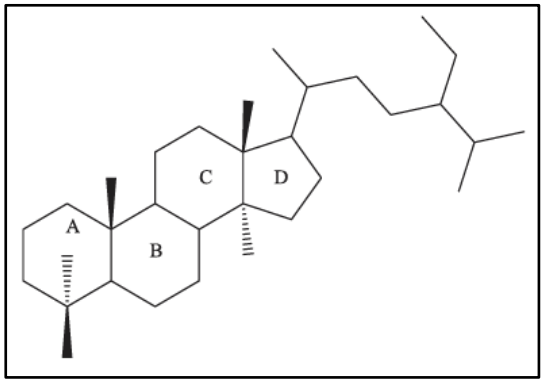

Figure 16. Structure of steroid [17]

4.2.4 Anthroquinones

Anthraquinones (**Figure 17**) are secondary metabolites belonging to the quinones class [18,19]. They are present in plants and abundant in fruits, flowers, roots, and rhizomes [18]. Anthraquinones have pharmacological properties, including antimicrobial (antifungal, antibacterial, and antimalarial), antioxidant, anticancer, anti-inflammatory, antiarthritic, and diuretic [20,21,22]. Anthraquinones responsible for antimicrobial activities include emodin, chrysophanol, rhein, alizarin, aloin, rubiadin, purpurin and mangiferin [23,24].

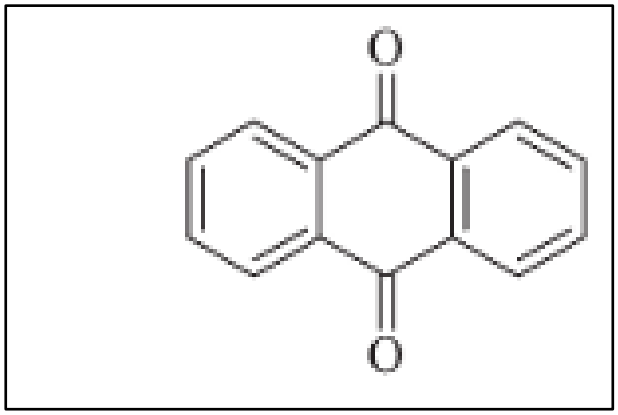

Figure 17. Structure of anthroquinone [23]

4.2.5 Terpenes

Terpenes (**Figure 18**) are found in many plant species, mostly in roots, stems, leaves and flowers [25,26]. They can be classified based on the number of isoprene units (n) in the molecule, namely monoterpenes ($C_{10}H_{16}$), diterpenes ($C_{20}H_{32}$), triterpenes ($C_{30}H_{48}$), tetraterpenes ($C_{40}H_{64}$), hemiterpenes (C_5H_8), sesquiterpenes ($C_{15}H_{24}$), and polyterpenes $(C_5H_8)n$ [27].

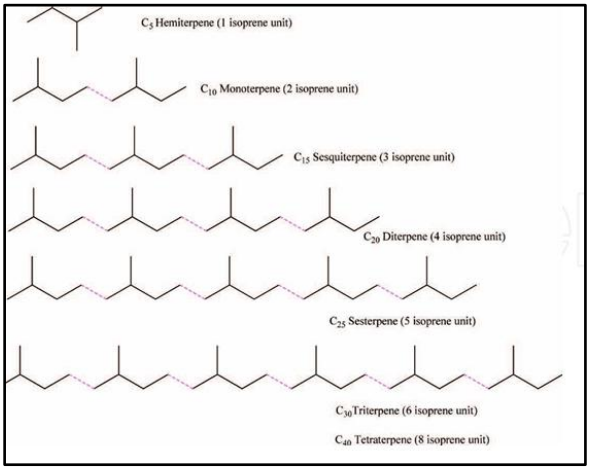

Figure 18. Structural classification of terpenes [28]

Diterpenoids are an abundant family of isoprenoid products formed from 2E, 6E, 10E-geranylgeranyl pyrophosphate [29]. They have pharmacological activities, including antifungal, antibacterial, anti-inflammatory, cytotoxic and anti-tumour activities [30]. Sesquiterpenes are 15 carbon terpenoids that consist of three isoprene units [31]. They have anti-proliferative, anti-tumour, anti-inflammatory, antimicrobial and cytotoxic activities [32].

In this chapter, secondary metabolites of the MPs used in the treatment of respiratory conditions were determined. This also directed the evaluation of pharmacological activities of these MPs in the next chapter (Chapter 5).

4.3 METHODS

4.3.1 Reagents and Chemicals

Defender was supplied by Cberkie Projects and Innovation (Pty) Ltd. *Searsia erosa* and *Felicia filifolia* were collected at Aliwal North and identified and verified by the Botanist at the Department of Plant Sciences, University of the Free State. The specimens are housed in the Geo Potts Herbarium (BLFU), *Searsia erosa* (M.Binyane.AN), and *Felicia filifolia* (ME.BinyaneAN.2). All standards and chemicals used were of analytic grade. Acetonitrile, methanol (MeOH), dichloromethane (DCM), ferric chloride, Folin-Ciocalteu reagent, formic acid, dilute ammonium solution, concentrated sulphuric acid, leucine enkephalin, gallic acid, and quercetin were obtained from Sigma-Aldrich Inc. (St Louis, MO, U.S.A.).

4.3.2 Sample preparation

Plant samples were prepared and extracted as mentioned in 1.4.1 for the qualitative phytochemical analysis, TFC and TPC tests. Furthermore, plant samples were each extracted with methanol (2 g + 15 ml solvent) with vortexing and sonication. After centrifugation, 1 ml of supernatant was blown to dryness and then reconstituted in 50% methanol and 0.1% formic acid. After centrifugation, the dissolved sample was transferred to a glass vial for LCMS analysis.

4.3.3 Qualitative phytochemical analysis of *Searsia erosa*, *Felicia filifolia* and Defender Herbal Mixture

4.3.1.1 Test for Tannins

A 0.1% ferric chloride was added to 1ml of the plant extract and observed for brownish green or blue, black colouration.

4.3.1.2 Test for Flavonoids

A 2.5 ml of dilute ammonia solution was added to 0.5 ml of the plant extract, followed by the addition of concentrated sulphuric acid along the sides of the tube, and observed for the appearance of yellow colouration.

4.3.1.3 Test for Steroids

A 10% sulphuric acid was added to the 1 ml plant extract and observed for green colour.

4.3.1.4 Test for Anthroquinones (Modified Borntrager's test for C-Glycoside of Anthoquinone)

5 ml of dilute hydrochloric acid was added to 0.1 gm of the powdered plant material. Thereafter, 5 ml of 5% ferric chloride solution was added, and the mixture was boiled for 5 min. Then, the mixture was cooled and filtered, and the filtrate was shaken with benzene. Following that, an equal volume of dilute ammonia solution was added and observed for pink colour.

4.3.4 Determination of Total Phenolic Content

Plant extracts (100µl) were added into different test tubes and mixed thoroughly with 100 µl of 50% Folin-Ciocalteu reagent. Thereafter, 2 ml of 7.5% sodium bicarbonate solution was added, and the obtained blue-coloured mixture was shaken well and incubated for 30 min at room temperature; the absorbance was measured at 720 nm against a blank using microplate reader spectrophotometers. The Folin–Ciocalteu reagent oxidises phenols in plant extracts and changes into the dark blue colour, which is then measured by a UV-visible spectrophotometer. All the experiments were carried out in triplicates, and the average absorbance values obtained at different concentrations (50, 100, 150, 200, 250 and 300 µg/ml) of gallic acid were used to plot the calibration curve. Standard gallic acid solution was prepared by dissolving 10 mg of it in 10 mL of methanol (1 mg/mL). Standard curve of gallic acid solution was prepared using the similar procedure described for plant extracts.

4.3.5 Determination of Total Flavonoid Content

Total flavonoid contents (TFC) in the extracts were determined by an aluminium chloride colorimetric assay. Stock solution (5 mg/mL) of quercetin was prepared by dissolving 5 mg of quercetin in 1 mL of methanol. Thereafter, the standard solution was diluted to make various concentrations of (50, 100, 150, 200, 250, and 300 mg/mL). A 600 µl of quercetin of each concentration was added to the test tubes, and after 5 min, 2% aluminium chloride was added. The mixture was prepared in triplicate and incubated for 60 minutes at room temperature. TFC was expressed as quercetin equivalents using the linear equation based on the calibration curve.

4.3.6 Determination of Phytochemical content of *Searsia erosa and Felicia* species *by* Liquid Chromatography/ Mass Spectrometry

A Waters Cyclic Quadrupole time-of-flight (QTOF) mass spectrometer (MS) connected to a Waters Acquity ultra-performance liquid chromatography (UPLC) (Waters, Milford, MA, USA) was used for high-resolution UPLC-MS analysis. Column eluate first passed through a Photodiode Array (PDA) detector before going to the mass spectrometer, allowing simultaneous collection of UV and MS spectra. Electrospray ionisation was used in negative mode with a cone voltage of 15 V, a desolvation temperature of 275 °C, desolvation gas at 650 L/h, and the rest of the MS settings optimised for best resolution and sensitivity. Data were acquired by scanning from m/z 150 to 1500 m/z in resolution mode as well as in MSE mode. In MSE mode, two channels of MS data were acquired, one at a low collision energy (4 V) and the other using a collision energy ramp (40−100 V) to obtain fragmentation data as well. Leucine enkephalin was used as lock mass (reference mass) for accurate mass determination, and the instrument was calibrated with sodium formate. Separation was achieved on a Waters HSS T3, 2.1 × 100 mm, 1.7 µm column. An injection volume of 2 µL was used, and the mobile phase consisted of 0.1% formic acid (solvent A) and acetonitrile containing 0.1% formic acid as solvent B. The gradient started at 98% solvent A for 0.5 min and changed to 22% B over 4 min in a linear way. It then went to 44% B after 9 minutes and 100% B after 13 minutes with a wash step of 1 min, followed by re-equilibration to initial conditions for 2 min. The flow rate was 0.3 mL/min, and the column temperature was maintained at 55 °C. Data was processed using MSDIAL and MSFINDER (RIKEN Centre for Sustainable Resource Science: Metabolome Informatics Research Team, Kanagawa, Japan).

4.4. RESULTS

4.4.1 Qualitative phytochemical analysis of *Searsia erosa*, *Felicia filifolia* and Defender Herbal Mixture

Results (**Table 2**) show the presence of tannins, flavonoids, steroids and anthoquinones in *S.erosa* water and methanol extracts and *F. filifolia* water and methanol extracts.

Anthraquinones and tannins were absent in the Defender, and the presence of flavonoids and steroids was observed.

Table 2. Qualitative phytochemical analysis of (1) *Searsia erosa* methanol extract, (2) *Searsia erosa* water extract, (3) *F.filifolia* methanol extract, (4) *F.filifolia* water extract, (5) Defender Herbal Mixture, (+) presence of secondary metabolite, (-) absence of secondary metabolites

#	Plant extracts	Phytochemical tests			
		Tannins	Flavonoids	Steroids	Anthroquinones
1	*S.erosa* MeoH	+	+	+	+
2	*S.erosa* water	+	+	+	+
3	*F.filifolia* MeoH	+	+	+	+
4	*F.filifolia* water	+	+	+	+
5	Defender	-	+	+	-

4.4.2 Determination of Total Flavonoid and Phenolic Contents

Results (**Figures 19 and 20, and table 3**) below show the graphs and estimated flavonoid and phenolic contents of *S. erosa, F. filifolia* and Defender. The quantitative determination of flavonoid contents in plant extracts was determined using aluminium chloride in a colorimetric method. The results were derived from the calibration curve (y = 0.0126x-0.0622, R^2 = 0.9876) of quercetin (0-300 mg/mL) and expressed in quercetin equivalents (QE) per gram of dry extract weight. *S. erosa* MeOH extract had a greater flavonoid content (257±3.3 mg QE/g) compared to *S. erosa* water extract (30±0.4 mg QE/g). *F. filifolia* MeOH extract had a greater flavonoid content (83±1.1 mg QE/g) compared to *F. filifolia* water extract (32±0.5 mg QE/g). Defender had a flavonoid content of 86±1.4 mg QE/g. Phenolic content was measured using the Folin-Ciocalteu reagent in each plant extract. The results were derived from a calibration curve (y = 0.0031x-0.0192, R^2 = 0.9967) of gallic acid (0-300 mg/mL) and expressed in gallic acid equivalents (GAE) per gram of dry extract weight. *S. erosa* MeOH extract had a greater phenolic content (426±0.1 mg GAE/g) compared to *S. erosa* water extract (100±0.0 mg GAE/g). *F. filifolia* water extract had a greater phenolic content (246±0.1 mg QE/g) compared to *F. filifolia*

MeOH extract (127±0.0 mg GAE/g). Defender had a phenolic content of 236±0.0 mg GAE/g. Higher flavonoid and phenolic contents of *Searsia erosa* compared to Defender could be a result that a different solvent of extraction is used, *Searsia erosa* is extracted with methanol and Defender is prepared in water. Secondly, *Searsia erosa* is not one of TMPs used in preparation of Defender.

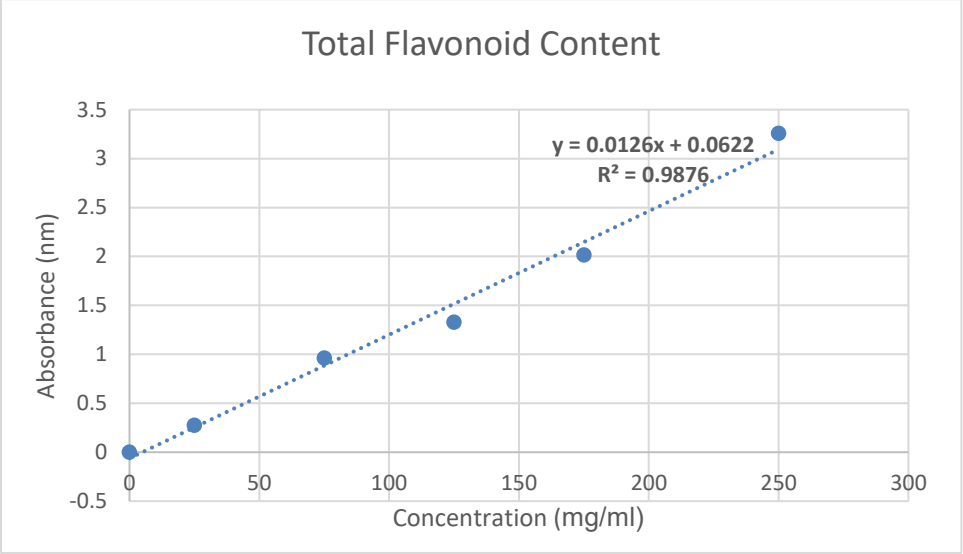

Figure 19. Total flavonoid content calibration graph used for *Searsia* erosa and *Felicia filifolia* extracts and Defender herbal medicine

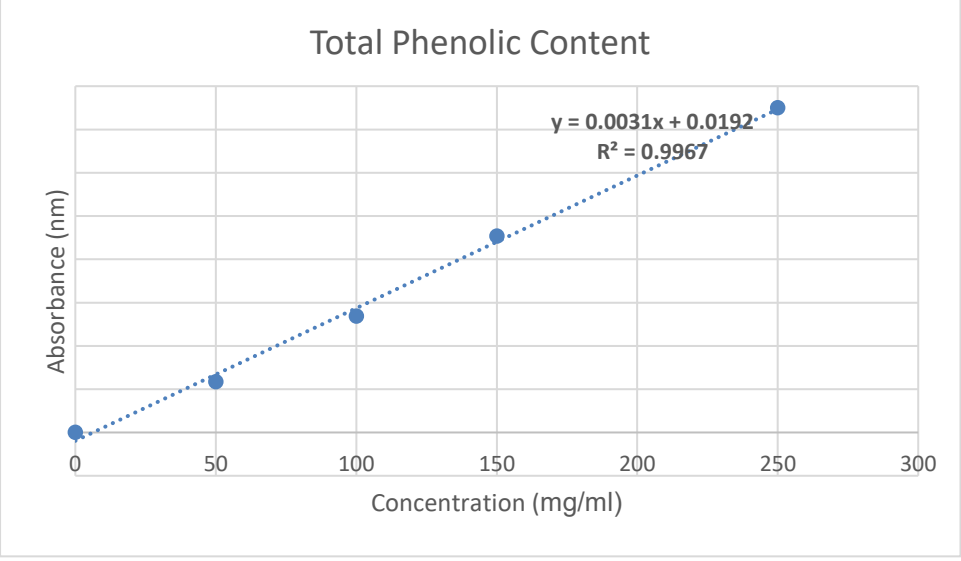

Figure 20. Total phenolic content calibration graph used for *Searsia* erosa and *Felicia filifolia* extracts and Defender herbal medicine

Table 3. Total flavonoid and phenolic contents of *Searsia* erosa, *Felicia filifolia* and Defender

Sample (1mg/ml)	TFC (mg QE/g sample)	TPC (mg GAE/g sample)
S. erosa water extract	30±0.4	100±0.0
S. erosa MeOH extract	257±3.3	426±0.1
Felicia spp water extract	32±0.5	246±0.1
Felicia spp MeOH extract	83±1.1	127±0.0
Defender Herbal Mixture	86±1.4	236±0.0

4.4.3 Determination of Phytochemical content of *Searsia erosa and Felicia filifolia by* Liquid Chromatography/ Mass Spectrometry

The phytocompounds found in *Felicia filifolia* methanol extract using LC-MS analysis are shown in **Figures 21a and b,** and the identified phytocompounds are tabulated in **Table 4** and **table 5** below. LCMS analysis using a negative mode identified 10 compounds, namely (1) Scandoside, (2) Lamiide, (3) Ranupenin 3-rutinoside,(4) Rutin, (5) Loniphenyruviridoside D, (6) Acerosin, (7) 5,7,4'-Trihydroxy-3,6,8,3',5'-pentamethoxyfla vone, (8) Dictamnoside B, (9) Prostaglandin E2, and (10) Aphidicolin. LCMS analysis of *Felicia filifolia* (**Table 5**) using a positive mode identified 6 compounds, including (1) Spinacetin 3-rutinoside, (2) Calendoflaside, (3) Zerumbone, (4) Nevadensin, (5) 7-Deacetoxyyanu thone A, and (6) Mactraxanthin.

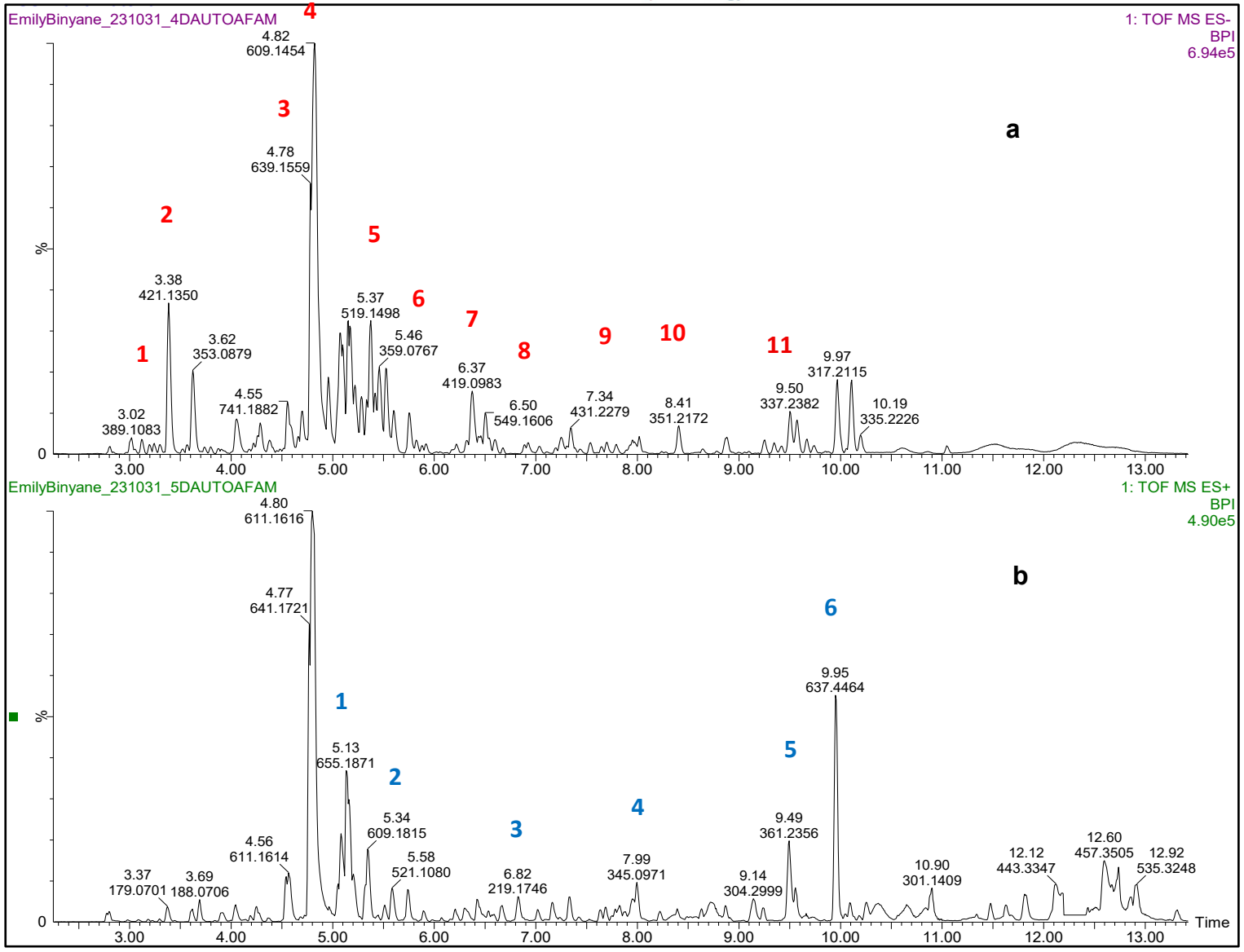

Figure 21. LCMS chromatograms of *Felicia filifolia* methanol extracts in (a) negative (-) and (b) positive (+) mode

Table 4. LCMS (negative mode) identification of phytochemical compounds in *Felicia filifolia* methanol extract

#	Identified compound	Formula	Phytochemical class	RT (min)	Average Mz	MS/MS spectrum
1	Scandoside	$C_{16}H_{22}O_{11}$	Iridoid O-glycosides	3.02	389	331,345,361,389
2	Lamiide	$C_{17}H_{26}O_{12}$	Iridoids and derivatives	3.38	421	169,213,329,411,421
3	Ranupenin 3-rutinoside	$C_{28}H_{32}O_{17}$	Flavonoid-3-O-glycosides	4.78	639	243,316,407,509,640
4	Rutin	$C_{27}H_{30}O_{16}$	Flavonoid-3-O-glycosides	4.82	609	491,591,600,609,610
5	Loniphenyruviridoside D	$C_{25}H_{28}O_{12}$	Terpene glycosides	5.37	519	381,433,478,501,519
6	Acerosin	$C_{18}H_{16}O_8$	8-O-methylated flavonoids	5.46	359	133,219,315,359
7	5,7,4'-Trihydroxy-3,6,8,3',5'-pentamethoxyflavone	$C_{20}H_{20}O_{10}$	8-O-methylated flavonoids	6.37	419	149,197,302,419
8	Avenacoside B	$C_{57}H_9O_{28}$	Steroidal saponin	6.50	549	300,417,609,731, 1224
9	Dictamnoside B	$C_{21}H_{36}O_9$	Terpene glycosides	7.34	431	187,209,314,431
10	Prostaglandin E2	$C_{20}H_{32}O_5$	Prostaglandins and related compounds	8.41	351	249,305,351
11	Aphidicolin	$C_{20}H_{34}O_4$	Aphidicolane and stemodane Diterpenoids	9.50	337	275,301,337

Table 5. LCMS (positive mode) identification of phytochemical compounds in *Felicia filifolia* methanol extract

#	Identified compound	Formula	Phytochemical class	RT (min)	Average Mz	MS/MS spectrum
1	Spinacetin 3-rutinoside	$C_{29}H_{34}O_{17}$	Flavonoid-3-O-glycosides	5.13	655	244,300,419,510,655
2	Calendoflaside	$C_{28}H_{32}O_{15}$	Flavonoid-3-O-glycosides	5.34	609	147,258,301,411,503, 609
3	Zerumbone	$C_{15}H_{22}O$	Sesquiterpenoids	6.82	219	203,219
4	Nevadensin	$C_{18}H_{16}O_7$	8-O-methylated flavonoids	7.99	345	219,345
5	7-Deacetoxyyanuthone A	$C_{22}H_{32}O_3$	Diterpenoids	9.49	361	159,243,361
6	Mactraxanthin	$C_{40}H_{60}O_6$	Xanthophylls	9.95	637	157,201,304,637
7	Diosgenin	$C_{63}H_{102}O_{31}$	Steroidal saponins	7.64	1356	277,351,424,591,622, 706,885,1194,1237, 1356

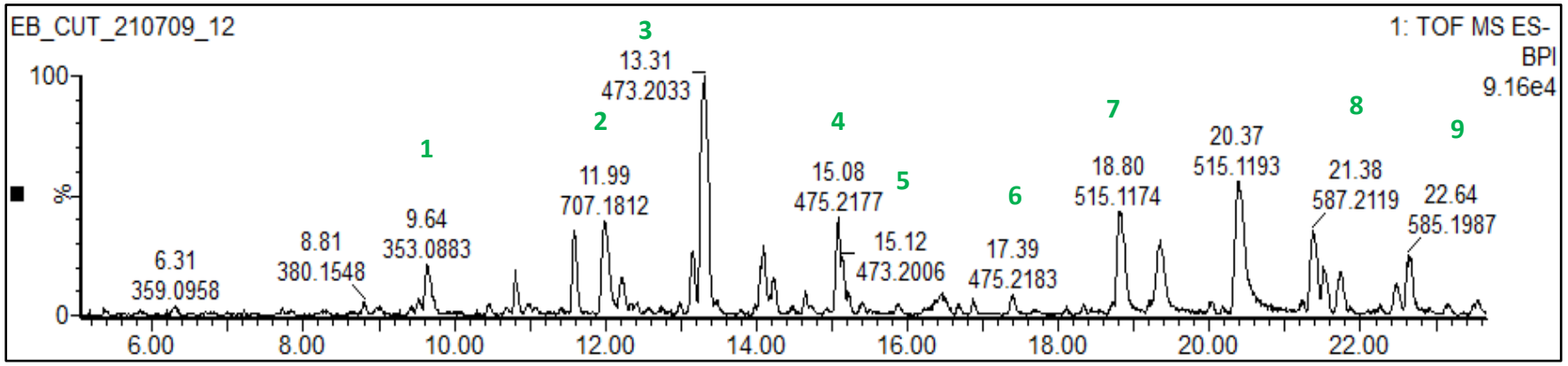

Figure 22. LCMS chromatogram of *S. erosa* methanol extract in (a) negative (-) mode

Table 6. LCMS (negative mode) identification of phytochemical compounds in *S. erosa* methanol extract

#	Identified compound	Formula	Phytochemical class	RT (min)	Average Mz	MS/MS spectrum
1	Caffeoylquinic acid	$C_{16}H_{18}O_9$	Quinic acids and derivatives	9.64	353	42,57,79,89,109,201, 353
2	Chlorogenic acid	$C_{16}H_{18}O_9$	Quinic acids and derivatives	11.99	707	47,59,67,77,87,108, 200,305,407,506,611, 707
3	UNPD79762	$C_{22}H_{34}O_{11}$	Terpene glycosides	13.31	473	42,59,71,85,99,206,

						304,473
4	UNPD60595	$C_{27}H_{30}O_{16}$	Saccharolipids	15.08	475	42,52,72,86,101,203, 301,407,475
5	UNPD63775	$C_{22}H_{36}O_{11}$	Terpene glycosides	17.39	475	58,71,85,99,210,310, 401,476
6	3,4-Dicaffeoylquinic acid	$C_{25}H_{24}O_{12}$	Quinic acids and derivatives	18.80	515	44,57,71,89,97,192, 293,363,497,515
7	UNPD219098	$C_{30}H_{36}O_{12}$	Flavonoid-7-O-glycosides	21.38	587	282,392,499,588
8	UNPD78176	$C_{30}H_{34}O_{12}$	Pentacarboxylic acids and Derivatives	22.64	585	54,75,81,92,101,202, 303,417,586

Figure 22 and Table 6 above **show** the results of LCMS (negative mode) of *Searsia erosa;* identified compounds include (1) Caffeoylquinic acid, (2) Chlorogenic acid, (3) Terpene glycoside (UNPD79762), (4) Saccharolipid (UNPD60595), (5) Terpene glycoside (UNPD79762), (6) Terpene glycoside (UNPD63775), (7) 3,4-Dicaffeoylquinic acid, (8) Flavonoid-7-O-glycoside (UNPD219098), and (9) Pentacarboxylic acids and derivatives (UNPD78176).

4.5 DISCUSSION

Plants contain a wide range of phytochemicals that can be used in the treatment of infectious diseases as well as chronic conditions [33]. Phytochemicals determine the therapeutic effects of MPs, and their identification in plant material assists in determining the potential pharmacological activity of the specific medicinal plant [34].

Felicia species are rich in terpenes [35]. Further identification of phytochemicals using LCMS positive mode identified in the current study revealed the presence of iridoids, scandoside and lamiide in *F. filifolia* methanol extract. Iridoids are a special group of cyclopentanol (c) pyran monoterpenoids which are mostly glycosides and are abundant in many plant families, including the *Apocynaceae*, *Lamiaceae*, *Rubiaceae*, *Loganiaceae*, *Scrophulariaceae*, and *Verbenaceae*, [36,37]. Pharmacological activities of iridoids include antimicrobial, anti-inflammatory, antioxidant, anticancer, antitumor, and anticoagulant [39]. Iridoid glycosides can protect plants against damage caused by the fungus *Cladosporium cucumerinum* [38]. The study conducted by He *et al*. (2018) described the inflammatory effect of scandoside to be because of the inhibition of the cytokines and mediators responsible for signalling pathways, including mitogen-activated protein kinase and nuclear transcription factor kappa-B [36]. Viljoen *et al*. (2012) discussed the anti-inflammatory effect of lamiide due to peroxidation membrane lipid inhibition in the phospholipid assay of rat brain [39].

The negative and positive LCMS modes have revealed the presence of diterpenoids, aphidicolin, and 7-deacetoxyyanuthone A, respectively, in *F. filifolia* methanol extract. Aphidicolin is a tetracyclic diterpenoid that possesses the antimicrobial activity, and it inhibits B-family DNA polymerases of bacteria and viruses [40]. Furthermore, aphidicolin possesses the anticancer activity and has the antimitotic activity in human cancer cell lines [41]. The study conducted by He *et al*. (2017) has revealed the antitubercular activity of 7-deacetoxyyanuthone A, a class 1 yanuthone [42].

The *in vitro* antibacterial study has shown that 7-deacetoxyyanuthone A has mild activity against multidrug-resistant and methicillin-resistant *Staphylococcus aureus* [43]. Moreover, sesquiterpenoids, Zerumbone, was present in *the F. filifolia* methanol extract. Zerumbone has been found to have pharmacological activities, including antimicrobial, anti-inflammatory, chemotherapeutic and anti-hypersensitivity [44]. Terpene glycosides such as loniphenyruviridoside D and dictamnoside B were also present in *F. filifolia* methanol extract. Loniphenyruviridoside D is reported to have inhibitory activity on the STAT-3 signalling pathway of HELF cells [45]. The study conducted by Chang *et al*. (2001) isolated dictamnoside A, B, D and G, and only dictamnoside A was found to have significant activity in stimulating T-cell proliferation [46].

Mactraxanthin, a hexahydroxy carotenoid, was found in *F. filifolia* methanol extract. The first report on a naturally occurring hexahydroxy carotenoid was described following the isolation of mactraxanthin from the Japanese edible surf clam [47]. Carotenoids have antioxidant activity [48]. Flavonoids such as rutin, ranupenin 3-rutinoside, acerosin, 5,7,4'-trihydroxy-3,6,8,3',5'-pentamethoxyflavone, spinacetin 3-rutinoside, and calendoflaside were also present in *F. filifolia* methanol extract. Rutin, also called vitamin P or rutoside, has numerous biological activities, including antimicrobial, anti-inflammatory, anticancer, antiulcer, analgesic and antidepressant [49].

Acerosin inhibited HIV-1 replication *in vitro* in the study conducted by Ticona *et al*. (2020) [51]. It also has anti-proliferative activity [48]. The 5,7,4'-trihydroxy-3,6,8,3',5'-pentamethoxyflavone belongs to the class of natural products called polymethoxyflavonoids. Polymethoxyflavonoids have pharmacological effects, including anti-inflammatory, antiviral and anticarcinogenic [50]. The 5'-Hydroxy-6, 7, 8, 3', 4'-pentamethoxyflavone has antioxidant activity [51]. Virtual molecular docking and Molecular dynamics simulation research have revealed that calendoflaside may inhibit the main protease of SARS-CoV-2 [52].

LCMS negative mode has revealed the presence of quinic acids and derivatives, caffeoyl quinic acid, chlorogenic acid, and 3,4-Dicaffeoyl quinic acid, and unspecified terpene glycosides, saccharolipids, flavonoid-7-O-glycoside, and pentacarboxylic acids and derivatives in *S. erosa* methanol extract.

The study conducted by More *et al*. (2025) has revealed that caffeoylquinic acids are potential inhibitors of the Rift Valley Fever Virus [53]. Caffeoylquinic acids have properties including antioxidant and anti-inflammatory [54]. Chlorogenic acid has pharmacological effects, including antimicrobial, anti-inflammatory, antioxidant and anti-tumour activities [55]. Saccharolipids, when modified chemically, can be used as adjuvants for cancer immunotherapy and wound healing [58]. Carboxylic acid-containing drugs are used as antibiotics, nonsteroidal anti-inflammatory drugs, cholesterol-lowering statins, and anticoagulants [56]. Lower benzene polycarboxylic acid and its derivatives are used as antifungals in the pharmaceutical industry [57].

4.6 CONCLUSIONS

Qualitative and quantitative analysis of TMPs, *F. filifolia*, *S. erosa*, and Defender have revealed the presence of secondary metabolites, including flavonoids, glycosides, terpenoids, xanthophylls, saccharolipids, quinic and pentacarboxylic acids, and their derivatives. These phytochemical compounds have biological properties and are crucial in drug development. It is, therefore, necessary to further investigate their pharmacological importance. Biological activities of these secondary metabolites were investigated and reported in Chapter 5.

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CHAPTER 5

ANTIOXIDANT, ANTI-INFLAMMATORY, AND CYTOTOXICITY ASSESSMENT AND ANTIFUNGAL ACTIVITY ANALYSIS OF *SEARCIA EROSA*, *FELICIA SPECIES* AND DEFENDER

ABSTRACT

Respiratory fungal infections caused by *C. neoformans*, *C.gatti*, *C. albicans* and *A. fumigatus* affect HIV and COVID-19 patients, and this has a severe impact on the diagnosis and treatment outcomes. Traditional medicinal plants (TMPs) have antifungal properties and are cheaper, easy to obtain, and considered safer than conventional therapies. *Searsia erosa* is traditionally used in South Africa to treat colds. *Felicia* species are used to treat respiratory symptoms, including headaches and fever. Defender herbal mixture is currently sold in the Eastern Cape and is used to boost the immune system and improve circulation. The main aim of the current study was to assess the antioxidant, anti-inflammatory, cytotoxic, and antifungal activities of *Searsia erosa* and *Felicia filifolia* methanol extracts, as well as the Defender. The antioxidant activity was evaluated using the diphenylpicrylhydrazyl (DPPH) radical scavenging method. The anti-inflammatory properties of the medicinal plant extracts and Defender were assessed by measuring their ability to inhibit nitric oxide production in RAW 264.7 macrophages activated by Lipopolysaccharide (LPS). Cytotoxicity of extracts and Defender was performed using the Hoechst 33342/Propidium iodide (PI) dual staining method and 3-(4,5-dimethylthiazol-2yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. The antifungal activity of extracts and defenders against *Cryptococcus neoformans*, *Cryptococcus gattii*, *Candida albicans*, and *Aspergillus fumigatus* was determined using a serial dilution assay. The antioxidant activity percentages of *Searsia erosa* methanol (74%) and aqueous (68%) extracts, *Felicia filifolia* aqueous extract (68%), and Defender (74%) were higher than those of the positive controls, trolox (64%) and ascorbic acid (53%), when tested at 250 µg/ml. *Searsia erosa* methanol (7.5 µg/mL) and aqueous (50 and 200 µg/mL) extracts, *Felicia filifolia* methanol extract (50 and 200 µg/mL), and Defender (500 µg/mL) showed potential anti-inflammatory activities. *Felicia filifolia* methanol extract exhibited the most cytotoxicity activity at 62.5-250 µg/mL on Vero cells, while its aqueous extract did not

show significant cytotoxicity at any analysed concentrations. *Searsia erosa* methanol extract showed the most cytotoxicity at the highest treatment concentration (250 µg/mL) on Vero cells. *Searsia erosa* aqueous extract (MIC=1 µg/mL) was effective against *C. albicans*, *A. fumigatus*, *C. neoformans*, and *C. gattii* at 1-100 µg/mL. *Searsia erosa* methanol extract (MIC=1 µg/mL) was effective against *C. albicans* and *A. fumigatus* at 1-100 µg/mL, while for *C. neoformans* and *C. gattii*, the MIC was 50 µg/mL at 50-100 µg/mL. *Felicia filifolia* aqueous extract did not show any antifungal activity against all pathogens, and its methanol extract (MIC=1 µg/mL) inhibited all tested fungal pathogens at 1-100 µg/mL. Defender inhibited *A. fumigatus* (MIC=1 µg/mL), *C. gattii* (MIC=1 µg/mL), *C. albicans* (MIC=50 µg/mL), and *C. neoformans* (MIC=10 µg/mL). *Searsia erosa* and *Felicia filifolia* extracts and Defender were found to possess antioxidant and anti-inflammatory activities with no toxicity against macrophages. Methanol extracts of both *Searsia erosa* and *Felicia filifolia* showed cytotoxic activities at 250 µg/mL and have potential for development as anticancer agents following continuous investigation. Both *Searsia erosa* and *Felicia filifolia* methanol extracts, and Defender were effective against the tested respiratory fungal pathogens, and further analysis *in vivo* is warranted to determine their safety and efficacy. Also, further isolation, characterisation and identification of pure compounds and their analysis thereafter is warranted to determine the active compounds responsible for antifungal activities of these MPs.

5.1 INTRODUCTION

Fungal infections burden millions of people globally due to antibiotic misuse, immunosuppressive therapy, and climate-associated pathogen proliferation [1,2]. *Cryptococcus*, *Aspergillus*, and *Candida* species cause respiratory fungal coinfections in COVID-19 and HIV patients [3,4]. The invasive lung mycosis, pulmonary cryptococcosis, is caused by *the Cryptococcus neoformans* and *Cryptococcus gattii* complex [5]. Its symptoms include cough, chest pain, fever, the production of sputum, and chronic inflammatory fibrosis [5,6].

Pulmonary candidiasis is caused by *Candida* species, and its clinical manifestation includes cough, *Candida* pneumonia, fever, inflammatory alveoli, hypoxaemia, purulent sputum and dyspnoea [7,8]. *Aspergillus* species causes CPA and IPA [9]. Pulmonary aspergillosis causes inflammation and lung damage, and CPA is a chronic

fungal infection which causes lung scarring damage, and IPA is a more serious and fatal form of infection [10]. Other symptoms of pulmonary aspergillosis include cough, fever, dyspnoea, pleuritic chest pain, and haemoptysis [11].

In SA, MPs have been used for the treatment of respiratory infections (RIs) and diseases for numerous centuries [12]. Many MPs are used as antifungals in Africa [13]. MPs produce a variety of secondary metabolites which have antifungal activity [14]. These secondary metabolites include flavonoids, alkaloids, essential oils and terpenoids [15]. Several techniques to determine antifungal activity include bioautography methods, dilution methods, and diffusion methods [16].

Respiratory diseases caused by *Cryptococcus*, *Candida* and *Aspergillus* species cause inflammation [5,6,7,8,9]. Inflammation is a natural response by the body's defence against infections caused by fungi, bacteria, and viruses [17]. It is a main factor for the progression of chronic conditions such as cancer [18]. The processes of inflammatory response depend on the exact nature of stimuli and the situation in the body, and the mechanism is common and involves the recognition of stimuli by cell surface pattern receptors, followed by the activation of inflammatory pathways and the release of inflammatory markers and finally the recruitment of inflammatory cells [19].

Numerous MPs contain phytochemicals with pharmacological properties effective against several acute and chronic conditions [20]. Phytochemical compounds containing anti-inflammatory properties include saponins, glycosides, terpenoids, flavonoids, tannins, alkaloids, and carotenoids [21]. *In vitro* anti-inflammatory assays measure the production of pro-inflammatory cytokines such as interleukins (IL) and tumour necrosis factor (TNF) and pro-inflammatory enzyme activity such as cyclooxygenase (COX) and lipoxygenase (LOX) [22].

Microorganisms such as *Cryptococcus*, *Candida*, and *Aspergillus* species increase production of reactive oxygen and reactive nitrogen species (ROS/RNS) and stimulate NADPH oxidase and Nitric Oxide Synthase (iNOS) pathways [23]. Increased ROS can cause degenerative diseases such as asthma, cancers, atherosclerosis, stroke, trauma, asthma, heart attack, liver injury, hyperoxia, dermatitis, hepatitis, arthritis, retinal damage, cataract genesis, and periodontitis [23]. Production of free radicals from various environmental and biological sources is a result of a lack of sufficient natural antioxidants, which further causes inflammation-related diseases [20].

Antioxidants are responsible for reducing tissue damage induced by free radicals in the body [21]. Phenolic compounds, including phenolic acids, flavonoids and tannins, are rich in plant-based antioxidants [22]. These secondary metabolites inhibit oxidative enzymes, chelate metal ions and scavenge free radicals by preventing lipid peroxidation, neutralising reactive oxygen species, preventing DNA damage and protein oxidation [22].

Several methods used to quantify antioxidant activity include the DPPH radical scavenging capacity assay, ferric reducing antioxidant power (FRAP) assay, 2,2′-azinobis-(3-ethylbenzthiazolin-6-sulfonic Acid (ABTS) assay, hydrogen-atom transfer (HAT), single electron transfer (SET), total peroxyl radical-trapping antioxidant parameter (TRAP) assay, oxygen radical absorbance capacity (ORAC) assay, and total oxyradical scavenging capacity (TOSC) assay [23].

Variety of medicinal plants (MPs) have been used since ancient times for their therapeutic effects, including antioxidant and anti-inflammatory properties associated with diseases such as cancer, atherosclerosis, cardiovascular diseases, diabetes, neurodegenerative diseases, inflammation, ischaemia and anaemia [17,24]. MPs have also been used traditionally to treat fungal infections [3], and their secondary metabolites may be crucial for the development of new antifungal drugs [22]. Flavonoids and phenols are responsible for the antioxidant and anti-inflammatory activities [17].

Terpenoids, flavonoids and alkaloids have antimicrobial activities [22]. These secondary metabolites, including alkaloids, terpenoids, tannins, saponins, amino acids and cyanogen, have pharmacological benefits, but some may also be toxic to animals and humans [25]. Since there is no easy distinction between the therapeutic and toxicity doses of MPs, research investigating their potential toxicities is highly recommended [26], because a very safe compound may at high doses be toxic [27]. Herbal medicines may have toxicity-related issues, including cytotoxicity, hepatotoxicity, carcinogenicity, genotoxicity, and mutagenicity [28,29]. Cytotoxicity assays are used to determine substance toxicity to several tissues [29].

This study evaluated the antioxidant, anti-inflammatory, antifungal, and cytotoxicity activities of the methanol and aqueous extracts of *Felicia filifolia*, *Searsia erosa*, and Defender.

5.2 METHODS

5.2.1 Chemicals and reagents

All standards and chemicals used were of analytic grade. Bis-benzamide H33342 trihydrochloride (Hoechst), propidium iodide (PI), dimethyl sulfoxide (DMSO), MeOH, DPPH, iodonitrotetrazolium chloride (INT), fluconazole, trolox, lipopolysaccharide (LPS) and aminoguanidine were purchased from Sigma-Aldrich Inc. (St. Louis, MO, U.S.A.). RAW 264.7 mouse macrophages were purchased from Cellonex (South Africa). Sulfanilamide and 1-naphthyl)ethylenediamine (NED) products were purchased from Promega, and solutions were made as per the manufacturer's instructions. RPMI1640 culture medium and foetal bovine serum (FBS) were obtained from GE Healthcare Life Sciences (Logan, UT, USA). Dulbecco's Modified Eagle Media (DMEM) and phosphate-buffered saline (PBS) with and without calcium (Ca^{2+}) and magnesium (Mg^{2+}) were purchased from Cytiva (Marlborough, MA, USA). Foetal Bovine Serum (FBS) and penicillin/streptomycin were purchased from Biowest (Nuaillè, France). A 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide was obtained from ThermoFisher Scientific, SA. *Aspergillus fumigatus* (ATCC® 204305™), *Candida albicans* (ATCC® 10231™), *Cryptococcus neoformans* (ATCC® 32045™), and *Cryptococcus gattii* (ATCC® 34877™) were obtained from Microbiologics, Inc. (St Cloud, USA).

5.2.2 Sample preparation

Antioxidant assay sample preparation included dissolving 7.89 mg of DPPH in 100 mL of methanol. The DPPH solution was kept in the dark for 2 hours. 1,000 µL of DPPH solution was added to 1,000 µL of methanol. Ascorbic acid (1mg/mL) and Trolox (1 and 10 mg/mL) were used as positive controls. Sample preparation for the anti-inflammatory assay was as follows: compounds were solubilised using DMSO to make a stock of 100 mg/mL. Samples were stored at 4°C until used. Aminoguanidine (AG) was used as a positive control to indicate anti-inflammatory activity. Cytotoxicity assay sample preparation included reconstitution of dried extracts in DMSO to produce a

final concentration of 100 mg/mL, and samples were sonicated if solubility was a problem and stored at 4°C until required. The African green monkey kidney cell line, Vero cells, was used for cytotoxicity screening. Complete growth medium consisted of DMEM supplemented with 10% FBS. Cells were maintained in 10 cm culture dishes in complete medium and incubated at 37°C in a humidified atmosphere with 5% CO_2. The antifungal assay sample preparation involved dissolving ATTC strains of *Aspergillus fumigatus*, *Candida albicans*, *Cryptococcus neoformans*, and *Cryptococcus gattii* in tryptic soy broth.

5.2.3 Determination of antioxidant activity using diphenylpicrylhydrazyl (DPPH) radical scavenging method

The antioxidant activity of the plant extracts and Defender against DPPH was determined using the methods by Baliyan *et al*. (2022), Phunyal *et al*. (2020), Xiao *et al*. (2020), Chaves *et al*. (2019), and Maizura *et al*. (2011) with slight modifications [13, 22,23,24,25]. 200 µL of methanolic dilution of DPPH was added to 100 µL of plant extracts (*Felicia filifolia* and *Searsia erosa* extracts) and Defender in a 96 well plate. The experiment was performed in triplicate. The mix was kept in the dark at room temperature for 30 min, and absorbance was measured at 517 nm using a UV-spectrophotometer. The blank was prepared with the methanolic dilution of DPPH. Percentage (%) of antioxidant activity was measured using the formula:

% Inhibition = [(Ac-As) /Ac] X100

where: Ac-Control reaction absorbance; As-Sample absorbance.

5.2.4 *In vitro* anti-inflammatory screening of *Searsia erosa* and *Felicia filifolia* extracts, and Defender

RAW 264.7 cells were seeded in RPMI1640 culture medium supplemented with 10% FBS (RPMI complete medium) into 96-well plates at a density of 1 x 10^5 cells per well and allowed to attach overnight. The following day, the previously prepared culture medium was removed, and 50 µL Defender aliquots diluted in RPMI complete medium were added to give final concentrations of 15.625, 31.25, 62.5, 125, 250 and 500 µg/mL. The 50 µL *Searsia erosa* methanol and aqueous extract aliquots diluted in RPMI complete medium were added to give final concentrations of 1, 2.5, 5, and 7.5 µg/mL and 50, 100 and 200 µg/mL. The 50 µL *Felicia filifolia* methanol extract aliquots diluted in RPMI complete medium were added to give final concentrations of 50, 100 and 200 µg/mL. To determine the anti-inflammatory activity, 50 µL of LPS (final

concentration of 500 ng/mL) containing medium was added to the corresponding wells. The 50, 100 and 200 µM of aminoguanidine (AG) were used as the positive control. Cells were incubated for a further 24 hours. To quantify NO production, 50 µL of the spent culture medium was transferred to a new 96-well plate, and 50 µL of sulfanilamide solution was added to the spent culture medium and incubated for 10 minutes in the dark at room temperature. Thereafter, 50 µL of NED solution was added to each well and further incubated for 5-10 minutes in the dark at room temperature. Absorbance was measured at 540 nm (BioTek® PowerWave XS spectrophotometer). A standard curve using sodium nitrite dissolved in culture medium was used to determine the concentration of NO in each sample.

5.2.5 *In vitro* cytotoxicity screening of *Searsia erosa* and *Felicia filifolia* extracts, and Defender

Cells were seeded in 96 well plates at a density of 4 x 10^3 cells/well in 100 µL aliquots and left overnight to attach. Treatment including *Searsia erosa* methanol and *Felicia filifolia* methanol and aqueous extracts was prepared in 15.63, 31.25, 62.5, 125, and 250 µg/mL in complete medium and added to cells. Melphalan (30 µM) was used as a positive control. Cells were then incubated for 48 hours. Thereafter, the culture or treatment medium was aspirated, and a 100 µL staining solution [Hoechst 33342 (5 µg/mL) in PBS with Ca^{2+} and Mg^{2+} was added to each well. Plates were then incubated for 30 minutes. Moreover, a 10 µL PI [(100 µg/mL) in PBS with Ca^{2+} and Mg^{2+} was added to each well. Fluorescent micrographs were captured immediately using an ImageXpress Micro XLS Widefield Microscope (Molecular Devices) with a 10x Plan Fluor objective and DAPI and Texas Red filter cubes. Acquired images were analysed using the MetaXpress software and Multi-Wavelength Cell Scoring Application Module. Cell viability was determined as a percentage of the untreated control using Scoring Profile 1 (number of live cells).

To confirm the absence of toxicity in plant extracts and Defender, cell viability was assessed using the MTT assay. For the MTT assay, at the end of the incubation period (24 hours), 20 µL of 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) solution (0.5 mg/mL) was added into each well. After 30 minutes of incubation at 37 °C, media containing MTT was removed, and 100 µL of DMSO was added to each well to solubilise the formazan crystals. Absorbance was measured at 540 nm

using a BioTek® PowerWave XS spectrophotometer (Winooski, VT, USA). Percentage of viable cells was obtained by dividing the mean absorbance of treated cells (for each concentration of extract) by the mean absorbance of its control cells. MTT assays were carried out in three independent experiments done in triplicate.

5.2.6 A micro-dilution technique / p-iodonitrotetrazolium chloride (INT) Minimum inhibitory concentration (MIC) assay

The *C. albicans*, *A. fumigatus*, *C. Neoformans* and *C. gattii* cultures were diluted in sterile tryptic soy broth to an approximate inoculum size of 1 x 10^8 colony forming units (CFU)/ml. Each 96 well microtiter plate was filled with 100 µL of inoculated broth. Then 100 µL of 1 mg/mL fluconazole (FCZ) and 5 mg/mL salicylic acid (SAC) were used as the positive controls. A 100 µL of aqueous and methanol extracts of *Searsia erosa* and *Felicia filifolia* and Defender at 1, 10, 50, and 100 µg/mL were added to other wells containing inoculated broth. Thereafter, microtiter plates were sealed with sterile adhesive and incubated for 24 hours. Then, 50 µL of a colour reagent, *p*-iodonitrotetrazolium violet (0.5 mg/mL), was transferred to all the plates. The microtiter plates were examined for colour changes after 6 hours.

5.3 STATISTICAL ANALYSIS

Data was analysed by non-parametric methods using the Graphpad™Statistical program. Therefore, parameters were reported as mean, percentages, and mean and standard deviation (SD), and the student's t-test was utilised to compare data between two groups, and One-way Analysis of Variance (ANOVA) was used further to compare between three and more groups, and the level of significance was set at $P<0.05$.

5.4 RESULTS

5.4.1 Results for antioxidant activity assay using diphenylpicrylhydrazyl radical scavenging method

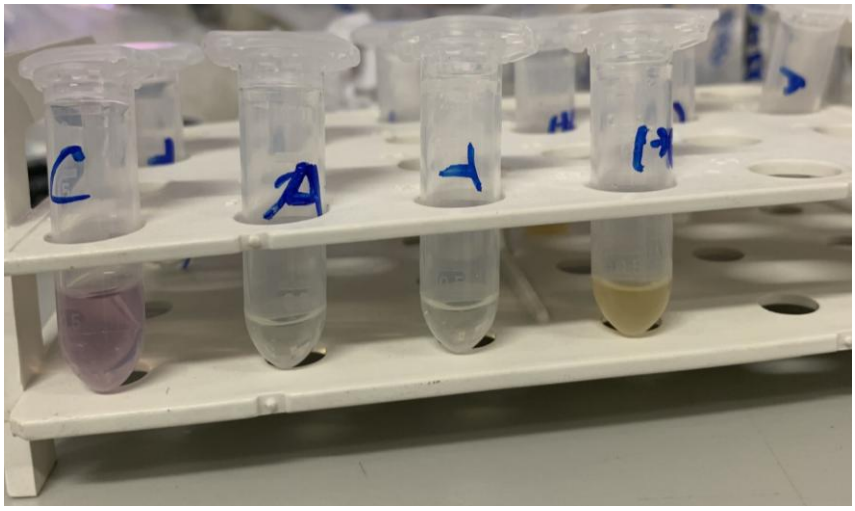

Figure 23. Picture of DPPH experiment (C=control, DPPH: Methanol, A= Ascorbic acid, T=Trolox, and H=Defender

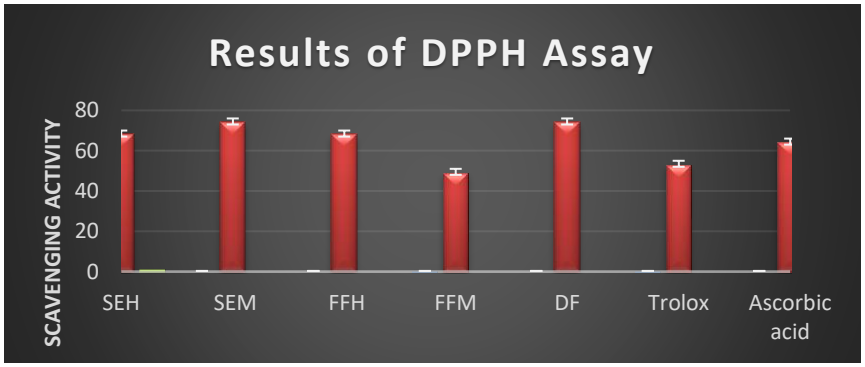

Figure 24. Results of DPPH Assay, SEH=*Searsia erosa* aqueous extract, SEM= *Searsia erosa* methanol extract, FFH= *Felicia filifolia* aqueous extract, FFM=*Felicia filifolia* methanol extract, DF= Defender

The DPPH free radical (**Figure 23**) above is an organic nitrogen radical with a purple colour. When a DPPH solution combines with an antioxidant, it changes from purple to yellow of the corresponding hydrazine [24]. **Figure 24** above shows the percentages of antioxidants of plant extracts and Defender. It can be observed that *Searsia erosa* methanol (74%±1) and aqueous (68%±1) extracts had higher percentages of antioxidant activity than positive controls, trolox (64%±1) and ascorbic acid (53%±2).

Felicia filifolia aqueous extract (68%±1) and Defender (74%±2) also had higher percentages of antioxidant activity than trolox and ascorbic acid at 250 µg/ml.

5.4.2 *In vitro* anti-inflammatory and cytotoxicity screening of *Searsia erosa* and *Felicia filifolia* extracts, and Defender

Anti-inflammatory activity of **Defender (Figures 25A and 26A below)** is indicated by the decrease in nitrite concentration in response to LPS activation of RAW macrophages with no effect on cell viability, as seen with the AG treated cells. Defender showed to have no significant cytotoxicity on the macrophages. At the highest concentration of treatment, 500 µg/mL, a decrease in nitrite concentration was evident, showing potential anti-inflammatory activity at this concentration. The anti-inflammatory activities **(Figures 25B and 26B)** of *Searsia erosa* aqueous and *Felicia filifolia* methanol extracts and *Searsia erosa* methanol extract (7) were assessed using RAW 264.7 macrophages and the Griess assay. The cytotoxic effect of extracts on RAWs was also determined to establish potential anti-inflammatory activity. Anti-inflammatory activity is indicated by the decrease in nitrite concentration in response to LPS activation of RAW macrophages with no effect on cell viability, as seen with the AG treated cells. All samples showed no toxicity, and thus all NO data can be considered. All three samples were shown to possess anti-inflammatory potential at the highest concentration tested.

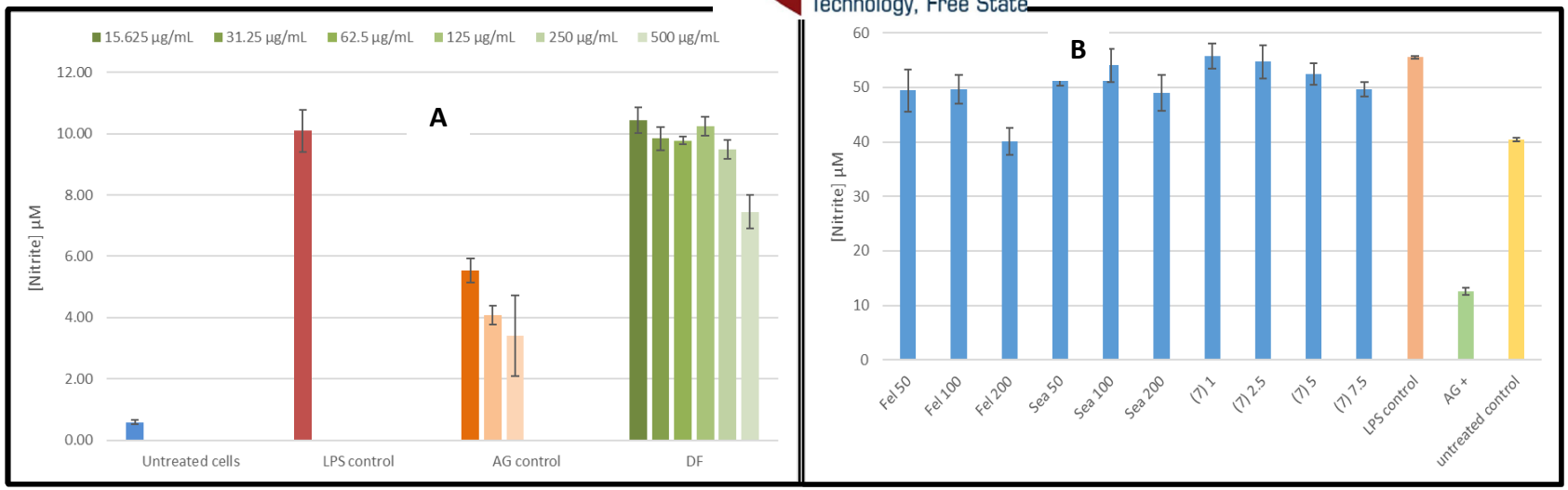

Figure 25: Nitric oxide production in LPS activated macrophages treated with extracts as indicated in the Figure. Bar graph represents quadruplicate values of one experiment. Error bars represent the standard deviation of the mean.

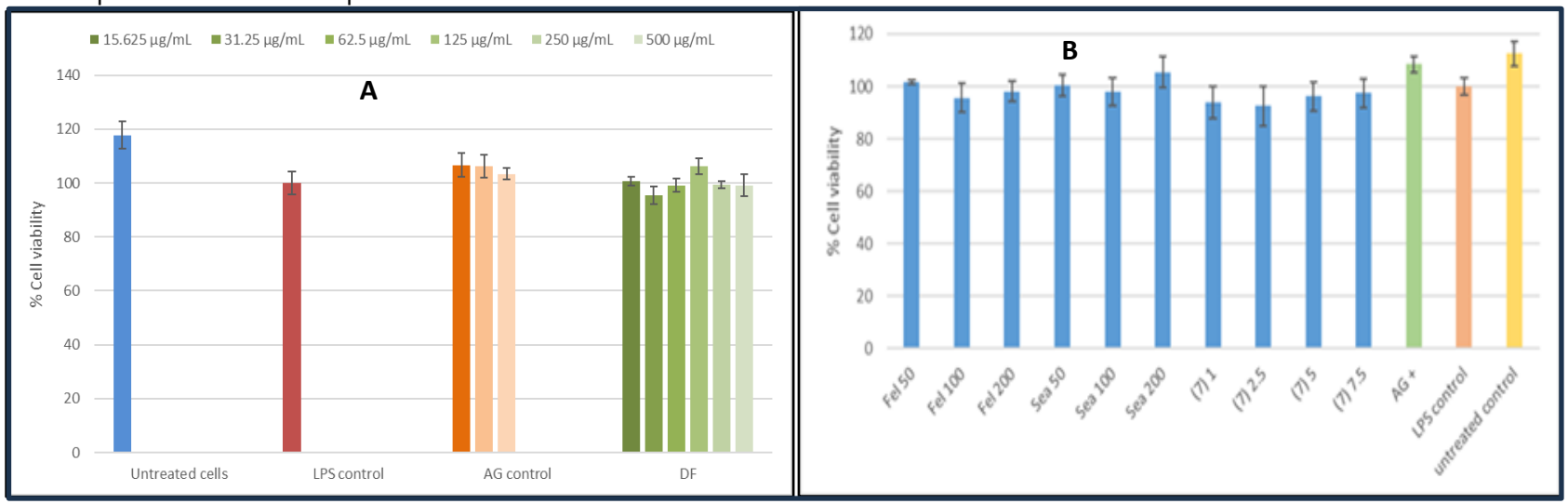

Figure 26. Cell viability (%) of LPS activated macrophages after 24 hours of exposure to treatments as indicated in the Figures. Bar graph represents quadruplicate values of one experiment. Error bars represent the standard deviation of the mean

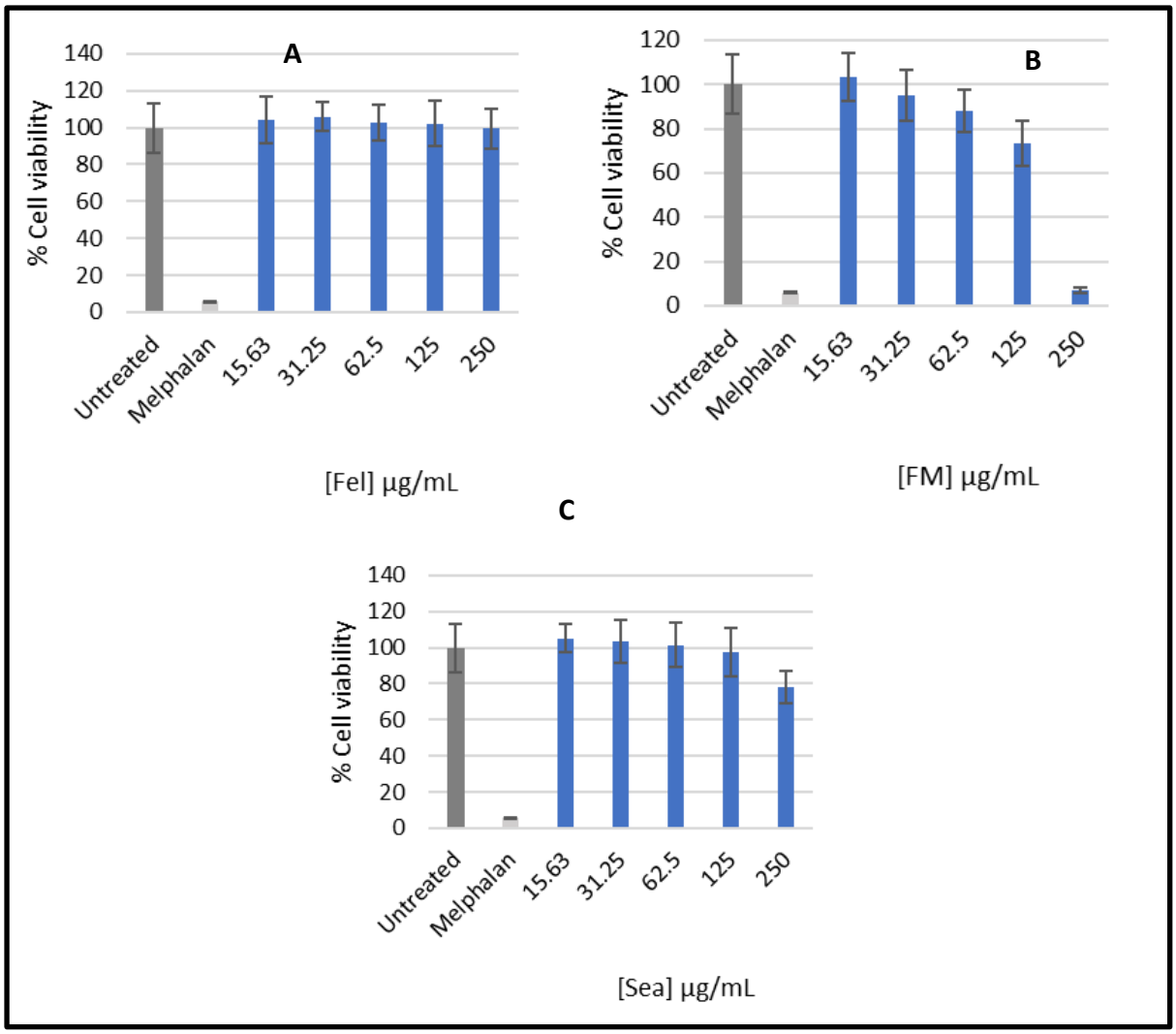

Figure 27. Cytotoxicity of *Felicia filifolia* aqueous (Fel) and methanol (FM) and *Searsia erosa* methanol (Sea) extracts in Vero cells after 48 hours of treatment. Melphalan (30 µM) was used as a positive control. Error bars indicate standard deviation of quadruplicate values obtained from a single experiment.

Felicia filifolia methanol extract (**Figure 27B)** exhibited the most significant cytotoxicity, with treatment concentrations of 62.5-250 µg/ml significantly reducing cell viability. Significant cytotoxicity was only observed at the highest treatment concentration (250 µg/mL) for sample *Searsia erosa* methanol extract (**Figure 27C**), whereas *Felicia filifolia* aqueous extract (**Figure 27A**) did not exhibit significant cytotoxicity at any of the tested concentrations.

5.4.3 A micro-dilution technique / p-iodonitrotetrazolium chloride (INT) Minimum inhibitory concentration (MIC) assay

Table 7 below shows the results of the antifungal microdilution (INT) assay, the results of positive controls, FCZ and SAC, were compared with those of *Searsia erosa* and *Felicia filifolia* extracts and Defender. *Searsia erosa* aqueous extract had a minimal inhibitory concentration (MIC) of 1 µg/mL and was effective against all the tested microorganisms, *C. albicans*, *A. fumigatus*, *C. neoformans*, and *C. gattii,* at 1-100 µg/mL. *Searsia erosa* methanol extract was effective against *C. albicans* and *A. fumigatus* at 1-100 µg/mL with the MIC of 1 µg/mL, while for *C. neoformans* and *C. gattii*, it was effective at 50-100 µg/mL with the MICs of 50 µg/mL. *Felicia filifolia* aqueous extract tested negative against all tested pathogens, and its methanol extract inhibited all tested microorganisms at 1-100 µg/mL with the MIC of 1 µg/mL. Defender was effective against *C. albicans* (MIC=50 µg/mL), *A. fumigatus* (MIC=1 µg/mL), *C. neoformans* (MIC=10 µg/mL), and *C. gattii* (MIC=1 µg/mL).

Table 7. Antifungal microdilution (INT) assay, FCZ=Fluconazole, SAC=Salicylic acid, SEH20=Searsia erosa aqueous extract, SEMeOH=Searsia erosa methanol extract, FFH20=Felicia filifolia aqueous extract, FFMeOH=Felicia filifolia methanol extract, DF=Defender, positive (+)=inhibition of microbial growth, negative (-)=microbial growth

Fungi	Controls		Concentration	SEH_20	SEMeOH	FFH_20	FFMeOH	DF
	FCZ	SAC	µg/mL					
C. albicans	+	+	1	+	+	-	+	-
			10	+	+	-	+	-
			50	+	+	-	+	+
			100	+	+	-	+	+
A. fumigatus	+	+	1	+	+	-	+	+
			10	+	+	-	+	+
			50	+	+	-	+	+
			100	+	+	-	+	+
C. Neoformans	+	+	1	+	-	-	+	-
			10	+	-	-	+	+
			50	+	+	-	+	+

			100	+	+	-	+	-
C. gattii	+	+	1	+	-	-	+	+
			10	+	-	-	+	+
			50	+	+	-	+	+
			100	+	+	-	+	-

5.5 DISCUSSION

The current study evaluated antioxidant, anti-inflammatory, cytotoxicity and antifungal activities of the *Searsia erosa* aqueous and methanol extracts, *Felicia filifolia* aqueous and methanol extracts, and Defender. The investigation of the antioxidant potential of medicine is a promising and important field of research with extensive human health implications [30]. This study demonstrated that *Searsia erosa* methanol and aqueous extracts, *Felicia filifolia* aqueous extract and Defender showed better antioxidant potential by DPPH radical scavenging assay when compared to Trolox and ascorbic acid (**Figure 24**). Phenols are the main plant compounds with antioxidant activity [31]. The results of total phenolic compound (**Chapter 4, Table 2**) agree with the antioxidant activity assay results in that *Searsia erosa* methanol extract had an antioxidant activity percentage of 74% and higher phenolic content than aqueous extract (antioxidant activity percentage of 68%). Similarly, *Felicia filifolia* aqueous extract had an antioxidant activity percentage of 68%, and the phenolic content was higher than methanol extract (68%).

Defender also had a high antioxidant activity percentage of 74%, corresponding with the high phenolic content. Furthermore, the study conducted by Yu *et al*. (2021) has reported strong relationships between total phenols and antioxidant capacities, signifying that phenolic compounds are responsible for the inhibition of oxidative processes [32]. Many investigations reveal that flavonoids and phenols play a key role in antioxidant activities of plants [17], and various flavonoids such as flavonoid-3-O-glycosides, flavonoid-7-O-glycosides, and 8-O-methylated flavonoids were observed in the LCMS results of *Searsia erosa* (**Chapter 4, Table 6**) and *Felicia filifolia* (**Chapter 4, Tables 4 and 5**) methanol extracts. *Felicia filifolia* methanol (**Chapter 4**) was also found to contain mactraxanthin, which possesses antioxidant activity. *Searsia erosa* methanol extract (**Chapter 4**) was found to contain caffeoylquinic acids, which are known to have antioxidant and anti-inflammatory activities [33].

Numerous studies have shown that phenols have excellent anti-inflammatory properties [17], and the observed potential anti-inflammatory activities (**Figure 25**) of *Searsia erosa*

aqueous and methanol extract, *Felicia filifolia* methanol extract and Defender could be attributed to the presence of phenols and terpenoids. Phenolic compounds NO scavenging activity has been reported in other studies [34]. Terpenoids, including diterpenoids, sesquiterpenoids, and terpene glycosides, were observed in LCMS analysis results of *Felicia filifolia* (**Chapter 4, Tables 4 and 5**) and *Searsia erosa* (**Chapter 4, Table 6**) methanol extracts. The results of *the Felicia filifolia* methanol extract in Chapter 5 further revealed the presence of scadoside and lamiide, which are reported to possess anti-inflammatory activities.

Results of cell viability percentages of LPS activated macrophages following 24 hours of exposure to treatments (**Figure 26**) revealed that all tested plant extracts and Defender showed no toxicity, and this suggests that these medicinal plants are safe when taken over a short period of time. Toxicity of medicinal plants in general has been reported following their long-term use [26], and it will be important to test these MPs for long-term administration at the tested concentrations. International Council for Harmonisation (ICH) guidelines recommend the importance of repeated dose toxicology studies for long-term treatments in safety pharmacological studies, animal and nonanimal models, genetic toxicity experiments, phototoxicity evaluations, immunotoxicity and immunogenicity assessments, developmental and reproductive toxicity analysis, and carcinogenicity evaluation [27].

Following 48 hours of treatment, cytotoxicity of *Searsia erosa* methanol extract (**Figure 27C)** and *Felicia filifolia* methanol extract (**Figure 27B**) was observed at the highest treatment concentration (250 µg/mL). Therefore, these medicinal plant extracts could serve as sources for potential natural anticancer agents at higher concentrations. Various studies on animal models and cell lines report on phytochemicals effective in both the cancer prevention and treatment, and these secondary metabolites could cause apoptosis, reduce cell proliferation, inhibit angiogenesis, and slow down metastasis [35]. The anticancer and anti-inflammatory activities of *Felicia filifolia* methanol extract could also be due to the 5,7,4'-trihydroxy-3,6,8,3',5'-pentamethoxyflavone as observed in **Chapter 4**.

Antifungal activity assay results revealed that *Searsia erosa* aqueous and methanol extracts, *Felicia filifolia* methanol extract and Defender inhibited the growth of *C. albicans*, *A. fumigatus*, *C. neoformans*, and *C. gattii*. According to the findings in Chapter 5, *Felicia methanol* extract contains iridoid glycosides, which are reported to have antioxidant, anti-inflammatory, anticancer and antimicrobial activities. *Felicia methanol* extract was also found to contain amphidicolin (**Chapter 4),** which is reported to have antimicrobial and anticancer activities. It was also found to have zerumbone (**Chapter 4),** which has anticancer, anti-inflammatory, and antimicrobial activities. Reports have revealed that rutin (**Chapter 4**) found in *Felicia methanol* extract is known to possess anticancer, anti-inflammatory, and antimicrobial activities. *Searsia erosa* methanol extract (**Chapter 4**) was found to have carboxylic acid and its derivatives, known to possess antifungal properties, and chlorogenic acid, which possesses properties such as antimicrobial, antioxidant, anti-inflammatory and anticancer activities.

The current study has identified that the tested medicinal plant extracts and Defender are potential antioxidant, anti-inflammatory, cytotoxic and antifungal agents. Fractionation that is bioactivity directed of these extracts is required to isolate and identify compounds which are bioactive [36]. Some compounds have been isolated from medicinal plants and are currently used as drugs for the treatment of diseases, including morphine, a cancer drug isolated from *Papaver somniferum* [36], and aspirin (acetylsalicylic acid), an anti-inflammatory, anticancer, and antipyretic drug isolated from the willow tree (species include *Salix alba* L., *Salix purpurea*, and *Salix pentandra L.)* [37]. Aspirin was also found to have antifungal activity against *C. albicans* [38], and *A. flavus* [39].

5.6 CONCLUSION

Plant extracts of *Searsia erosa* and *Felicia filifolia* methanol extract are potential antioxidants, anti-inflammatory, and cytotoxic (anticancer) agents and antifungal treatments against *C. albicans*, *A. fumigatus, C. neoformans,* and *C. gattii*, and Defender is a potential antioxidant and anti-inflammatory agent and antifungal treatment against *C. neoformans*, *C. gattii, C. albicans*, and *A. fumigatus.* The pharmacological activities of these medicinal plant extracts and Defender are attributed to phytochemical constituents,

including phenolics, flavonoids and terpene compounds. There is a need to further isolate, identify and determine the activities of individual compounds and conduct *in vivo* studies to better understand their mechanism of action as antioxidant, anti-inflammatory, cytotoxic and antifungal agents. Furthermore, the *in vivo* pharmacokinetic and clinical studies are warranted to determine the safety, efficacy, bioavailability, and drug interactions of these plant extracts and Defender.

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CHAPTER 6

DETERMINATION OF THE EFFECT OF *SEARSIA EROSA*, *FELICIA FILIFOLIA* AND DEFENDER ON ERGOSTEROL AND CYP3A4 ACTIVITY

ABSTRACT

Medicinal plants (MPs) are extensively used because they are easily accessible, cheap, and safe. However, cases of adverse effects, including herb-drug interactions and toxic and allergic effects, have been reported. Cytochrome P450 3A (CYP3A) is a crucial member of the CYP450 superfamily in metabolising drugs. It is, therefore, important to conduct research on drug interactions involving metabolic substrate drugs of CYP3A4 for precision and personalized medicine. Moreover, the most important antifungal target for fungal survival is the ergosterol pathway. Therefore, the ergosterol biosynthetic pathway is essential for drug targeting and survival of fungus. This study aimed at determining the effect of methanol plant extract of *Searsia erosa* and *Felicia filifolia* and Defender on ergosterol and CYP3A4 activity. Plant extracts and Defender were tested against *C. neoformans* and *C. gattii* in the presence (100-800 µg/ml) and absence of exogenous ergosterol, and their Minimum Inhibitory Concentrations (MICs) were determined after 24h of incubation at 35°C. The potential inhibition of human P450 3A4 was determined using a Vivid® CYP450 Screening Kit, and the experiment was carried out according to the manufacturer's instructions. MICs of the tested extracts and Defender increased in the presence of ergosterol, and the increase was concentration dependent. A decrease in substrate production was observed for samples tested with extracts, and it was concentration dependent. The results suggest that these tested compounds act by inhibiting the ergosterol biosynthesis and that both *Searsia erosa* and *Felicia filifolia* extracts have CYP3A4 inhibitory activity.

6.1 INTRODUCTION

MPs are widely used for treatment in several parts of the world because they are safe [1]. However, there is little experimental and clinical data available for most herbal medicines, and numerous adverse cases have been reported, mostly on long-term treatments, which include allergic reactions, toxic effects and herbal-drug interactions [1]. Herb-Cytochrome P450 interactions have crucial toxicological and clinical consequences [2]. Cytochrome P450 3A4 (CYP3A4) plays a vital role in drug metabolism, accounting for 30-50% of known drugs [3]. Ergosterol biosynthetic pathway is a primary target for most antifungal drugs used for the treatment of severe fungal infections in humans [4].

Cytochrome P450 (CYP) enzymes are mainly found in the liver and can also be found in other tissues such as the brain, lungs, intestines, and kidneys [5]. They are the most crucial Phase I drug-metabolising enzymes that metabolise numerous xenobiotics, including essential steroids and therapeutic drugs [2]. CYPs can be induced and inhibited by exposure to numerous natural compounds and using *in vitro* and *in vivo* experiments, many natural compounds have been isolated from herbs and identified as inhibitors and inducers of numerous CYP enzymes [3]. All CYPs can be induced or inhibited by numerous herbal medicines and drugs [6]. Induction or inhibition of CYP enzymes by herbal medicines can lead to the altered drug metabolism, and one example is CYP3A4 induction by St John's wort in humans [6].

MPs can interact with western drugs including anticoagulants, sedatives and antidepressants, anti-HIV agents, cardiovascular drugs, immunosuppressants and anticancer drugs [6]. They can interfere with the pharmacokinetics or pharmacodynamics of such drugs and cause life-threatening adverse reactions, and due to the clinical importance of such drug interactions with herbal medicines, it is crucial to identify compounds and drugs under development that might interact with medicinal plants [6]. *Searsia erosa* and *Felicia filifolia* are found to be potential treatments for various conditions in Chapter 6, and it is important to determine their effect on CYP450 enzymes, and particularly CYP3A4.

Discovering the mechanism of action of natural products is essential and will maximise their effect, either by optimising their formulation or by active ingredient concentration [7]. The mechanism of action of most currently used antifungal drugs targets the ergosterol biosynthesis pathway or fungal plasma membrane [4,8]. Ergosterol is vital for protecting the fluidity and integrity of the fungal cell membrane [9]. Azoles act mainly on ergosterol biosynthesis by inhibiting 14α-lanosterol demethylase responsible for the conversion of lanosterol to ergosterol and thereby compromising the integrity of the membrane [8,10]. Polyenes act by binding directly to ergosterol on the fungal cell membranes and create pores in the membrane, resulting in cell death [8,10]. Allylamines, thiocarbamates, fenpropimorph and amorolfine target genes of ergosterol biosynthesis [8]. The current study determined whether the mechanism of action of *Searsia erosa*, *Felicia filifolia* and Defender involves targeting the ergosterol biosynthesis pathway.

6.2 METHODS

6.2.1 Chemicals and reagents

Ergosterol was purchased from Sigma-Aldrich Inc. (St. Louis, MO. U.S.A). The Vivid® CYP3A4 Red Kit was obtained from Thermo Fisher Scientific (Carlsbad, CA, USA).

6.2.2 Sample preparation

Searsia erosa and *Felicia filifolia* methanol extracts and Defender were reconstituted in dimethyl sulfoxide (DMSO) to a final concentration of 100 mg/mL. Samples were sonicated if insoluble and stored at 4°C until required.

6.2.3 Determination of the effect of *Searsia erosa*, *Felicia filifolia* and Defender on ergosterol using Ergosterol Effect Assay

The MICs of *Searsia erosa and Felicia filifolia* methanol extracts were determined against *C. neoformans* and *C. gattii* in the presence (100, 200, 300, 600, and 800 µg/mL) and absence of exogenous ergosterol. Salicylic acid and fluconazole were used as controls. The MICs were determined after 24h of incubation at 35°C. The results were determined by the increase in the MIC of the tested substances in the presence of ergosterol. When combined with ergosterol, a substance that has affinity to it quickly forms complexes, thereby inhibiting interactions with ergosterol in the fungal membrane [11].

6.2.4 Determination of the effect of *Searsia erosa*, and *Felicia filifolia* on CYP3A4 activity

The Vivid® CYP3A4 Red Kit reagents and Vivid® BOMR Red substrate were prepared and used as per the manufacturer's instructions. Test samples (Plant extracts and Defender; 40 µl, diluted in reaction buffer) and Master Pre-Mix (CYP450 BACULOSOMES® Plus Reagent and Vivid® Regeneration System, 50 µL) were added to a black 96 well plate in quadruplicate. Thereafter, samples were incubated at room temperature for 10 minutes. Reaction was then started by adding 10 µL substrate (Vivid® BOMR/NADP+). Following that, samples were incubated at room temperature for 60 minutes (Endpoint Assay Mode), after which 50 µL of stop reagent (0.5 M Tris base) was added. Fluorescence intensity was measured at 550/590 (Ex/Em) nm using a BioTek® PowerWave XS spectrophotometer (Winooski, VT, USA). Results were determined using a Vivid® Fluorescent Standard curve and are expressed as [substrate produced] (nM).

6.3 RESULTS

6.3.1 Determination of the effect of *Searsia erosa*, *Felicia filifolia* and Defender on ergosterol using Ergosterol Effect Assay

Table 7 below shows the results of the ergosterol assay. It was used to determine whether *Searsia erosa*, *Felicia filifolia* and Defender induce changes in the fungal membrane by interacting directly with ergosterol. This assay uses exogenous ergosterol and has been used to determine substances that bind ergosterol in fungal membranes [11]. The MICs of the tested medicinal plant extracts and Defender increased in the presence of different concentrations (100 to 800 µg/mL) of exogenous ergosterol (**Table 8**). Thus, inhibition of *C. neoformans* and *C. gattii* by these medicinal plants and herbal medicines involves interaction with ergosterol.

6.3.2 Determination of the effect of *Searsia erosa*, and *Felicia filifolia* on CYP3A4 activity

Figure 29 below shows the effect of *Searsia erosa* and *Felicia filifolia* on CYP3A4 activity. A concentration-dependent decrease in substrate production was observed for *Searsia erosa* and *Felicia filifolia* methanol extracts, suggesting that both samples have CYP3A4 inhibitory activity. Fluconazole was used as a positive control.

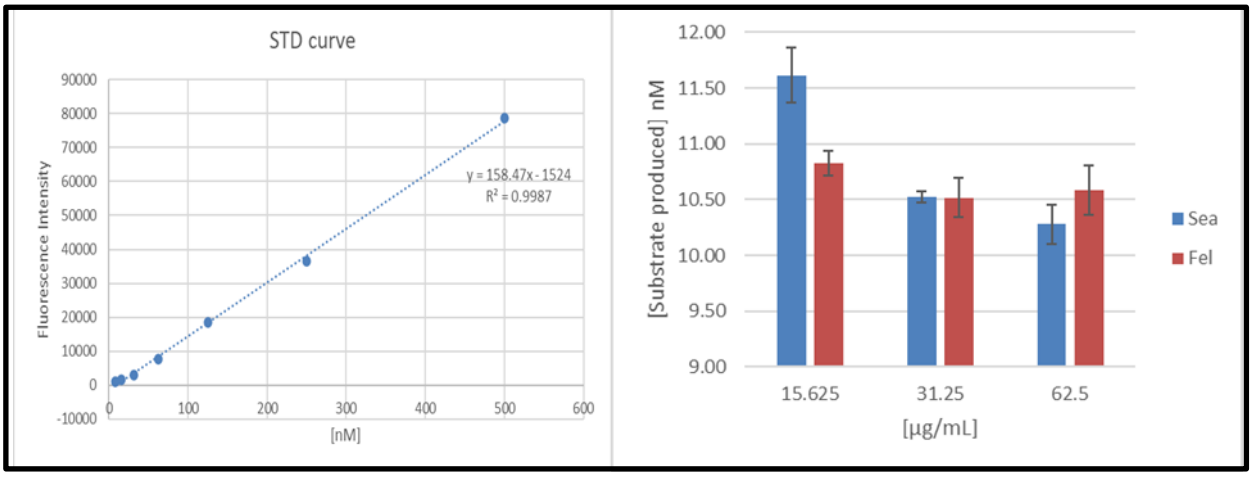

Figure 28: CYP3A4 inhibition of *Searsia erosa* and *Felicia filifolia*. Error bars indicate the standard deviation of the mean of 4 replicates from a single experiment. Sea=*Searsia erosa* methanol extract, Fel=*Felicia filifolia* methanol extract.

Table 8. Effect of ergosterol (100-800 µg/ml) on the MICs of fluconazole, *Searsia erosa*, *Felicia filifolia*, and Defender, against *Cryptococcus neoformans* and *Cryptococcus gattii*

Test substance	**Absence of ergosterol**	**Presence of ergosterol**						
	MIC (µg/mL)	**MIC (µg/mL)**					**Mean ±SD**	**P-value**
		100	**200**	**300**	**600**	**800**		**(<0.05)**
Cryptococcus neoformans								
Fluconazole	56	119	124	124	133	133	126±6	0.02
Searsia erosa methanol extract	66	136	131	135	138	146	137±5	0.0
Felicia filifolia methanol extract	64	136	129	135	142	150	138±7	0.03
Defender	66	120	125	126	129	136	127±51	0.04
Between groups								0.01
Cryptococcus gattii								

Fluconazole	59	119	127	133	143	149	134±13	0.08
Searsia erosa	72	134	135	139	154	144	141±8	0.30
Felicia filifolia	63	127	132	139	147	162	141±14	0.99
Defender	62	128	128	129	135	144	133±7	0.12
Between groups								0.45

6.4 DISCUSSION

Medicinal plants and western medicines are being utilised widely for the treatment of various diseases and conditions, including cancer, HIV, pain, diabetes mellitus, hypertension, and asthma [12]. Co-administration of medicinal plants and western medicines is becoming more common [2], and without sufficient knowledge about the mechanisms of action of medicinal plants, safety profiles, adverse effects, and possible interactions with western medicines, healthcare providers will not be able to improve the populations' health [12]. This study determined the possible CYP3A4 interactions of *Searsia erosa* and *Felicia filifolia* (**Figure 28**) and found that these medicinal plants are possible inhibitors of CYP3A4, responsible for the metabolism of numerous drugs. Other medicinal plants known to inhibit CYP3A4 include Grapefruit, kava-kava, *Acacia catechu*, *Andrographis paniculata*, *Bupleurum marginatum*, *Arctium lappa*, *Dysosma versipellis*, *Areca catechu*, *Chrysanthemum indicum*, *Spatholobus suberectus*, *Hydrastis canadensis*, and *Camila sativa* [13,14]. Concomitant administration of these medicinal plants with other drugs that are metabolised by CYP3A4 could increase plasma concentration of these medicines and cause unforeseen adverse effects [14].

The current study also determined the possible mechanism of *Searsia erosa*, *Felicia filifolia* and Defender as the possible antifungal treatment against *C. neoformans* and *C. gattii* (**Table 8**). The increased MICs of these medicinal plants and herbal medicine demonstrated that they were able to bind directly to ergosterol and form immediately a complex, and thereby preventing the cellular membrane from interacting with ergosterol [7]. The positive Affinity Ergosterol Assay results of these MPs and Defender strongly suggest that their mechanism of action is associated with binding on ergosterol and immediately causing disruption of fungal cell membranes [7].

The comparison between groups (fluconazole, *Searsia erosa*, *Felicia filifolia* and Defender) revealed a highly statistically significant results, with P-value=0.01 for binding to ergosterol against *C. neoformans*. P-value>0.05 was observed for the comparison between groups for binding to ergosterol against *C. gattii*, indicating that the results are

not statistically significant. This could mean that the high MIC values observed might be due to other factors, and this can be investigated in future studies.

There are challenges associated with the three most used classes in clinical fungal infections: azoles, polyenes, and echinocandins, and that includes toxicity, drug-drug interactions, and the development of tolerance [15]. Azole drugs, such as fluconazole, act by inhibiting the enzyme involved in ergosterol synthesis, fungal lanosterol 14-α-demethylase (CYP51) [15]. However, the long-term use of azoles may lead to the development of drug resistance due to mutations in genes that encode CYP51, resulting in overexpression of these genes or increased efflux by membrane pumps [15]. The tested MPs and Defender provide a better alternative to azoles because they act directly on ergosterol and can overcome the challenge of the resistant CYP51 encountered by azoles.

6.5 CONCLUSION

Searsia erosa and *Felicia filifolia* are possible inhibitors of CYP3A4, and this finding has dose implications. It will therefore be essential to adjust doses of substrates of CYP3A4 in coadministration with these MPs. Tested MPs and Defender are possible antifungal treatments for infections caused by *C. neoformans and C. gattii*. These MPs bind to ergosterol, and this could result in the creation of pores on the membrane and cell death. They, therefore, provide a better alternative to azoles by overcoming the CYP51 resistance challenges.

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CHAPTER 7

CONCLUSIONS, STUDY LIMITATIONS AND FUTURE STUDIES

7.1 CONCLUSIONS

The objectives of the study were achieved as follows: (1) Qualitative and quantitative analysis of *Felicia filifolia*, and *Searsia erosa* plant extracts, and Defender were performed and showed the presence of phytochemicals, including flavonoids, glycosides, saccharolipids, terpenoids, quinic and pentacarboxylic acids and their derivatives, and xanthophylls; (2) further testing of the pharmacological activities of these MPs revealed potential antioxidant, cytotoxic, anti-inflammatory and antifungal activities of *Searsia erosa* and *Felicia filifolia* plant extracts, and Defender, (3) the study determined possible CYP3A4 drug interaction between *Searsia erosa* and *Felicia filifolia* with substrates of CYP3A4, and also determined the possible mechanism of action of antifungal activity against *C. neoformans*, and *C. gattii* to be due to binding on ergosterol.

Therefore, the current study has successfully identified potential treatments for respiratory diseases caused by fungal pathogens *C. albicans*, *C. neoformans*, *C. gattii*, and *A. fumigatus*. *Searsia erosa*, *Felicia filifolia*, and Defender can be used as potential antifungal treatments in coinfections caused by these fungal species in COVID-19 and HIV patients. The study has also identified other pharmacological benefits of these MPs, *in vitro* safety and drug interactions with CYP3A4.

7.2 STUDY LIMITATIONS

- *In vivo* testing and clinical trials were not possible because these test plant extracts, and Defender had no pre-clinical data of phytochemical components and pharmacological activities.
- Plant isolation, identification and investigation of single compounds, and testing of these plant extracts and Defender against various CYP450 enzymes was not possible at this stage due to limited financial resources and time.

7.3 FUTURE STUDIES

There is a need to further isolate, identify and investigate activities of individual compounds. Furthermore, also to perform *in vivo* pharmacokinetic and clinical studies to investigate the bioavailability, efficacy, safety, and drug interactions of these plant extracts and Defender. Moreover, further testing of CYP3A4 *in vivo* interactions of these medicinal plants and Defender is warranted.

APPENDICES

A. Published article 1

Chapter

A Review of South African Traditional Medicinal Plants Used for Treating Fungal Coinfections in COVID-19 Patients with Respiratory Diseases

Moleboheng Emily Binyane, Sitheni Samson Mashele and Polo-Ma-Abiele Hildah Mfengwana

Abstract

Fungal infections are still most prevalent in the South African population. Fungal respiratory infections and diseases are the cause of severe clinical challenges and mortality in patients with compromised immune systems. Clinical signs of coronavirus disease of 2019 (COVID-19) such as lung injury, hyperglycemia due to diabetes, host iron and zinc depletion, hypoxia, immunosuppression, steroid therapy, and long-term hospitalization predispose patients to opportunistic fungal infections. Fungal pathogens, including *Cryptococcus*, *Aspergillus*, and *Candida* species, cause coinfections in patients infected with (COVID-19), and this has a negative impact on the patients' pharmacological management goals. *Cryptococcus*, *Aspergillus*, and *Candida* species cause respiratory infections and illnesses including pneumonia, pulmonary aspergillosis, pulmonary candidiasis, and pulmonary cryptococcosis. South African traditional medicinal plants have been used in the treatment of respiratory symptoms and diseases caused by these fungal pathogens. Medicinal plants contain secondary metabolites possessing antifungal activity against *Cryptococcus*, *Aspergillus*, and *Candida* species. Moreover, medicinal plants are cheaper and easily accessible and are believed to be safe. This review documents the use of South African traditional medicinal plants including *Artemisia absinthium*, *Artemisia afra*, *Dicoma anomala*, *Felicia* species, *Mentha* species, *Ruta graveolens*, and *Seasia erosa* in the treatment of fungal infections and diseases caused by these pathogens.

Keywords: fungal coinfections, traditional medicinal plants, COVID-19, cryptococcosis, aspergillosis

1. Introduction

Coronavirus disease of 2019 (COVID-19) patients with asymptomatic, mild, moderate, severe, and critical disease states are at risk of developing coinfection with

pathogenic fungal species including *Aspergillus*, *Candida*, and *Cryptococcus* [1, 2]. Research reports suggest that COVID-19 predisposes patients to fungal, and other viral coinfections, and superinfections [3]. Concurrently occurring coinfections pose a massive challenge because it complicates diagnoses and COVID-19 management [3]. COVID-19 by severe acute respiratory coronavirus 2 (SARS-CoV-2) [1–4] causes respiratory symptoms such as shortness of breath, fever, fatigue, runny nose, headache, chest pain, congestion, anosmia, ageusia, sore throat, confusion, and vomiting [3, 5, 6], similar to those caused by *Aspergillus*, *Candida*, and *Cryptococcus* species infections [3]. An estimated 15% of COVID-19 patients admitted to the hospital's intensive care units (ICU) become coinfected by *Aspergillus* [7]. *Aspergillus* causes pulmonary aspergillosis including allergic bronchopulmonary aspergillosis (ABPA), chronic pulmonary aspergillosis (CPA), and invasive pulmonary aspergillosis (IPA) [8]. COVID-19-associated pulmonary aspergillosis (CAPA) is reported to have a 52% death rate [9]. *Aspergillus fumigatus/A. fumigatus* and *A. flavus* are the most common *Aspergillus* species causing coinfection in COVID-19 patients [4]. Conducted cohort studies on COVID-19-associated pulmonary aspergillosis have described its incidence to be between 2 and 33% [2, 10]. Aspergillosis is treated by the antifungal drug class, triazoles [1, 11], voriconazole, and isavuconazole being the first-line therapies [7, 9]. However, there are challenges associated with treatment therapy including the occurrence of azole-resistant *A. fumigatus* [11] and drug-drug interactions associated with the use of voriconazole, which lead to increased cardiotoxic effects of anti-SARS-CoV-2 agents [1]. The study conducted on COVID-19 patients who were severely and critically ill has revealed that dexamethasone is associated with increased pulmonary aspergillosis risk and death [12]. COVID-19-associated candidiasis (CAC) has occurred in various hospitals across countries [3]. CAC is an opportunistic infection caused by fungal species of *Candida* genus [3, 13]. Studies conducted in various countries, including the UK, Italy, Egypt, China, Iran, India, Gharbia, and Cairo, have revealed that *Candida* species including *C. albicans*, *C. tropicalis*, *C. glabrata*, *C. auris*, and *C. parapsilosis* are implicated in CAC [4, 13, 14]. Treatment of *Candida* infections includes azoles, echinocandin, Amphotericin B, and its liposomes [15]. However, there is an emergence of multidrug-resistant *Candida* species, including *C. glabrata*, *C. auris*, inherently resistant *C. krusei*, *C auris*-resistant fluconazole, and Amphotericin B, and fluconazole-resistant *C. parapsilosis* and *C. tropicalis* [4, 15]. Moreover, COVID-19 patients receiving treatment therapy, including tocilizumab, interferon type 1β, and lopinavir-ritonavir, are at an elevated risk of developing coinfections with *Candida* spp. [16]. Chloroquine, hydroxychloroquine, azithromycin, and protease inhibitors can cause direct myocardial toxicity, arrhythmias, and death [1]. COVID-19 patients coinfected with human immunodeficiency virus (HIV) or those with compromised immune systems are at risk of developing cryptococcosis [15]. The literature reveals a growing number of cryptococcosis cases in COVID-19 patients who were receiving corticosteroids and immunomodulators [17–19]. Pulmonary cryptococcosis is caused by two cryptococcal pathogenic species, namely *C. neoformans* and *C. gattii* [20, 21]. The recommended treatment therapy for cryptococcosis includes initial treatment with Amphotericin B in combination with flucytosine, followed by maintenance therapy with fluconazole [15, 22]. However, fluconazole-resistant *Cryptococcus* has been reported, and there is also an increased risk of antifungal toxicity [19]. Phytotherapy is an important solution for treating respiratory infections and diseases in adults and children [23]. Research reports that medicinal plants contain a variety of active secondary metabolites including alkaloids, saponins, and terpenoids with antifungal activity [24]. In South Africa

(SA), the majority of people utilize traditional medicinal plants (TMPs) more than Western medicines because TMPs are cheaper, widely available, and considered to be more effective [25]. South African TMPs such as *Artemisia absinthium*, *Artemisia afra*, *Dicoma anomala, Felicia* species, *Mentha* species, *Ruta graveolens*, and *Searsia erosa* have been shown to possess antifungal activity against fungal pathogens, including *Cryptococcus*, *Aspergillus*, and *Candida* species [19, 26–29].

2. South African traditional medicinal plants used in the treatment of respiratory diseases caused by fungal pathogens

2.1 *Artemisia* species

Artemisia is the most widely distributed genus belonging to the Asteraceae family [26, 27]. It consists of over 500 plant species of small herbs and shrubs, which are classified as annual, biennial, and perennial natural plants [27, 30]. These plants are used as traditional medicines [26]. Among all 500 *Artemisia* species, two species, *Artemisia afra* and *Artemisia absinthium* are the most used in SA [30]. *Artemisia afra* Jacq. ex Willd (**Figure 1**), also known as Wilde als in Afrikaans, African wormwood in English, Lengana in Sesotho, Umhlonyane in isiXhosa, and Mhlonyane in isiZulu, is a South African medicinal plant commonly used to treat respiratory symptoms and conditions such as bronchitis, asthma, colds, coughs, fever, pneumonia, sore throat, chills, whooping cough and headache [6, 19, 28, 30, 31]. *A. afra* is also used in combination with other TMPs such as *E. globulus* and *Lippia asperifolia* as prophylaxis for lung inflammation and to treat influenza [28]. The crude extract of *A. afra* has shown antifungal activity against *Candida albicans*, *Cryptococcus neoformans*, and *Aspergillus* species including *Aspergillus ochraceus*, *Aspergillus niger*, and *Aspergillus parasiticus* (**Table 1**) [19, 28, 32]. The leaves of *A. afra* contain numerous phenolic compounds with antimicrobial activity [33]. *A. afra* methanolic crude extract contains scopoletin, betulinic acid, and acacetin with good antimicrobial activity [34]. Other secondary metabolites including alkaloids, tannins, saponins, steroids, cardiac glycosides, and anthraquinones, are found in the crude extract and essential oil of *A. afra* [35]. Toxicity testing results of *A. afra* extract on McCoy fibroblast cell lines indicated moderate toxicity [19].

Figure 1.
Artemisia afra.

South African TMPs	Venicular names	Traditional uses in respiratory conditions	Inhibited fungal pathogens implicated in coinfections in COVID-19 patients	Secondary metabolites responsible for the antifungal activity
Artemisia afra	Wild als, African wormwood, Lengana, Umhlonyane, Mhlonyane [6, 19, 28, 30, 31]	Asthma, bronchitis, colds, coughs, sore throat, chills, fever headaches, lung, inflammation, influenza, whooping cough, pneumonia [6, 19, 28, 30, 31]	*C. albicans*, *C. neoformans* [19, 28, 32]	Phenolic compounds, scopoletin, betulinic acid, acacetin, alkaloids, tannins, saponins, steroids, cardiac glycosides, anthraquinones [33–35]
Artemisia absinthium	Wormwood, Green ginger, Absinthium, Absinthe [27, 29]	Fever [29]	*C. albicans*, *A. niger*, *A. flavus* [27, 29]	Lactones, terpenoids, flavonoids, flavonoid glycosides, organic acids, tannins, phenols [27]
Dicoma anomala	Fever bush, Hloenya, Maagbitterwortel, Inyongana, Isihlabamakho-ndlwane [36, 37]	Cold, cough, fever, sore throat [36–38]	*C. albicans*, *A. niger* [36, 39]	Phenolic acids, flavonoids, tannins, saponins, triterpene, phytosterols, acetylenic compounds, sesquiterpene, lactones, diterpene [40]
Felicia muricata	White Felicia, Ihbosisi [41–43]	Headaches, fever [41, 43–45]	*A. niger*, *A. flavus* [41]	Phenols, proanthocyanidins, flavonols, sesquiterpene, lactones, triterpenoids flavonoids [41, 46]
Mentha spicata	Spearmint, brown mint, Garden mint, Lady's mint, Imboza [47, 48]	Asthma, cold, fever, flu [48–50]	*A. niger*, *C. neoformans*, *C. albicans* [48, 51–53]	Biopeptides, flavonoids, tannins, sterols, polyphenols, sterols, triterpenes, glycosides [53, 54]
Mentha longifolia	Wild mint, Horsemint, Silver mint, Koena, Inxina, Inzinziniba [49, 55–59]	Common cold, cough, sore throat, fever, headache, flu [60, 61]	*C. albicans* *C. glabrata*, *A. flavus*, *A. fumigatus*, *A. niger* [55, 57, 62]	Flavonoids, ceramides, cinnamates, ester, ketones, monoterpenes, phenols, polyene, sesquiterpenes [60]
Ruta graveolens	Ruta, rue, Garden rue, Herb of grace, Wynruit [63–66]	Fever, headache, colds, influenza [64, 66]	*C. albicans*, *C. tropicalis* *C. parapsilopsis*, *C. glabrata* *A. flavus*, *A. fumigatus*, *A. niger* *C. neoformans* [67–70]	Coumarins, coumarin dimers, dihydrofuranocoumarins, quinolone, furoquinoline, dihydrofuroquinoline, phenolic acids, alkaloids, flavonoids [71]

South African TMPs	Venicular names	Traditional uses in respiratory conditions	Inhibited fungal pathogens implicated in coinfections in COVID-19 patients	Secondary metabolites responsible for the antifungal activity
Searsia erosa	Broom karee, Besembos, Tšilabele [68, 72, 73]	Colds [72, 73]	*C. neoformans* [74]	Alkaloids, flavonoids, terpenoids, saponins, tannins [74]
Searsia lancea	African sumuc, Willow rhus, [68]	Colds, influenza [68]	*A. flavus* [75]	Flavonoids, tannins, phenols [76]
Searcia natalensis	Natal rhus [76]	Influenza [76]	*C. albicans*, *A. Niger* [75]	epicatechin, 3β-sitosterol, 3β-sitosterol glucoside stigmasterol, lupeol [75]

Table 1.
Traditional medicinal plants used in respiratory diseases caused by fungal pathogens causing coinfections in COVID-19 patients.

Figure 2.
Artemisia absinthium.

Figure 3.
Dicoma anomala.

A. absinthium (**Figure 2**), also known as Wormwood, Green ginger, Absinthium, or Absinthe in English, is used traditionally to treat fever [27, 29]. However, when used for a long period, *A. absinthium* is reported to be responsible for the central nervous system associated-adverse effects in patients such as convulsions, hallucination, and insomnia [27]. It contains secondary metabolites including lactones, terpenoids, flavonoid

glycosides, organic acids, tannins, and phenols [27]. Moreover, *A. absinthium* has antifungal activity against *C. albicans*, *A. niger*, and *A. flavus* (**Table 1**) [29]. *A. absinthium* is reported to be nontoxic when tested on Wistar Hannover rats for 13 weeks [77].

2.2 *Dicoma anomala*

Dicoma anomala (**Figure 3**) is a herbaceous plant belonging to the Asteraceae family of plants [36, 37]. It is known as Maagbitterwortel in Afrikaans, Fever bush in English, Hloenya in Sesotho, Inyongana in isiXhosa, and Isihlabamakhondlwane in isiZulu [36, 37]. In SA, *Dicoma anomala* is distributed in various provinces including the Free State, Limpopo, Gauteng, Northwest, Northern Cape, and Kwazulu natal [36, 38, 78]. Two subspecies, *Dicoma anomala* and *Dicoma gerrardi* are found in SA [37]. *Dicoma anomala* is used traditionally to treat respiratory symptoms and diseases including coughs, colds, and fever [36–38]. It has antifungal activity against *C. albicans*, and *A. niger* (**Table 1**) [36, 39]. *Dicoma anomala* produces bioactive compounds including phenolic acids, flavonoids, tannins, saponins, triterpenes, phytosterols, acetylenic compounds, sesquiterpene, lactones, and diterpene [40]. Results of acute and subchronic oral toxicity assessment of aqueous root extract of *Dicoma anomala* in rats for 14-day acute and 90-day subchronic toxicity testing have revealed that *Dicoma anomala* is not toxic at 0.5 to 2000 mg/kg [39]. *Dicoma anomala* dichloromethane: Methanol extract was found to be nontoxic at concentrations below 200 μg/ml when tested on Chang liver cells [79].

2.3 *Felicia muricata*

The genus *Felicia* consists of small shrubs of 85 known species of annual and perennial herbaceous plants [80]. *Felicia muricata* (**Figure 4**) is an aromatic herb belonging to the Asteraceae family [41, 42]. It is known as white *Felicia* in English and Ihbosisi or Ubosisi in isiXhosa [41–43]. *Felicia muricata* is widely distributed in SA, and in the Eastern Cape province, it is used traditionally to treat respiratory symptoms including headaches and fever [41, 43–45]. It has antifungal activity against *Aspergillus* species including *A. niger* and *A. flavus* (**Table 1**) [41]. *Felicia muricata* contains secondary metabolites including phenols, proanthocyanidins, flavonols, sesquiterpene lactones, triterpenoids, and flavonoids [41, 46]. The study conducted in Wistar rats using *Felicia muricata* aqueous leaf extract at 50, 100, and 200 mg/kg body weight for 14 days revealed that the plant is mildly toxic and safe for oral use, and requires further investigation [81].

Figure 4.
Felicia muricata.

glycosides, organic acids, tannins, and phenols [27]. Moreover, *A. absinthium* has antifungal activity against *C. albicans*, *A. niger*, and *A. flavus* (**Table 1**) [29]. *A. absinthium* is reported to be nontoxic when tested on Wistar Hannover rats for 13 weeks [77].

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Figure 4.
Felicia muricata.

2.4 *Mentha* species

Genus *Mentha* is a perennial and annual plant belonging to the Lamiaceae family [82, 83]. *Mentha spicata* (**Figure 5**) is also known as Spearmint, Brown mint, Garden mint, Lady's mint in English, and Imboza in isiXhosa [47]. It is a creeping rhizomatous and perennial herb cultivated in various tropical to temperate regions including SA [47]. *Mentha spicata* is used traditionally to treat respiratory symptoms and conditions such as asthma, flu, cold, and fever [48–50]. It has antifungal activity against *A. niger*, *Cryptococcus neoformans*, and *Candida albicans* (**Table 1**) [48, 51–53]. *Mentha* extracts and oils contain biopeptides responsible for their antifungal activity [54, 84]. *Mentha spicata* contains secondary metabolites including flavonoids, tannins, sterols, polyphenols, sterols, triterpenes, and glycosides [53]. Toxicity investigational study of *Mentha spicata* methanolic extract in mice using 500, 1000, 2000, and 5000 mg/kg for 24 hours to 14 days revealed that the plant is safe for oral administration [53]. *Mentha longifolia* (**Figure 6**), also known as Wild mint, Silver mint, and Horsemint in English, Koena in Sesotho, Inxina, and Inzinziniba in isiXhosa, is naturally present

Figure 5.
Mentha spicata.

Figure 6.
Mentha longifolia.

in SA [49, 55–59]. It is traditionally used to treat respiratory conditions including the common cold, cough, sore throat, headache, flu, and fever [60, 61]. *Mentha longifolia* has antifungal activity against *Candida albicans*, *Candida glabrata*, *A. flavus*, *A. fumigatus*, and *A. niger* (**Table 1**) [55, 57, 62]. The essential oil of *Mentha longifolia* contains a terpenoid and methanol, that has fungistatic and fungicidal activities [85]. *Mentha longifolia* possesses other secondary metabolites such as flavonoids, ceramides, cinnamates, ester, ketones, monoterpenes, phenols, polyene, and sesquiterpenes [60]. A toxicity testing study of *Mentha longifolia* methanolic extract in rats revealed that the acute oral dose was nontoxic [86].

2.5 *Ruta graveolens*

Ruta graveolens (**Figure 7**) belongs to the *Rutaceae* family [63]. It is commonly known as Ruta, Rue, Garden rue, and Herb of grace in English, and Wynruit in Afrikaans [63–66]. *Ruta graveolens* is distributed worldwide including in SA [64, 66]. It is used traditionally to treat respiratory symptoms and diseases including fever, headache, colds, and influenza [64, 66]. *Ruta graveolens* has antifungal activity against *Candida albicans*, *Candida tropicalis*, *Candida parapsilosis*, *Candida glabrata*, *Aspergillus flavus*, *Aspergillus fumigatus*, and *Cryptococcus neoformans* (**Table 1**) [67–70]. The essential oil of *Ruta graveolens* contains ketones responsible for antimicrobial activity [67]. *Ruta graveolens* is rich in bioactive compounds including coumarins, coumarin dimers, dihydrofuranocoumarins, quinolone, furoquinoline, dihydrofuroquinoline, phenolic acids, alkaloids, and flavonoids [71]. Toxicity investigation of *Ruta graveolens* in Wistar rats has shown that the plant's seeds extract at 50 mg/kg/day was not toxic after oral administration for 4 weeks [87].

2.6 *Seasia* species

The genus *Searsia* (previously known as *Rhus*) belongs to the family *Anacardiaceae*. It is widely distributed in tropics and subtropics areas globally mostly in the African continent, especially southern Africa [88, 89]. Most *Searsia* species such as *Searsia*

Figure 7.
Ruta graveolens.

erosa, *Searsia divaricate*, *Searsia lancea*, *Searcia natalensis*, and *Searsia undulata* are traditionally used to treat respiratory illnesses including colds, influenza, and microbial infections [68, 90]. *Searsia* species have pharmacological activities including anti-inflammatory, anticancer, antiviral, antimalarial, antidiarrheal, and antioxidant activities [91]. *Searsia erosa* (**Figure 8**), also known as Broom karee, Besembos in English, and Tśilabele in Sesotho [68, 72, 73], is used traditionally to treat respiratory diseases including colds [72, 73]. It has antifungal activity against *Cryptococcus*

Figure 8.
Searsia erosa.

Figure 9.
Searsia lancea.

Figure 10.
Searsia natalensis.

neoformans (**Table 1**) [74]. Aqueous extracts of *Searsia erosa* were found to be non-toxic when tested using the brine shrimp lethality assay [74]. *Searsia lancea* (**Figure 9**) also known as African sumuc, and Willow rhus in English is used to treat colds and influenza [68]. It contains bioactive compounds including flavonoids, tannins, and phenols [75]. *Searsia lancea* has antifungal activity against *A. flavus* (**Table 1**) [76]. *Searsia natalensis* (**Figure 10**), also known as Natal rhus in English is used to treat influenza [76], possesses secondary metabolites including epicatechin, 3β-sitosterol, 3β-sitosterol, glucoside stigmasterol, lupeol [75]. *Searsia natalensis* has antifungal activity against *C. albicans* and *A. Niger* [75]. There are no studies documenting the toxicity analysis of reported *Searsia* species, and further studies are warranted to determine the safety of these medicinal plants.

Table 1 shows the traditional use of South African TMPs in respiratory conditions including, asthma, bronchitis, colds, coughs, sore throat, headaches, lung inflammation, influenza, chills, whooping cough, pneumonia, and fever [6, 19, 28–31, 63, 70, 85]. These TMPs are also reported to possess antifungal activity against *Aspergillus*, *Candida*, and *Cryptococcus* species, which are implicated in coinfections with COVID-19.

3. Conclusions

This review has summarized TMPs commonly used in the treatment of respiratory diseases caused by fungal pathogens such as *Aspergillus*, *Candida,* and *Cryptococcus* species implicated in coinfection in COVID-19 patients. *Artemisia absinthium*, *Artemisia afra*, *Dicoma anomala*, *Felicia* species, *Mentha* species, *Ruta graveolens*, and *Searsia erosa* have been used in SA for the treatment of respiratory symptoms and diseases including asthma, bronchitis, colds, coughs, sore throat, headaches, lung inflammation, influenza, chills, whooping cough, pneumonia, fever, and flu. These TMPs contain secondary metabolites responsible for their antifungal activities. *In vitro* and *in vivo* toxicity studies have confirmed that these TMPs are nontoxic for oral administration. However, further testing using animal models and clinical studies are required to profile the pharmacokinetics and pharmacodynamics of these TMPs before recommendations to use in coinfections in COVID-19 patients.

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Conflict of interest

The authors declare no conflict of interest.

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B. Published article 2

Chapter

A Review on the Prevalence, Risk Factors, and Management of COVID-19 Disease in South African Children in Comparison to the World

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Abstract

The first case of coronavirus disease of 2019 (COVID-19) in South Africa (SA) was first reported at the beginning of March 2022, and then further spread from Gauteng, Western Cape, and KwaZulu Natal to the rest of the provinces. It is caused by severe acute respiratory syndrome coronavirus 2. In SA, COVID-19 is less prevalent in children less than 18 years. Only a few studies describe the epidemiology, risk factors, and clinical manifestation of COVID-19 among children in SA in comparison to other countries including China, North America, and Europe. South African children are affected by conditions including poverty, tuberculosis, and human immunodeficiency virus which predispose them to COVID-19. Overcrowding and limited healthcare facilities and resources also complicated the diagnosis and clinical and pharmacological management of COVID-19 in SA. The current review discusses the prevalence, risk factors, and management of COVID-19 in South African children in comparison to other continents in the world.

Keywords: COVID-19, epidemiology, children, management, South Africa

1. Introduction

The World Health Organization (WHO) reports that virus-causing diseases are still a serious public health issue [1]. Apart from the global pandemic caused by the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), the twenty-first century has experienced a flood of viral infectious diseases [2]. The severe acute respiratory syndrome coronavirus outbreak occurred in 2003, followed by the 2009 swine flu pandemic, the Middle East respiratory syndrome coronavirus 2012 outbreak, the West Africa Ebola Virus disease epidemic between 2013 and 2016, and the 2015 Zika virus disease epidemic in various countries [2]. WHO declared the Coronavirus disease 2019 (COVID-19) a

global emergency on January 30, 2020 [3]. The highly communicable respiratory disease COVID-19 is caused by SARS-CoV-2 belonging to the family Coronaviridae capable of causing infections in humans and vertebrate animals [4–6]. A bat is reported to be the natural host for SARS-CoV-2 but the pandemic-causing infections that occurred in humans were because of the intermediate host, the pangolin [7, 8]. The COVID-19 pandemic began in Wuhan, the capital of Hubei province, China on December 31, 2019 [8], and within five months had progressed worldwide and reached South Africa (SA) in March 2020 [8, 9]. Currently, there are a limited number of studies reporting on the epidemiology and clinical manifestations of COVID-19 in South African children [10]. However, most studies focus on children residing in China, Europe, Australia, North America, South America, Iran, and the Democratic Republic of Congo. Therefore, their findings might not be generalizable to SA due to the differences in risk factors predisposing children to COVID-19. Furthermore, identified risk factors in South African children include, human immunodeficiency virus (HIV), tuberculosis (TB), malnutrition, childhood obesity, overcrowding, and limited access to quality healthcare facilities for effective prevention and management of COVID-19 [10]. In SA, changes in the epidemiology of SARS-CoV-2 among children and adolescents less than 18 years in comparison to adults were reported [11]. During the first wave, the rates of infection were higher in infants, and the rates increased in all age groups during the second and third waves. However, significant changes were observed during the omicron BA.1/BA.2 wave where the number of infections dropped in individuals in less than one year and increased in those over one year [10]. These changes could be attributed to the variation in the population's immunity from natural infection, vaccination, and the characteristics and potential effects of the emerging variants on SARS-CoV-2-related illness in children [11]. SARS-CoV-2 infection in children is in most cases asymptomatic or causes milder symptoms than in adults, and as a result, children are less likely to be tested or receive clinical management [10, 12]. Other studies have reported that SARS-CoV-2-infected children can also be seriously ill and manifest the signs of the multisystem inflammatory syndrome in children such as persistent fever, severe gastrointestinal (GIT) symptoms, systemic excessive inflammation, multiple organ involvement, and symptoms like toxic shock syndrome (TSS) [13]. Treatment of COVID-19 in children includes supportive care and pharmacological management with antiviral drugs, vitamins, corticosteroids, anticoagulants, and antibiotics based on the condition of the child [13]. Recently, the US Food and Drug Administration (FDA) has approved the use of the Pfizer-BioNTech COVID-19 vaccine in children aged 12 years, and older, and children between 5 and 11 years are also covered for the prevention of COVID-19 [14].

2. Comparison between the prevalence of COVID-19 disease in South African children and children from other countries

COVID-19 is reported to have been transmitted by the intermediate host pangolin to an adult human being in China and the rest of the world and South Africa [7–9, 15]. COVID-19 in children is mainly transmitted through contact with those infected with SARS-CoV-2 (**Figure 1**) and the period of virus incubation is from 24 hours to 14 days [13, 16]. The virus spreads through contact with respiratory droplets, and COVID-19 in most children can also be excreted through urine, feces, aerosols, and body fluids that also contaminate the environment and continue the circle of infection (**Figure 1**) [13]. Children with COVID-19 have milder symptoms affecting the respiratory, gastrointestinal tract, and neurological systems [13, 16]. COVID-19 symptoms and signs in children

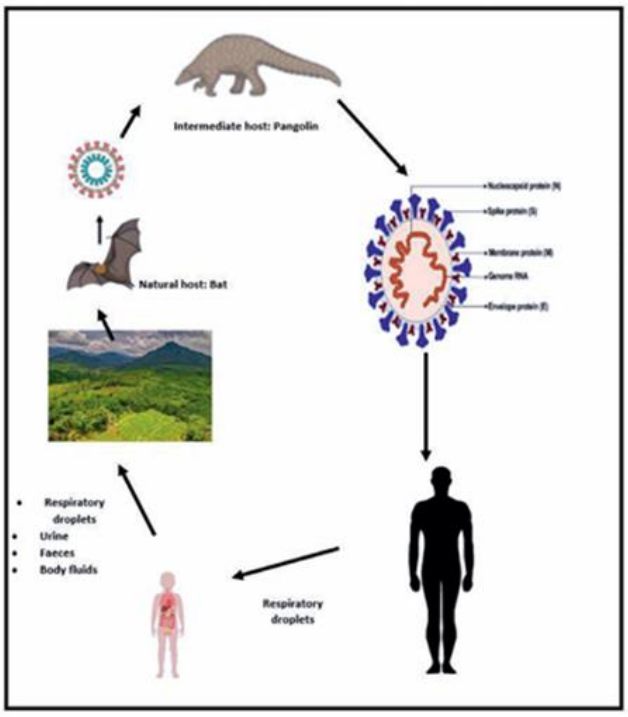

Figure 1.
SARS-CoV-2 transmission from the environment, animals, humans, and children.

include fever, cough, nasal congestion, headache, dyspnea, sore throat, ageusia, anosmia, abdominal pain, diarrhea, nausea, vomiting, lack of appetite, malaise, and myalgia [17]. Other children can become critically ill, and require hospitalization, intensive care, or mechanical ventilation or die, or in rare cases develop multisystem inflammatory syndrome (MIS) [13, 15, 16]. MIS is characterized by hypotension, pulmonary edema, and edema of other organs, necessitating intensive care to support the heart and lungs [16]. The first case of MIS-C in SA was reported in August 2020 in Cape Town [17]. COVID-19 is more prevalent in adults than in children less than 18 years [11, 16, 18].

2.1 Worldwide distribution and SA

There is still the continuation of the COVID-19 pandemic in Africa and globally [9]. WHO global COVID-19 reports on January 06, 2023 indicated that there are 657,977,736 cases of morbidity and 6,681,433 cases of mortality, and Europe has the highest number of confirmed cases of COVID-19, whereas Africa has the lowest cases [18]. The first positive case of COVID-19 in the African continent was confirmed on February 14, 2020 in Egypt, followed by Nigeria on February 28, 2020, and in SA on March 5, 2020 [5, 9]. There are currently 9,453,366 confirmed cases of COVID-19, 4,049,319 cases of morbidity, and 102,568 cases of mortality in SA as on January 06, 2023 [19, 20].

2.2 COVID-19 distribution in SA children and children from other countries 2020-2023

The prevalence of COVID-19 in children is lower than in adults worldwide [13, 21–24]. However, severe cases of morbidity and mortality have occurred in children [23]. In comparison to adults, there are few studies on COVID-19 in children [25]. There are gaps in the knowledge of the epidemiology of COVID-19 among children and adolescents worldwide [26]. In SA, on September 2020, the total laboratory-confirmed COVID-19 cases in South African children were 228 per 100,000 and were lower than the 829 per 100,000 children in the United States. COVID-19 cases in children in SA were higher compared to rates of less than 100 per 100,000 children in Norway and Australia [10]. UNICEF report from 96 countries in 2020 has indicated that children and adolescents less than 20 years have accounted for 21% of the COVID-19 cases and 33% of the 2020 population [27]. According to the UNICEF data, at the beginning of January 2023, there were 4,400,000 mortality reports globally. From those reports, 17,200 deaths occurred in children and adolescents less than 20 years, and 53% of those cases occurred in adolescents aged 10 to 19 years, whereas 47% of the cases occurred among children aged 0 to 9 years [28].

3. Risk factors predisposing South African children to COVID-19 disease compared to other countries

There are few studies conducted on the epidemiology and clinical manifestation of COVID-19 in African children in comparison to continents such as China, Europe, and North America [10, 21, 29]. The findings of studies from other countries cannot be generalizable to South African children because of the differences in the risk factors predisposing children to COVID-19, mostly the burden of infectious diseases [10]. SA children are affected by HIV, TB, malnutrition, childhood obesity, overcrowding, chronic kidney disease, malignancy, heart conditions, asthma, diabetes, and limited access to quality health care [10]. However, the study conducted in six African countries namely SA, Congo, Ghana, Kenya, Nigeria, and Uganda from March 01, 2020, to December 31, 2020 on COVID-19 children and adolescents has revealed the highest mortality and morbidity rates due to comorbidity with non-infectious diseases [21]. In the United States, and North America, important risk factors in children include hypertension, obesity, neuropsychiatric disorders, cardiac or circulatory anomalies, chronic lung disease, and immunosuppression [21, 22]. In China, children are at risk of developing severe COVID-19 cases due to underlying

	SA	China	North America
Risk factors	HIV, TB, malnutrition, childhood obesity, overcrowding, limited access to quality healthcare, diabetes, asthma, heart conditions, malignancy, chronic, kidney disease, and hypertension [10, 30, 31]	Circulatory or cardiac congenital anomalies, obesity, essential hypertension, epilepsy, malnutrition, asthma, Down syndrome, neuropsychiatric disorders, leukemia, hydronephrosis, and intussusception [13, 22].	Hypertension, obesity, diabetes, neuropsychiatric disorders, cardiac, or circulatory anomalies [21]

Table 1.
Comparison of the risk factors predisposing South African children to COVID-19 in comparison to other countries.

conditions such as circulatory or heart congenital anomalies, obesity, essential hypertension, epilepsy, malnutrition, asthma, Down syndrome, neuropsychiatric disorders, hydronephrosis, leukemia, and intussusception (**Table 1**) [13, 22].

4. Diagnosis, prevention, and treatment of COVID-19 disease in children in South Africa and other countries

4.1 Diagnosis of COVID-19 disease in children in South Africa and other countries

COVID-19 diagnosis is defined based on clinical manifestations, laboratory testing, and chest radiograph imaging, including asymptomatic infection, as mild, moderate, severe, or critical [24]. According to WHO, clinical diagnosis depends on disease severity, where (1) non-severe indicates the absence of signs of severe or critical disease, and (2) severe by oxygen saturation less than 90% on room air, signs of pneumonia, or respiratory distress, and (3) critical, the patients require treatment and presents with acute respiratory distress, sepsis, or shock [32]. The laboratory diagnosis of COVID-19 in SA (**Table 2**) is by using the SARS-CoV-2 reverse-transcription real-time polymerase chain reaction (rRT-PCR) on a respiratory sample obtained from a nasopharyngeal or oropharyngeal swab and SARS-CoV-2 antigen-based testing [10, 11, 30]. In other

Continent/Country	**Diagnosis** **Laboratory diagnosis**
1. SA	rRT-PCR, SARS-CoV-2 antigen-based test [10, 11, 33]
2. China	RT-PCR, viral antigen test, and serology test [12, 16, 23, 32]
3. America	RT-PCR, viral antigen test, and serology test [12, 16, 23, 32]
Continent/Country	**Prevention** **COVID-19 Vaccines**
1. SA	Pfizer-BioNTech, BNT162b2 [11, 14, 34]
2. China	BNT162b2 [34]
3. America	Pfizer-BioNTech, BNT162b2, Sinovac [14, 25, 26, 34]
4. Argentina	BNT162b2, Sinopharm [34, 35]
5. Colombia	AstraZeneca, Moderna, Sinopharm, Johnson, and Johnson [35]
6. El Salvador	Sinovac [35]
7. Ecuador	Sinovac [35]
8. Brazil	BNT162b2 [34]
9. Finland	BNT162b2 [34]
10. Poland	BNT162b2 [34]
11. Turkey	BNT162b2 [34]
12. Spain	BNT162b2 [34]
13. Germany	BNT162b2 [34]
14. Europe	Spikevax [34]

Table 2.
Diagnosis and prevention of COVID-19 for South African children and adolescents versus children and adolescents from other countries/continents.

places such as China and the American continent, laboratory diagnosis (**Table 2**) is by reverse transcription-polymerase chain reaction (RT-PCR) of nasopharyngeal and oropharyngeal swabs, viral antigen, and serology test [12, 16, 23, 33].

4.2 Prevention of COVID-19 disease in children in South Africa and other countries

The worldwide COVID-19 vaccine nation strategies had initially focused mainly on adults because children were less affected [14]. However, the emergence of mutations in the SARS-CoV-2 genome such as delta and omicron variants increased the risk of infections in children and adolescents in various countries [11, 14]. In SA, various variants emerged during the first and fourth waves of the COVID-19 epidemic; the Wuhan-Hu in the first wave between weeks 24 and 34 of 2020, the Beta variant during the second wave between week 47 of 2020 and week 5 of 2021 [11, 36]. The Delta variant caused the third wave between weeks 19 and 37 of 2021, and the omicron variant was responsible for the fourth wave between week 48 of 2021 and week 5 of 2022. Currently, several countries have approved the use of COVID-19 vaccines in children and adolescents (**Table 2**) [14]. The FDA has approved the use of the Pfizer-BioNTech COVID-19 vaccine in children aged 5 to 12 years [14, 25, 26]. Furthermore, SA approved the use of the Pfizer-BioNTech COVID-19 vaccine in children aged 12–17 years on October 20, 2021 (**Table 2**) [11, 14]. The Spikevax vaccine is approved by the European Medicines Agency to be used in adolescents aged 12 to 17 years (**Table 2**) [34]. FDA has authorized the emergency use of the BNT162b2 vaccine in children and adolescents aged 12 years and above in countries including, the United States, China, Finland, Spain, Turkey, Poland, Germany, Brazil, Argentina, and SA (**Table 2**) [34]. Other licensed vaccines for children and adolescents in Latin America include the Sinovac COVID-19 vaccine in Chile for children over 6 years, and in El Salvador for children aged 6 to 11 years, the Sinovac COVID-19 vaccine (**Table 2**) [35]. Sinopharm COVID-19 vaccine is licensed in Argentina for children as young as three years old and the Sinovac vaccine is used in Ecuador for children from six years old (**Table 2**) [35]. COVID-19 vaccines from AstraZeneca, Moderna, Sinopharm,

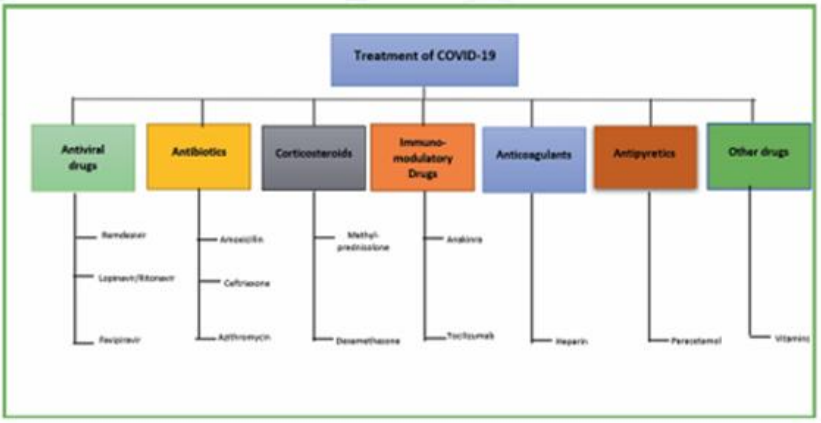

Figure 2.
Drugs used in the treatment of COVID-19 in adults and children worldwide.

and Johnson and Johnson are used in Colombia for children 12 years and older, and vaccination in Costa Rica is from 12 years (**Table 2**) [35].

4.3 Treatment of COVID-19 disease in children in South Africa and other countries

The treatment of COVID-19 is still evolving, and the clinical trials are ongoing, and the current recommendations depend on previous experiments and clinical trials [31]. Moreover, pharmacological management in children and adolescents is extrapolated from adult studies [31]. Management of COVID-19 is based on categories including (1) no treatment for asymptomatic cases, (2) antipyretic therapy in moderate and mild cases, and (3) for critical cases several drugs [37]. COVID-19 pharmacological management in children includes antiviral drugs (remdesivir, lopinavir/ritonavir, and favipiravir), antibiotics (amoxicillin, ceftriaxone, and azithromycin), vitamins, corticosteroids (methylprednisolone and dexamethasone), immunomodulatory drugs (anakinra and tocilizumab), anticoagulant (heparin), antipyretic drug (paracetamol), and hydroxychloroquine, based on the condition of the child (**Figure 2**) [13, 37].

5. Conclusions

The current review documents the prevalence, risk factors, and management of COVID-19 disease in South African children and adolescents in comparison to other countries in other continents. There is limited literature, and few studies covering the epidemiology of COVID-19 in SA and other countries in Africa and other continents. COVID-19 is less prevalent in children across the countries covered in this review when compared to adults. The burden of COVID-19 is higher in children in countries like the United States when compared to SA. There are similarities in the risk factors which predispose South African children to COVID-19 and children in other countries, except that the burden of bacterial and viral infection is higher in Africa and SA. Moreover, living conditions, poverty, and quality and access to healthcare facilities are still a challenge in SA and other African countries. Clinical and laboratory diagnosis is similar and laboratory diagnosis in SA and other countries is mainly through rRT-PCR, RT-PCR, SARS-CoV-2 viral antigen, and serology tests. Various vaccines including Pfizer-BioNTech COVID-19 vaccine, Spikevax, BNT162b2, AstraZeneca, Moderna, Sinopharm, and Johnson and Johnson are approved and licensed for use in children and adolescents in various countries including SA. There are limited studies defining country-based pharmacological management of COVID-19 in children.

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Conflict of interest

The authors declare no conflict of interest.

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C. Published article 3

Chapter

Traditional Medicinal Plants as the Potential Adjuvant, Prophylactic and Treatment Therapy for COVID-19 Disease: A Review

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and Polo-Ma-Abiele Hildah Mfengwana

Abstract

Coronavirus disease 2019 (COVID-19) is a respiratory disease caused by a severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2). In an effort to combat the pandemic caused by COVID-19 disease, researchers have identified several traditional medicinal plants (TMPs) as potential adjuvant, prophylactic, and treatment for COVID-19. TMPs reported in this paper were identified based on the findings of molecular docking research and the documented traditional use of these plants for COVID-19-related symptoms, such as fever, coughing, headaches, and tiredness. Secondary metabolites with antiviral, anti-inflammatory, and immunomodulatory activity against various SARS-CoV-2 proteases were also identified from the list of South African medicinal plants. This review discusses secondary metabolites of TMPs with pharmacological benefits, which contribute to the management of COVID-19, and these include *Acacia Senegal*, *Artemisia afra*, *Aspalathus linearis*, *Clerodendrum splendens*, *Dioscorea batatas* decne, *Echinacea purpurea*, *Hypoxis hemerocallidea*, *Xysmalobium undulatum*, *Tinospora crispa*, *Sutherladia frutescens,* and *Zingiber officinale*.

Keywords: traditional medicinal plants, COVID-19, adjuvant, antiviral, immunomodulatory

1. Introduction

Coronavirus disease 2019 (COVID-19) that caused pandemic started in December 2019 in Wuhan, China [1–3]. The novel coronavirus responsible for this respiratory disease was identified to be the member of the Coronaviridae family known to cause infections in humans called severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) [1]. SARS-CoV-2 is reported to be found in bats, and the infections occurred in humans because of the intermediate host, the pangolin [2]. SARS-CoV-2 is the third coronavirus reported to cause the respiratory disease pandemic after severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS) [2]. COVID-19 disease

spreads from human to human by respiratory droplets [4], and the symptoms include dry cough, fever, fatigue, body aches, dyspnea, chills and shivering, sputum production, diarrhea, nausea, nasal congestion, rhinorrhea, and loss of speech or movement [3, 5–7]. To date, COVID-19 is still the cause of morbidity and mortality worldwide, accounting to 409 confirmed positive cases and 5.8 million deaths between 7 and 13 February 2022 [8]. Among the African countries, South Africa (SA) reported the highest numbers of new mortality between 7 and 13 February 2022 [8].

Vaccines were quickly developed for the prevention of COVID-19 pandemic [9], but there is no specific treatment available [4] as vaccinated individuals can still contact and transmit the COVID-19 virus. COVID-19 symptom management is mainly supported with oxygen therapy, steroids, antivirals, antibiotics, and anti-inflammatory agents, including chloroquine and hydroxychloroquine [5, 6]. However, antibiotics, antiviral, and anti-inflammatory drugs are reported to be the cause of health problems due to their toxicities [6]. Africa has a long historic record on the use of traditional medicinal plants (TMPs), and phytomedicine is preferred as 80–90% of rural population rely on medicinal plants for primary healthcare [10]. Fortunately, the World Health Organization (WHO) promotes the use of traditional, complementary, and alternative medicine on condition that their efficacy, safety, and quality are scientifically reported [1, 11]. Therefore, considering the potential of TMPs as alternative and complementary conventional drugs for COVID-19 management is an important research topic during the current situation of COVID-19 pandemic [12]. Several studies were conducted on TMPs and their pharmacological activities against COVID-19 [10, 13–15], and this review is, therefore, aimed at the documentation of TMPs that can be used in adjuvant, prophylactic, and management therapy of COVID-19.

2. Potential use of TMPs in adjuvant, prophylactic, and management therapy for COVID-19 disease

TMPs have become the subject of interest in the era of COVID-19 pandemic, and various researchers have conducted studies based on selecting TMPs commonly used traditionally to treat fever, cold, and flu symptoms [13, 14]. *Echinacea purpurea* and *Zingiber officinale* were identified among TMPs with promising adjuvant symptomatic therapy [14]. A number of secondary metabolites isolated from TMPs were identified to have immunomodulatory, antiviral, and anti-inflammatory activities against SARS-CoV-2 [15]. TMPs with immunomodulatory effect could be used in COVID-19 patients as a prophylactic and treatment therapy [16]. Immunomodulation agents identified as potential therapy against infectious diseases, including COVID-19, are, among others, *Dioscorea batatas* decne, *Clerodendrum splendens,* and *Tinospora crispa* [17].

Active secondary metabolites of these TMPs have immunomodulatory effect and can reduce cytokine production against viral infections [6]. TMPs with potential antiviral activity against SARS-CoV-2 were identified as *Artemisia afra*, *Acacia Senegal*, *Aspolathus linerias*, *Hypoxis hemerocallidea, Sutherladia frutescens*, and *Xysmalobium undulatum* [15, 18, 19]. Madagascar's *Artemisia afra* was found to have inhibitory effect against SARS-CoV-2 [18]. Although the safety and dosage of this medicinal plant was determined *in vitro*, clinical studies must still be conducted to evaluate the use of this medicinal plant for COVID-19 prevention and treatment in COVID-19 patients [18]. Molecular docking research using the list of South African TMPs identified plants with

antiviral activity against SARS-CoV-2 included *Acacia Senegal*, *Aspolathus linerias*, *Hypoxis hemerocallidea*, *Sutherladia frutescens*, *and Xysmalobium undulatum* [19].

TMPs reported in this review article target various stages in viral life cycle starting from the prevention of viral entry to the E6 cells, halting the fusion of the S protein, the inhibition of SARS-CoV-2 receptor-binding domain, the prevention of viral replication, and the transcription by targeting SARS-CoV-2 RNA-dependent RNA polymerase and major proteases [6, 15, 17, 19–25]. The diagrams in **Figure 1** show strategies for the prevention and management of COVID-19 using TMPs.

2.1 *Acacia senegal*

A. senegal (**Figure 2**), also known as white gum tree, belongs to the *Mimosoideae* family of plants and is widely distributed in Senegal, Cameroon, and Sudan [26–28]. Exotic *A. Senegal* is found in South Africa and is called siKhambophane and umKhala in isiZulu [29]. *A. senegal* is traditionally used to treat respiratory symptoms and infections, such as flu and sore throat (**Table 1**), and other conditions including, sinusitis, toothaches, stomach ulcer, colic, diarrhea, and dysentery [19, 26]. This medicinal plant has pharmacological activities, which include anti-inflammatory, antibacterial, antifungal, and antioxidant [26]. Secondary metabolites identified from *A. senegal's*

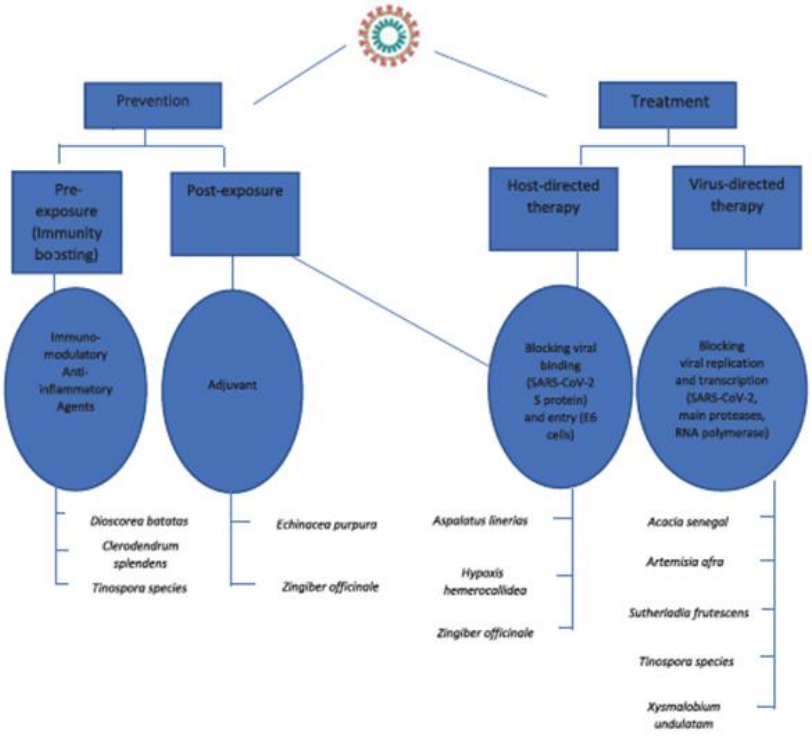

Figure 1.
Identified strategies for prevention and treatment of COVID-19 using TMPs.

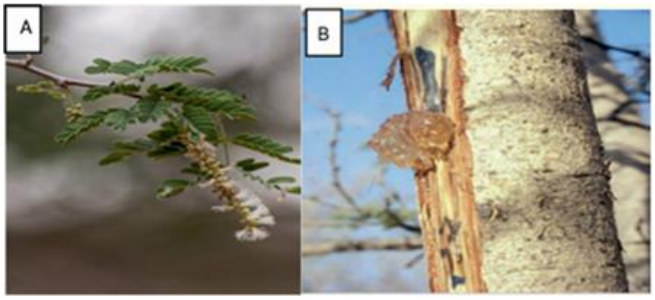

Figure 2.
Acacia Senegal *leaves in picture A and bark in picture B.*

TMPs	Traditional uses in respiratory symptoms and diseases
Acacia senegal	Flu, sore throat [19]
Artemisia afra	Cold, cough, headache, influenza, sore throat, asthma, pneumonia [30, 31]
Aspalathus linearis	Asthma [32]
Clerodendrum splendens	Asthma, cough, [20, 33]
Dioscorea batatas decne	Asthma [21]
Echinacea purpurea	Common cold [34]
Hypoxis hemerocallidea	Tuberculosis [35, 36]
Sutherladia frutescens	Influenza, fever [37]
Tinospora species	Fever [23]
Xysmalobium undulatum	Headache [19]
Zingiber officinale	Cold, cough, asthma, influenza, headache, fever, sore throat [14, 38–40]

Table 1.
Traditional uses of TMPs in respiratory symptoms and diseases.

plants extracts include glycosides, alkaloids, flavonoids, and arabic acid [15, 19, 26]. Arabic acid was determined to have a higher docking score (−5.2 kcal/mol) against 3CLpro, suggesting that *A. senegal* is a medicinal plant with antiviral activity against SARS-CoV-2 3C-like major protease (**Table 2**) [15, 19]. Thus, testing *A. senegal in vitro* might help to characterize new treatment and/or prophylactic strategies against SARS-CoV-2.

2.2 *Artemisia afra*

Artemisia afra (**Figure 3**), also known as African wormwood, belongs to the *Asteraceae* family [44, 45]. It is indigenous to Africa and is widely distributed in South Africa, Namibia, Zimbabwe, Kenya, Tanzania, Uganda, and Ethiopia [30, 44]. *Artemisia afra* is called Umhlonyane in Xhosa and Lengana in Sesotho [44]. It is used traditionally for the treatment of respiratory symptoms and diseases including cold, cough, headache, influenza, sore throat, asthma, and pneumonia (**Table 1**), and other disease conditions such as diabetes, colic, dyspepsia, bladder and kidney disorders,

TMPs	Secondary metabolites with a Adjuvant, prophylactic, and anti-COVID-19 activity
Acacia senegal	Arabic acid, Anti-SARS-CoV-2 3C-like major protease activity [15, 19]
Artemisia afra	Flavonoids, Anti-SARS-CoV-2 activity [15]
Aspalathus linearis	Flavonoids, quercetin, luteolin, Anti-SARS-CoV activity [41]
Clerodendrum splendens	Type II arabinogalactam, immuno modulatory activity [17, 20]
Dioscorea batatas decne	Allantoin, batatas, choline, dioscorin, diosgenin, gracillin, glycoproteins, L-arginine, mucopolysaccharides, prosapogenin, protein, polysaccharide, saponins, Immunomodulatory activity [17, 21, 22]
Echinacea purpurea	Chicoric acid, polysaccharide, alkamides, immunomodulatory activity [14] and Extracts, Anti-coronavirus activity [42]
Hypoxis hemerocallidea	Hypoxide, Anti-SARS-CoV-2 receptor binding domain activity [19]
Sutherladia frutescens	L-canavanine, Anti-SARS-CoV-2 3C-like main protease activity [19]
Tinospora crispa	hydroxy-5-cholen-24-oic acid, androstan-17-one, 3-ethyl-3-hydroxy-(5.alpha), camphenol, (−)-globulol, yangambin, nordazem, TMS derivative, benzene ethanamide, Anti-SARS-CoV-2 main protease activity [25]
Tinospora cordifolia	Amritoside, apigen-6-C-glucosyl7-O-glu-coside, 20a hydroxy ecdysone, tinosporine B, epicatechin, Anti-SARS CoV-2 main protease activity [6]
Xysmalobium undulatum	Uzarin, Anti-SARS-CoV-2 RNA dependent RNA polymerase activity [19]
Zingiber officinale	10-paradol, 8-paradol, scopoletin, 10-shogaol, 8-gingerol, 10-gingerol, Anti-SARS-CoV-2 activity [43]

Table 2.
Secondary metabolites of TMPs with adjuvant, prophylactic, and anti-COVID-19 activity.

constipation, malaria, and rheumatism [30, 31]. *Artemisia afra* contains secondary metabolites including tannins, alkaloids, terpenoids, cardiac glycosides, and saponins [30]. Pharmacological activities of *Artemisia afra* include antioxidant, antiviral, antiplamodial, antifungal, and antibacterial [30, 31, 44]. *Artemisia afra* aqueous and ethanolic extracts, as well as teas, were shown to inhibit SARS-CoV-2 plaque formation *in vitro* [15]. The antiviral activity of this medicinal plant is reported to have been as a result of flavonoids present in *Artemisia* species (**Table 2**) [15]. The extracts showed some toxicity at higher concentrations with the selectivity index of 10, which opened a therapeutic window that is required to be further investigated in clinical trial [15]. There is still a need to prove whether *Artemisia afra* extracts can reach the serum levels required to completely inhibit the virus in COVID-19 patients.

2.3 *Aspalathus linearis*

A. linearis (**Figure 4**), also known as Rooibos in Afrikaans, belongs to the *Fabaceae* family and is an endemic South African species cultivated to produce a tea [46–48]. It is used commonly for the treatment of respiratory disease such as asthma (**Table 1**) and other diseases including cardiac arrhythmias, colic, diarrhea, and hypertension [32]. Rooibos contains flavonoids including aspalathin, isoorientin,

Figure 3.
Artemisia afra *leaves.*

Figure 4.
Aspalathus linearis.

isovitexin, nothofagin, orientin, quercetin, rutin, and vitexin [41]. Other present secondary metabolites include polyphenols and phenolic compounds such as dihydro-chalcones, flavonols, flavonones, and proanthocyanadins [41, 46, 47]. Rooibos has pharmacological activities including antioxidant, antiviral, immunomodulatory, anti-inflammatory, cardioprotective, and nephroprotective effects [41]. Flavonoids, quercetin, and luteolin (**Table 2**) present in Rooibos were found to inhibit SARS-CoV infection by preventing entry of virus into E6 cells, and luteolin acts by binding to SARS-CoV S proteins, thereby interfering with the S protein function [41]. However, more experiments must be conducted to validate the clinical relevance of Rooibos in treating COVID-19 and other respiratory diseases [41]. Although other studies have highlighted the drug interactions associated with the

Rooibos derived phytochemicals [42], more research is required in determining the safety of Rooibos in patients.

2.4 *Clerodendrum splendens*

Clerodendrum splendens (**Figure 5**), also known as bag flower, bleeding-heart, and glory bower in English [33, 49], belongs to the *Lamiaceae* family of plants [20]. It is distributed in tropical Africa, Southern Asia, America, and Northern Australasia [33]. *Clerodendrum splendens* is used traditionally to treat respiratory diseases such as asthma and coughs (**Table 1**) and other diseases including anorexia, leucoderma, leprosy, malaria, skin diseases, ulcers, uterine fibroids, wounds, burns, and sexually transmitted diseases such as syphilis and gonorrhea [20, 33]. Phytochemical constituents present in *Clerodendrum splendens* include alkaloids, cyanogenic glycosides, diterpenes, flavonoids, phenolic compounds, saponins, steroids, tannins, terpenoids, and volatile compounds [17, 20]. It has pharmacological activities including antibacterial, antifungal, anti-inflammatory, antiproliferative, antioxidant, and hepatoprotective [17, 20, 33]. *Clerodendrum splendens* contains a polysaccharide, type II arabinogalactam (**Table 2**), that has been shown to have immunomodulatory activity both *in vitro and in vivo* [17, 20]. Its antiproliferative activity is reported to be as a result of clerodane diterpenes and phenyl propanoids found in aerial parts this plant [17]. The methanol extract of *Clerodendrum splendens* was reported to have *in vitro* anti-inflammatory activity (**Table 2**) [24]. The findings reported on *Clerodendrum splendens* form the basis for further research into the efficacy and safety of this plant as potential COVID-19 treatment and anti-inflammatory agents [17, 24].

2.5 *Dioscorea batatas* decne

D. batatas decne (**Figure 6**), commonly called Chinese yam [50, 51], belongs to the *Dioscoreaceae* family of plants [21, 52]. *Dioscoreaceae* plant species are widely distributed in West Africa, Southeast Asia, and Tropical America [52]. *D. batatas* decne is used traditionally for the treatment of respiratory disease such as

Figure 5.
Clerodendrum splendens *leaves and flowers.*

Figure 6.
Dioscorea batatas *decne leaves and fruits.*

asthma (**Table 1**) and other conditions including, abscesses, cancer, inflammation, hypertension, ulcer, chronic diarrhea, and diabetes [21]. It has antioxidant and anti-inflammatory activities [22, 50]. *D. batatas* decne contains various active components, such as allantoin, batatasins, choline, dioscorin, diosgenin, gracillin, glycoproteins, L-arginine, mucopolysaccharides, prosapogenin, protein, polysaccharides, and sapogenins (**Table 2**) with immunomodulation effects when orally administered [17, 21, 22]. The immunomodulatory activity of tuber protein and dioscorin occurs through the activation of TLR4-induced macrophage due to the stimulation of signaling molecules such as NF-kB, JNK, p38, and ERK, and by TNF-a and IL-6 cytokines expression [17, 21, 22]. The immunomodulation effect of tuber extract on inflamed and normal skin was reported to be due to the enhancement of granulocyte-macrophage colony-stimulating factor promoter [17]. The tuber extract of *D. batatas* was found to be the potent inhibitor of SARS-CoV (**Table 2**) at concentrations between 25 and 200 μg/mL [53].

2.6 *Echinacea purpurea*

E. purpurea (**Figure 7**), also known as Eastern Purple Coneflower, belongs to the *Asteraceae* family [54–56]. It is native to eastern North America [55]. *E. purpurea* is used for the treatment of respiratory conditions such as common cold (**Table 1**) and other conditions including pain, cancer, toothache, seizures, arthritis, and skin disorders [14, 34, 54]. *E. purpurea* has been approved by the European Medicine Agency Herbal Medicinal Product Committee to be used as prophylactic therapy for the maximum of 10 days for immunostimulation and to prevent cold and other respiratory infections [14]. Pharmacological activities of *E. purpurea* include antiviral, antioxidant, antibacterial, immunomodulatory, antitumor, and anti-inflammatory [54, 55]. *E. purpurea* contains phytochemicals such as alkamides, betaine, phenolic compounds, polysaccharides, lipoproteins, saponins, sesquiterpenes, and polyacetylene [55]. *Echinacea* species exerts a soothing effect and could be useful in the relief of respiratory symptoms and common cold [14]. The immunomodulatory activity of *E. purpurea* was reported to be as a result of chicoric acid, polysaccharide, and alkamides (**Table 2**) in a rat study [14]. The use of *Echinacea*

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Figure 7.
Echinacea purpurea. *(A) Flowers and (B) leaves.*

for supplementation is reported to decrease the duration of acute respiratory tract infections and the severity of the disease [57]. The extract of *E. purpurea* (L.) Moench has shown direct antiviral activity against coronaviruses, and the preliminary published findings on human clinical trials covering antiviral activity of *Echinacea* against SARS-CoV-2 further support the use of this plant species against this particular coronavirus [58].

2.7 *Hypoxis hemerocallidea*

Hypoxis hemerocallidea (**Figure 8**), also known as African Potato, belongs to the *Hypoxidaceae* family [35, 36, 59]. It is called inkomfe in Zulu and Lotsane in Tswana [60]. *Hypoxis hemerocallidea* is widely distributed in Southern Africa including, South Africa, Lesotho, Mozambique, and Zimbabwe and is also found in East Africa [36]. It is used traditionally to treat HIV/acquired immunodeficiency syndrome, arthritis, diabetes mellitus, testicular tumors, cancers, infertility, urinary infection, cardiovascular diseases, and respiratory disease such as tuberculosis (**Table 1**) [35, 36]. *Hypoxis hemerocallidea* contain phytochemicals, such as sterols, sterolins, norlignan, daucosterol, and rooperol, responsible for its therapeutic benefits [35]. Hypoxide is the main glycoside isolated from *Hypoxis* species [36]. Molecular docking analysis identified hypoxide (**Table 2**) as a potent inhibitor of SARS-CoV-2 receptor-binding domain with the docking score of −6.9 kcal/mol [19]. The study conducted on rats has demonstrated that *Hypoxis hemerocallidea* has the ability to impair kidney function. There is a need for more *in vitro* and *in vivo* research on the toxicity, safety, and efficacy of *Hypoxis hemerocallidea* [61].

2.8 *Sutherladia frutescens*

Sutherlandia frutescens (**Figure 9**), also known as cancer bush, belongs to *Fabaceae* family of plants [6, 62]. It is an indigenous medicinal plant commonly used in South Africa to treat respiratory symptoms and disease such as fever and influenza (**Table 1**) and other diseases including cancers, diabetes, kidney and liver problems, rheumatism, depression, wounds, hemorrhoids, gonorrhea, urinary tract infections, and back pain [37]. Various *Sutherlandia* formulations are available in pharmacies and herbal shops and include capsules and tablets, gels, creams, liquid extracts, and ointments [37]. *S. frutescens* has been scientifically reported to have anticancer, antidiabetic, and anti-HIV properties [37, 62]. It has phytochemical constituents including sutherlandioside A, B, C and D, D-pinitol, gamma (γ) aminobutyric acid, and L-canavanine responsible for its biological

Figure 8.
Hypoxis hemerocallidea *leaves.*

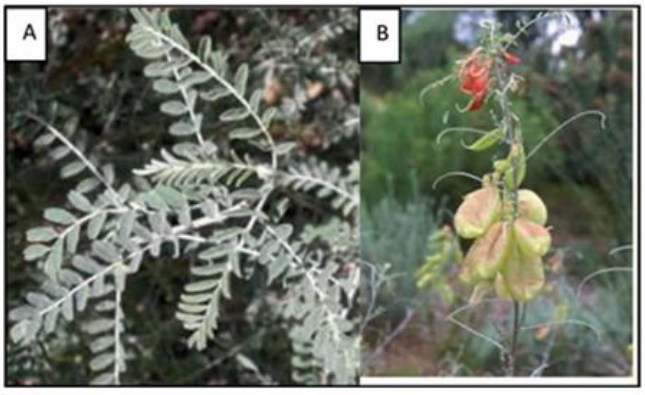

Figure 9.
Sutherlandia frutescens. *(A) Leaves and (B) flowers.*

activities [37]. The results of molecular docking analysis identified L-canavanine (**Table 2**) present in *S. frutescens* as a potential inhibitor of SARS-CoV-2 3C-like main protease [19]. The results of a randomized, double-blind, placebo-controlled trial of *Sutherlandia* leaf powder in healthy adults revealed that 800 mg/day of *Sutherlandia* leaf powder capsules were safe for consumption twice per day for three months in healthy adults [63].

2.9 *Tinospora species*

Tinospora crispa (**Figure 10A**) is also known as Seruntum in Malaysia, Brotawali in Indonesia, Makabuhay in Philippines, Boraphet in Thailand, Da ye ruanjinteng in China, Banndol Pech in Cambodia, Golonchi in Bangladesh, and Lyann span Zeb kayenn in Martinique island [23, 64]. *Tinospora cordifolia* (**Figure 10B** and **C**), also known as heart leaved Moonseed plant in English, Giloy in Hindi, and Guduchi in Sanskrit [65]. *Tinospora* species belongs to the family *Menispermaceae* [23, 65, 66]. *Tinospora crispa* is found in South East Asia and the Pacific [23, 66], and *Tinospora cordifolia* is found throughout India and certain parts in China [65]. Traditionally, *Tinospora* species are used to treat respiratory diseases and symptoms such as fever (**Table 1**) and other conditions including muscle pain, immune system associated inflammatory disorders, rheumatism, muscle pain, diabetes, and abdominal pain, septicemia, scabies, and ulcer-related disorders, hypertension, jaundice, paralysis, skin disease, leprosy, flatulence, dyspepsia, and diarrhea [6, 23, 66]. Phytochemical constituents of *Tinospora crispa* include alkaloids, flavonoids, furanoditerpenes, lignans, lactones, and steroids [66]. *Tinospora crispa* has pharmacological activity including antioxidant [23]. Active constituents such as boldine, cardioside, eicosenoic acid, quercetin, magnoflorin, and syringin are reported to have the antioxidant potential higher than that of ascorbic acid [23]. The same constituents are also reported to have the ability to increase the expression of IL-6, IL-8, and INF-g, thereby activating the immune system [17]. *Tinospora cordifolia* contains secondary metabolites including folioside A, tinocordiside, magnoflorine, and syringin with immunomodulatory activity [6]. The results of the molecular docking study on *Tinospora crispa* have revealed nine potential anti-SARS-CoV-2 lead molecules, namely, imidazolid-4-ne, 2-imino-1-(4-methoxy-6-dimethylamino-1,3,5-triazin-2-yl), spiro [4, 8] dec-6-en-1-ol, 2,6,10,10-tetramethyl, 3.beta-hydroxy-5-cholen-24-oic acid, androstan-17-one, 3-ethyl-3-hydroxy-(5.alpha), camphenol, (−)-Globulol, yangambin, nordazem, TMS derivative, and benzeneethanamide (**Table 2**). Three of these molecules have demonstrated some biological activity, which led to further optimization and drug development research for COVID-19 disease [25]. Molecular docking analysis also revealed that *Tinospora cordifolia* contains bioactive compounds, including amritoside, 20a hydroxy ecdysone, apigen-6-C-glucosyl7-O-glucoside, tinosporine B, and epicatechin (**Table 2**), with promising anti-SARS CoV-2 main protease activity [6]. The acute toxicity study conducted on rats has revealed that the ethanol extract of *Tinospora crispa* stem is not toxic and did not cause animal

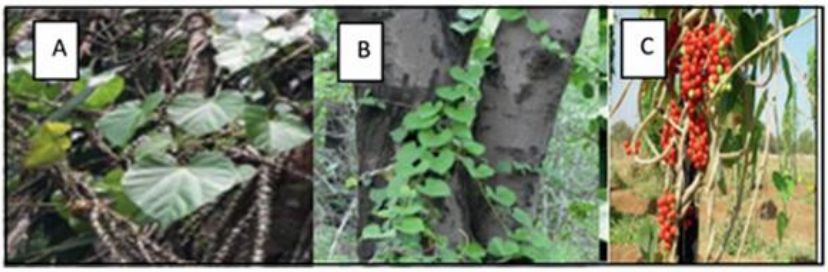

Figure 10.
(A) Tinospora crispa. (B) and (C) Tinospora cordifolia.

death at a dose of 4.0 g/kg of body weight (g/kg BW). However, six-month chronic toxicity study has reported hepatic and renal toxicities of the ethanol extract at a dose of 9.26 g/kg BW/day [64].

2.10 *Xysmalobium undulatum*

Xysmalobium undulatum (**Figure 11**) also known as Uzara wild cotton and milk bush, belongs to the family *Apocynaceae* [67–70]. Genus *Xysmalobium* is endemic to Africa and there are about 18 plant species occurring in SA [67]. Uzara is used traditionally to treat respiratory symptoms such as headaches (**Table 1**) and other disease conditions including, diarrhea, stomach cramps, afterbirth cramps, dysmenorrhea, wounds, sores, abscesses, and hysteria and has a diuretic effect [68]. Uzarin and its isomers allouzarin, xysmalorin, and alloxysmalorin are the main compounds isolated from Uzara [67]. Pharmacological activities of Uzara include antidiarrheal and antidepressant [68]. Uzarin (**Table 2**) was identified as the potential inhibitor of SARS-CoV-2 RNA-dependent RNA polymerase, and it showed favorable docking score of −3.5 kcal/mol in a molecular docking study conducted from the list of South African TMPs [19].

2.11 *Zingiber officinale*

Z. officinale (**Figure 12**), also known as Ginger, belongs to the *Zingiberaceae* family which comprises of close to two hundred species [38, 71]. *Z. officinale* is used for the treatment of respiratory symptoms and diseases including common cold, cough,

Figure 11.
Xysmalobium undulatum leaves and flowers.

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Figure 12.
Zingiber officinale *whole plant showing roots, stem, and leaves.*

asthma, influenza, headaches, sore throats, and fever (**Table 1**) and other diseases such as arthritis, rheumatism, nausea, flatulence, muscular aches, pains, cramps, constipation, hypertension, dementia, infectious diseases, helminthiasis, colic, and diarrhea [14, 38–40]. It has pharmacological activities including immunomodulatory, antitumorigenic, anti-inflammatory, antiapoptotic, antihyperglycemic, antilipidemic, antiemetic, antipyretic, antioxidant, antibacterial, and analgesic [38–40]. Active compounds in ginger include phenolic and terpene compounds, and phenolic compounds in ginger include gingerols, paradols and shogaols, and paradols [39]. The profile and chemistry of *Z. officinale* makes it a perfect anti-inflammatory therapy in the context of upper respiratory affections [39]. Molecular docking *in silico* studies suggested that phytochemical compounds, such as 10-Paradol, 8-Paradol, Scopoletin, 10-Shogaol, 8-Gingerol, and 10-Gingerol, in *Z. officinale* (**Table 2**) have potential in reducing viral load and detaching of SARS-CoV-2 in the nasal passages [43].

Future aspects include the extraction of the medicinal plants listed in **Table 1**, the isolation of pure compounds as well as their fingerprinting and identification, and the confirmation of their mechanisms of action [72]. Further testing of extracts in animal models and investigations of effective and safe dosages, route administration, drug administration intervals, pharmacokinetics, and mechanisms of action are required before the use of medicinal plants discussed in this review can be advocated to be used for COVID-19 patients [7].

3. Conclusions

The current review has summarized TMPs commonly used in the treatment of respiratory symptoms and diseases, which possess potential adjuvant, prophylactic, and therapeutic properties against SARS-CoV-2 including *Acacia Senegal*, *Artemisia afra*, *Aspalathus linearis*, *Clerodendrum splendens*, *D. batatas* decne, *E. purpurea*, *Hypoxis hemerocallidea*, *Xysmalobium undulatum*, *Tinospora crispa*, *Sutherladia frutescens,* and *Z. officinale*. Secondary metabolites present in selected TMPs are responsible for the pharmacological activities of these medicinal plants. TMPs identified by molecular docking analysis should be investigated experimentally as potential SARS-CoV-2 treatment. Further studies are warranted to isolate and test secondary metabolites with inhibitory properties against SARS-CoV-2. Safety and efficacy profiles of these TMPs must be explored *in vitro* and *in vivo*. Animal studies and human clinical trials are required for further testing of these TMPs before recommendations to use in COVID-19 patients.

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Conflict of interest

The authors declare no conflict of interest.

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D. International conference certificate 1

WCP2023
2 - 7 JULY 2023
GLASGOW | SCOTLAND

Certificate of Attendance

This is to certify that

Ms Moleboheng Binyane

has attended the event

19TH WORLD CONGRESS OF BASIC & CLINICAL PHARMACOLOGY 2023

held on

Sunday, July 2, 2023

to

Friday, July 7, 2023

Professor David Webb Professor Amrita Ahluwalia

E. International conference certificate 2

CERTIFICATE

OF APPRECIATION

PRISM

Awarded to

Mr./Mrs./Ms. **Moleboheng Emily Binyane,** Walter Sisulu University, South Africa

for presenting poster on *Phytochemical Analysis and Toxicity Assessment of Traditional Medicinal Plants commonly used in Treatment of Respiratory Diseases in South Africa*

at

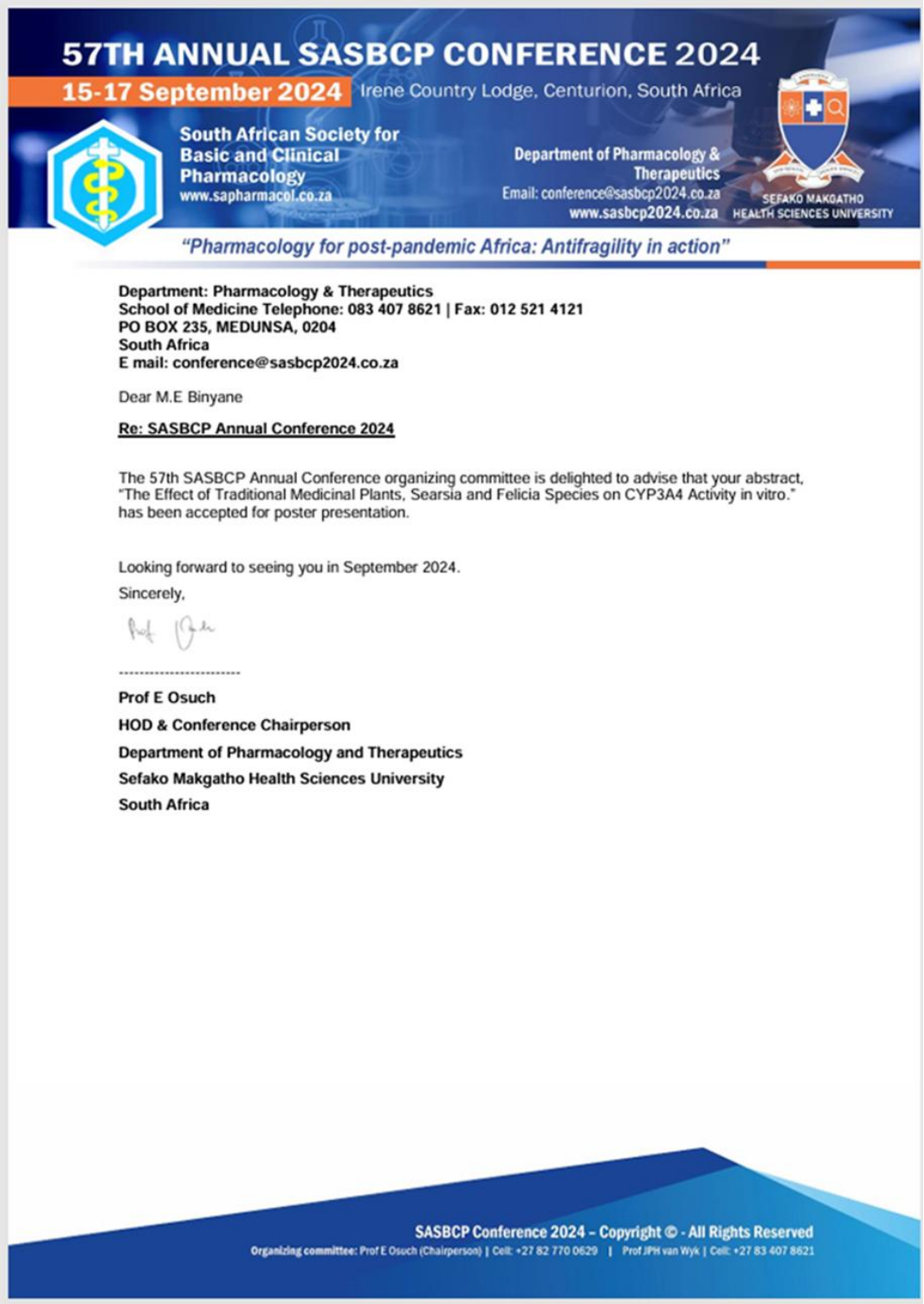
International Conference on Traditional Medicine and Phytochemistry

July 12-14, 2021 | Virtual

INTERNATIONAL CONFERENCE ON TRADITIONAL MEDICINE AND PHYTOCHEMISTRY 2021 | VIRTUAL

Dr. Nitin Mantri
RMIT University, Australia
Conference Chair
TMED-2021

F. Abstract acceptance letter 1 (National conference)



57TH ANNUAL SASBCP CONFERENCE 2024

15-17 September 2024 Irene Country Lodge, Centurion, South Africa

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PO BOX 235, MEDUNSA, 0204
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E mail: conference@sasbcp2024.co.za

Dear M.E Binyane

Re: SASBCP Annual Conference 2024

The 57th SASBCP Annual Conference organizing committee is delighted to advise that your abstract, "The Effect of Traditional Medicinal Plants, Searsia and Felicia Species on CYP3A4 Activity in vitro." has been accepted for poster presentation.

Looking forward to seeing you in September 2024.

Sincerely,

Prof E Osuch
HOD & Conference Chairperson
Department of Pharmacology and Therapeutics
Sefako Makgatho Health Sciences University
South Africa

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Organizing committee: Prof E Osuch (Chairperson) | Cell: +27 82 770 0629 | Prof JPH van Wyk | Cell: +27 83 407 8621

G. Abstract acceptance letter 2 (National conference)

South African Society for Basic and Clinical Pharmacology

Moleboheng Emily Binyane

Walter Sisulu University

Dear Moleboheng Emily Binyane

Thank you for submitting your abstract entitled "***Antifungal Activity of a Traditional Medicinal Plant Used for Treatment of Respiratory Diseases in South Africa***" for consideration for presentation at the 54th SASBCP Annual Conference which will take place on the **22nd October 2021**. It is my great pleasure to inform you that your abstract has been accepted for **oral presentation** in the **basic pharmacology** category. Congratulations!

Please be advised that this year's conference will be a **fully virtual event** managed/administered by University of Cape Town (UCT)'s Conference Management Centre (CMC). For this reason, Roxanne Adams and her team at CMC require that your final presentation be sent to them by no later than **Monday, 18 October 2021**. Detailed instructions on how to record your presentation are outlined at the end of this letter.

Each speaker in your session will have a 12-minute slot to discuss their work. Abstract presentations will be pre-recorded and played to a live audience during the allocated session. When preparing your presentation, we ask you to ensure that it is not longer than 10 minutes, leaving time for some engagement. Presentations will be played for exactly 10 minutes and stopped if longer. Please try to provide a concise description of the background, methods, and key findings, discussion and conclusion in this time. There will be a live question and answer session at the end of each talk. Therefore, speakers are expected to be logged in at the time of their presentations. Furthermore, we'd like to invite you to stay on to participate in the half-day event.

We are looking forward to your participation in the 54th SASBCP Annual Conference. Do not hesitate to contact us further if you have any questions.

Yours sincerely,

Phumla Sinxadi, on behalf of the 54th SASBCP Annual Conference Committee