Anti-fungal, anti-mycotoxigenic activity and phytochemical spectra of selected South African plants

Submitted by

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Abstract

Inhibition of *in vitro* mycelial growth against six mycotoxigenic fungal pathogens *in vitro* of six medicinal plants leaves extracts to check potential of anti-mycotoxigenic activity . Organic extract from *Strychnos mitis* exhibited a noteworthy antifungal activity yielding lowest minimum inhibitory concentration (MIC) value of 0.01 mg/ml against three *Furasium* species (*Furasium vercitilloides*, *Furasium oxysporum* and *Furasium graminareum*) and a further MIC value of 0.08 mg/ml against *Aspergillus parasiticus*, *Aspergillus flavus* and *Aspergillus ochraceous* at 24 hr incubation period. At both 24 and 48 hr incubation period, aqueous extract from *Mystroxylon aethiopicum* yielded MIC value of 0.02 mg/ml against *A. parasiticus* while aqueous extract from *Spirostachys africana* exhibited MIC values of 0.04 and 0.02 mg/ml against *F. vercitilloides* and *F*. *graminareum* at 48 hr respectively. In the mycelial growth inhibition (MGI) studies, the extracts showed varying degree of inhibition in a dose dependent manner. *M. aethiopicum* showed the highest mycelial growth percentage inhibition of 44.38 against *Fusarium verticiloides* in a three-day incubation period. Furthermore, the organic extracts were also evaluated for cytotoxicity effects against both African green monkey (Vero) and Bovine dermis (skin) cell lines using the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay. Organic extract from *S. africana* exhibited the lowest lethal concentration (LC_{50}) value of 0.10 mg/ml against both cell lines used in the study, while *S. mitis* exhibited the highest selectivity index (SI) value of 88.0 against *F. graminareum*. In the antioxidants assays, only extracts from *S. africana* and *M*. *aethiopicum* exhibited a noteworthy activity against 2,2-Diphenyl-1-picrylhydrazyl (DPPH), 2-2′-Azino-di-[3-ethylbenzthiazoline sulfonate (ABTS) and chelation of iron.The extracts, with potent biological activity, were also subjected to Gas-chromatography-mass spectrometry (GC-MS) analysis to identify the presence and prevalence of phytocompounds. *M. aethiopicum* extract exhibited the presence of tetradecane (15.74%) and heptadecane (14.96%), while *S. africana* yielded *n*-hexadecanoic acid (12.14%) and nonadecane,2-methyl (12.14%). These results suggest that bio-compounds from plants can be further explored *in vivo* and used to develop a bio-fungicide with less side effects compared to synthetic fungicides. Elsewhere, tetradecane, heptadecane and n-decanoic acid were reported to inhibit the mycelial growth inhibition of various mycotoxigenic fungal strains.

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DECLARATION

I, Khashane Piet Molele hereby declare that the thesis, which I hereby submit for the degree of Maters of Science (Life Sciences) at the University of South Africa, is my work and has not previously been submitted by me for a degree at this or any other university.

I declare that the thesis does not contain any written work presented by other persons whether written, pictures, graphs or data, or any other information without acknowledging the source.

I declare that where words from a written source have been used the words have been paraphrased and referenced and where exact words from a source have been placed inside quotation marks and referenced.

I declare that I have not copied and pasted any information from the internet, without specifically acknowledging the source and have inserted appropriate references to these sources in the reference section of the thesis.

I declare that during my study I adhered to the Research Ethics Policy of the University of South Africa, received ethics approval for the duration of my study before the commencement of data gathering, and have not acted outside the approval conditions.

I declare that the content of my thesis has been submitted through an electronic plagiarism detection program before final submission for examination.

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

1. Introduction

Mycotoxins are the low-molecular-weight toxic chemical compounds with reduced volatility. Such compounds are formed as secondary metabolites by some filamentous fungi that colonize crops, in the field (pre-harvest) and or after harvest (post-harvest), and are capable of causing many devastating diseases and may well result in death in both humans and animals through the ingestion of food products derived from these crops (Mongalo *et al*., 2018). The term *mycotoxin* is derived from the Greek word *mycos*, for fungus, and the Latin word *toxicum*, for poison. Crops that are mostly infected by mycotoxin producing fungi includes maize, wheat, rice potatoes and many other staple foods in many African countries (Dikhoba *et al*., 2019).

A number of intrinsic, chemical environmental and physical factors affect the mycotoxin occurrence in food and feed chains (Figure 1.1). Mycotoxins may develop due to poor handling of foods during harvesting, infections during growth and development of such foods. Heavy rains, high moisture content and very high temperature may as well favor the growth of many mycotoxin producing fungal strains (Darwish *et al*., 2014).

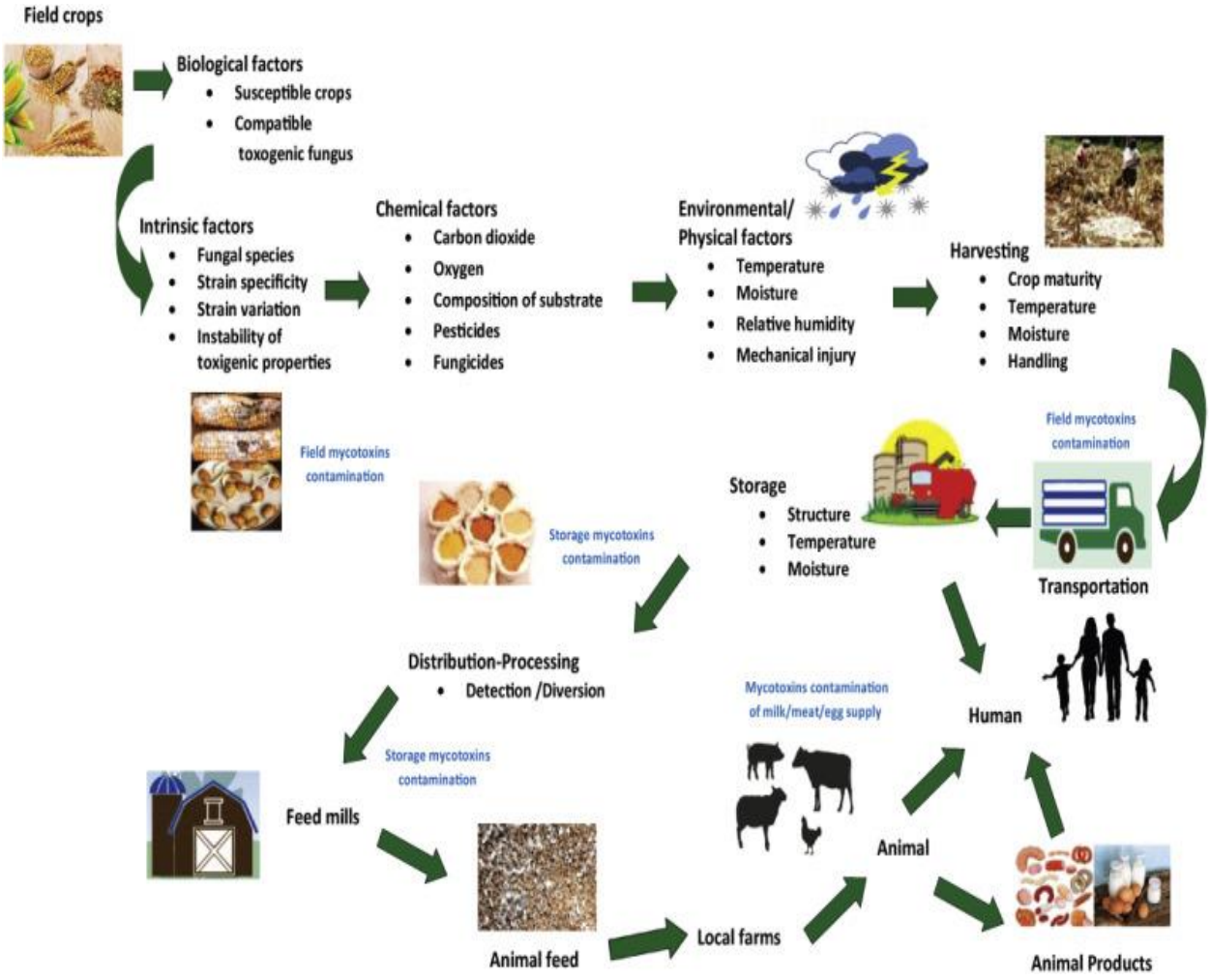

Figure 1.1 Factors affecting mycotoxin occurrence in the food and feed (Haque *et al*., 2020).

These mycotoxins have a negative impact on the growth of such important crops used as major foods. It is estimated that the global food production must increase by 50% to meet the demands of the total world's population by 2050 (Conijn *et al*., 2018). However, these may not be achieved as there is an enormous increase in uncontrollable mycotoxin producing fungal strains and these hampers the economy and farming in many countries (Bhat *et al*., 2010; Chakraborty *et al*., 2011). It is also important to notice that levels of

mycotoxins are regulated differently in different countries and these alone may be a hazard to many other countries that barely depend on farming with less or no quality control of such toxins (Alberts *et al*., 2016)

1.1 Mycotoxins and mycotoxicosis

Mycotoxins are common contaminants of staple food in many developing countries. They are secondary fungal metabolites produced by a number of fungi including members of the genera *Aspergillus, Penicillium, Fusarium, Claviceps* and *Alternari* (Diener *et al*., 1987, Cleveland *et al*., 2003; Dawson *et al*., 2004). The intake of foodstuff contaminated with mycotoxins causes mycotoxicoses (Etcheverry *et al*., 2002; Bullerman and Bianchini, 2007; Emri *et al*., 2013;). There are four primary types of toxicity in mycotoxins specifically; acute, chronic, mutagenic and teratogenic (Pitt, 2000). These contaminants have adverse effects on the health of animals and human. Acute mycotoxin poisoning is characterised by deterioration of liver and kidney functions which in many cases triggers death. Add all the other primary types Mycotoxins enters the body mainly through ingestion of food which is contaminated, poor by inhalation of organic dust containing moulds (Brera *et al*, 1998).

1.2 Classes of mycotoxins

Aflatoxin B_1, trichothecense, zearalenone, and fumonisin B_1 are the main mycotoxins that induce toxicities in animals and human (Coulombe, 1993). The crucial mycotoxins are aflatoxins, ochratoxin A, fumonisins, and trichothecenes. Aflatoxins are succession of metabolites produced by *Aspergillus flavus* (Adye and Mateles, 1964). Ochratoxin A is considered first important mycotoxin due to its nephrotic effects (Cabanes *et al*., 2002). Fumonisins are the mycotoxins produced by *Fusarium moniliforme*. The important fumonisins in terms of their toxicity are fumonisins B_1 and B_2 although fumonisins B_3, B_4, A_1, and A_2 take place in small quantities (Periaca *et al*., 1999). Trichothecence are mycotoxins that are produced by different kind of moulds. They represent the greater cluster of mycotoxins specifically belonging to the *Fusarium* class. Toxic effects induced by trichothecenes results from the prevention of eukaryotic protein synthesis. According to the epidemiological surveys the prevalent trichothecence A and B kind are extensively disseminated in cereals as natural contaminants, while in general constant trichothecenes are hardly found in food and feed (Binder, 2007).

1.3 Effects of mycotoxins on animal and human health

The intake of aflatoxins by animals results in liver, kidney damage, decrease tissue strength, delay blood clotting and exposure to bruising. The aflatoxin also reduces tolerance to diseases like bacterial, fungal, viral and parasitic diseases in pigs by interfering with the cellular and humoral immune system (Diekman and Green, 1992). Fumonisins causes several effects on animals, which include, increase relative weight of

gizzard bursa of fabricus and heart, it decreases feed consumption, oral lesions in broiler chickens. In cattle fumonisins may cause variety of illnesses that includes anorexia, gastro-intestinal lesions, diarrhoea, and reduce milk production. Fumonisins may also lower food intake in lambs and may result in death (Montiel *et al.,* 2003).

The regular ingestion of high quantities of food contaminated with aflatoxin by human and animals results in aflatoxicosis, liver cancer and aspergillosis. It also causes Turkey X disease in poultry (Franseschi *et al*., 1990; Hussein and Brasel, 2001). The intake of food contaminated with aflatoxin has been associated with hepatotoxicity, teratogenicity, immunotoxicity and it has been proved to cause death in animals and human (Huwig *et al*., 2001; Duran *et al.,* 2007*)*. *Ochratoxin* causes lymphoid necrosis in domestic animals *(*Cooray, 1984).

1.4 Exposure to mycotoxins in developing countries

Mycotoxins affect the diet of large proportions of the world's population. In most developing countries, mycotoxins contaminate staple foods, which include groundnuts (peanuts), maize (corn), cereals, nuts, and the exposure is constant and often at high levels (Ncube, 2008; Waalwijk *et al*., 2008; Ekwomadu *et al*., 2018). In rural areas where the dietary staple foods are cereal grains, continuous consumption of contaminated food by human and animals results in impaired growth and development, gastro-interstinal pain, inflammation, diarrhoea and immune dysfunction (Stark *et al*., 2013). The indirect exposure to mycotoxin through the consumption of animal products that are exposed to

contaminated feed result in health risk leading to many other illnesses and death (Peraica *et al*., 1991 and 2001; Bankole and Adebanjo, 2004; Petska, 2007). High mycotoxins incidents such as acute poisoning outbreaks are common in developing countries and may cause diarrhoea in many instances (Bryden, 2007).

The high exposure to mycotoxin in developing countries is primarily due to lack of adequate regulation of foods in local markets and because food safety and quality assurance legislation is not fully enforced by the authorities. Most foods that are grown in rural areas are consumed regardless of their quality because of food insecurities and the excessive consumption of same diet (Shephard, 2008). The knowledge of risk and implication that results from the exposure to mycotoxins is not well documented and communicated with the people in developing countries (Wild and Gong, 2010).

The approaches which are needed to control mycotoxin contamination are regarded as possibly, complex; which requires numerous intervention points pre-and post-harvest. The main problem with the mycotoxins is that they interfere with agriculture, health as well as economic perspective (Campbell and Stollof, 1974). The highest exposures to mycotoxins normally occur in areas that produce and consume their own food and this may results in an extreme ineffectiveness in the regulatory measures to control mycotoxin exposure.

1.5 Control and management of mycotoxigenic fungi

Several methods are employed to reduce the contact of human and animals with mycotoxins. This includes examination of cultivars, which are not at risk to toxin production, control of insects and weeds and cultural practice at different farms (Kung *et al*., 1997; Torres *et al*., 2003). Lemongrass oil has been identified as fungi toxicant used after the harvesting process, used on higher plant, which is suitable for the protection of foodstuffs against storage fungi (Mishra and Dubey, 1994). The primary means to which postharvest diseases are controlled is through synthetic fungicides. There are other alternatives in terms of approaches globally, for safety and eco-friendliness which is the use of the microbial antagonists such as yeast, fungi and bacteria. The microbial antagonists are naturally found in the surface on fruits and vegetables and after separation are used to control the post-harvest diseases in fruits and vegetables (Madeiros *et al*., 2004; Sharma *et al*., 2009). Yet, in contrast, there are vaccination programmes which can be used to reduce the mycotoxin; though in low-income countries may be reasonably low (Fotakis and Timbrel, 2006; Milus and Parsons, 1994).

One of the approaches is the usage of antagonistic microorganisms, which aims at controlling mycotoxigenic fungi. This technique has been applied from the previous decades and success has been achieved regarding the management of these pathogens (Velmourougane *et al*., 2011). More effective fungicides are needed for the control of mycotoxins. The rate at which fungicides must be applied needs to be well monitored as it may lead to an increase in mycotoxins (Matthies *et al*., 1999; Richard, 2007). Though

not fully effective, application of fungicides are commonly used methods (Nicholson *et al*., 2003).

1.6 Plants as sources of antifungal compounds

Plants are considered to be a rich source of secondary metabolites which are pharmacologically active compounds recognised by pharmacologists to have reactions towards sickness. South Africa has a rich plant diversity of approximately 24 000 plant species comprising more than 10% of the world's plant flora. The enormous chemical diversity of plant secondary metabolites presents a valuable resource for possible development of new pharmaceuticals (Cox and Balick, 1994). Plants offer excellent perspectives for the discovery of new therapeutic products, some of which can be developed as bio-fungicides (for prevention of contamination) and nutraceuticals (for prevention of mycotoxicosis). These plant-based bio fungicides may have low to no toxic effects (Manker, 2012). The nutraceuticals can be used in functional foods not only for the prevention and treatment of mycotoxicosis but also for the prevention of mutation related diseases.

Plants play a major in the treatment and or management of human and animal diseases (Principle, 1991). Plants have been targets for the discovery of new drugs and also the synthesis of useful compounds (Thirumalai *et al.*, 2009; Rates, 2001). Over the years, it was discovered that human beings have relied vastly on medicinal plants, as sources of medicine and food storage until the middle of 20th century, where a turnaround was met as researchers favoured the use of synthetic chemicals for curing diseases (Hossain *et*

al., 2015). However, in modern times, people are now in favour of the usage of medicinal plants as they contain natural products, which are believed to be having fewer side effects compared to synthetic chemicals (Sultana *et al*., 2015). Moreover, such plants are readily available in most communities and are inexpensive compared to modern drugs.

1.7 The potential of medicinal plants in preventing mycotoxigenic fungal growth.

In recent years, there has been an enormous interest in the investigation of possible plant-based compounds which could inhibit the growth of mycotoxigenic fungi, thereby mitigating their devastating economic and health effects (Cragg and Newman, 2013; Makhuvele *et al.,* 2020). In our laboratories, in our quest to create a major library of possible plant species that could be a source of compounds that could inhibit the growth of such fungal strains, we investigated several plants species randomly selected from the wild and protected areas (Molele *et al*., 2016; Mongalo and Makhafola, 2018; Dikhoba *et al.,* 2019). Shortly, in these studies we found that the acetone and 1:1 methanol: dichloromethane extracts exhibited a potential anti-mycotoxigenic activity against a plethora of fungal strains. Furthermore, the extracts contained a variety of phytochemicals which includes triterpenes, alkaloids, terpenes, fatty acids and other classes of compounds.

There is a notable interest on research in finding plant-based sources as antifungal agents against agricultural crops (Gakhukar, 2018; Makhuvele *et al*., 2020). A number of African medicinal plants have been evaluated for antifungal activity against crop infecting

fungal strains (Thompson, 1994; Afolayan *et al*., 2002; Kotze *et al*., 2002; Eloff *et al*., 2007; Satish *et al.,* 2007; Askun *et al*., 2008; Waalwijk *et al*., 2008; Mahlo *et al*., 2010; Thembo *et al*., 2010; Ncube *et al*., 2011; Sittisart *et al.,* 2017; Alotibi *et al*., 2020; Seepe *et al*., 2020; Sulub-Tun *et al.,* 2020). Various extracts from *Harpephyllum caffrum* and *Combretum erythrophyllum* showed strong synergistic antifungal activity against *Fusarium graminearum*, *Fusarium proliferatum* and *Fusarium verticillioides* exhibiting MIC values ranging from 0.001 to 0.02 mg/ml (Seepe *et al*., 2020). Besides plant extracts, essential oils may also serve as possible inhibitors of mycotoxigenic fungi (Soliman and Badeaa, 2002; Bluma *et al*., 2008; Atif *et al.,* 2020).

Although there is a growing interest in studying mycotoxigenic fungal strains, most research work focuses of *in vitro* studies, not *in vivo* (Favre-Godal *et al*., 2014). Furthermore, the safety efficacy and standards of such potential medicinal plants extracts, essential oils and other botanicals have been overlooked (Mongalo *et al*., 2018; Dikhoba *et al*., 2019). According to Makhafola *et al*. (2014), the genotoxicity of such plant species needs to be explored as well.

Some authors have further explored the possible use of metabolomics and molecular interventions in finding phytocompounds with anti-mycotoxigenic potential (Choudhary *et al.,* 2021).

1.8 Selected mycotoxigenic fungi for this study

The selected mycotoxigenic fungi for this study are *Aspergillus flavus, Aspergillus ochraceus, Aspergillus vercitilloides, Fusarium oxysporum, Fusarium graminearum, and Fusarium verticillioides. These fungal strains* are of a great health, agricultural and economic importance (Giorni *et al*., 2007; Giorni *et al*., 2008). The focus is on the carcinogenic mycotoxigenic fungi that produce the most potent hepatocarcinogenic mycotoxins. These includes fungal species that produce ochratoxin A, which is known to be carcinogenic in rodents and nephrotoxic in humans, although its genotoxic power has so far not been definitively established (Klich and Mullaney, 1987; Klich and Pitt, 1988; Bilal *et al.,* 2014). Zearalenone is produced by various species of *Fusarium,* in particular *F. graminearum,* which has anestrogenous action and is significantly toxic to the reproductive system of animals; thetricothecens are a group of numerous metabolites produced by *Fusarium species* (Boonen *et al*., 2012).

1.9 Selected medicinal plants species

The six indigenous plant tree species (Table 1.1), from five different families have been randomly selected in the Lowveld Botanical garden (30˚57´58.16˝E 25˚26´42.61˝S Long 30.96800 Lat -2544669) in the Mpumalanga province, South Africa, in March 2016. The plants were collected with the help of a senior curator (MR Willem Froneman) and a junior technician, MR Mandla Nkosi (National Biodiversity Institute, RSA). The leaves were collected, and the voucher specimen were deposited into University of South Africa Herbarium (Unisa, Florida Science Campus). The photographs of the plant species used

in the current work were taken by Molele P.K at the Lowveld Botanical Gardens. Others were obtained from the herbarium at the Botanical Garden through MR Mandla Nkosi.

Table 1.1 The selected medicinal plant species and their voucher numbers.

Plant Scientific names	**Family**	**Common names**	**Voucher number**
Strychnos mitis S. Moore	Loganiaceae	Mokwakwa (S); Umanono (X); Umnono (Z); Umqalothi (Z); Yellow Bitterberry (E)	MPK-01
Spirostachys afracana Sond.	Euphorbiaceae	Tamboti (E); Tambotie (A); umThombothi (Z); Modiba (S); Ndzopfori (T); Muonze (V)	MPK-02
Loxostylis alata A. Spreng. ex Rchb.	Anacardiaceae	Isibara (Z); Isibara (X); Tigerwood (E), Wildepeperboom (A), Breekhout (A)	MPK-03
Maytenus undata (Thunb.) Blakelock	Celestraceae	Koko Tree (E); Idohame (Z); Indabulaluvalo (Z); Inqayi-elibomvu (X); Koko (A); Kokoboom (A)	MPK-04
Mystroxylon aethiopicum (Thunb.) Loes. *subsp. Aethiopicum*	Celestraceae	Barsbessie (A); Bushveld Cherry (E); Cape Cherry (E); Monamane (S); Mukwatikwati (V); Mukwatule (V); Umgxube (X); Umnqayi (X)	MPK-05
Dracaena mannii Baker	Ruscaceae	Insundwana (Z); Kleinblaar-drakeboom (A); Kosi Dragon Tree (E); Small-leaved Dragon Tree (E)	MPK-06

Key: S-Sepedi; A-Afrikaans; T, Tsonga; E-English; X-Xhosa; Z-Zulu; E, English; V, Venda (TshiVenda)

1.9.1 *Strychnos mitis* S. Moore

It is a medium sized to tall tree of about 7 to 40 meters in height, occurring mostly in bushvelds, evergreen forest and rocky woodland areas, with its elliptic to obovate leaves which are shiny dark and leathery (Van Wyk *et al.,* 1997). The leaves are also base tapering, margin entire and possess a petiole which may be 2-5 cm long (Figure 1.2). The stem bark is greyish and may be multi-stemmed at times. The fruit if greenish when raw and brownish to yellow when ripe and its edible.

Figure 1.2 *Strychnos mitis* leaves and fruiting bodies.

1.9.2 Spirostachys afracana Sond.

It is a semi-deciduous to deciduous tree of up to 18 meters in height, with all its body parts exuding a toxic milky latex (Deutschländer *et al.,* 2009). Its distributed from Tanzania to South of KwaZulu-Natal and its fruiting time is from October to February. Its leaves are alternate, simple and posess toothed margins (Figure 1.3). The stem is dark grey and posses some rectangular blocks which are arranged in rows when matured.

Figure 1.3 *Spirostachys africa* leaves, fruits its full tree.

1.9.3 *Loxostylis alata* A. Spreng. ex Rchb.

It is a small tree with a height of up to 5 m in height, a pale grey stem bark with some fissures and imparipinately compound leaves (Palgrave, 2015). Its flowers are different in color and explains whether it's a male or female tree. (Figure 1.4).

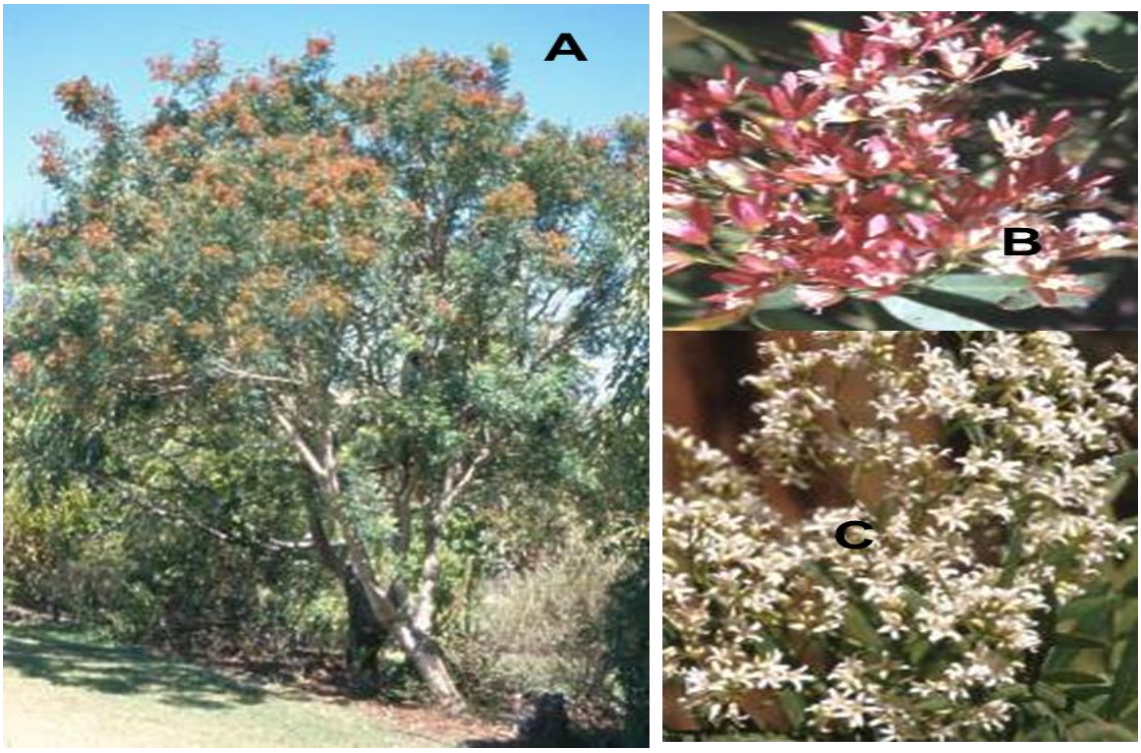

Figure 1.4 Photograph of *Loxostylis alata* tree (A), female flowers (B) and male flowers (C).

The plant species is distributed mostly in KwaZulu-Natal and Eastern cape provinces with its small fleshy fruits and brightly colored sepals.

1.9.4 *Maytenus undata* (Thunb.) Blakelock

The plant species commonly known as Kokoboom is a multi-stemmed shrub with an average height of 2 to 3 m, almost circular toothed leaves that are pale green (Palgrave, 2015) and possess a three lobed fruit capsule which is whitish to yellow in color when ripe (Figure 1.5).

Figure 1.5. *Maytenus undata* fruits and leaves.

It is important to notice that although completely serrate, the plant species possess different forms of leaf arrangement.

1.9.5 Mystroxylon aethiopicum (Thunb.) Loes. subsp. Aethiopicum

It is an evergreen shrub or a tree of about 6 meters in height with a roundish crown. Its leaves are simple, elliptic to obovate in shape, alternate, finely toothed margins and usually opposite on larger trees with a pointed tip, leathery light green and a bit glossy above and darker underneath (Figure 1.6). The stem bark is dark grey, fissured in larger trees and rough, while the flowers are yellowish and appear on short stalks in clusters (Meyer, 2003; Deutschländer *et al.,* 2009). Its fruit is a drupe, edible and very sweet but often sour and bitter. The plant species is distributed along the coastal belt from Western

Cape Province to Limpopo Province and extends further to Mozambique, Swaziland and Zimbabwe (Palgrave, 2015).

Figure 1.6 *Mystroxylon aethiopicum* (Thunb.) Loes. subsp. *Aethiopicum*

1.9.6 Dracaena mannii Baker

It is a shrub or small tree with a maximum height of 12 meters. Its leaves are narrow, dark green, shiny and ends in branches (Figure 1.7). The stem is whitish, smooth and develop some papery peel (Palgrave, 2015). The flowers are creamy in colour and appear and loose spikes or branched while the fruits are fleshy and bright red when matured.

Figure 1.7 *Dracaena mannii* leaves and fruits

1.10 Problem statement

Major health problems in animals and human are associated with the ingestion and contact with mycotoxins. Mycotoxins have different acute and chronic effects on the animals and human in relation with the species and susceptibility of the animal or human within the species (Zain, 2011). Exposure to *Fusarium verticillioides* on human diet results in oesophageal cancer and the neural tube defect in some maize consuming population. Aflatoxin in humans cause hepatocellular carcinoma which in turn results in hepatotoxicity

and ochratoxin causes kidney diseases in animals by deterioration of proximal tubules cells in the interstitial nephritis and hyalinization (Wild and Gong, 2010).

Aflatoxins have been linked with the human primary liver cancer which acts together with Hepatitis B Virus infection (Binder, 2007; Shephard, 2008). Ergotism is a disease cause by mycotoxin that affects animals and human consuming the contaminated food with mycotoxin. Animals such as cattle, sheep, pigs, and chickens are the most principal animals at risk. The known clinical symptoms of ergotism in animals include gangrene, abortion, convulsions, and suppression of lactation, hypersensitivity and ataxia. Mycotoxins cause severe animal diseases such as equine leukoencephalomalacia in horses, and hydrothorax and porcine pulmonary edema in swine (PPE).

The use of synthetic drugs in the management of crop infecting is prohibited in most countries through legislation. Furthermore, a number of mycotoxigenic fungi have been found to be highly resistant to such synthetic drugs (Mongalo *et al*., 2018). There is therefore a need to search for alternative sources for the prevention of mycotoxicosis, prevention of contamination of food stuff and feeds with mycotoxins, and agents for the decontamination and detoxification of food commodities. Consumption of feed contaminated with aflatoxin results in the reduced meat quality of farm animals and also the production (Rustom, 1997).

1.11 Objectives of the current study

The current research works aims at

- Investigating the antifungal activity of the selected medicinal plants against a plethora of mycotoxigenic fungal strains.
- To compare the antifungal activity of aqueous and organic extracts of the selected plant species.
- To determine the total activity (TA) of the selected medicinal plants.
- To investigate the *in vitro* cytotoxicity and efficacy of the selected medicinal plants extracts against selected cell lines.
- To determine the selectivity index of the selected plant extracts.
- To determine the possible mode of action of such medicinal plants extracts against pathogens. Investigating the mycelial growth inhibition of the plant extracts with potent antifungal activity.
- To determine the antioxidant activity of the selected medicinal plants extracts.
- To detect and identify the possible compounds responsible for such biological activity.

In the current work, Chapter 2 will present the materials and methods, Chapter 3 reports the results obtained from our laboratory experiments, Chapter 4 discusses the importance and relevance of the results of the current work, chapter 5 reports the general conclusions and recommendations while chapter 6 cites the relevant bibliography used the manuscript.

CHAPTER 2

MATERIALS AND METHODS

2.1 Collection of plant materials

The selected medicinal plants (Table 1.1) were collected from Lowveld botanical Garden, Mpumalanga Province. Vouchers were collected and matched with those in the Herbarium, deposited into University of South Africa herbarium. The collection was for leaves samples only.

2.2 Extraction of plant materials

The dried plant materials of the selected medicinal plants were separately extracted with distilled hot water and 1:1 methanol: dichloromethane (AR Grade) respectively. Aqueous extracts were prepared by boiling plant materials at 1:5 w/v, cooled off on a laboratory bench and filtered through Whatman's No. 1 paper (GE Healthcare Life Sciences, Amersham place, UK). The resulting liquid was frozen and freeze dried in a tabletop freeze drier fitted with sucking pump (Labconco Corporation, Kansas City, Moussouri, USA). The organic extracts were extracted at 1:10 w/v, kept on a Desk shaking incubator (Scientific, USA) overnight at 110 rpm and filtered through Whatman's no1 paper and then concentrated using Buchi rotary evaporator (Bibby Scientific Limited, Stone Staffordshire,

UK). The resulting plant extracts of both aqueous and organic origin were weighed

and then kept in the refrigerator at 4 ºC.

2.3 Antifungal assays

2.3.1 Preparation and maintenance of fungal strains

The selected fungal strains were obtained from Agricultural Research Council as slants. The fungi were inoculated into freshly prepared Potato Dextrose broth (Lab Limited, Heywood, United Kingdom) in a sterile environment for few days until confluent growth is obtained. The strains were further plated and maintained into sterile labelled petri dishes containing Potato Dextrose Agar (Lab Limited, Heywood, United Kingdom) until needed for experimental purposes.

2.3.2 *In Vitro* antifungal activity using microplate assay

The antifungal activity of both aqueous and organic extracts was determined by microplate-dilution method developed by Eloff (1998) with slight modifications. The extracts were tested at 10 mg/ml plant extracts dissolved in 10 % Dimethyl sulphoxide (DMSO) against selected mycotoxigenic pathogenic fungi. Briefly, 100µl of plant extracts were transferred into the first row of wells in a flat bottom sterile 96 well plate (Merck, RSA) laid with 100 µl autoclaved distilled water. The contents in the wells were serially diluted two-fold from wells A-H, while the final 100 µl was decanted. The fungal pathogens were spectrophotometrically adjusted (OD_{625}=0.08 to1) Aliquot of 100 µl of quantified inoculum of each fungal strain was added into all the wells. Amphotericin B and 10 % DMSO were prepared as positive and negative controls respectively. All the wells were

then loaded with 40 µl of 2 mg/ml *p*-Iodonitroterazolium (INT) chloride (Sigma-Aldrich, Germany). The plates were sealed with parafilm and incubated at 30˚C for 48 hours under 100% humidity. Minimum inhibitory concentration was determined as the lowest concentration of plant extract that inhibits microbial growth after 48 hours of incubation. The extracts were tested in triplicates. Results were reported as the mean of the independent replicates.

2.3.2 Determination of Total activity (TA)

The total activity of the extracts was calculated by dividing quantity extracted in milligrams from 1g of plant material by MIC value (mg/ml), as proposed by Masoko *et al.* (2005).

2.3.3 *In Vitro* antifungal activity using mycelial growth inhibition (MGI) assay

The fungal mycelia growth inhibition of the organic extracts of the selected medicinal plants was evaluated by using a method previously applied by Shukla *et al.* (2012) with slight modifications. Briefly, three biological replicates of varying concentrations of plant extracts and Amphotericin B were added into potato dextrose agar petri dishes during preparationto yield the final concentrations of MIC values (i.e MIC, ½ MIC and ¼ MIC concentrations), PDA plates without amendments were used as controls for each tested concentration. The discs of 4mm diameter cut from periphery of fresh and actively growing 4-5 days old fungal strains were aseptically inoculated upside down at the centre of all treatments and control set and a drop (10µl) spore suspensions of all fungal species

was pipetted at the centre of the plates. The plates were then incubated for nine days at 30˚C and the mycelial growth diameter was measured after every three days. Percentage MGI was determined from growth diameter using the formula:

$\%MGI = (\Delta Dc - \Delta Dt)/\Delta Dc \times 100$

Where ΔDc = Average mycelial growth diameter measured at three points on control sets and ΔDt = Average mycelial growth diameter measured at three points in the treatment sets.

2.4 Cytotoxicity studies

2.4.1 *In vitro* cytotoxicity using MTT assay

The cytotoxic effects of the selected medicinal plants organic extracts were evaluated against Vero and Bovine dermal (BD) cell lines using the MTT assay as described by Mosmann (1983). Cells of a sub-confluent culture of each cell line were grown on Minimal Essential Medium (MEM, Whitehead Scientific) supplemented with 0.1% gentamicin (Virbac) and 5% foetal calf serum (Highveld Biological). A cell suspension of about 200 µl containing $5X10^4$ cells per ml was pipetted into each well of columns 2 to 11 of a sterile 96-well micro-titre plate in a Laminar Flow. MEM (200 µL) was added to wells of columns 1 and 12 to minimize the “edge effect” and maintain humidity.

The plates were incubated for 24 h at 37 °C in a 5% CO_2 incubator, until the cells were in the exponential phase of growth. The MEM was aspirated from the cells which were then

washed with 150µL phosphate buffered saline (PBS, Whitehead Scientific) and replaced with 200 µL of the fractions at different concentrations ranging from 7.5 to 1000 µg/mL. The serial dilutions of the test fractions were prepared in MEM. The micro titre plates were incubated at 37 °C in a 5% CO2 incubator for 48 h with test fractions. Untreated cells and positive control (doxorubicin chloride, Pfizer Laboratories) were included in the assay. Each experiment was repeated three times independently. The LC_{50} (concentration of the plant extract that inhibit 50% of cell growth) values were determined from the graphs of the concentration vs % inhibition using the formula below,

Percentage cell inhibition =100 -Abs (Sample) / Abs (Control) x 100 (Mongalo *et al*., 2019). Results were recorded as mean±SE. Selectivity index (SI) values were calculated using formula below.

2.4.2 Determination of selectivity index

The selectivity index of the extracts was calculated using the formula below as proposed by (Fadipe *et al*., 2015).

SI= LC_{50}/ MIC (both units in mg/ml).

The MIC values were obtained from data as evaluated in 2.3.1 (Microdilution assay) while LC_{50} data was extrapolated from 2.4.1 (MTT assay).

2.5 *In Vitro* antioxidant activity

2.5.1 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay

Free radical scavenging activity of the organic extracts from the selected medicinal plants was determined against 2,2-diphenyl-1-picrylhydrazyl (DPPH) using method adopted from Opoku *et al.* (2007) with slight modification. DPPH solution was prepared by dissolving DPPH into methanol (Sigma Aldrich, Germany) to make a working solution of 2 mg/100 ml. Shortly, plant extracts at 10 mg/100 ml (150 µl) were serially diluted with methanol in 96 well plates. Later, DPPH solution (150 µl) was added to all the wells and the plate was placed on a shaker at 80 motions per minute (IKA® KS, Laboratory equipment, Germany) for 10 minutes covered with a foil and ten incubated in the dark for 50 minutes. Wells containing DPPH and methanol were used as sample blank while Ascorbic acid was as a positive control. Absorbance was read at 517 nm using microplate reader (Varioshskan Flash, Thermo Scientific Amsterdam).

2.5.2 2-2′-Azino-di-[3-ethylbenzthiazoline sulfonate (ABTS) assay

Free radical scavenging activity of the organic extracts from the selected medicinal plants was determined against 2-2′-Azino-di-[3-ethylbenzthiazoline sulfonate] (ABTS), using method adopted from Re *et al.* (1999) with slight modification. Shortly, 7 mM of ABTS tablet was diluted with 3 ml sterile distilled water and 2.45 mM potassium persulfate and the mixture which was then incubated at room temperature in the dark for 16 hours. Equal amounts of serially diluted fractions as in DPPH assay were reacted with 150 µl of ABTS

solution and absorbance was measured at 734 nm using micro plate reader (Varioshskan Flash, Thermo Scientific Amsterdam) after 6 minutes of vigorous shaking at 80 motions per minute (IKA® KS, Laboratory equipment, Germany). Ascorbic acid was used as positive control.

2.5.3 Iron chelating activity

The Fe2+ chelating effect of the of the organic extracts from the selected medicinal plants was evaluated according Decker and Welch (1990), with slight modifications. Plant extracts as in DPPH assay were serially diluted with methanol. The extracts in all wells were further diluted with 100 µl of sterile distilled water, 10 µl of $FeCl_2$ (2 mM) and 20 µl of disodium; 4-[3-pyridin-2-yl-6-(4-sulfonatophenyl)-1,2,4-triazin-5-yl]benzenesulfonate (5 mM). The mixture was covered with foil and placed on a shaker at 80 motions per minutes (IKA® KS, Laboratory equipment, Germany) for 10 mins and the absorbance was measured at 562 nm using microplate reader (Varioshskan Flash, Thermo Scientific, Amsterdam). Azelaic acid was used as a positive control.

Percentages of inhibition in all the three antioxidant assays was calculated as

% Scavenging Inhibition = $[1-A_t/A_0] \times 100$,

Where At represents the absorbance of the test sample, while A0 represent absorbance of blank solution. The IC_{50}, concentrations of plant sample that inhibit 50% of test radical, were extrapolated from graphs drawn from concentrations VS % inhibition using Graph Pad Prism software 7.

2.6 Phytochemical analysis of the selected medicinal plants

2.6.1 Gas-chromatography-mass spectrometry (GC-MS) analysis

The GC-MS analysis of organic extracts of the selected medicinal plants species was carried out using method adopted from (Mongalo *et al*., 2019) with slight modification. The little amount of about 0.05 mg/ml of the selected medicinal plants extracts were completely dissolved in acetonitrile (GC-MS grade, Sigma Aldrich, Germany) to a lowest concentration. The separation of compounds was performed on a gas chromatography (7890N GC-MS, Agilent Technologies, Santa Clara, California, USA) coupled to a LECO Pegasus HT Flight Mass Spectrometry Time (TOFMS) obtained from LECO Corporation, Michigan, USA. In short, the prepared samples were loaded into a Gerstel MPS2 Liquid/HS/SPME Auto-sampler. For the chromatographic separation, AJ and W capillary column HP-5MS 30 X 0.25mm I.D with the film thickness of about 0.25 µM was used.

The following conditions were applied in the chromatographic separation: About 1 µL of the sample was injected at 250 ºC with a splitless injector. The GC oven was programmed at 80 º for 1 min, then ramped of at 10 ºC per min to 280 ºC for 20 mins. Helium obtained from Afrox (Johannesburg, RSA) at 99.99% purity was used as a carrier at a constant flow of 1 ml/min. The interface temperature of the GC/TOFMS was set at 280 ºC and the mass spectra were obtained in full scan mode at 70ev (m/z scan varying from 50 to 550). The collection of data was obtained using ChromaTOF, which possess a NIST 95 library for compounds matches.

CHAPTER 3

RESULTS

3.1 Data analysis

In the antifungal assays, the results are reported as mean of the three independent experiments (n=3), while in the cytotoxicity and mycelial growth inhibition studies the results are reported as mean±SE. In the antioxidant activity, the results were analysed using Graph Pad Prism Version 7. *P*-values ≤0.05 were referred to as significant. In the phytochemical analysis, the results were populated and analysed by ChromaTOF, which possess a NIST 95 library for compounds matches.

3.2 Percentage yields, antifungal and total activity

The aqueous extract from *Maytenus undata* and *Dracaea mannii* exhibited the highest % yields of 31.09 and 29.33 respectively, compared with organic extracts (Figure 1.1). The plant materials exhibited higher percentage yields when extracted with water compared to 1: 1 methanol: dichloromethane. Organic extracts from *Loxoxstylis alata* and *Dracaea mannii* exhibited higher % yields of 17.74 and 15.65 respectively compared to organic extracts from other selected medicinal plants.

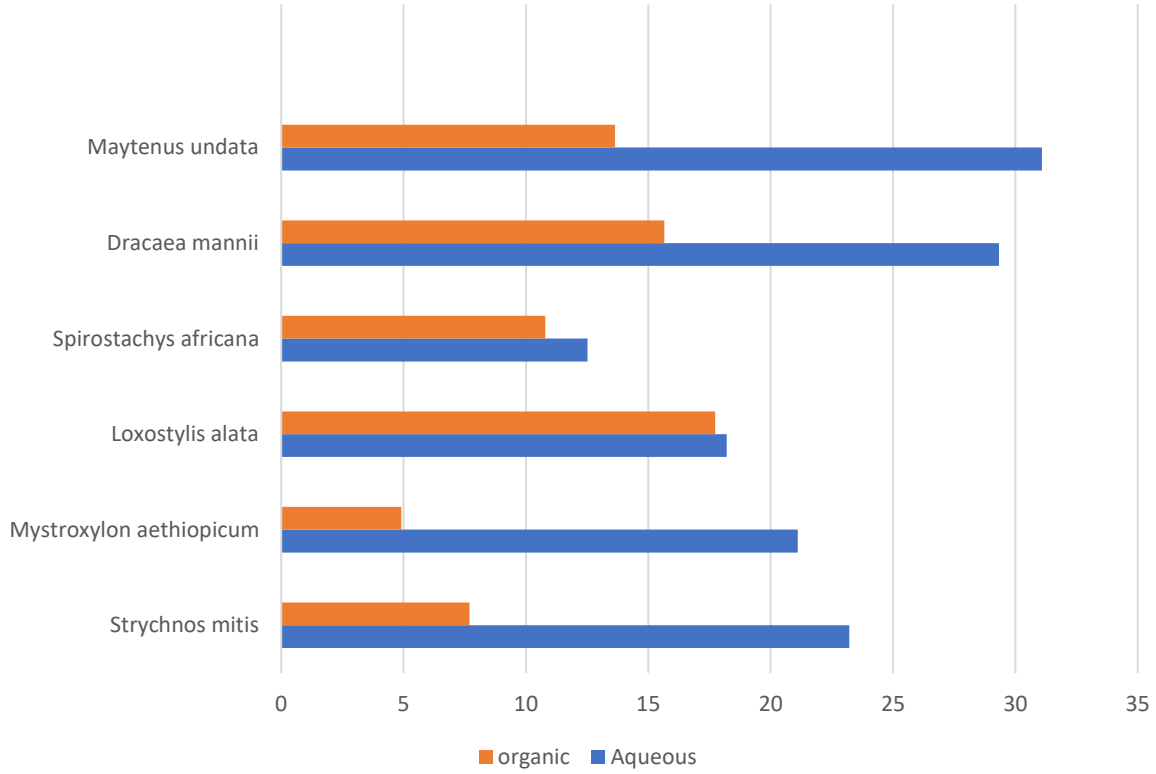

Figure 3.1 Percentage yields of the selected medicinal plants extracted with hot water and 1:1 methanol: dichloromethane

Organic extracts from *Strychnos mitis* exhibited a notable lowest minimum inhibitory concentration (MIC) value of 0.01 mg/ml against *Furasium oxysporum*, *Furasium verticilloides* and *Furasium graminareum* at 24 hr incubation period (Table 3.1). Organic extract from *Loxoxstylis alata* exhibited a similar MIC value of 0.01 against A. parasiticus and MIC value of 0.04 mg/ml against *F. oxysporum, F. graminareum and A. parasiticus* at 48 hr incubation period. Furthermore, organic extracts from *Strychnos mitis* similar extract further exhibited MIC value of 0.08 mg/ml against *Aspergillus parasiticus*, *Aspergillus flavus* and *Aspergillus ochraceous* at similar incubation period. Organic

extracts from *Dracaea mannii* and *Mystroxylon aethiopicum* exhibited an MIC value of 0.04 and 0.02 against *A. parasiticus* at 24 hr incubation period respectively.

Aqueous extract from *Maytenus undata* exhibited an MIC value of 0.04 mg/ml against both *F. vercitilloides* and *A. parasiticus* at 24 hr incubation time, while aqueous extract from *S. africana* exhibited an MIC value of 0.04 against *F. vercitilloides* and A. *parasiticus* at 48 hr incubation time. Aqueous extract from *S. mitis* exhibited an MIC value of 0.02 against *F. graminareum* at 48hr incubation time, while *M. undata* exhibited an MIC value of 0.04 mg/ml against both *F. vercitilloides* and *A. parasiticus* at 24 hr incubation time.

The total activity (TA) of the organic extracts against the selected mycotoxigenic fungal strains range from 8 to 9375, while the overall highest TA of the selected medicinal plants was obtained against *A. parasiticus* at 24 hr incubation period, ranging from 962 to 9375 (Table 3.2). S. mitis exhibited a notable TA ranging from 99 to 7692 against the selected mycotoxigenic pathogens at both 24 and 48 hr incubation period.

Table 3.1 Antifungal activities (MIC in mg/ml) of selected medicinal plant extracts

Medicinal plants	Extracts	Selected mycotoxigenc fungal strains												Average MIC value
		Furasium verticilloides		*Furasium graminareum*		*Furasium oxysporum*		*Aspergillus parasiticus*		*Aspergillus flavus*		*Aspergillus ochraceous*		
		24 hr	**48 hr**	**24 hr**	**48 hr**	**24 hr**	**48 hr**	**24 hr**	**48 hr**	**24hr**	**48 hr**	**24 hr**	**48 hr**	
Dracaena mannii	Organic	1.56	0.78	0.39	0.39	0.78	0.78	**0.04**	**0.04**	0.39	0.39	1.56	6.25	1.11
	Aqueous	**0.08**	0.16	0.16	0.78	3.13	6.25	0.16	0.78	3.13	6.25	6.25	6.25	2.78
Mystroxylon aethiopicum	Organic	**0.08**	0.39	0.39	0.78	0.39	0.39	**0.02**	0.78	0.16	6.25	3.13	6.25	1.58
	Aqueous	0.39	3.13	6.25	12.5	1.56	3.13	**0.02**	**0.02**	3.13	6.25	3.13	3.13	3.55
Maytenus undata	Organic	0.78	0.78	0.78	0.78	1.56	0.78	0.16	0.39	0.16	0.39	0.78	0.78	0.68
	Aqueous	**0.04**	**0.04**	0.20	0.78	1.56	1.56	**0.04**	0.78	1.56	6.25	**0.08**	0.39	1.11
Loxostylis alata	Organic	0.39	0.39	0.16	**0.04**	0.16	**0.04**	**0.01**	**0.04**	**0.08**	0.39	0.39	0.39	**0.21**
	Aqueous	3.13	3.13	0.78	3.13	**0.04**	00.39	0.16	0.78	6.25	6.25	0.39	6.25	2.56
Spirostachys afracana	Organic	0.39	0.16	0.63	0.16	0.16	0.78	**0.04**	**0.04**	0.16	6.25	0.39	0.39	0.80
	Aqueous	**0.04**	**0.04**	**0.01**	**0.02**	0.39	0.78	**0.02**	**0.04**	0.78	0.78	0.78	3.13	0.57
Strychnos mitis	Organic	**0.01**	**0.08**	**0.01**	**0.01**	**0.01**	**0.06**	**0.08**	**0.04**	**0.08**	0.78	**0.08**	0.78	**0.17**
	Aqueous	0.16	0.78	**0.08**	**0.02**	6.25	6.25	0.39	6.25	0.78	0.78	0.16	0.16	1.84
Control drug (Amphotericin B)		0.006	0.004	0.004	0.004	0.002	0.004	0.008	0.008	0.002	0.002	0.008	0.008	0.005

Key: Bold-faceted data shows plant extract with noteworthy activity against selected microbes at different incubation periods.

3.3 Cytotoxicity studies and selectivity index

The cytotoxicity studies of selected medicinal plants against Bovine dermis (BD) and Vero cells (VC) are reported in Table 3.2. The extracts exhibited some varying degrees of toxicity, with *Spirostachys africana* yielding a lowest LC_{50} value of 0.10 mg/ml against the selected two cell lines. Both *Strychnos mitis* and *Dracaena mannii* exhibited the highest LC_{50} value of 0.88mg/ml against VC and BD respectively, while organic extract from *Maytenus undata* yielded LC_{50} value of 0.55±0.22 mg/ml against BD cells. The BD cells were more susceptible to doxorubicin, a control drug used in the experimental setup, with LC_{50} value of 0.002±0.00 mg/ml compared to VC with an LC_{50} value of 0.004±0.00 mg/ml.

The selectivity index (SI) values of the selected medicinal plants were reported (Table 3.4 and 3.5). *S. mitis* against *Furasium graminareum* (VC) exhibited the highest SI value of 88.0 compared to other plant species, while *Mystroxylon aethiopicum* revealed lowest SI value of 0.00 against both *Aspergillus flavus* and *Aspergillus ochraceous* when using LC_{50} obtained against VC. In the BD cells, *Dracaea mannii* and *S. mitis* exhibited the higher SI values of 22.0and 18.0 against *Aspergillus parasiticus* and *F. graminareum* respectively.

Table 3.2 Cytotoxicity studies (LC_{50} in mg/ml) of the selected organic extracts against the selected cell lines

Selected cell lines	**Medicinal plants**						
	Dracaena mannii	*Mystroxylon aethiopicum*	*Maytenus undata*	*Loxostylis alata*	*Spirostachys afracana*	*Strychnos mitis*	**Doxorubucin (Positive control)**
Bovine dermis cells	0.88±0.12	0.49±0.34	0.64±0.01*	0.55±0.22	0.10±0.01*	0.18±0.02*	0.002±0.00*
Vero cells	0.77±0.01*	0.73±0.09	0.11±0.00*	0.19±0.07	0.10±0.00*	0.88±0.01*	0.004±0.00*

P-values ≤0.05 were significant and marked (*)

Table 3.3 Total activity of organic extracts of the selected medicinal plants

Medicinal plants	**Selected mycotoxigenc fungal strains**											
	Furasium verticilloides		*Furasium graminareum*		*Furasium oxysporum*		*Aspergillus parasiticus*		*Aspergillus flavus*		*Aspergillus ochraceous*	
	24 hr	**48 hr**	**24 hr**	**48 hr**	**24 hr**	**48 hr**	**24 hr**	**48 hr**	**24hr**	**48 hr**	**24 hr**	**48 hr**
Dracaena mannii	100	200	400	400	200	200	**3900**	**3900**	400	400	100	25
Mystroxylon aethiopicum	**613**	126	126	63	126	126	**2452**	63	307	8	16	8
Maytenus undata	174	174	174	174	87	174	**850**	348	**850**	348	174	174
Loxostylis alata	240	240	586	**2344**	586	**2344**	**9375**	**2344**	**1172**	240	240	240
Spirostachys afracana	277	675	171	675	675	138	**2699**	**2699**	675	17	277	277
Strychnos mitis	**7692**	**962**	**7692**	**7692**	**7692**	**1282**	**962**	**1923**	**962**	99	**962**	99

Table 3.4 Selectivity index (SI) of the selected medicinal plants at 48 hr incubation period against Bovine dermis cell line.

	Dracaena mannii	*Mystroxylon aethiopicum*	*Maytenus undata*	*Loxostylis alata*	*Spirostachys afracana*	*Strychnos mitis*
Bovine dermis cells	0.88±0.12	0.49±0.34	0.64±0.01*	0.55±0.22	0.10±0.01*	0.18±0.02*
Fungal strains and SI values (Bovine dermis)						
Fusarium verticilloides	1.13	1.26	0.82	1.41	0.63	**2.25**
Furasium graminareum	**2.26**	0.63	0.82	**13.75**	0.63	**18.0**
Furasium oxysporum	1.13	1.26	0.82	**13.75**	0.13	**3.0**
Aspergillus parasiticus	**22.0**	0.63	1.64	**13.75**	**2.5**	**4.50**
Aspergillus flavus	**2.26**	0.08	1.64	1.41	0.02	0.23
Aspergillus ochraceous	0.14	0.08	0.82	1.41	0.26	0.23

Bold-faceted data shows noteworthy SI values(>2.0).

Table 3.5 Selectivity index (SI) of the selected medicinal plants at 48 hr incubation period against Vero cells.

	Dracaena mannii	*Mystroxylon aethiopicum*	*Maytenus undata*	*Loxostylis alata*	*Spirostachys afracana*	*Strychnos mitis*
Vero cells	0.77±0.01	0.73±0.09	0.11±0.00	0.19±0.07	0.10±0.00	0.88±0.01
Fungal strains and SI values (Bovine dermis)						
Fusarium verticilloides	0.99	1.87	0.14	0.49	0.63	**11.0**
Furasium graminareum	1.97	0.94	0.14	**4.75**	0.63	**88.0**
Furasium oxysporum	0.99	1.87	0.14	**4.75**	0.13	**14.67**
Aspergillus parasiticus	**19.25**	0.94	0.28	**4.75**	**2.5**	**22.0**
Aspergillus flavus	1.97	0.00	0.28	0.49	0.02	1.13
Aspergillus ochraceous	0.12	0.00	0.14	0.49	0.26	1.13

Bold-faceted data shows noteworthy SI values(>2.0).

3.4 Mycelial growth inhibition

The mycelial growth inhibition (MGI) of the selected organic extracts was evaluated against most susceptible mycotoxigenic fungal strains such as *Fusarium vercitilloides* (Table 3.6), *Fusarium graminareum* (Table 3.7), *Aspergillus parasiticus* (Table 3.8) and *Aspergillus ochraceous* (Table 3.9). *Mystroxylon aethiopicum* exhibited the highest MGI values of 44.38±0.01, 33.73±0.08 and 33.73±0.08 percent against *F. vercitilloides, F. graminareum* and and *A parasiticus* respectively at 3 days incubation period MIC. At 0.5 MIC, the extract further exhibited a noteworthy MGI value of 25.21±0.08 against both A. parasiticus and *F. graminareum* in a 3 days incubation period.

Spirostachys africana and *Strychnos mitis* exhibited a notable percent MGI against *Aspergillus ochraceous* in a 3 days incubation period at MIC, 0.5 MIC and 0.25 MIC. S. Africana further exhibited notable percent MGI values of 30.01±0.08 and 21.21±0.06 against *A. ochraceous* at MIC and 0.5 MIC in 6 days incubation period. Overall, the percentage MGI decreased with an increase in incubation period at all levels. All the selected pathogens were susceptible to Amphotericin B, which exhibited the highest % MGI of 76.99±0.44 against *F. graminareum* at MIC in a three days incubation period.

Table 3.6 Percentage Mycelia Growth Inhibition of varying concentrations of acetone extracts on *Furasium vercitilloides.*

Medicinal plants	**3 days incubation**			**6 days incubation**			**9 days incubation**		
	MIC	**0.5 MIC**	**0.25 MIC**	**MIC**	**0.5 MIC**	**0.25 MIC**	**MIC**	**0.5 MIC**	**0.25 MIC**
Dracaea mannii	8.22±0.22	5.91±0.20	5.67±0.99	5.24±0.01	4.44±0.33	1.94±0.10	1.11±0.05	0.20±0.85	0.11±0.00
Mystroxylon aethiopicum	**44.38±0.01**	**28.66±0.01**	11.92±0.52	**30.08±0.02**	10.11±1.00	5.55±0.79	7.33±0.96	7.33±0.10	2.95±0.06
Maytenus undata	**34.66±0.01**	**34.12±0.77**	**21.33±1.22**	15.33±1.81	15.44±0.01	12.11±0.88	8.16±0.00	1.99±0.00	1.11±0.00
Loxostylis alata	15.02±0.37	14.55±0.05	18.55±0.09	**20.66±1.25**	**20.44±0.18**	19.84±1.01	15.81±0.71	8.13±0.69	12.40±0.77
Spyrostachys Africana	5.82±0.08	6.66±0.11	4.12±0.11	1.88±0.16	1.14±0.00	1.33±0.00	1.1±0.01	1.1±0.01	1.11±0.00
Strychnos mitis	**36.66±0.04**	**34.68±0.88**	**33.91±0.01**	**31.22±1.11**	**28.14±0.08**	**26.84±0.01**	19.88±0.01	16.21±0.17	13.66±0.09
Amphotericin B	**69.99±0.08**	**63.12±0.44**	**54.45±0.22**	**53.33±0.00**	**42.81±0.40**	**41.78±0.11**	**39.78±0.16**	**39.44±0.17**	**36.56±0.19**

Bold faceted data shows noteworthy mycelial growth inhibition

Table 3.7 Percentage Mycelia Growth Inhibition of varying concentrations of acetone extracts on *Furasium graminareum.*

Medicinal plants	**3 days incubation**			**6 days incubation**			**9 days incubation**		
	MIC	**0.5 MIC**	**0.25 MIC**	**MIC**	**0.5 MIC**	**0.25 MIC**	**MIC**	**0.5 MIC**	**0.25 MIC**
Dracaea mannii	18.66±0.00	14.33±0.17	11.88±0.21	11.34±0.09	4.99±0.96	3.21±0.88	2.82±0.44	2.05±0.08	1.88±0.04
Mystroxylon aethiopicum	**33.73±0.08**	**25.21±0.08**	**20.04±0.60**	15.11±0.90	10.98±0.77	8.40±0.09	7.05±0.10	7.00±0.04	2.11±0.71
Maytenus undata	12.99±0.01	12.11±0.12	10.33±0.91	10.33±0.01	6.11±0.31	5.11±0.18	1.22±0.34	1.99±0.20	1.11±0.10
Loxostylis alata	9.22±0.22	8.11±0.06	8.05±0.00	6.96±0.20	5.21±0.08	2.14±0.01	2.11±0.01	1.11±0.09	1.10±0.07
Spyrostachys Africana	9.12±0.04	8.96±0.01	7.72±0.00	4.08±0.06	3.14±0.00	3.03±0.00	3.0.1±0.00	1.15±0.01	1.01±0.00
Strychnos mitis	**22.60±0.01**	**21.08±0.88**	16.91±0.81	11.22±0.11	8.94±0.02	5.81±0.11	2.18±0.00	1.84±0.10	0.71±0.01
Amphotericin B	**76.99±0.04**	**73.22±0.44**	**66.44±0.44**	**56.38±0.06**	**46.88±0.10**	**44.69±0.31**	**44.22.±0.26**	**40.34±0.08**	**39.66±0.99**

Bold faceted data shows noteworthy mycelial growth inhibition

Table 3.8 Percentage Mycelia Growth Inhibition of varying concentrations of acetone extracts on *Aspergillus parasiticus.*

Medicinal plants	**3 days incubation**			**6 days incubation**			**9 days incubation**		
	MIC	**0.5 MIC**	**0.25 MIC**	**MIC**	**0.5 MIC**	**0.25 MIC**	**MIC**	**0.5 MIC**	**0.25 MIC**
Dracaea mannii	18.66±0.00	14.33±0.17	11.88±0.21	11.34±0.09	4.99±0.96	3.21±0.88	2.82±0.44	2.05±0.08	1.88±0.04
Mystroxylon aethiopicum	**33.73±0.08**	**25.21±0.08**	**20.04±0.60**	15.11±0.90	10.98±0.77	8.40±0.09	7.05±0.10	7.00±0.04	2.11±0.71
Maytenus undata	12.99±0.01	12.11±0.12	10.33±0.91	10.33±0.01	6.11±0.31	5.11±0.18	1.22±0.34	1.99±0.20	1.11±0.10
Loxostylis alata	9.22±0.22	8.11±0.06	8.05±0.00	6.96±0.20	5.21±0.08	2.14±0.01	2.11±0.01	1.11±0.09	1.10±0.07
Spyrostachys Africana	9.12±0.04	8.96±0.01	7.72±0.00	4.08±0.06	3.14±0.00	3.03±0.00	3.0.1±0.00	1.15±0.01	1.01±0.00
Strychnos mitis	**22.60±0.01**	**21.08±0.88**	16.91±0.81	11.22±0.11	8.94±0.02	5.81±0.11	2.18±0.00	1.84±0.10	0.71±0.01
Amphotericin B	**66.44±0.11**	**59.77±0.08**	**52.90±0.55**	**57.44±0.13**	**56.32±0.55**	**54.10±0.88**	**46.66±0.01**	**42.44±0.01**	**4.33±0.34**

Bold faceted data shows noteworthy mycelial growth inhibition

Table 3.9 Percentage Mycelia Growth Inhibition of varying concentrations of acetone extracts on *Aspergillus ochraceous*

Medicinal plants	3 days incubation			6 days incubation			9 days incubation		
	MIC	**0.5 MIC**	**0.25 MIC**	**MIC**	**0.5 MIC**	**0.25 MIC**	**MIC**	**0.5 MIC**	**0.25 MIC**
Dracaea mannii	**33.26±0.77**	18.93±0.09	17.77±0.11	18.33±0.44	10.22±0.09	6.99±0.85	6.73.±0.84	6.55±0.44	1.09±0.07
Mystroxylon aethiopicum	15.95±0.42	15.23±0.88	11.83±1.00	11.21±0.41	11.11±0.42	8.34±0.11	9.33±0.22	6.22±0.43	3.92±0.05
Maytenus undata	17.44±0.16	17.11±0.02	10.99±0.11	10.66±0.71	8.44±0.11	8.10±0.04	0.00±0.00	0.00±0.00	0.00±0.00
Loxostylis alata	15.24±0.12	15.11±0.01	11.55±0.12	9.96±0.10	4.61±0.12	4.44±0.66	1.0.1±0.00	0.00±0.00	0.00±0.00
Spyrostachys Africana	**34.12±0.02**	**30.66±0.03**	**31.22±0.01**	**30.01±0.08**	**21.21±0.06**	16.03±0.06	17.01±0.0	12.10±0.01	8.01±0.00
Strychnos mitis	**29.10±0.06**	**22.04±0.08**	**20.08±0.11**	19.29±0.21	15.44±0.12	10.11±0.31	4.12±0.06	4..04±0.10	2.11±0.01
Amphotericin B	**61.94±0.31**	**57.67±0.02**	**52.30±0.47**	**54.94±0.73**	**53.12±0.15**	**51.10±0.69**	**42.67±0.62**	**42.66±0.31**	**43.33±0.84**

Bold faceted data shows noteworthy mycelial growth inhibition

3.5 Antioxidant activity

The percentages of inhibition of the selected medicinal plants extracts against 2-2′-Azino-di-[3-ethylbenzthiazoline sulfonate (ABTS) are reported (Figure 3.2). At the highest concentration of 2.5 mg/100ml, the organic extracts from *Spirostachys africana* and *Mystroxylon aethiopicum* exhibited the highest percentage of inhibition of 75.39 and 73.84 respectively. *Strychnos mitis* and *Loxostylis alata* exhibited the lowest percentages of inhibition of 24.62 and 23.08 at a similar concentration respectively. In the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay, a similar trend was observed where the organic extracts from *Spirostachys africana* and *Mystroxylon aethiopicum* exhibited the highest percentage of inhibitions yielding 75.28 and 73.84 respectively, while the extracts from *Dracaea mannii*, *Maytenus undata*, *L. alata* and *S. mitis* exhibited less than 30 % inhibition of DPPH at all the five different concentrations tested (Figure 3.3).

In an iron chelation assay (Figure 3.4), *S. africana* and *M. undata* exhibited the highest percentage inhibitions of 74.33 and 74.0 at the highest tested concentration of 0.52 mg/100ml respectively, while *M. aethiopicum* exhibited a notable % inhibition of 64.80 at a similar concentration. Except *L. alata*, the extracts of all the selected plant species exhibited % iron inhibition of greater than 50 % at the highest concentration tested.

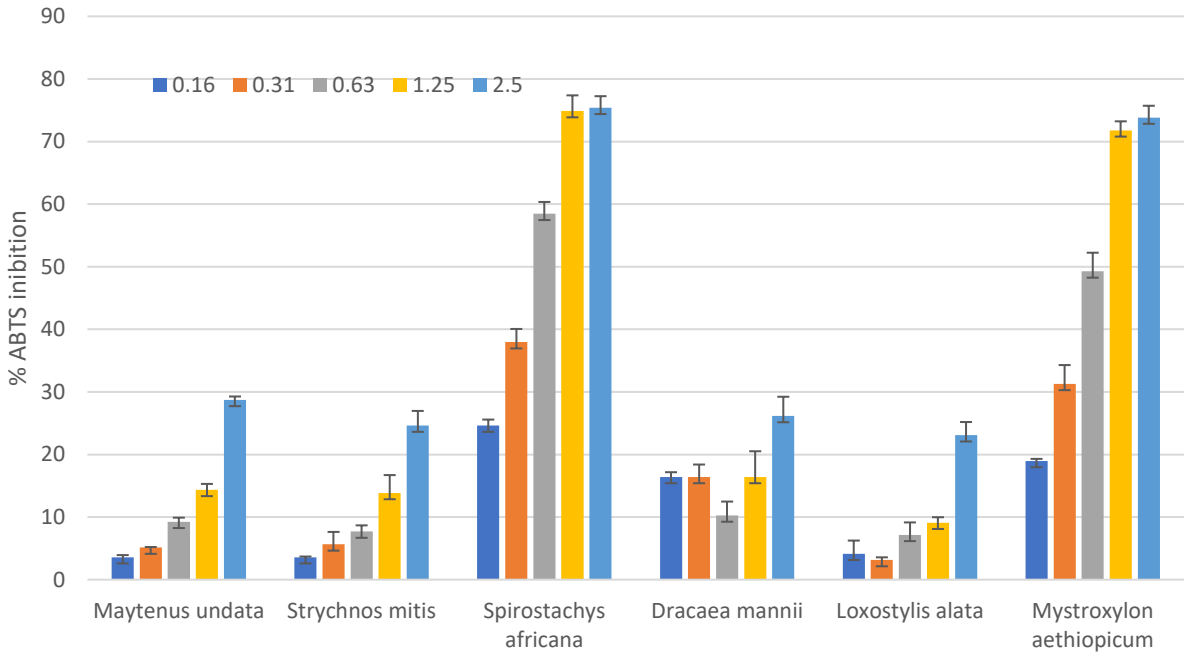

Figure 3.2 % Inhibition of ABTS of selected medicinal plants at diffrerent concentrations.

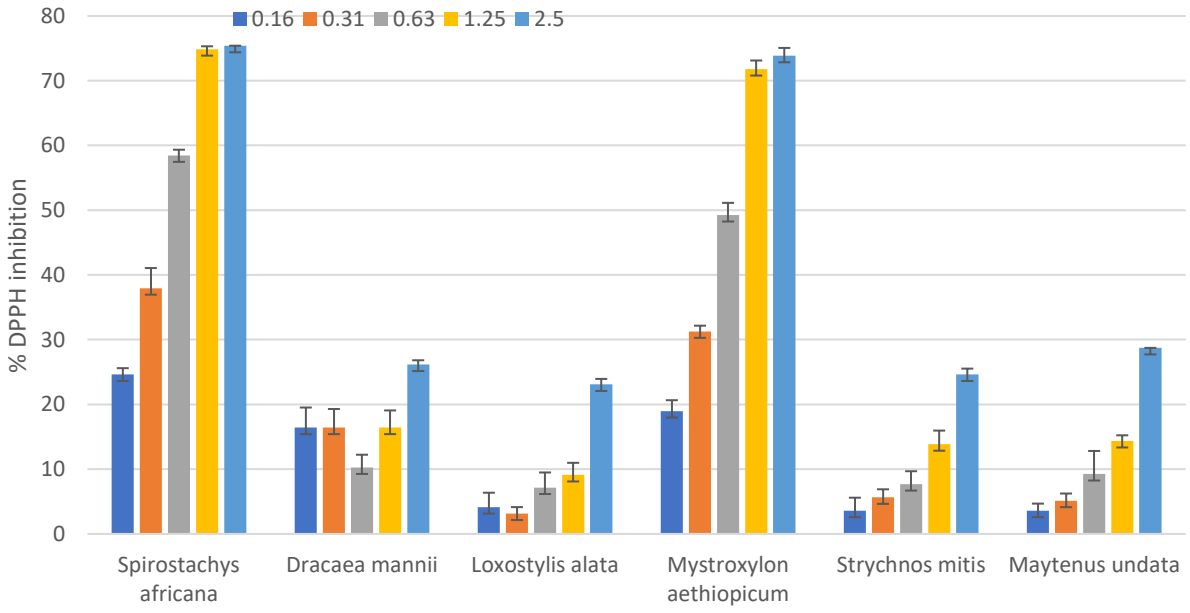

Figure 3.3 % Inhibition of DPPH selected medicinal plants at different concentrations.

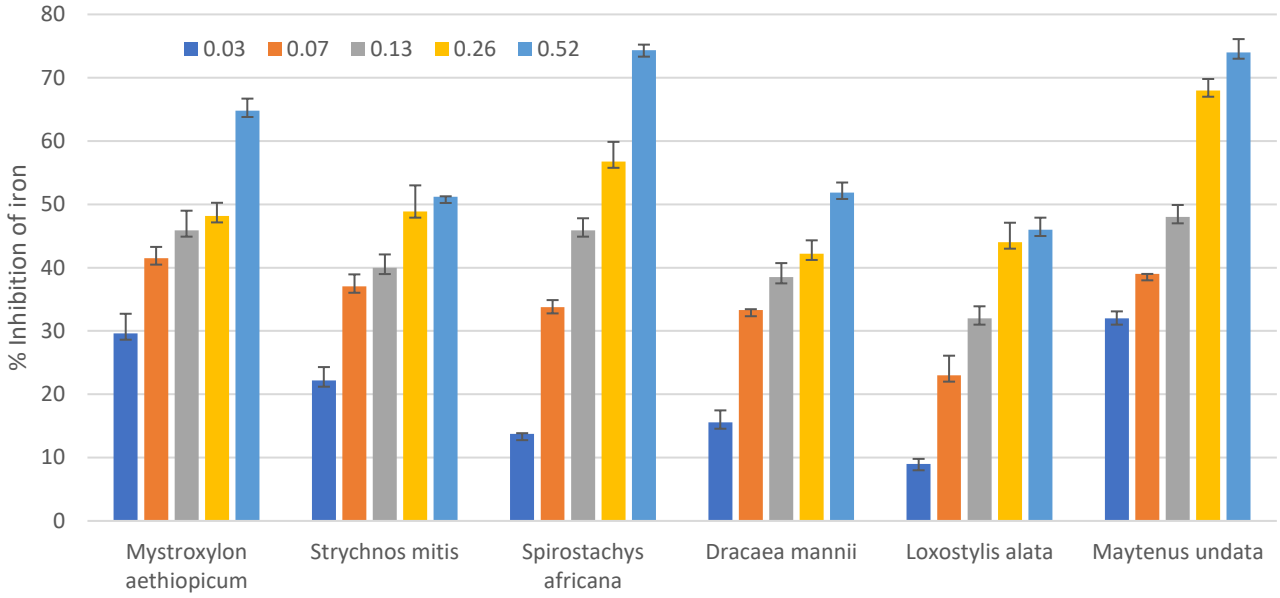

Figure 3.4 % Inhibition of iron selected medicinal plants at different concentrations.

The concentrations of plant sample that inhibit 50 % of test radical or chelation of iron (IC_{50}) of the organic extracts from selected medicinal plants species, extrapolated using Graph Pad Prism Version 7, are reported below (Table 3.10). *Mystroxylon aethiopicum* and *Spyrostachys africana* exhibited a notable IC_{50} values across the assays selected in the current study. Furthermore, the extracts exhibited better chelation of iron compared to their scavenging of free radicals in both ABTS and DPPH assays, with extract from *Maytenus undata* yielding lowest IC_{50} value of 0.14±0.00 mg/100ml against the Fe^{2+}.

S. africana exhibited the lowest IC_{50} in both DPPH and ABTS assay compared to the extracts from other selected medicinal plants extracts yielding 0.51±0.02 and 0.41±0.04 mg/100ml respectively. The extracts from *D. mannii*, *S. mitis, M. undata* and *L. alata* exhibited IC_{50} as high as >2.5 mg/100 mg/ml in both ABTS and DPPH assays.

Table 3.10 Antioxidant activity (IC_{50} in mg/100 ml) of selected medicinal plants extracts

Medicinal plants	**DPPH**	**ABTS**	**Fe^{2+}**
Dracaea mannii	>2.5	>2.5	0.45±0.01
Mystroxylon aethiopicum	0.74±0.01	0.54±0.01	0.24±0.04
Maytenus undata	>2.5	>2.5	0.14±0.00
Loxostylis alata	>2.5	>2.5	>0.52
Spyrostachys Africana	0.51±0.02	0.41±0.04	0.21±0.04
Strychnos mitis	>2.5	>2.5	0.43±0.09
Ascorbic acid	0.14±0.02	0.31±0.01	-
Azealic acid	-	-	0.04±0.02

3.6 GC-MS Analysis

The chromatogram and variety compounds, a total of 66, were detected from *Mystroxylon aethiopicum* organic extract is presented below in Figure 3.5 and Table 3.11 respectively. Ten major compounds with higher percentage areas were identified. These includes Tetradecane (15.742 %), Heptadecane (14.963 %), (Eicosane 9.3475 %), 1,1,6-trimethyl-3-methylene-2-(3,6,9,13-tetramethyl-6-ethenye-10,14-dimethylene-pentadec-4-enyl)cyclohexane (7.8283 %), Tridecane (6.0613 %), Eicosane, 2-methyl (4.6093 %), 11-Hexadecynal (4.2974 %), n-Hexadecanoic acid (3.2896), 2-Methoxypyridine-3-carbonitrile (2.4456 %) and Eicosane, 2-methyl- (2.1909 %). It is important to notice that Eicosane, 2-methyl was detected twice at 15:24.9 and 17:15.0 time intervals with similar unique mass of 57.

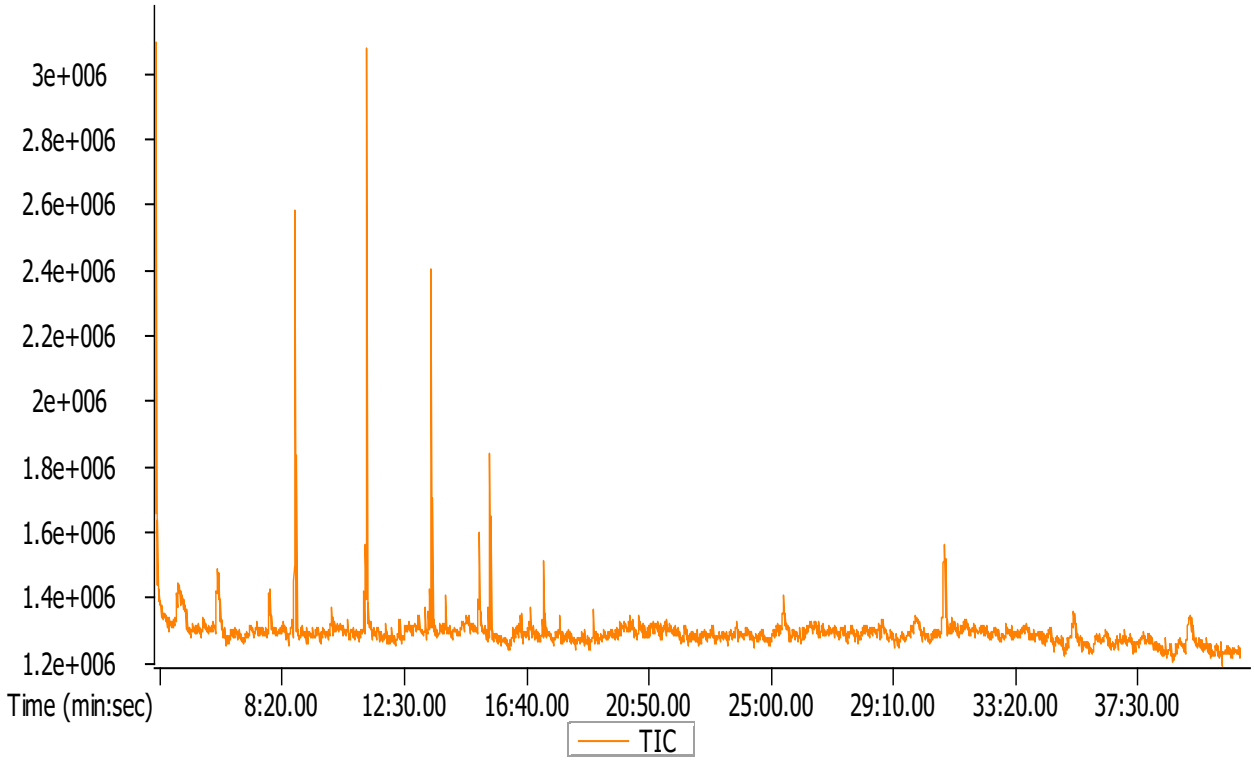

Figure 3.5 GC-MS spectra of *Mystroxylon aethiopicum* organic crude extract

Table 3.11 GC-MS analysis of *Mystroxylon aethiopicum* crude organic extract

NO	Name of compound detected	**Retention time** (min:sec)	Area %	Unique mass
1	Undecane	04:45.3	1.0525	71
2	Decane	04:49.2	1.6024	71
3	**Tridecane**	**06:08.1**	**6.0613**	**71**
4	Phenol, 4-ethenyl-, acetate	06:24.9	0.088421	65
5	Hexadecane	07:27.9	0.39278	57
6	**2-Methoxypyridine-3-carbonitrile**	**07:53.8**	**2.4456**	**134**
7	7-Hexadecene, (Z)-	08:40.0	1.5472	83
8	**Tetradecane**	**08:46.0**	**15.742**	**57**
9	Tetradecane, 4-methyl-	09:29.9	0.095471	70
10	Heptadecane, 2,6,10,14-tetramethyl-	09:33.0	0.068232	57
11	Pentanoic acid	09:50.9	0.058561	73
12	Pentadecane	10:00.6	1.1023	57
13	Propoxyphene	10:06.6	0.57616	58
14	Heptadecane, 2-methyl-	10:51.6	0.1034	57
15	Cetene	11:06.9	1.1202	83
16	**Heptadecane**	**11:12.2**	**14.963**	**57**
17	D-Fucose	11:17.1	0.37461	60
18	Hexadecane, 4-methyl-	11:51.6	0.57958	71
19	Behenic alcohol	12:04.1	0.38552	57
20	Eicosane	12:19.7	0.70764	57
21	Citalopram	12:25.2	0.026308	58
22	Tetradecanoic acid	12:58.3	0.32302	60
23	6-Hydroxy-4,4,7a-trimethyl-5,6,7,7a-tetrahydrobenzofuran-2(4H)-one	13:11.9	0.5395	111
24	5-Eicosene, (E)-	13:20.2	1.0998	83
25	**Eicosane**	**13:24.6**	**9.3475**	**57**
26	2-Pentadecanone, 6,10,14-trimethyl-	13:53.9	1.0019	58
27	Octadecane, 4-methyl-	14:00.4	0.078995	71
28	Ketone, methyl 2,2,3-trimethylcyclopentyl	14:21.2	0.051573	84
29	Eicosane	14:25.9	0.19691	57
30	Undecanal, 2-methyl-	14:37.0	0.45107	58
31	8-Hexadecenal, 14-methyl-, (Z)-	14:40.9	0.41956	95
32	1,13-Tetradecadien-3-one	14:46.8	0.07813	70
33	**n-Hexadecanoic acid**	**15:02.9**	**3.2896**	**60**
34	Dibutyl phthalate	15:07.0	0.33995	149

35	4H-1-Benzopyran-4-one, 5,7-dihydroxy-2-methyl-	15:15.3	0.20272	192
36	1-Docosene	15:21.3	0.48302	97
37	**Eicosane, 2-methyl-**	**15:24.9**	**4.6093**	**57**
38	7-Octenal, 3,7-dimethyl-	15:28.3	0.13882	97
39	Octadecane, 4-methyl-	15:57.7	0.20699	71
40	Hexadecen-1-ol, trans-9-	16:12.4	0.44987	83
41	Ethanone, 1-(3-butyloxiranyl)-	16:15.1	0.2121	85
42	Bis(2-ethylbutyl) diselenide	16:24.9	0.33422	85
43	1-Hexadecyn-3-ol, 3,7,11,15-tetramethyl-	16:28.2	0.58408	84
44	1-Hexadecyn-3-ol, 3,7,11,15-tetramethyl-	16:46.9	0.93243	84
45	Tetradecanoic acid	16:54.7	0.25175	60
46	1-Docosene	17:11.9	0.53151	97
47	**Eicosane, 2-methyl-**	**17:15.0**	**2.1909**	**57**
48	1-Bromo-3-(2-bromoethyl)-nonane	17:47.8	0.90332	83
49	2-Octanone, 1-nitro-	18:31.3	0.2863	113
50	2-Pentene-1,4-dione, 1-(1,2,2-trimethylcyclopentyl)	18:32.6	0.2863	111
51	4,8,12,16-Tetramethylheptadecan-4-olide	18:37.4	0.51532	99
52	Eicosane, 2-methyl-	18:56.2	0.91652	57
53	Hexanedioic acid, mono(2-ethylhexyl)ester	18:57.3	0.91652	129
54	Octacosane	19:43.9	0.084846	57
55	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	19:49.9	0.27146	98
56	Bis(2-ethylhexyl) phthalate	20:09.9	0.42153	149
57	Eicosane, 2-methyl-	20:29.8	0.48592	57
58	2-methyloctacosane	21:14.5	0.11016	57
59	Hexacosane	22:03.1	0.39522	57
60	Sulfurous acid, butyl hexadecyl ester	22:59.6	0.66503	57
61	Cholesta-4,6-dien-3-ol, (3á)-	25:24.6	1.9516	143
62	Heneicosane	25:27.2	0.34658	99
63	**1,1,6-trimethyl-3-methylene-2-(3,6,9,13-tetramethyl-6-ethenye-10,14-dimethylene-pentadec-4-enyl)cyclohexane**	**30:54.0**	**7.8283**	**95**
64	**11-Hexadecynal**	**35:18.1**	**4.2974**	**69**
65	Tricyclo[20.8.0.0(7,16)]triacontane, 1(22),7(16)-diepoxy-	39:15.8	0.74148	133
66	Farnesol isomer a	39:16.5	1.3496	95

Bold faceted data shows compounds with % Area >2.0

The chromatogram and variety compounds, a total of 127, were detected from *Spirostachys africana* organic extract is presented below in Figure 3.6 and Table 3.12 respectively. *n*-Hexadecanoic acid and Nonadecane, 2-methyl- were identified as major compounds yielding % area 12.142 and 6.3336 respectively while Nonadecane, 2-methyl- and D-Allose yielded notable % area of 4.8632 and 4.7836 respectively. β-Sitosterol with a unique mass of 120 was detected at % area of 4.285, while Myo-Inositol, 2-C-methyl- and Cyclooctane, methyl- were also prevalent with % area 4.1079 and 3.9467 respectively.1,2,3,5-Cyclohexanetetrol, (1à,2á,3à,5á)- exhibited % area of 3.6486 with unique mass 83, while both Tetradecane and Di(1-methyl-1-silacyclobutyl)amine exhibited similar % area of 2.816. Tetratetracontane and Heptacosyl acetate also yielded a notable % area of 2.7386 and 2.0289 respectively.

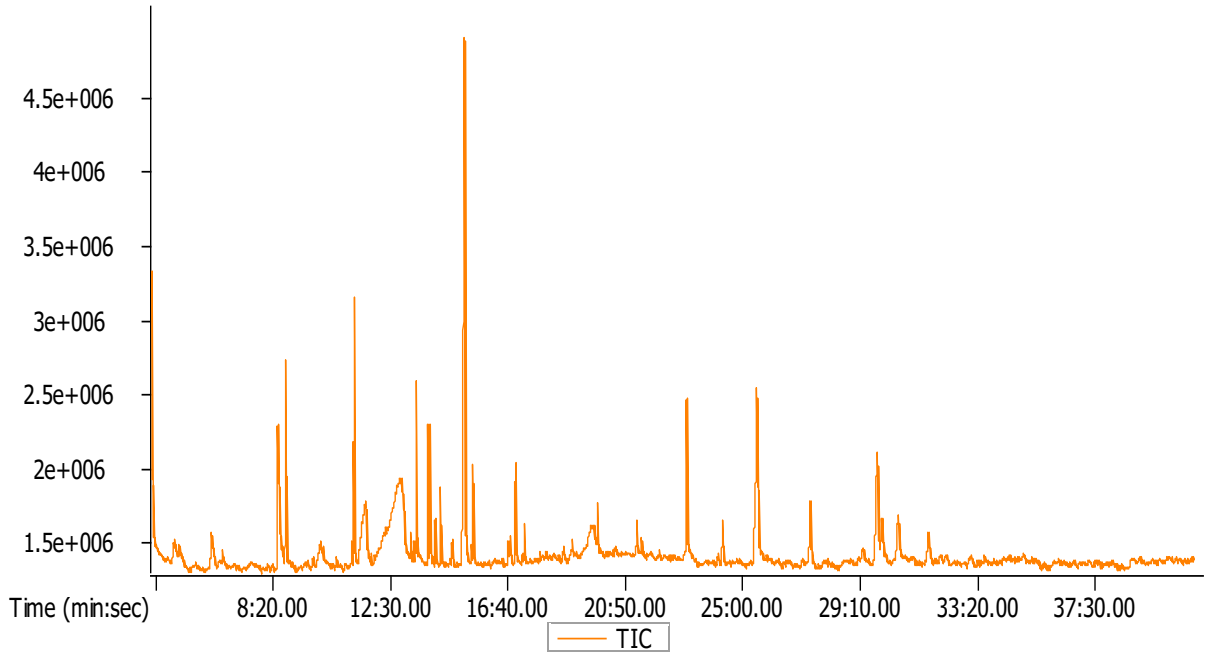

Figure 3.6 GC-MS spectra of *Spirostachys africana* organic crude extract

Table 3.12 GC-MS analysis of *Spirostachys africana* crude organic extract

NO	Name of compound detected	Retention time (min:sec)	Area %	Unique mass
1	1-Penten-3-yne, 2-methyl-	04:00.7	0.38679	79
2	Propionaldehyde, diethylhydrazone	04:11.3	0.5432	128
3	Thymine	04:34.5	0.024963	126
4	Decane	04:45.9	0.11058	71
5	Decane	04:49.1	0.4405	85
6	Ethyl à-d-glucopyranoside	04:59.9	0.4893	75
7	Levoglucosenone	05:06.3	0.10757	68
8	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	05:28.3	0.015472	72
9	Undecane	06:08.1	1.5419	57
10	Catechol	06:09.0	1.5419	110
11	Phenol, 4-ethenyl-, acetate	06:24.4	0.11589	120
12	5-Hydroxymethylfurfural	06:31.5	0.47434	97
13	1H-Pyrrole-2,5-dione, 3-ethyl-4-methyl-	06:35.7	0.086577	139
14	Undecane	07:28.1	0.030302	57
15	Thiophene, 2-butyltetrahydro-	07:30.8	0.048719	87
16	**1,2,3-Benzenetriol**	**08:29.6**	**4.8632**	**80**
17	**D-Allose**	**08:30.0**	**4.7836**	**60**
18	7-Hexadecene, (Z)-	08:40.0	0.11322	56
19	**Tetradecane**	**08:46.2**	**2.8165**	**57**
20	**Di(1-methyl-1-silacyclobutyl)amine**	**08:47.8**	**2.8163**	**142**
21	Bisphenol C	09:00.5	0.086017	241
22	Tridecane, 4-methyl-	09:30.0	0.10758	70
23	Decane, 2,3,5,8-tetramethyl-	09:33.1	0.19646	71
24	9-Oxononanoic acid	09:45.3	0.24298	111
25	cis-Hexahydrophthalide	09:52.5	0.016085	68
26	Benzoic acid, 3-hydroxy-	09:56.2	0.46467	121
27	á-D-Glucopyranose, 1,6-anhydro-	10:00.6	1.3461	56
28	Heptadecane	10:00.7	1.0481	85
29	2,5-Difluorobenzoic acid, 2,3-dichlorophenyl ester	10:05.4	0.37366	141
30	3-Pentadecanol	10:05.8	0.37366	199
31	Isobutyl 4-hydroxybenzoate	10:14.0	0.16899	121
32	1-Dimethylaminohexane	10:15.1	0.051676	58
33	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-	10:34.3	0.26761	111
34	Dodecanoic acid	10:43.5	0.061552	60
35	Fumaric acid, ethyl 2,4,5-trichlorophenyl ester	10:54.5	0.10626	127
36	Cetene	11:06.9	0.31758	83
37	(-)-Spathulenol	11:07.7	0.31758	91

38	**Heptadecane**	**11:12.2**	**2.6752**	**57**
39	1-Methylcyclohexylcarboxylic acid	11:15.4	0.077011	87
40	**1,2,3,5-Cyclohexanetetrol, (1à,2á,3à,5á)-**	**11:35.6**	**3.6486**	**83**
41	6-Ethoxy-6-methyl-2-cyclohexenone	11:47.6	0.10776	219
42	Tridecane, 4-methyl-	11:51.7	0.002936	70
43	2-n-Octylfuran	11:55.0	0.065735	81
44	endo-Borneol	12:15.1	0.090636	199
45	3-Buten-2-one, 4-(4-hydroxy-2,2,6-trimethyl-7-oxabicyclo[4.1.0]hept-1-yl)-	12:17.4	0.065566	123
46	Heneicosane	12:19.7	0.12202	99
47	**Cyclooctane, methyl-**	**12:40.6**	**3.9467**	**70**
48	**Myo-Inositol, 2-C-methyl-**	**12:52.9**	**4.1079**	**74**
49	6-Hydroxy-4,4,7a-trimethyl-5,6,7,7a-tetrahydrobenzofuran-2(4H)-one	12:54.8	1.202	109
50	4-methyl-6-ethoxylcoumarin	12:57.5	0.43679	204
51	Tetradecanoic acid	12:58.7	0.30909	185
52	6-Hydroxy-4,4,7a-trimethyl-5,6,7,7a-tetrahydrobenzofuran-2(4H)-one	13:12.8	0.2954	111
53	5-Eicosene, (E)-	13:20.1	0.17188	83
54	Heneicosane	13:24.6	1.7906	57
55	Fumaric acid, 4-methoxyphenyl dodec-2-en-1-yl ester	13:24.8	1.7906	124
56	Hydroperoxide, 1,4-dioxan-2-yl	13:27.5	0.047921	73
57	Neophytadiene	13:49.9	1.3399	68
58	2-Pentadecanone, 6,10,14-trimethyl-	13:54.0	1.4381	58
59	Heneicosane, 11-(1-ethylpropyl)-	14:00.4	0.1117	70
60	Neophytadiene	14:05.1	0.42412	82
61	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	14:10.8	0.009932	149
62	Neophytadiene	14:16.3	0.7819	82
63	Ketone, methyl 2,2,3-trimethylcyclopentyl	14:21.3	0.075173	84
64	Octadecane, 2-methyl-	14:25.9	0.022485	57
65	2-Pentadecanone, 6,10,14-trimethyl-	14:36.9	0.067764	58
66	8-Hexadecenal, 14-methyl-, (Z)-	14:40.8	0.42296	84
67	Dodecanoic acid, methyl ester	14:41.9	0.42296	74
68	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	14:43.6	0.041132	205
69	1-Hexadecen-3-ol, 3,5,11,15-tetramethyl-	14:55.8	0.008741	71
70	**n-Hexadecanoic acid**	**15:07.2**	**12.142**	**149**
71	1-Docosene	15:21.5	0.20136	97
72	Nonadecane, 2-methyl-	15:25.0	1.1906	57
73	2-n-Hexylcyclopentanone	15:25.5	1.1906	84
74	Heptadecane, 2,6,10,14-tetramethyl-	15:57.8	0.03605	113
75	Nonanoic acid	15:59.2	0.086445	60
76	Hexadecen-1-ol, trans-9-	16:12.6	0.077728	83
77	Ethanone, 1-(3-butyloxiranyl)-	16:24.9	0.066065	85
78	cyclopentanone, 2-decyl-	16:28.2	0.16762	84
79	Isophytol	16:30.0	0.16762	71

80	Tridecanoic acid, methyl ester	16:36.3	0.035337	74
81	Linoelaidic acid	16:40.4	0.33039	81
82	Oleic Acid	16:43.2	0.44447	84
83	9,12,15-Octadecatrienal	16:44.3	0.44447	79
84	Hexanamide, N-(2-hydroxyethyl)-	16:44.9	0.44447	85
85	cyclopentanone, 2-octadecyl-	16:46.9	0.12224	84
86	Octadecanoic acid	16:55.9	1.3759	60
87	Bicyclo[3.3.1]nonan-9-one, 1,2,4-trimethyl-3-nitro-, (2-endo,3-exo,4-exo)-(.+-.)-	17:10.8	0.17429	109
88	1-Docosene	17:11.9	0.17429	97
89	Eicosane, 2-methyl-	17:15.0	0.48117	57
90	9,12-Octadecadienal	17:27.5	0.041997	67
91	1-Bromo-3-(2-bromoethyl)-nonane	17:48.1	0.098938	83
92	Palmidrol	18:16.0	0.058212	98
93	Dodecanamide, N-(2-hydroxyethyl)-	18:18.6	0.085075	85
94	4,8,12,16-Tetramethylheptadecan-4-olide	18:37.6	0.27602	99
95	n-Hexadecanoic acid	18:38.8	0.27602	60
96	Octacosane	18:56.3	0.26232	57
97	Hexanedioic acid, mono(2-ethylhexyl)ester	18:57.4	0.26232	129
98	Urs-12-en-28-ol	19:43.0	0.62834	117
99	Methoxyacetic acid, 3-tetradecyl ester	19:43.9	0.58801	85
100	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	19:50.4	0.4639	98
101	Bis(2-ethylhexyl) phthalate	20:10.0	0.055087	149
102	Dodecanoic acid, 3-hydroxy-	20:25.4	0.06034	84
103	Octacosane	20:29.9	0.13403	57
104	Hexacosane	21:14.7	0.55482	57
105	9,12,15-Octadecatrienal	21:16.9	0.10312	79
106	Octadecanoic acid, 2,3-dihydroxypropyl ester	21:24.6	0.20188	98
107	Hexadecanamide	21:56.2	0.3211	59
108	Hexatriacontane	22:03.1	0.1064	57
109	**Tetratetracontane**	**23:00.5**	**2.7386**	**57**
110	Eicosane, 2-methyl-	24:06.9	0.18554	57
111	Heptacosyl acetate	24:17.0	0.77783	61
112	Cholesta-4,6-dien-3-ol, (3á)-	25:24.8	0.35755	143
113	**Nonadecane, 2-methyl-**	**25:29.9**	**6.3336**	**71**
114	1-Eicosanol	25:32.7	1.1951	82
115	Vitamin E	26:10.6	1.7105	165
116	Octacosane	27:06.5	0.13761	71
117	**Heptacosyl acetate**	**27:24.1**	**2.0289**	**61**
118	Octacosane	29:07.8	0.15998	71
119	Octacosanol	29:16.8	0.34518	97
120	**β-Sitosterol**	**29:46.4**	**4.285**	**120**
121	9,19-Cyclolanostan-3-ol, acetate, (3á)-	29:56.7	1.603	381
122	á-Amyrin	30:30.8	1.8531	218

123	Lupeol	31:35.6	1.0944	93
124	Heptacosyl acetate	32:02.5	0.12082	97
125	Pregnan-3,11-diol-20-one	32:14.6	0.35712	105
126	Ethanone, 1,1'-(6-hydroxy-2,5-benzofurandiyl)bis-	33:08.3	0.1575	218
127	Octacosanol	34:58.6	0.19432	57

Bold faceted data shows compounds with % Area >2.0

CHAPTER 4

DISCUSSIONS

4.1 Antifungal, cytotoxicity and total activity of selected plants extracts

The antifungal activity of organic and aqueous plant extracts tested against phytopathogenic fungal members of *Fusarium* and *Aspergillus* are presented in (Table 3.1) as minimum inhibitory concentrations (MIC) values. MIC is the lowest concentration of plant extract that inhibits the microbial proliferation. The MIC values against the investigated mycotoxigenic fungal strains ranged between 0.01 and 6.25 mg/ml. This was indicated by varying color intensity in the wells where fungal proliferation is observed by reduction of yellowish *p*-iodonitrotetrazolium (INT) chloride to reddish-pink colored formazan during incubation. Organic extract from *Strychnos mitis* exhibited a noteworthy inhibition of majority of the selected fungal strains, compared to extracts from other medicinal plants yielding potent MIC values of 0.08, 0.01, 0.06 and 0.04 mg/ml against *Furasium vercitilloides*, *Furasium graminareum*, *Fusarium oxysporum* and *Aspergillus parasiticus* at 48 h incubation period respectively. These findings may well suggest that the extract prevalently possess phytocompounds which inhibit the growth a variety of *Fusarium* species compared to *Aspergillus*. Although most authors are corroborating on the standard MIC value of 0.1 mg/ml to be potent (Makhafola *et al*., 2014; Fadipe *et al*., 2015 and 2020; Mongalo *et al*., 2018; Mongalo *et al*., 2019), there is no validated endpoint criteria for *in vitro* testing of plant extracts. Therefore, a proposed classification as proposed by Souza *et al.* (2007) is adopted in the current work. That is, plant extracts yielding MIC values below 0.5 mg/ml are considered as strong inhibitors of fungal growth,

0.5 mg/ml to1.6 mg/ml (moderate inhibitors) and greater than 1.6 mg/ml as poor fungal inhibitors. Marking by the above standard, organic extract from *Strychnos mitis* and aqueous extract from *Spirostachys africana* leaves prevalently suppress the growth of a wide variety of fungal strains, yielding an average MIC value of 0.17 mg/ml and 0.57 mg/ml respectively, against all the selected pathogens at two different growth intervals of 24 and 48 h incubation period. However, *Aspergillus flavus* and *Aspergillus ochraceous* developed some resistance against the *S. mitis* organic extract after 48 hours incubation, yielding MIC value of 0.78 mg/ml. A decrease in activity could imply that the fungi either became resistant to the extracts or the active compound(s) became unstable during the incubation.

Elsewhere, acetone extracts from *Strychnos mitis* leaves exhibited MIC value of 0.31 and 1.25 mg/ml against *Aspergillus fumigatus* and *Aspergillus flavus* respectively (Adamu *et al.,* 2012; Dikhoba *et al*., 2019). In the current study, organic extract exhibited MIC value of 0.8 mg/ml against both A. flavus and A. ochraceous. It is not easy to compare our findings with those from other sources due to differences in extracting solvent. However, the findings of our study suggest that the phytocompounds responsible for antifungal inhibition might be more soluble in 1:1 methanol: dichloromethane compared to acetone. It is important to note that many literature sources support the use of acetone in extracting antifungal compounds from plant sources (Dai and Mumper, 2010; Abubakar and Haque, 2020).

Fusarium species were more sensitive to organic extracts compared to *Aspergillus* species, with MIC values ranging between 0.01 and 0.08 mg/ml. Such MIC values are proposed to show outstanding to very good microbial activity. In a similar study, *Fusarium verticiloides* and *Fusarium proliferatum* were also found to be sensitive to organic extracts compared to *Aspergillus flavus* and *Aspergillus Parasiticus* (Thembo *et al*., 2010). The average MIC values show that organic extract from *Loxostylis alata* had a moderate activity with MIC values of 0.21 while while *Mystroxylon aethiopicum* had the weaker average MIC value of 3.55 mg/ml. Elsewhere, the crude leaf extracts of *Loxostylis alata* tested against *Aspergillus fumigatus* showed a potential as antifungal agent against avian aspergillosis (Eloff *et al.,* 2007). It is important to note that aqueous extract from *Spirostachys africana* exhibited promising average MIC value of 0.57 mg/ml against selected fungal strains. This may be relevant as aqueous solutions are less likely to have a devastating effect on crops. In the current study, negative controls showed that 1:1 methanol: dichloromethane was not harmful to the plant pathogens at the highest percentage tested, confirming previous results (Mongalo *et al*., 2018; Dikhoba *et al.,* 2019).

Total activity (TA) of the extracts from the selected plant extracts are reported in Table 3.2. *Strychnos mitis* exhibited the highest total activity against *Fusarium vercitilloides* and *Furasium graminareum* at 24 and 48 hr incubation period respectively. TA indicates the largest volume to which the biologically active compounds in one gram of plant material can be diluted and still inhibit the growth of bacteria and is measured in ml/g (Fadipe *et al*., 2015). This means that one gram of the extract from *S. mitis* could be diluted 7692 ml

of water and still inhibit the growth of fungi. In the current work, S. mitis exhibited a higher TA against *A. ochraceous*, *A. aspergillus*, *F. oxysporum*, *F. graminareum* and *F. vercitilloides* compared to other medicinal plant extracts at 24 hr incubation period. The TA is dependent on several factors which includes the antimicrobial effect of the plant extract and the solubility of the phytocompounds from the plant material in a solvent of choice.

4.2 Cytotoxicity and selectivity index of the selected medicinal plants

Although plants are regarded as safe, especially those used in folk medicine, many are potentially toxic. It is therefore very important to investigate the level of toxicity of before recommendation. In addition, the toxicity study helps to ensure that the observed biological activity of plant extract is not due to general metabolic toxic effect (Dzoyem *et al*., 2016). The MTT cytotoxicity assay which asses the cells mitochondrial competence was used to evaluate the toxicity of the extracts. This assay is widely used to assess the viability and the metabolic state of the cells (Soyingbe *et al*., 2018). Table 3.3 presents the cytotoxic effects of selected medicinal plants against Bovine dermis and Vero Monkey cells. The LC_{50} is the concentration of an extract that is lethal to 50% of the cells exposed to it. The extracts showed varying degree of cytotoxicity against tested cell lines with LC_{50} values ranging between 0.10 to 0.88 mg/ml. *Dracaena mannii* was also found to be less toxic to both Bovine dermis and Vero cells yielding LC_{50} values of 0.88±0.12 and 0.77±0.01 mg/ml respectively. The higher LC_{50} implies that it would take a huge quantity of the extract to cause a toxic response, while small LC_{50} values are likely to be highly toxic and could be dangerous to human health (Fadipe *et al*. 2017; Okeleye *et al*., 2013).

Although some authors refer to a LC_{50} value of less than 0.10 mg/ml to be toxic (Mthethwa *et al*., 2014; Fadipe *et al*., 2015), the American National Cancer Institute refer to an LC_{50} of less than 0.03 mg/mL to be toxic (Talib *et al*., 2010; Tiwary *et al*., 2015). Besides exhibiting a potent antioxidant and antimycobacterial activity, it is highly concerning that *Spirostachys africana* leaves extracts exhibited a lowest LC_{50} value of 0.10 mg/ml against both Vero and Bovine dermis cell lines (Elisha *et al*., 2016). However, there is a need to further explore the toxicological aspects of the plant species. It is important to note that [ent-2,6ά-dihydroxy-norbeyer-1,4,15-trien-3-one and lupeol isolated from *S. africana* stem bark exhibited LC_{50} values of >0.03 mg/ml against Vero cells in a sodium-2,3-bis-[2-methoxy-4-nitro-5-sulfophenyl]-2H-tetrazolium-5-carboxanilide (XTT) assay (Mathabe *et al*., 2008).

To determine the effectiveness and the safety of the extracts the selectivity index of the selected medicinal plant extracts was determined against Bovine dermis and Vero cells. The results are presented in Table 3.5. Using Bovine dermis cell line, *Strychnos mitis* exhibited highest SI value of 18.0 against *Furasium graminareum*, while *Loxostylis alata* exhibited SI value of 13.75 against *Furasium graminareum*, *Furasium oxysporum* and *Aspergillus parasiticus*. In the Vero cells, *Strychnos mitis* extracts exhibited potent SI values ranging from 11.0 to 88.0 against all the three *Furasium* selected species. The higher the selectivity index, the higher the safety margin (Mongalo *et al*., 2018). Although *Strychnos mitis* extracts exhibited a lower antioxidant capacity, its higher selectivity index is commended and gives a higher safety margin compared to other plant species.

4.3 Mycelial growth inhibition and antioxidant activity

The *in Vitro* MGI was evaluated on the tested phytopathogenic fungal strains to determine the fungistatic and fungicidal potential. This was done by amending the extracts into potato dextrose agar growth media to a concentration equivalent to the MIC, 0.5 MIC and 0.25 MIC values and the results show some varied degree of mycelial growth inhibition in a concentration dependent manner (Tables 3.6-3.9). Extracts of *Mystroxylon aethopicum*, *Maytenus undata*, *Strychnos mitis*, *Dracea mannii*, and *Spyrostachys africana* exhibited good, and varying percentage inhibition of mycelial growth against the tested fungal strains.

In comparing members of the genus *Fusarium*, the mycelial growth of *Fusarium verticilloides* and *Fusarium graminareum* were significantly suppressed by amended extracts from *Mystroxylon aethopicum*, *Maytenus undata* and *Strychnos mitis* recording the highest percentage mycelial growth inhibitions of 44.38%, 34.66% and 36.66% respectively after three days incubation. Similar findings and comparable results on *Mystroxylon aethopicum* tested against *Fusarium verticilloides* were recorded by Dikhoba *et al* (2019). *Aspergillus ochraceous* mycelial growth was also more suppressed by extracts of *Dracaena mannii*, *Spyrostachys africana* and *Strychnos mitis* with *Spyrostachys africana* recording the highest mycelial growth inhibition percentage of 34.12% while 33.73% mycelial growth inhibition was recorded by *Strychnos mitis* against *Aspergillus parasiticus*. However, it is important to note that mycelial growth inhibition of the organic extracts was not comparable to the control drug, Amphotericin.

The mode of action of the plant species in inhibiting the growth of the selected mycotoxigenic activity is proposed to be through mycelial growth inhibition. However, further studies need to be explored to evaluate other possible modes of inhibition. Furthermore, there is a need to further quantify such mycotoxins in experimental setup where crops such as maize would be infected with known fungal strains.

Free radical-scavenging activity of DPPH, ABTS and Iron chelation potential of the extracts results are summarized in (Figure 3.2-3.4) as percentages of inhibition and in Table 3.10 presented as inhibitory concentration leading to 50% reduction of free radicals (IC_{50}). The beneficial phytochemicals are known to possess antioxidant properties and could well counteract unstable molecules called free radicals which may accumulate and result in damage of DNA, cell membrane and other parts of the cell (Tepe *et al*., 2007). These free radicals are implicated in causing a decay, thereby allowing prominent growth of mycotoxigenic fungal strains, in many crops and have a negative impact on the yield of crops, particularly at postharvest stages (Dikhoba *et al*., 2019). Extracts with antioxidant compounds are perceived to well possess some protective effect on living cells and protect the crops against various pathogenic infections (Makhuvele *et al*., 2020). The extracts of *Mystroxylon aethopicum* and *Spirostachys africana* exhibited a good antioxidant activity across the selected antioxidant assays, i.e DPPH, ABTS and Iron free radicals, compared to other medicinal plant extracts. According to McGaw *et al* [35], a good antioxidant plant should exhibit a reasonable antioxidant activity in at least three different assays. *M. aethiopicum* extract exhibited IC_{50}s ranging from 0.24±0.04 to

0.74±0.01 while *S. africana* exhibited IC_{50}s ranging from 0.21±0.04 to 0.51±0.02 in all three assays. These may well suggest that *S. africana* extracts exhibited a slightly better antioxidant activity compared to *M. aethiopicum* in all the three assays. According to Mongalo *et al* (2012), a potent antioxidant plant extract should exhibit the IC_{50} value of <0.88 mg/100ml. Elsewhere, the leaves and twigs from *S. africana* exhibited 96.6 % inhibition of DPPH at 100 µg/ml (Amoo *et al*., 2012). It is difficult to compare these results with the findings of the current study due to differences in plant sample used, extracting solvent, concentrations used and the various environmental conditions relating to the area of plant collection. Although *S. africana* extract exhibited a noteworthy antioxidant activity, there is a need to explore the antioxidant activity of the extracts against other *in vitro* antioxidant assays. Furthermore, the *in vivo* studies still need to be explored

4.4 Phytocompounds detected from plant species with potent antifungal and antioxidant activity.

Organic extracts of *Mystroxylon aethiopicum* and *Spirostachys africana* were subjected to GC-MS analysis to identify and determine the presence of phytocompounds in the extracts which can then further be associated to the biological activity of plant extract. *Mystroxylon aethiopicum* and *Spirostachys africana* revealed the total of 66 and 127 phytocompounds respectively.

Of the total phytocompounds that were found in *Spirostachys africana*, n-hexadecanoic acid (12.14 %), Nonamethane-2-methyl- (6.33 %), 1,2,3-benzenetriol (4.86) and were found to be available at the highest concentrations. The GC-MS results suggest that the antifungal, antioxidant activity and cytotoxicity of the extracts may be attributed to the presence of different phytochemicals acting together in a synergistic or additive manner.

Mystroxylon aethiopicum indicated the presence of eleven phytocompounds that were present in abundance. Tetradecane (15.74 %), heptadecane (14.96 %) and Eicosane (9.35 %) were present in abundance compared to other compounds. Tetradecane, which is a volatile alkane, was found to inhibit the growth and spore germination of agricultural important strain of *Fusarium verticilloides* (Yuan *et al*., 2012). This is corroborating our findings where *Fusarium verticilloides* was found to be highly sensitive to *Mystroxylon aethiopicum* organic extracts, yielding MIC value of 0.08 mg/ml, and a further notable MGI value of 44.38 % at 3 days incubation period. Elsewhere, heptadecane was implicated in

the mycelial growth inhibition of various mycotoxigenic fungal strains such as *Botrytis cinerea, Penicillium expansum, Colletotrichum fulcatum NCBT 146, Pyricularia oryzae Rhizoctonia solani* and most importantly *Aspergillus niger* as one of the fungal strains used in the current study (Sheikh *et al*., 2018; Abubacker *et al*., 2015; Pourakbar *et al*. 2021).

Although the anti-mycotoxigenic activity of many of these detected compounds is not reported in the literature, we are of the opinion that these compounds may synergistically inhibit the growth of these selected fungal strains *in vitro*. However, the *in vivo* studies still need to be explored and such mycotoxins needs to be quantified as well.

CHAPTER 5

CONCLUSSIONS AND RECOMMENDATIONS

The food additives are used to diminish the microbial contamination which may be associated with spoilage due to fungal attack and subsequent contamination with toxins known as mycotoxins which are unhealthy for human and animal consumption. Consumption of contaminated food stuff also results in adverse health effects. The use of synthetic chemical additives and physical control techniques such as thermal treatment (which may alter the nutritional or organoleptic properties) are used to preserve food. However, this is perceived as unhealthy practice by consumers due to negative side effects of synthetic chemicals and necessitates the need for a development of a biologically safe alternative. The plant metabolites are gaining increasing interest as food additives and preservatives. This is due to known antifungal mechanisms of action of phytochemicals which include inhibition of cellular biosynthesis, spore germination and alteration of cellular membrane permeability which results in reduction of fungal fitness cell death, or both. The selected medicinal plants in the current work, serves as a start point in finding possible plant based antimycotoxigenic agents that are also safe for human consumption. As a result, phytochemicals that exhibit some antifungal, antioxidant activities and shows less toxicity to mammalian cells, Such as *M. aethiopicum* and *S. africana* should further be explored *in vivo* for possible application as food preservatives. The biological activity observed may be due to the presence of tetradecane and heptadecane synergisticall

References

1. Abubacker, M.N., Devi, P.K. 2015. *In-Vitro* Antifungal efficacy of bioactive compounds heptadecane, 9- hexyl and octadecane, 3-ethyl-5-(2- ethylbutyl) from *Lepidagathis cristata* willd. (Acanthaceae) root extract. *European Journal of Pharmacology Medical Res*earch, 2: 1779-1787.

2. Abubakar A.R., Haque M. 2020. Preparation of Medicinal Plants: Basic Extraction and Fractionation Procedures for Experimental Purposes. *Journal of Pharmacy and Bioallied Sciences,*12(1):1-10.

3. Adamu, M., Naidoo V., Eloff J.N. 2012. Some southern African plant species used to treat helminth infections in ethnoveterinary medicine have excellent antifungal activities. *BMC Complementary Alternative Medicine,*12: 1-13. Adye, J.A., Mateles, R.I. 1964. Incorporation of labelled compounds into aflatoxins. *Biochimica et Biophysica Acta (BBA)-General Subjects*, 86(2): 418-420.

4. Afolayan, A.J., Grierson, D.S., Kambizi, L., Madamombe, I., Masika, P.J. 2002. *In Vitro* antifungal activity of some South African medicinal plants. *South African Journal of Botany* 68: 72-76.

5. Alberts, J.F., Van Zyl, W.H., Gelderblom, W.C. 2016. Biologically based methods for control of fumonisin-producing *Fusarium* species and reduction of the fumonisins. *Frontiers in Microbiology*, 7: 1-33.

6. Alotibi, F.O., Ashour, E.H., Al-Basher, G., 2020. Evaluation of the antifungal activity of *Rumex vesicarius* L. and *Ziziphus spina-christi* (L.) Desf. Aqueous extracts and assessment of the morphological changes induced to certain myco-phytopathogens. *Saudi Journal of Biological Sciences,* 27: 2818-2828.

7. Amoo, S.O., Aremu, A.O., Moyo, M., Van Staden, J. 2012. Antioxidant and acetylcholinesterase-inhibitory properties of long-term stored medicinal plants. *BMC Complementary Alternative Medicine*, 12: 1-9.

8. Askun, T., Tumen, G., Satil, F., Kilic, T., 2008. Effects of some *Lamiaceae* species methanol extracts on potential mycotoxin producer fungi. *Pharmaceutical Biology* 46(10-11): 688-694.

9. Atif, M., Ilavenil, S., Devanesan, S., AlSalhi, M.S., Choi, K.C., Vijayaraghavan, P., Alfuraydi, A.A., Alanazi, N.F. 2020. Essential oils of two medicinal plants and protective properties of jack fruits against the spoilage of bacteria and fungi. *Industrial Crops and Products,* 147: 1-9.

10. Bankole, S. A., Adebanjo, A. 2004. Mycotoxins in food in West Africa: current situation and possibilities of controlling it. *African Journal of Biotechnology* 2(9): 254-263.

11. Bhat, R., Rai, R.V., Karim, A.A. 2010. Mycotoxins in food and feed: present status and future concerns. *Comprehensive Reviews in Food Science and Food Safety,* 10:57-81.

12. Bilal, T., Aksakal, D.H., Sünnetci, S., Keser, O., Eseceli, H. 2014. Detection of aflatoxin, zearalenone and deoxynivalenol in some feed and feedstuffs in Turkey. *Pakistan Veterinary Journal,* 34: 459-463.

13. Binder, E.M. 2007. Managing the risk of mycotoxins in modern feed production. *Animal Feed Science and Technology,* 133(1), 149-166.

14. Bluma, R. V., Etcheverry, M.G., 2008. Application of essential oils in maize grain: Impact on *Aspergillus flavus* growth parameters and aflatoxin accumulation. *Food Microbiology,* 25(2): 324-334.

15. Boonen, J., Malysheva, S.V., Taevernier, L., Di Mavungu, J.D., De Saeger, S., De Spiegeleer, B. 2012. Human skin penetration of selected model mycotoxins. *Toxicology,* 301(1): 21-32.

16. Brera, C., Miraglia, M., Colatosti, M., 1998. Evaluation of the impact of mycotoxins on human health: sources of errors. *Microchemical Journal,* 59(1): 45-49.

17. Bryden, W.L. 2007. Mycotoxins in the food chain: human health implications. *Asian Pacific Journal of Clinical Nutrition,* 16(1): 95-101.

18. Bullerman, L.B., Bianchini, A. 2007. Stability of mycotoxins during food processing. *International Journal of Food Microbiology,* 119(1): 140-146.

19. Campbell, T. C., Stoloff, L. 1974. Implication of mycotoxins for human health. *Journal of Agricultural and Food Chemistry*, 22(6): 1006-1015.

20. Chakraborty, S., Newton, A.C. 2011. Climate change, plant diseases and food security: An overview. *Plant Pathology,* 60: 2-14.

21. Cleveland, T. E., Dowd, P.F., Desjardins, A.E., Bhatnagar, D., Cotty, P.J. 2003. United States Department of Agriculture- Agricultural Research Service research on pre-harvest prevention of mycotoxins and mycotoxigenic fungi in US crops. *Pest Management Science,* 59(6-7): 629-642.

22. Conijn, J.G., Bindraban, P.S., Schröder, J.J., Jongschaap, R.E.E. 2018. Can our global food system meet food demand within planetary boundaries*? Agriculture, Ecosystems and Environment,* 251: 244-256.

23. Cooray, R. 1984. Effects of some mycotoxins on mitogen-induced blastogenesis and SCE frequency in human lymphocytes. *Food and Chemical Toxicology,* 22(7): 529-534.

24. Coulombe, R.A. 1993. Biological action of mycotoxins. *Journal of Dairy Science,* 76(3): 880-891.

25. Cox, P.A., Balick, M.J. 1994. The ethnobotanical approach to drug discovery. *Scientific American,* 6: 60-65.

26. Cragg, G.M., Newman, D.J. 2013. Natural products: a continuing source of novel drug leads. *Biochimica et Biophysica Acta,* 1830(6): 3670-3695.

27. Dai, J., Mumper RJ. 2010. Plant phenolics: extraction, analysis and their antioxidant and anticancer properties. *Molecules*,15(10): 7313-7352.

28. Darwish, W.S., Ikenaka, Y., Nakayama, S.M.M., Ishikuza, M. 2014. An overview on mycotoxin contamination of foods in Africa. *Journal of Veterinary and Medical Sciences,* 67: 789-797.

29. Dawson, W.A.J.M., Jestoi, M., Rizzo, A., Nicholson, P., Bateman, G.L. 2004. Field evaluation of fungal competitors of *Fusarium culmorum* and *F. graminearum*, causal agents of ear blight of winter wheat, for the control of mycotoxin production in grain. *Biocontrol Science and Technology,* 14(8): 783-799.

30. Decker, E.A., Wielch, B. 1990. Role of ferritin as a lipid oxidation catalyst in muscle food. *Journal of Agricultural and Food Chemistry,* 38: 674-677.

31. Deutschländer, M.S., Lall, N. and Van De Venter, M. 2009. Plant species used in the treatment of diabetes by South African traditional healers: An inventory. *Pharmaceutical Biology*, *47*(4): 348-365.

32. Diekman, M.A., Green, M.L. 1992. Mycotoxins and reproduction in domestic livestock. *Journal of Animal Science,* 70(5): 1615-1627.

33. Diener, U.L., Cole, R.J., Sanders, T.H., Payne, G.A., Lee, L.S., Klich, M.A. 198. Epidemiology of aflatoxin formation by *Aspergillus flavus*. *Annual review of Phytopathology,* 25(1): 249-270.

34. Dikhoba, P.M.; Mongalo, N.I.; Elgorashi, E.E.; Makhafola. T.J. 2019. Antifungal and anti-mycotoxigenic activity of selected South African medicinal plants species. *Heliyon*, *5*, e02668.

35. Duran, R.M., Cary, J.W., Calvo, A.M. 2007. Production of cyclopiazonic acid, aflatrem, and aflatoxin by *Aspergillus flavus* is regulated by veA, a gene necessary for sclerotial formation. *Applied Microbiology and Biotechnology*, 73(5): 1158-1168.

36. Dzoyem, J.P.; Aro, A.O.; McGaw, L.J.; Eloff, J.N. 2016. Antimycobacterial activity against different pathogens and selectivity index of fourteen medicinal plants used in southern Africa to treat tuberculosis and respiratory ailments. *South African of Botany*, 102: 70-74.

37. Ekwomadu, T.I., Gopane, R.E., Mwanza, M. 2018. Occurrence of filamentous fungi in maize destined for human consumption in South Africa. *Food Science and Nutrition,* 6(4): 884-890.

38. Elisha, I.L., Dzoyem, J.P., McGaw, L.J. 2016. The anti-arthritic, anti-inflammatory, antioxidant activity and relationships with total phenolics and total flavonoids of nine South African plants used traditionally to treat arthritis. *BMC Complementary and Alternative Medicine*, 16: 1-10.

39. Eloff, J.N. (1998). A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. *Planta Medica*, 64(8): 711-713.

40. Eloff, J.N., Mdee, L.K., Masoko, P. 2007. Invasive and weedy species can be used as a source of antifungal compounds to control plant fungal pathogens. *South African Journal of Botany,* 73(2): 287.

41. Emri, T., Majoros, L., Tóth, V., Pócsi, I. 2013. Echinocandins: production and applications. *Applied Microbiology and Biotechnology,* 97(8): 3267-3284.

42. Etcheverry, M., Torres, A., Ramirez, M.L., Chulze, S., Magan, N. 2002. *In vitro* control of growth and fumonisin production by *Fusarium verticillioides* and *F. proliferatum* using antioxidants under different water availability and temperature regimes. *Journal of Applied Microbiology,* 92(4): 624-632.

43. Fadipe, V.O., Mongalo, N.I., Opoku, A.R. 2015. *In Vitro* evaluation of the comprehensive antimicrobial and antioxidant properties of *Curtisia dentata* (Burm.f) C.A. Sm: Toxicological effect on the Human Embryonic Kidney (HEK293) and Human Hepatocellular carcinoma (HepG2) cell lines. *Experimental and Clinical Sciences Journal,* 14: 971-983.

44. Fadipe, V.O., Mongalo, N.I., Opoku, A.R., Dikhoba, P.M., Makhafola, T.J. 2017. solation of anti-mycobacterial compounds from *Curtisia dentata* (Burm.f.) C.A.Sm (Curtisiaceae). *BMC Complementary Alternative Medicine,* 17: 1-6.

45. Fadipe, V.O.; Opoku, A.R.; Singh, M.; Pereira, A.R.; Rijo, P.; Mongalo, N.I**. 2020.** Antimycobacterial, antiplasmodial studies and cytotoxicity of oleanolic acid and its derivative from *Syzygium aromaticum* Linn (Myrtaceae). *Journal Biopharmaceutics Sciences,* 17: 1-12.

46. Favre-Godal, Q., Dorsaz, S., Queiroz, E.F., Conan, C., Marcourt, L., Wardojo, B.P.E., Wolfender, J.L. 2014. Comprehensive approach for the detection of antifungal

compounds using a susceptible strain of *Candida albicans* and confirmation of *in vivo* activity with the Galleria mellonella model. *Phytochemistry,* 105: 68-78.

47. Fotakis, G., Timbrell, J.A. 2006. *In vitro* cytotoxicity assays: comparison of LDH, neutral red, MTT and protein assay in hepatoma cell lines following exposure to cadmium chloride. *Toxicology Letters,* 160(2): 171-177.
48. Giorni, P., Magan, N., Pietri, A., Bertuzzi, T., Battilani, P. 2007. Studies on *Aspergillus flavus* isolated from maize in northern Italy. *International Journal of Food Microbiology,* 113(3): 330-338.
49. Giorni, P., Battilani, P., Pietri, A., Magan, N. 2008. Effect of a w and CO 2 level on *Aspergillus flavus* growth and aflatoxin production in high moisture maize post-harvest. *International Journal of Food Microbiology,* 122(1): 109-113.
50. Haque, M.A., Wang, Y., Shen, Z., Li, X., Saleemi, M.K. and He, C. 2020. Mycotoxin contamination and control strategy in human, domestic animal and poultry: A review. *Microbial pathogenesis,* 142: 1-12.
51. Hossain, M.T., Modise, D.M., Rong, I.H., Karodia, A.M. 2015. The mycoflora associated with diseased plants and seeds of *Oryza sativa* (Rice): Exemplifying the importance of effective disease control management. *Asian Journal of Science and Technology,* 6:1523-1532.
52. Hussein, H.S., Brasel, J.M. 2001. Toxicity, metabolism, and impact of mycotoxins on humans and animals. *Toxicology,* 167(2): 101-134.
53. Huwig, A., Freimund, S., Käppeli, O., Dutler, H. 2001. Mycotoxin detoxication of animal feed by different adsorbents. *Toxicology Letters,* 122(2), 179-188.

54. Klich, M.A., Mullaney, E.J.1987. DNA restriction enzyme fragment polymorphism as a tool for rapid differentiation of *Aspergillus flavus* from *Aspergillus oryzae*. *Experimental Mycology,* 11(3): 170-175.

55. Klich, M.A., Pitt, J.I. 1988. Differentiation of *Aspergillus flavus* from *A. parasiticus* and other closely related species. *Transactions of the British Mycological Society* 91(1): 99-108.

56. Kotze, M., Eloff, J.N., Houghton, P.J. 2002. Extraction of antibacterial compounds from *Combretum microphyllum* (Combretaceae). *South African Journal of Botany,* 68(1): 62-67.

57. Kung, H.C., Chipley, J.R., Latshaw, J.D., Kerr, K.M., Wilson, R.F. 1977. Chronic mycotoxicosis in chicks caused by toxins from Phomopsis grown on soybeans. *Journal of Comparative Pathology,* 87(3): 325-333.

58. Mahlo, S.M., McGaw, L.J., Eloff, J.N. 2010. Antifungal activity of leaf extracts from South African trees against plant pathogens. *Crop Protection*, *29*(12):1529-1533.

59. Makhafola, T.J., McGaw, L.J., Eloff, J.N. 2014. *In vitro* cytotoxicity and genotoxicity of five *Ochna* species (Ochnaceae) with excellent antibacterial activity. *South African Journal of Botany,* 91: 9-13.

60. Makhuvele, R., Naidu, K., Gbashi, S., Thipe, V.C., Adebo, O.A., Njobeh, P.B., 2020. The use of plant extracts and their phytochemicals for control of toxigenic fungi and mycotoxin. *Heliyon* 6, e05291.

61. Manker, D.C. 2012. Biopesticides: The next line of defense for resistance management. *Outlooks on Pest Management,* 23(3): 136-139.

62. Masoko, P., Picard, J., Eloff, J.N. 2005. Antifungal activities of six south African *Terminalia* species (Combretaceae). *Journal of Ethnopharmacology,* 99(2): 301-308.

63. Mathabe, M.C.; Hussein, A.A.; Nikolova, R.V.; Basson, A.E.; Meyer, J.J.M.; Lall, N. 2008. Antibacterial activities and cytotoxicity of terpenoids isolated from *Spirostachys africana. Journal of Ethnopharmacolog*y,116:194-197.

64. Matthies, A, Walker, F., Buchenaur, H. 1999. Interference of selected fungicides, plant growth retardants as well as piperonyl butoxide and 1-aminobenzotriazole in trichothecene production of *Fusarium graminearum* (strain 4528) *in vitro*. *Zeitschrift f¨ur Pflanzenkrankheiten und Pflanzenschutz,* 106:198-212.

65. Meyer, N.L. 2003. Plants of southern Africa: an annotated checklist. Pretoria: National Botanical Institute.

66. Milus, E.A., Parsons, C.E. 1994. Evaluation of foliar fungicides for controlling *Fusarium* head blight of wheat. *Plant Disease,* 78: 697-699.

67. Mishra, A.K., Dubey, N.K. 1994. Evaluation of some essential oils for their toxicity against fungi causing deterioration of stored food commodities. *Applied and Environmental Microbiology,* 60(4): 1101-1105.

68. Molele, P.K., Mongalo, N.I., Dikhoba, M.P., Makhafola, T.J. 2016. Antifungal and antioxidant properties of ten medicinal plants collected from Kwa-Dlangezwa area, KwaZulu-Natal Province. *South African Journal of Botany,* 103: 334.

69. Mongalo, N.I., Opoku, A.R., Zobolo, A.M. 2012. Antibacterial and antioxidant activity of the extracts of *Waltheria indica* Linn. collected from Capricorn District, Limpopo Province. *Journal* of *Medicinal Plants Research*, 6: 5593-5598.

70. Mongalo, N.I., Dikhoba, P.M., Soyingbe, S.O., Makhafola, T.J. 2018. Antifungal, antioxidant activity and cytotoxicity of South African medicinal plants against mycotoxigenic fungi. *Heliyon, 4,* e00973.

71. Mongalo, N.I. and Makhafola, T.J. 2018. Ethnobotanical knowledge of the lay people of Blouberg area (Pedi tribe), Limpopo Province, South Africa. *Journal of Ethnobiology and Ethnomedicine*, *14*(1): 1-23.

72. Mongalo, N.I., Soyingbe, S.O., Makhafola, T.J. 2019. Antimicrobial, cytotoxicity anticancer and antioxidant activities of *Jatropha zeyheri* Sond. Roots (Euphorbiaceae). *Asian Pacific Journal of Tropical Biomedicine,* 9(7): 307-314.

73. Montiel, D., Dickinson, M.J., Lee, H.A., Dyer, P.S., Jeenes, D.J., Roberts, I.N., Archer, D. B. 2003. Genetic differentiation of the *Aspergillus* section Flavi complex using AFLP fingerprints. *Mycological Research,* 107(12): 1427-1434.

74. Mosmann, T. 1983. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *Journal of Immunological Methods*, 65(1): 55-63.

75. Mthethwa, N., Oyedeji, B.A.O., Obi, C.L., Aiyegoro, O.A. 2014. Anti-staphylococcal, anti-HIV and cytotoxicity studies of four South African medicinal plants and isolation of bioactive compounds from *Cassine transvaalensis* (Burtt. Davy) codd. *BMC Complementary Alternative Medicine,* 14: 1-9.

76. Ncube, E. 2008. Mycotoxin levels in subsistence farming systems in South Africa. Doctoral Thesis, Stellenbosch University.

77. Ncube, E., Flett, B.C., Waalwijk, C., Viljoen, A. 2011. *Fusarium* spp. and levels of fumonisins in maize produced by subsistence farmers in South Africa. *South African Journal of Science*, 107(1): 1-7.

78. Nicholson, P., Turner, J.A., Jenkinson, P., Jennings, P., Stonehouse, J., Nuttall, M., Thomsett, M. 2003. Maximising control with fungicides of Fusarium ear blight (FEB) in order to reduce toxin contamination of wheat. HGCA Project Report.

79. Okeleye, B.I., Mkwetshana, N.T., Ndip, R.N. 2013. Evaluation of the antibacterial and antifungal potential of *Peltophorum africanum*: Toxicological effect on human chang liver cell line. *The Scientific World Journal,* Article ID 878735. DOI: 10.1155/2013/878735.

80. Opoku, A.R., Ndlovu, I.M., Terblanche, S.E. and Hutchings, A.H. 2007. In vivo hepatoprotective effects of *Rhoicissus tridentata* subsp. *cuneifolia*, a traditional Zulu medicinal plant, against CCl4-induced acute liver injury in rats. *South African Journal of Botany*, *73*(3): 372-377.

81. Palgrave, K.C. 2015. Palgrave's trees of southern Africa. Penguin Random House South Africa.

82. Peraica, M., Radic, B., Lucic, A., Pavlovic, M. 1999. Toxic effects of mycotoxins in humans. *Bulletin of the World Health Organization*, 77(9): 754-766.

83. Peraica, M., Domijan, A.M. 2001. Contamination of food with mycotoxins and human health. *Arhiv Za Higijenu Rada I Toksikologiju,* 52(1): 23-35.

84. Pitt, J.I., 2000. Toxigenic fungi and mycotoxins. *British Medical Bulletin,* 56(1): 184-192.

85. Pourakbar, L., Moghaddam, S.S., Enshasy, H.A.E., Sayyed, R.Z. 2021. Antifungal Activity of the Extract of a Macroalgae, *Gracilariopsis persica*, against four plant pathogenic fungi. *Plants,* 10: 1-13.

86. Rates, S.M.K. 2001. Plants as source of drugs. *Toxicon,* 39(5): 603-613.

87. Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., Rice-Evans, C. 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine*, *26*(9-10): 1231-1237.

88. Richard, J.L. 2007. Some major mycotoxins and their mycotoxicosis- An overview. International *Journal of Food Microbiology,* 119(1), 3-10.

89. Rustom, I.Y. 1997. Aflatoxin in food and feed: occurrence, legislation and inactivation by physical methods. *Food Chemistry,* 59(1), 57-67.

90. Satish, S., Mohana, D.C., Ranhavendra, M.P., Raveesha, K.A. 2007. Antifungal activity of some plant extracts against important seed borne pathogens of *Aspergillus* sp. *An International Journal of Agricultural Technology,* 3(1): 109-119.

91. Seepe, H.A., Amoo, S., Nxumalo, W., Adeleke, R.A. 2020. Sustainable use of thirteen South African medicinal plants for the management of crop diseases caused by *Furasium* species- An in Vitro study. *South African Journal of Botany*, 130: 456-464.

92. Sharma, R.R., Singh, D., Singh, R. 2009. Biological control of postharvest diseases of fruits and vegetables by microbial antagonists: A review. *Biological Control,* 50(3): 205-221.

93. Sheikh, H., El-Naggar, A., Al-Sobahi, D. 2018. Evaluation of antimycotic activity of extracts of marine algae collected from Red Sea Coast, Jeddah, Saudi Arabia. *Journal of Bioscience and Medicine,* 6: 51-68.

94. Shephard, G.S. 2008. Impact of mycotoxins on human health in developing countries. *Food Additives and Contaminants,* 25(2): 146-151.

95. Shukla, R., Singh, P., Prakash, B., Dudey, N.K. 2012. Antifungal, aflatoxin inhibitory and free radical-scavenging activities of some plant extracts. *Journal of Food Quality,* 35: 182-189.

96. Sittisart, P., Yossan, S., Prasersan, P. 2017. Antifungal property of chili, sharlot and garlic extracts against pathogenic fungi, *Phomopsis* spp. isolated from infected leaves of para rubber (*Hevea brasiliensis Muell*. Arg.). *Agriculture and Natural Resources,* 51: 485-491.

97. Soliman, K.M., Badeaa, R.I. 2002. Effect of oil extracted from some medicinal plants on different mycotoxigenic fungi. *Food and Chemical Toxicology,* 40(11): 1669-1675.

98. Souza, E.L., Stamford T.L.M., Lima E.O., Trajano V.N. 2007. Effectiveness of *Origanum vulgare* L. essential oil to inhibit the growth of food spoiling yeasts. *Food Control,*18: 409-413.

99. Soyingbe, O.S., Mongalo, N.I. and Makhafola, T.J. 2018. In vitro antibacterial and cytotoxic activity of leaf extracts of Centella asiatica (L.) Urb, *Warburgia salutaris* (*Bertol*. F.) *Chiov* and *Curtisia* dentata (Burm. F.) CA Sm-medicinal plants used in South Africa. *BMC Complementary and Alternative Medicine*, *18*(1): 1-10.

100. Stark, T.D., Mtui, J.D., Balemba, O.B. 2013. Ethno-pharmacological survey of plants used in the traditional treatment of gastrointestinal pain, inflammation and diarrhea in Africa: future perspectives for integration into modern medicine. *Animals*, 3: 58-227.

101. Sultana, S., Asif, H.M., Akhtar, N. Ahmad, K. 2015. Medicinal plants with potential antipyretic activity: A review. *Asian Pacific Journal of Tropical Disease,* 5(1): 202-208.

102. Sulub-Tun, R.A., Rodriguez-Garcia, C.M., Peraza-Echeveria, L., Torres-Tapia, L.W., Peraza-Sanchez, S.R., Perez-Brito, D., Vera-KU, B.M. 2020. *South African Journal of Botany,* 131: 484-493.

103. Talib, W.H., Mahasheh, A.M. 2010. Anti-proliferative activity of plant extracts used against cancer in traditional medicine. *Scientia Pharmaceutica*, 78: 33-45.

104. Tepe, B., Dimitra, D., Arzuhan-Sihoglu, T., Moschos, P., Atalay, S. 2007. Antioxidant activity of the essential oil and various extracts of *Nepeta flavida* Hub. -Mor. from Turkey. *Food Chemistry,*103: 1358-1364.

105. Thembo, K.M., Vismer, H.F., Nyazema, N.Z., Gelderblom, W.C.A., Katerere, D.R. 2010. Antifungal activity of four weedy plant extracts against selected mycotoxigenic fungi. *Journal of Applied Microbiology*, 109(4): 1479-1486.

106. Thirumalai, T., Kelumalai, E., Senthilkumar, B., David, E. 2009. Ethnobotanical study of medicinal plants used by the local people in Vellore district, Tamilnadu, India. *Ethnobotanical Leaflets,* 2009(10): 10.

107. Thompson, D.P. 1994. Minimum inhibitory concentration of esters of p-hydroxybenzoic acid (paraben) combinations against toxigenic fungi. *Journal of Food Protection,* 57(2):133-135.

108. Tiwary, B.K., Bihani, S., Kumar, A., Chakraborty, R. Ghosh, R. 2015. The in vitro cytotoxic activity of ethnopharmacological important plants of Darjeeling district of West Bengal against different human cancer cell lines. *BMC complementary and Alternative Medicine*, 15(1): 1-10.

109. Torres, A.M., Ramirez, M.L., Arroyo, M., Chulze, S.N., Magan, N. 2003. Potential use of antioxidants for control of growth and fumonisin production by *Fusarium*

verticillioides and *Fusarium proliferatum* on whole maize grain*. International Journal of Food Microbiology*, 83(3): 319-324.

110. Van Wyk, B. E., Oudtshoorn, B. V., Gericke, N.,1997. Medicinal Plants of South Africa. Briza.

111. Velmourougane, K., Bhat, R., Gopinandhan, T.N., Panneerselvam, P. 2011. Management of *Aspergillus ochraceus* and *Ochratoxin*-A contamination in coffee during on-farm processing through commercial yeast inoculation. *Biological Control,* 57(3): 215-221.

112. Waalwijk, C., Koch, S.H., Ncube, E., Allwood, J., Flett, B., De Vries, I., Kema, G.H.J. 2008. Quantitative detection of Fusarium spp. and its correlation with fumonisin content in maize from South African subsistence farmers. *World Mycotoxins Journal,* 1: 39-47.

113. Wild, C.P., Gong, Y.Y. 2010. Mycotoxins and human disease: a largely ignored global health issue. *Carcinogenesis,* 31(1): 71-82.

114. Yuan, J., Raza, W., Shen, Q., Huang, Q. 2012. Antifungal Activity of *Bacillus amyloliquefaciens* NJN-6 volatile compounds against *Fusarium oxysporum* f. sp. Cubense. *Plant Microbiology,* 78: 5942-5944.

115. Zain, M.E. 2011. Impact of mycotoxins on humans and animals. *Journal of Saudi Chemical Society,* 15(2): 129-144.

ANNEXURES

APPENDIX I: ARTICLE NUMBER 1 (SUBMITTED)

South African Journal of Botany

GC/TOF-MS based phytochemical analysis and anti-mycotoxigenic activity of South African medicinal plants collected from Lowveld Botanical Garden (LBG), Nelspruit-South Africa

--Manuscript Draft--

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Abstract:	The anti-mycotoxigenic activity six medicinal plants extracts and their ir growth inhibition against six mycotoxigenic fungal pathogens have beer vitro . Antifungal activity was carried out using microdilution assay. Extr further assessed for their antioxidant activity. The cytotoxicity was explo African green monkey (Vero) and Organic extracts from Strychnos mitis lowest minimum inhibitory concentration (MIC) value of 0.01 mg/ml agai Fusarium species and further MIC value of 0.08 mg/ml against three A species at 24 hr incubation period. In the mycelial growth inhibition (MG aethiopicum showed the highest mycelial growth inhibition yielding 44.3 Fusarium verticiloides in a three-day incubation period. Organic extract africana exhibited the lowest lethal concentration (LC 50) value of 0.1 against both cell African green monkey (Vero) and bovine dermis cell lir from S. africana and M . aethiopicum exhibited a noteworthy antioxi against 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2-2'-Azino-di-[3-ethylbenz sulfonate (ABTS) and chelation of iron. In the Gas-chromatography time spectrometry (GC/TOF-MS) analysis, M. aethiopicum extract exhibited of tetradecane (15.74%) and heptadecane (14.96%), while S. africana hexadecanoic acid (12.14%) and nonadecane,2-methyl (12.14%). Elsev compounds were reported to inhibit mycelial growth inhibition of various fungal strains, particularly Aspergillus species. These results suggest t compounds from these plants should be further explored in vivo and us a bio-fungicide with less side effects compared to synthetic fungicides.
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