ISOLATION AND CHARACTERISATION OF ANTIFUNGAL COMPOUNDS FROM MEDICINAL PLANTS THAT ARE ACTIVE AGAINST SELECTED *FUSARIUM* SPECIES

by

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THESIS

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DECLARATION

I declare that the thesis hereby submitted to the University of Limpopo, for the degree of Doctor of Philosophy, Natural Products Chemistry has not previously been submitted by me for a degree at this or any other university; that it is my work in design and in execution, and that all materials contained herein has been duly acknowledged.

19 April 2021

Seepe, HA (Mr.) Date

DEDICATION

I dedicate this work to my grandparents, to whom my debt is great. May your understanding, kindness and generosity towards me extend to everyone in our community.

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Seepe, H.A., Amoo, S.O., Nxumalo, W. Compounds isolated from medicinal plants may be potential scaffold molecules in crop protection. The South African Chemical Institute (SACI), North Section Young Chemists Symposium. 31 October 2019. Research Conference Centre, University of Venda, Thohoyandou, South Africa.

Seepe, H.A., Amoo, S.O., Nxumalo, W. Application of medicinal plant extracts against maize seeds *Fusarium* pathogens and their effects on seeds germination. Combined Congress. 20–23 January 2020, University of the Free State, Bloemfontein, South Africa.

LIST OF ABBREVIATIONS

AC	Acetone
ANOVA	Analysis of Variance
ARC	Agricultural Research Council
$CDCl_3$	Deuterated chloroform
COSY	Correlation Spectroscopy
d	Doublet
DAS	Diacetoxyscirpenol
dd	doublet of doublets
DEPT	Distortionless Enhancement by Polarization Transfer
DMRT	Duncan's Multiple Range Test
DMSO-d6	Deuterated dimethyl sulfoxide
DON	Deoxynivalenol
EA	Ethyl acetate
ESI	Electrospray Ionization
ESI-MS	Electrospray Ionization-Mass Spectrum
FAO	Food and Agriculture Organization
FIC	Fractional Inhibitory Concentration
FICI	Fractional Inhibitory Concentration Index
GC-MS	Gass Chromatography-Mass Spsctrophotometer
HMBC	Heteronuclear Multiple Bond Correlation
HMQC	Heteronuclear Multiple Quantum Coherence
HPLC	High Performance Liquid Chromatography
INT	*p*-iodonitro-tetrazolium violet

LC	Liquid Chromatography
LC-MS	Liquid Chromatography-Mass Spectrometer
LC-PDA	Liquid Chromatography- Photodiode Array Detector
m/z	Mass-to-charge ratio
MAPP	Multi-Country Agriculture Productivity Programme
MIC	Minimum Inhibitory Concentration
MID	Minimum Inhibitory Dilution
MRSA	Methicillin-resistant *Staphylococcus aureus*
MS	Mass Spectrophotometer
na	Not applicable
NIV	Nivalenol
NMR	Nuclear Magnetic Resonance
NRF	National Research Foundation
PDA	Potato Dextrose Agar
PDB	Potato Dextrose Broth
PE	Petroleum ether
ppm	Parts per million
PPRI	Agricultural Reserach Council-Plant Health and Protection
RF	Retention factor
R.I	Relative impact.
SA	South Africa
SAAB	South African Association of Botanists
SACI	South African Chemical Institute
SADC	Southern African Development Community

SADC-FANR	Southern African Development Community-Food, Agriculture and Natural Resources
TLC	Thin layer chromatography
UK	United Kingdom
UNIN	University of the North
UV	Ultraviolet
v/v	Volume per volume
WA	Water
WHO	World Health Organization
ZEN	Zearalenone
Δ	Chemical shift
IUPAC	International Union of Pure and Applied Chemistry
IC_{50}	The half maximal inhibitory concentration

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ABSTRACT

Fusarium species are among pathogenic organisms responsible for massive yield and quality losses in crop production. They cause crop diseases in the field and during storage, and some species are capable of producing mycotoxins which contaminate products and threaten consumer s' health. Conventional synthetic fungicides are available for the control of *Fusarium* pathogens, however, their applications have been restricted or discouraged due to their harmful effect on the environment, livestocks and human health. There are also reports about fungal-resistance to available fungicides. Moreover, the synthetic chemicals are not affordable to smallholder farmers and to some extent, they are not recommended for applications in organic farming. As an alternative to these fungicides, selected medicinal plant species were investigated as sources of natural chemicals or compounds with potential to be developed into plant-based fungicides to control *Fusarium* pathogens. This study aimed to identify antifungal extracts among the selected medicinal plant species which could be used to develop plant-based fungicides to control *Fusarium* diseases. It also focused on isolation and characterization of antifungal compounds from selected medicinal plant species. Thirteen medicinal plant species (*Combretum erythrophyllum* (Burch.) Sond , *Melia azedarach* L, *Solanum mauritianum* Scop, *Nicotiana glauca* Graham, *Schotia brachypetala* Sond, *Lantana camara* L, *Combretum molle* R. Br. ex G. Don, *Quercus acutissima* Carruth, *Olea europaea* L, *Vangueria infausta* Burch, *Withania somnifera* (L.) Dunal, *Harpephyllum caffrum* Bernh and *Senna didymobotrya* (Fresen.) H.S. Irwin & Barneby) were selected from literature based on their reported strong antimicrobial activity against human and/or animal pathogens. The leaves of these plant species were collected, shade-dried and extracted with water, petroleum ether, ethyl acetate and acetone. Extractant yield was recorded and each extract was evaluated for antifungal activity using a micro-dilution assay against nine *Fusarium* pathogens (*Fusarium verticillioides, Fusarium proliferatum, Fusarium subglutinans, Fusarium graminearum, Fusarium solani,*

Fusarium oxysporum, *Fusarium semitectum*, *Fusarium chlamydosporum* and *Fusarium equiseti*). Similar solvent extracts from different plant species that demonstrated MIC value of less than 0.1 mg/ml against the same pathogen were combined and evaluated for antifungal activity. The interation effect of combined extracts was determined by calculating their fractional inhibitory concentration index (FICI) in order to determine their possible synergistic, additive, indifference or antagonistic antifungal activity against tested pathogens. Plant extracts demonstrating synergistic and or additive interaction were further evaluated in combination and individually for *in vivo* antifungal activity against maize seed *Fusarium* pathogens. At least, one of the extracts obtained from these medicinal plant species showed strong antifungal activity with minimum inhibitory concentration (MIC) of less than 0.1 mg/ml against at least one of the tested pathogens. Of the four solvent extracts evaluated, acetone and ethyl acetate extracts showed stronger antifungal activity compared to petroleum ether and water extracts. Of the nine pathogens tested, *F. proliferatum* was the most susceptible and was strongly inhibited (MIC < 0.1 mg/ml) by 41 plant extracts whilst *F. equisite* was found to be resistant with MIC < 0.1 mg/ml by only three plant extracts. In total, each pathogen was tested against 52 plant extracts. There were 17, 16 and 15 extracts from *C. erythrophyllum, S. mauritianum* and *Q. acutissima*, respectively, with MIC values less than 0.1 mg/ml. These species were the most active when tested individually. *Schotia brachypetala* was found to be the least active medicinal plant with only seven extracts demonstrating very strong activity (MIC < 0.1 mg/ml) against the tested pathogens. Minimum inhibitory dilution (MID) or total activity was also calculated and it was found that water and acetone extracts had the highest MID, followed by ethyl acetate extracts while petroleum ether extracts recorded the lowest. Of all plant extracts tested against the nine pathogens, 59 plant extracts demonstrated MID values of more than 1000 ml/g. Out of the 348 extract combinations evaluated, 116 and 87 extract combinations demonstrated synergistic and additive antifungal activity, respectively. The strongest activity

recorded for the combined extracts resulted from synergistic interaction with MIC value of 0.001 mg/ml against *F. proliferatum* and *F. verticilloides*. Combined acetone extract of *C. erythrophyllum* and *Q. acutissima* was very active (95.75% inhibition) against *F. verticilloides* inoculated on maize seeds while individual preparation from *M. azedarach* acetone extract demonstrated 97.10% inhibition against *F. proliferatum*. The extracts showing good antifungal activity (≥ 50% inhibition) were further tested for phytotoxicity on maize seed germination and the lowest recorded seed germination was 86.25%, resulting from *Q. acutissima* ethyl acetate extract. Combined acetone extract of *C. erythrophyllum* and *Q. acutissima* did not significantly affect maize seedling growth when compared to negative control (water treatment). All plant extracts that showed strong activity (MIC < 0.1 mg/ml) when tested using micro-dilution assay were spotted on thin layer chromatography (TLC) bioautographic assay to establish and determine the number of active compounds or bands. The white spots observed on the chromatograms indicated the presence of antifungal compounds. *Combretum erythrophyllum*, *W. somnifera* and *L. camara* exhibited the presence of antifungal compounds against 7, 5 and 4 pathogens, respectively. Hence, these plant species were selected for isolation of antifungal compounds where open column chromatography and preparative TLC were used for compound purification. At least, three isolated fractions from the three plant species were found to be active (MIC values ranging from 0.0098 to 0.625 mg/ml) against more than five pathogens. The fractions were also found to contain different levels of phytochemicals such as glycosides, flavonoids, steroids, and terpernoids. The structures of isolated compounds or fractions were determined using nuclear magnetic resonance (NMR) and mass spectroscopic (MS) techniques. A mixture of apeginin (*4',5,7-trihydroxyflavone*) and salvigenin (*5-hydroxy-6,7,4'-trimethoxyflavone*) isolated from the leaves of *C. erythrophyllum* showed strong antifungal activity (MIC values ranging from 0.01 mg/ml to 0.63 mg.ml) against 5 tested *Fusarium* pathogens. Also isolated from *C. erythrophyllum* was a derivative of maslinic acid and it has

shown antifungal activity with MIC values ranging from 0.08 mg/ml to 0.63 mg/ml against 6 tested pathogens. On the other hand, lantadene A (*22- angeloyloxy-9-hydroxy-3-oxo-olean-12-en-28-oic acid*), boswellic acid (*11-keto-β-boswellic acid*) and boswellic acid glycoside isolated from the leaves of *Lantana camara* showed good activity (MIC values ≤ 0.63 mg/ml) against one or more *Fusarium* pathogens. Withaferin A (*4β,27-dihydroxy-1-oxo-5β,6β-epoxywitha-2-24-dienolide*) glycoside isolated from the leaves of *Withania somnifera* showed antifungal activity with MIC value of 0.16 mg/ml against *F. verticilloides*. This study demonstrated potential applications of medicinal plant extracts as cheap, accessible and sustainable source of eco-friendly pesticides for fighting crop diseases in organic and smallholder farming. The extracts can be used as treatment agents to control maize seed spoilage during post-harvest storage. Additionally, characterised antifungals may serve as scaffold compounds during commercial synthesis of plant-based fungicides.

CHAPTER 1

GENERAL INTRODUCTION

1.1. BACKGROUND

Despite the availability of information and initiatives to address food insecurity challenges, many people in developing countries still do not have enough food (Mbow *et al*., 2014; Mungai *et al*., 2016). The demand for more nutritious and safe food is also fueled by increasing human population whilst climate change, drought, pest and diseases remain among major factors that negatively affect agricultural production (Godfray *et al*., 2010). Most people in sub-Saharan countries rely on smallholder farming as a source of food to sustain life and well-being (Singh *et al*., 2002; Salami *et al*., 2010). As it was acknowledged by the Southern African Development Community (SADC, 2004) and Southern African Development Community-Food, Agriculture and Natural Resources (SADC-FANR, 2008), agriculture has the potential to improve economic growth, create employment opportunities and reduce poverty. Hence, many governments in SADC countries embarked on programmes to subsidize poor-resourced smallholder farmers with agricultural inputs such as fertilizers and seeds (Levy, 2005; Rubey, 2005; Dorward and Chirwa, 2011; SADC-FANR, 2012). Major crops produced in this farming system include cassava, maize, cowpea, pumpkin, groundnuts and soybeans. Vegetable crops such as green beans, tomatoes, cabbage, sweet potatoes and potatoes are also produced in the backyards or household gardens. These crops are vulnerable to attack by pathogenic *Fusarium* diseases both in the field and during storage. *Fusarium* pathogens are among notorious microorganisms responsible for crop diseases and spoilage that render products unsuitable for consumption and may result in massive yield losses (Mao *et al*., 1996; Oren *et al*., 2003).

Maize seeds distributed to farmers through subsidizing programmes are usually treated with pesticides to control pests and diseases (Maude, 1996; Mikkelson *et al*., 2003). However, as

much as can be acsertained, there is a paucity of report, if any, regarding subsidizing farmers with fungicides to manage crop diseases during planting or post-harvest storage. We speculated that lack of suitable application technologies, costs and safety issues associated with these chemicals in smallholder farming might be the reasons. These farmers consume the leaves of some cultivated crops as vegetables or '*morogo*' and they also rely on utilization of stored produce for survival during off-season.

Effective or not, traditional rudimentary methods such as application of plant ashes and destroying pests' colonies or formation are among options available to control crop pest and diseases in smallholder farming since farmers cannot afford conventional pesticides (Demissie *et al*., 2008). On the other hand, commercial farming is faced with challenges of pesticide resistance and chemical residues remaining in the produce and environment after pesticide treatment (McKim, 1994; Ward *et al*., 1997). Therefore, it is important to search and develop affordable, sustainable and environmental friendly pesticides for the control of diseases in crop production. A search for fungicides, especially from medicinal plant species may provide mankind with new classes of fungicides.

1.2. PROBLEM STATEMENT

Most conventional synthetic fungicides used in the agricultural sector to control crop diseases including those caused by *Fusarium* pathogens are considered to be relatively unsafe to human and livestocks. These chemicals have longer half-lives and can remain in the environment for an extended period of time, causing negative effect such as contaminating water, soil and affecting non-target organisms (Aktar *et al*., 2009; Martínez, 2012; Wisniewski *et al*., 2016). Another major challenge is resistance biuld-up; pathogens have developed or will develop resistance towards available synthetic fungicides and this will result in more yield loss. To some extents, conventional fungicides are not recommended to control diseases in organic

farming and they are also not readily accessible and affordable to poor-resource smallholder farmers (Thembo *et al*., 2010; Hubert *et al*., 2015).

1.3. MOTIVATION

The above mentioned challenges necessitate a search for a different class of crop protection chemistry. There are few classes of fungicides with different mode of action available for the treatment of plant pathogens, which are also affordable to smallholder farmers. The use of medicinal plant species may serve as a natural alternative source of environmental friendly fungicides. Plants produce a wide variety of secondary metabolites and these constituents may possibly inhibit the development of fungal resistance. The leaves are sustainable part of the plants and they are also accessible, therefore, smallholder farmers can make plant-based fungicides without any complications. The active compounds may be isolated and their chemical structures explored as starting materials during design and discovery of new fungicides which can be synthesized in large quantities. Medicinal plant species have been used for many years by different ethnic groups to treat aliments affecting human and domesticated animals (Masika and Afolayan, 2002). Therefore, they are arguably, relatively safe, environmental friendly and probably suitable for applications as fungicides in organic farming.

1.4. AIMS

This study aimed to identify antifungal extracts among the selected thirteen medicinal plant species which could be used to develop plant-based fungicides to control *Fusarium* diseases. It also focused on isolation and characterization of antifungal compounds from selected medicinal plant species.

1.5. OBJECTIVES

- To evaluate *in vitro* antifungal activity of selected plant extracts using micro-plate dilution assay.
- To determine potential synergistic antifungal activity of selected plant extracts.
- To evaluate *in vivo* antifungal activity of plant extracts against pathogens inoculated artificially on maize seeds.
- To determine phytotoxicity of active plant extracts on maize seed germination and seedling growth.
- To isolate and structurally characterize or identify antifungal compounds from selected plant species.
- To evaluate antifungal activity of the isolated compounds using micro-plate dilution assay.

REFERENCES

Aktar, Md.W., Sengupta, D., Chowdhury, A., 2009. Impact of pesticides use in agriculture: Their benefits and hazards. Interdisciplinary Toxicology 2, 1–12.

Demissie, G., Tefera, T., Tadesse, A., 2008. Efficacy of silicosec, filter cake and wood ash against the maize weevil, *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) on three maize genotypes. Journal of Stored Products Research 44, 227–231.

Dorward, A., Chirwa, E., 2011. The Malawi agricultural input subsidy programme. International Journal of Agricultural Sustainability 9, 232–247.

Godfray, H.C., Beddington, J.R., Crute, I.R., Haddad, L., Lawrence, D., Muir, J.F., Pretty, J., Robinson, S., Thomas, S.M., Toulmin, C., 2010. Food security: the challenge of feeding 9 billion people. Science 327, 812–818.

Hubert, J., Mabagala, R.B., Mamiro, D.P., 2015. Efficacy of selected plant extracts against *Pyricularia grisea*, causal agent of Rice Blast Disease. American Journal of Plant Sciences 6, 602–611.

Levy, S. Starter Packs: A strategy to fight hunger in developing countries? Lessons from the Malawi Experience 1998-2003. Wallingford. United Kingdom: CABI Publishing; 2005.

Mao, W., Lewis, J.A., Hebbar, P.K., Lumsden, R.D., 1996. Seed treatment with a fungal or a bacterial antagonist for reducing corn damping-off caused by species of *Pythium* and *Fusarium*. Plant Disease 81, 450–454.

Martínez, J.A. Natural Fungicides Obtained From Plants. In: Dhanasekaran, D., Thajuddin, N., Panneerselvam, A., Editors. Fungicides for Plant and Animal Diseases. London, United Kingdom: IntechOpen; 2012.

Masika, P.J., Afolayan, A.J., 2002. Antimicrobial activity of some plants used for the treatment of livestock disease in the Eastern Cape, South Africa. Journal of Ethnopharmacology 84, 129–134.

Maude, R.B. Seedborne Diseases and Their Control: Principles and Practice. Wallingford, United Kingdom: CAB International; 1996.

Mbow, C., van Noordwijk, M., Luedeling, E., Neufeldt, H., Minang, P.A., Kower, G., 2014. Agroforestry solutions to address food security and climate change challenges in Africa, Current Opinion in Environmental Sustainability 6, 61–67.

McKim, F., 1994. Fungicide resistance re-examined. Plant Science 102, 221–223.

Mikkelson, M., Riesselman, J., Mathre, D., Johnston, B., Blodgett, S. Small grain seed treatment Guide. MontGuide MT199608AG Revised 8/12. Department of Agriculture (USDA) and Montana State University. Agriculture and Natural Resources, Plant Diseases; 2003.

Mungai, L.M., Snapp, S., Messina, J.P., Chikowo, R., Smith, A., Anders, E., Richardson, R.B., Li, G., 2016. Smallholder farms and the potential for sustainable intensification. Frontiers in Plant Science 7, 1720.

Oren, L., Smadar, E., David, C., Amir, S., 2003. Early events in the *Fusarium verticillioides* maize interaction characterized by using a green fluorescent protein-expressing transgenic isolate. Applied Environmental Microbiology 69, 1695–1701.

Rubey, L. Malawi's food crisis: Causes and solutions. Report for US Agency for International Development (USAID). Lilongwe; 2005.

SADC, 2004. Enhancing Agriculture and Food Security for Poverty Reduction in the SADC Region. SADC/EOCM/1/5/2004/1. Gaborone, Botswana.

SADC-FANR, 2008. SADC Multi-Country Agriculture Productivity Programme (SADC MAPP), vol. 1. Gaborone, Botswana.

SADC-FANR, 2012. Food Security Early Warning System: Update-July 2012. Gaborone, Botswana.

Salami, A.; Kamara, A.B.; Brixiova, Z. Smallholder agriculture in East Africa: trends, constraints and opportunities. Working Papers Series No. 105. Tunisia: African Development Bank Group; 2010.

Singh, R.; Kumar, P.; Woodhead, T. Smallholder farmers in India: Food Security and Agricultural Policy. 1st Edition. Bangkok: FAO Regional Office for Asia and the Pacific; 2002.

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, 1479–1486.

Ward, J.M.J., Laing, M.D., Nowell, D.C., 1997. Chemical control of maize grey leaf spot. Crop Protection 16, 265–271.

Wisniewski, M., Droby, S., Norelli, J., Liu, J., Schena, L., 2016. Alternative management technologies for postharvest disease control: The journey from simplicity to complexity. Postharvest Biology and Technology 122.

CHAPTER 2

LITERATURE REVIEW

2.1. *Fusarium* and crop diseases

Fusarium species are a group of ascomycete or filamentous fungi distributed widely in nature and their spores can travel through wind, water, agricultural equipment or infected seeds (Headrick and Pataky, 1991; Munkvold and Carlton, 1997). They can be isolated from plants or soil as pathogens, endophyte and saprobes (Bacon and Hinton, 1996; Summerell *et al*., 2010). Different *Fusarium* species or strains are responsible for various agricultural diseases, including head or seed blights, vascular wilts, stem, ear and root rots (Lew *et al*., 1991; Nelson *et al*., 1994; Logrico *et al*., 2002).

Although infection from other species exist, *F. oxysporum*, *F. solani*, *F. verticilloides*, *F. subglutinans*, *F. proliferatum* and *F. graminearum* are common pathogens infecting cereals, fruit and vegetable crops in most part of the world (Munkvold and Desjardins, 1997; Xu and Nicholson, 2009). They usually infect the crop through injuries due to emerging roots, insects, nematodes and other factors (Lewandowski *et al*., 2006; Incremona *et al*., 2014). In addition to crop diseases encountered in the fields and spoilage of products during storage, some *Fusarium* pathogens are also capable of producing mycotoxins such as deoxynivalenol (DON), nivalenol (NIV), diacetoxyscirpenol (DAS), zearalenone (ZEN) and fumonisins (Marasas *et al*., 1984; Nelson *et al*., 1993; Desjardins, 2006).

These fungal secondary metabolites have deleterious effects on human health and domesticated animals resulting from consumption of contaminated products or grains (Voss *et al*., 1998; Abbas *et al*., 2006; Leslie and Summerell, 2006). Various disease control strategies (crop rotation, resistant cultivars, application of biological controls or conventional synthetic

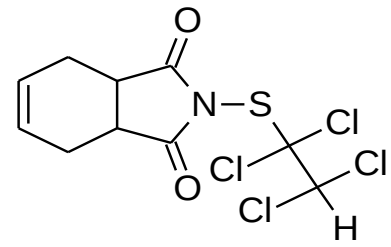
fungicides) have been proposed and implemented in many parts of the world, in order to mitigate the damages or losses caused by these fungal species (Smith, 1986; Sumner, 1990).

2.2. The impact of fungicides on the environment and human health

Fusarium species can live in the soil as oospores or chlamydospores for extended period of time and for that reason crop rotation become a less effective control strategy (Umaerus *et al*., 1989; Panth *et al*., 2020). Generally, commercial farmers relies on utilization of resistant cultivars or varieties and application of conventional synthetic fungicides.

Historically, synthetic fungicides have been used in commercial agriculture to control crop diseases and are still key components of several disease management methods worldwide (Panth *et al*., 2020). Although they have benefited farmers, their applications have also caused negative effects and promoted a series of problems including poisoning farmers, contamination of drinking water, aquatic system and soil (Igbedioh, 1991; Harris *et al*., 2001; Aktar *et al*., 2009). They can also affect non-target organisms and are liable to remain in vegetables, fruits and food following their application, thus posing health risks to the consumers and livestock (Aktar *et al*., 2009; Martínez, 2012; Wisniewski *et al*., 2016).

The period between 1960 and 1980, marked great successful years in crop protection chemistry with an introduction of chemicals such as captafol, thiabendazole, benomyl, carboxanilides, morpholines and mancozeb as fungicides used to control crop diseases (Russell, 2005; Morton and Staub, 2008). These fungicides were used to control diseases including those caused by *Fusarium* pathogens in vegetables, cereals and fruits. Another major discovery was fosetyl-Al, carbamate and benzimidazole fungicides (Klittich, 2008). The chemical structures of few fungicides used in agriculture to control crop diseases are illustrated in Figure 2.1.



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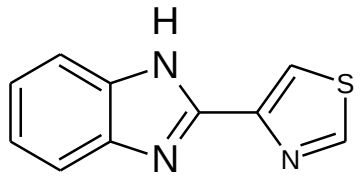

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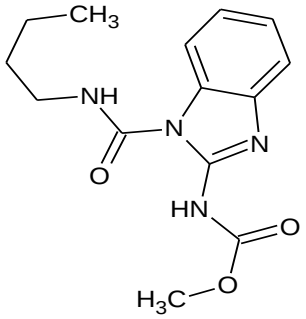

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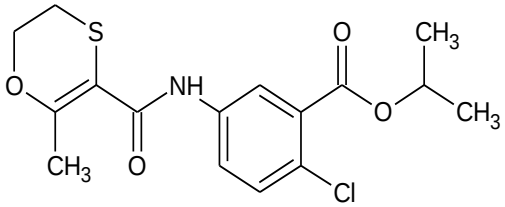

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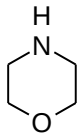
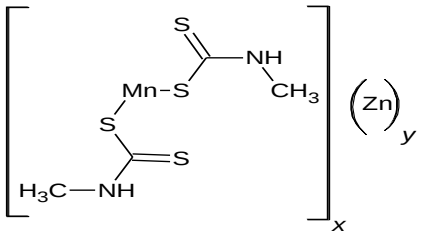
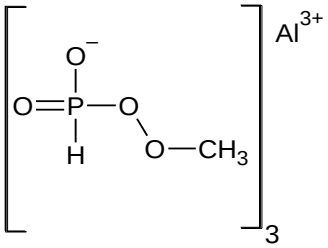


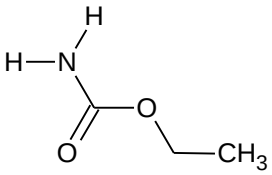
Morpholine



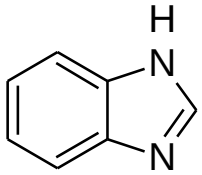
Mancozeb



Fosetyl-Al



Carbamate



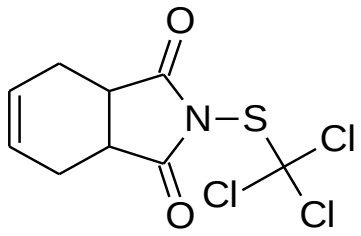
Benzimidazole

Figure 2.1. Fungicides used to control crop diseases.

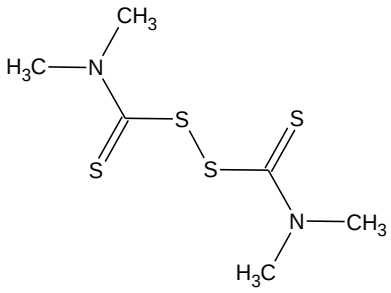
Benzimidazole fungicides inhibit the growth of wide variety of fungi and are applied in the soil or directly on the crop; to some extent they are used as seed treatment during pre-or post-harvest (Wu *et al*., 2009). Fungicides in the strobilurin family were also discovered and introduced in agrochemical industry in the 1990s and were found to be active against many

fungal species and pathogens causing common rusts (Bartlett *et al*., 2002). Different fungicides such as captan, thiuram, probenazole, thiabendazole and pyroquilon are also available as seed treatment strategies to control seed rot and seedling blight (Adenle and Cardwell, 2000; Agrios, 2005).

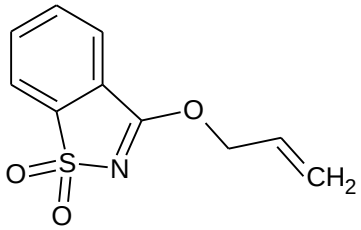
Maize ear infections and other diseases caused by phytopathogenic *Fusarium* species have been succcessfully controlled with the application of fungicides such as tebuconazole, chlorothalonil, carbendazim and metconazole (Magan *et al*., 2002; Freije and Wise, 2015; Zhang and Jeyakumar, 2018). The structures of some fungicides used as seed treatment to control *Fusarium* species and other pathogens are presented in Figure 2.2. Despite the importance of fungicides in crop protection, most of these compounds such as thiabendazole analoques, benzimidazole and carbamates persist in the environment for years following their applications and may lead to degradation of soil microbes and cause imbalance in ecosystem (Adepoju *et al*., 2014; Mishra *et al*., 2018).



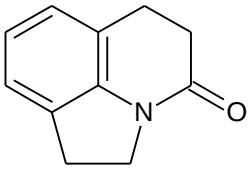
Captan



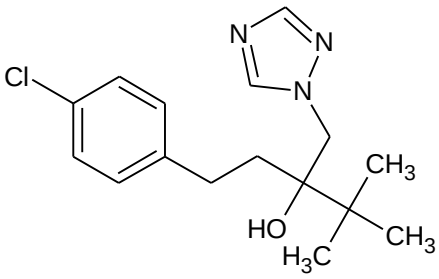
Thiuram



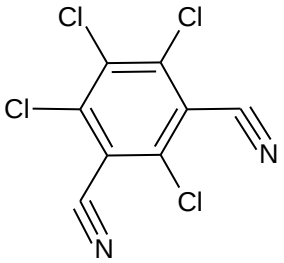
Probenazole



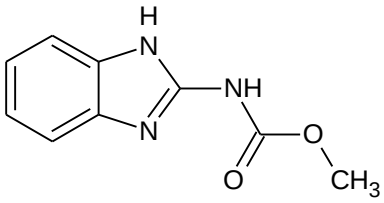
Pyroquilon



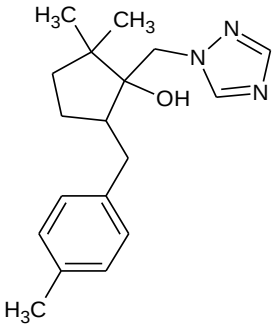
Tebuconazole



Chlorothalonil



Carbendazim



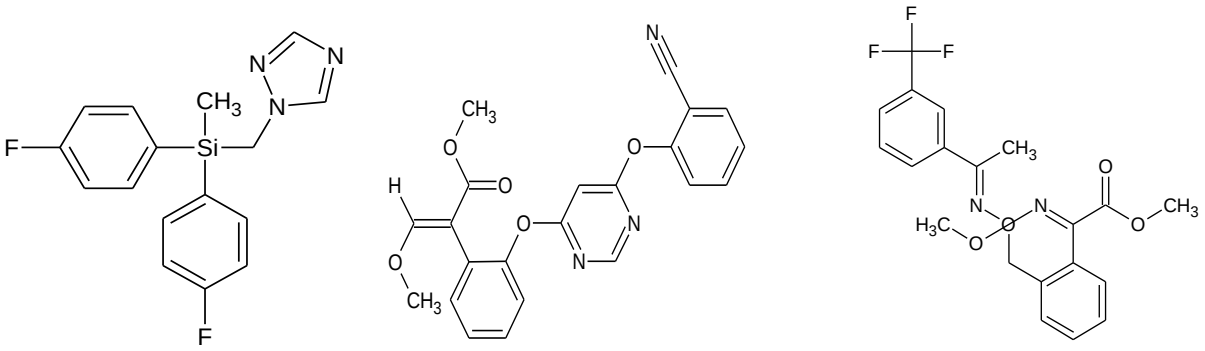
Metconazole

Figure 2.2. Fungicides used to control seed rot, seedling blight and maize ear diseases caused by *Fusarium* pathogens.

2.3. Fungal resistance in agricultural sector

In addition to the environmental and health issues arising from the applications of fungicides, agrochemical industry is faced with challenges of fungicide-resistant pathogens resulting from prolonged and/or misuse of these chemicals (Wilson *et al*., 1997; Daferera *et al*., 2003; Ramaiah and Garampalli, 2015). Many fungicides are at risk that the target pathogen (s) will develop resistance over time. There are reports about existence of resistance to carbendazim by *F. proliferatum*, *F. graminearum* and *F. verticilloides* (Yan and Dickman, 1996; Chen and Zhou, 2009; Chen *et al*., 2014; Xu and Nicholson, 2019). There is resistant strains of *F. graminearum* to fungicides such as tebucanozole, benzimidazole, flusilazole, azoxystrobin, trifloxystrobin and captan (Jackson-Ziems *et al*., 2017; Popiel *et al*., 2017; Zhang and

Jeyakumar, 2018). Moreover, resistant strains of *F. solani* was rep orted against mancozeb, captan and benomyl (Padvi *et al*., 2018). As presented in Figure 2.3, fungal strains may be resistant to fungicides, which belong to the same family.



Flusilazole Azoxystrobin Trifloxystrobin

Figure 2.3. Fungicides showed to be inactive against *F. graminearum* strains when evaluated *in vitro* studies (Jackson-Ziems *et al*., 2017; Popiel *et al*., 2017; Zhang and Jeyakumar, 2018*).*

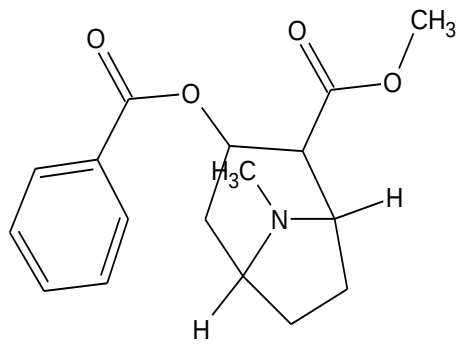
Generally, fungicides work by blocking metabolic pathways in the fungus and then prevent spore germination or mycelium growth (Yang *et al*., 2011). Conventional synthetic fungicides have benefited commercial farmers for many years and their reduction of crop diseases cannot be underestimated. However, the development of fungicide resistance by many pathogens, as well as environmental and health concerns necessitate the need to search for new classes of chemicals, which are environmental-friendly and affordable. Since poor-resource farmers are also responsible for more than 50% of food production in most sub-Saharan Africa, it is important to consider affordability and sustainability while searching for fungicides or any agents that can be used to protect crops (Spencer, 2002; Salami *et al*., 2010). The current synthetic fungicides are expensive and not easily accessible to smallholder farmers in many

African countries and to some extent, they are not applicable in organic farming (Thembo *et al*., 2010; Hubert *et al*., 2015).

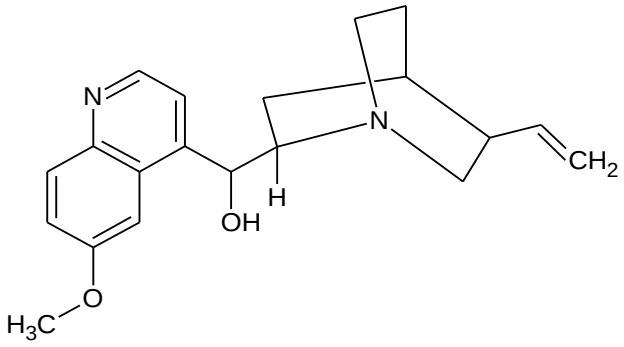
2.4. Medicinal plant species as natural source of fungicides

Medicinal plant species have a long history of use by many ethnic groups for the treatment of various diseases in both human and domestic animals (Masika and Afolayan, 2002; Ribeiro *et al*., 2010). Although traditional knowledge on the use of medicinal plant species is passed on from generation to generation by oral communication, it is important to document it before it vanishes (Maregesi *et al*., 2007). Many ethnobotanical studies conducted in southern Africa are focused mainly pathogens affecting humans and/ or animals, while a few deals with plant pathogens (Samie *at al*., 2010; Luseba and Tshisikhawe, 2013).

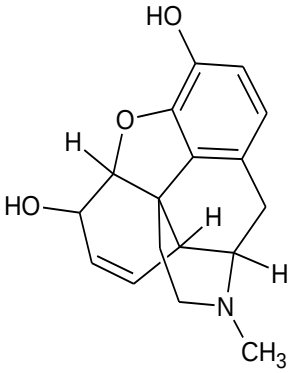
Availability of information for the treatment of human or animal infections or aliments as compared to the treatment of agricultural crops might be a reason behind this. Nevertheless, medicinal plant species have demonstrated ability to be used as fungicides in the agricultural sector to protect crops against pathogens (Mdee *et al*., 2009; Amadioha, 2000; Mahlo *et al*., 2010). They have also formed the basis of many applications in pharmaceuticals, food and cosmetic industries (Karuppusamy e*t al*., 2009; Mahmoudi, 2017). Medicinal plants are a primary source of natural products and many drugs such as cocaine, quinine, morphine, aspirin presented in Figure 2.4, and others had been discovered as led from medicinal plant species (Newman *et al*., 2000; Portillo *et al*., 2001; Samuelsson 2004; Gilani and Rahman 2005).



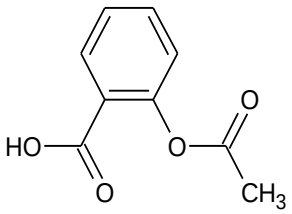
Cocaine



Quinine



Morphine



Aspirin

Figure 2.4. Some pharmaceutical drugs discovered from medicinal plant species.

The idea behind discovery of fungicides from plant species is based on their ability to synthesize diverse array of secondary metabolites or compounds which function to defend the plant against microbes, insects and herbivores (Harborne *et al*., 1995; Ahmad and Beg, 2001). Plant-based fungicides may be developed as products from the leaves and used as emulsion, extract or powder. However, they may also be discovered from other parts of the plant through isolation of active compounds that can be used as starting materials during retrosynthesis. Utilization of leaf-based products may benefit smallholder farmers since the leaves are a renewable part which are easily accessible. Additionally, medicinal plant species can be found

on their farms or can be secured from the surrounding areas and utilized as required. This approach of using the plant (emulsion, extract or powder), may be limited to smallholder farmers because they usually own small portion of cultivated land compared to commercial farmers. Development of plant leave-based fungicides in a very large quantity for commercial farmers requires that the target species be cultivated (Mdee *et al*., 2009).

Alternatively, antifungal compounds or chemical moieties may be isolated and structurally characterized and therefore, used to design novel synthetic compounds. Retrosynthesis forms the basis in the industrial discovery of plant-based fungicides since the compounds isolated from the plants are usually present in very small amount and are difficult to purify on a large scale (Martínez, 2012). Large quantity of synthesized antifungals may be further evaluated in the glasshouse, field and possibly applied in large scale crop farming and organinc farming.

The use of plant products against fungal pathogens may inhibit the development of resistance due to the presence of different constituent antifungal compounds and their synergisms (Fandohan *et al*., 2004; Calvo *et al*., 2011). Products from medicinal plant species are arguably safe, showed low human toxicity and are eco-friendly (Brinker, 1998). They are easily biodegradable because natural products particularly from plants are inherently unstable to elevated temperature and consequently they will not persist in the environment for a long time compared to synthetic fungicides (Martínez, 2012).

2.5. Distribution and antimicrobial activity of medicinal plant species investigated in this study

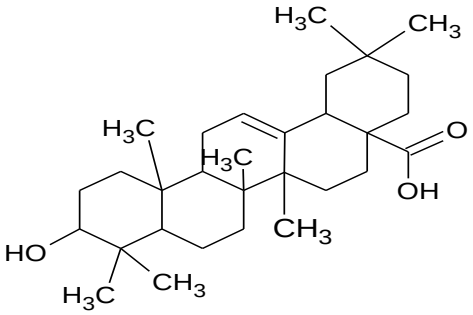
2.5.1. Lantana camara L.

It is a shrub, which belongs to the Verbenaceae family. It is commonly known as wild or red sage, bunchberry, bird's brandy, cherry pie and tick-berry. The plant is native to America

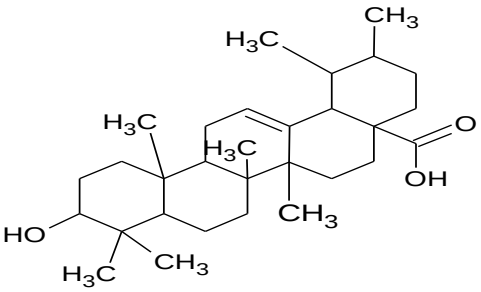
tropics and was introduced in many countries as a decorative or ornamental plant (Parajapati, 2003; Kalita *et al*., 2012). In South Africa, this plant can be found in almost every province, particularly in Gauteng, Limpopo, Mpumalanga and KwaZulu-Natal (Henderson, 2001). Currently, this plant is regarded as notorious invasive species as well as an agricultural weed that may poison livestock (Lonare *et al*., 2012). It can reach a height of up to 2 to 3 m, forms dense thickets and produce flowers with varied colours (red, pink, white, yellow and violet) depending on the environment (Figure 2.5). It can flower throughout the year and produces dark blue or purplish-black fruits. The stem and branches are usually square-shaped and have spines (Thamotharan, 2010). In many areas or locations, *Lantana camara* is an evergreen plant with aromatic, ovate, chartaceous and hairy leaves. Ethanol extraction prepared from the leaves of this plant showed efficient antifungal activity against *Trichophyton rubrum* and *Candida albicans* (Das and Godbole, 2015). On average, its antifungal activity was stronger than similar extracts preparation obtained from *Azadirachta indica*, *Citrus limonum* and *Hibiscus rosasinensis* leaves (Das and Godbole, 2015).

Figure 2.5. The leaves, fruits and flowers of *Lantana camara* growing naturally at the Agricultural Research Council, Roodeplaat, Pretoria, Gauteng Province.

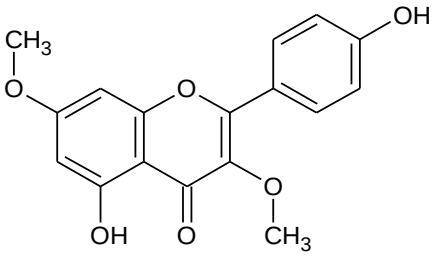
Antifungal evaluation of different solvents obtained from the leaves of *Lantana camara* showed that methanolic extract had stronger activity (80.74 and 79.07 % inhibition) against *Aspergillus flavus* and *Aspergillus niger*, respectively (Fayaz *et al.*, 2017). In a study conducted by Sailaja (2014), the extract obtained from *Lantana camara* had also exhibited good antifungal activity (0.7 mg/ml) against *Aspergillus niger*. *Aspergillus flavus* and *Aspergillus niger* are among fungal pathogens causing series of diseases on vegetables, fruits and cereal crops. *Aspergillus niger* can cause black mould on onions and crown rot of groudnut while *Aspergillus flavus* is usually associated with spoilage of corn, peanuts and wheat (Varaprasad *et al*., 2009; Belewa *et al*., 2011; Omidpanah *et al.*, 2015). An essential oil obtained from *Lantana camara* was found to be active against the strains for *Fusarium solani* (Deena and Thoppil, 2000). In another study, an extract from the leaves of *Lantana camara* showed good activity (0.08 mg/ml) against *Fusarium oxysporum* (Mdee *et al*., 2009). The same pathogen was found to be sensitive to the extracts obtained from medicinal plants such as *Bucida buceras, Breonadia salicina, Harpephyllum caffrum, Olinia ventosa, Vangueria infausta* and *Xylotheca kraussiana* (Mahlo *et al*., 2010). Chemical compounds isolated from *Lantana camara* include ursolic acid, oleanonic acid, camaroside, penduletin, linaroside and verbascoside as can be seen in Figure 2.6. These compounds and many more had been isolated from *Lantana camara* and their biological activity such as antifungal, anticancer and antibacterial activities were reported (Hart *et al*., 1976; Rwangabo, 1988; Sathish *et al*., 2011; Begum *et al*., 2014).



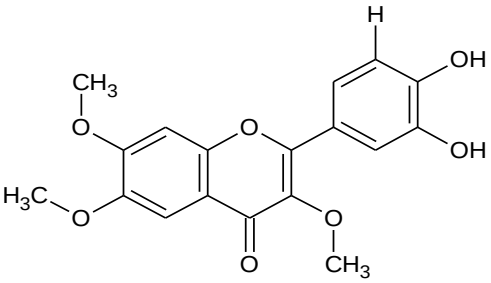
Oleanolic acid



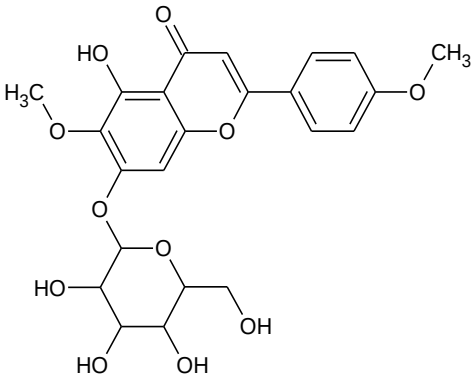
Ursolic acid



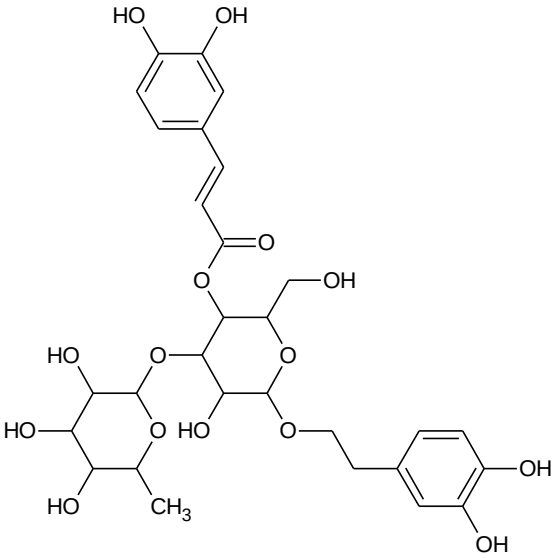
Camaroside



Penduletin



Linaroside



Verbascoside

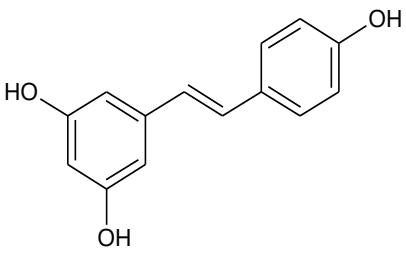
Figure 2.6. Chemical structures of the compounds isolated from *Lantana camara*.

2.5.2. Senna didymobotrya (Fresen.) H.S. Irwin & Barneby

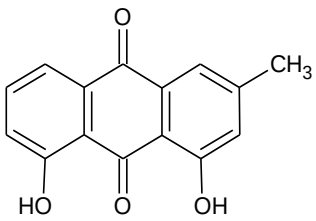
Senna didymobotrya has various common names; including peanut butter cassia, African senna, candelabra tree, popcorn senna and peanut butter tree. It belongs to the family Fabaceae (Leguminosae) and is indigenous to tropical Africa, including Kenya, Rwanda and Tanzania (Cherono and Akoo, 2011). In South Africa, this plant is widespread mostly in the Eastern Cape, KwaZulu-Natal, Gauteng and Limpopo provinces. It has been cultivated for its attractive flowers (Figure 2.7). The plant can reach a height of up to 3 m and has dark green pinnate leaves with 8 to 21 pairs of leaflets. The flowers are bright yellow with darker veins, its pods turns brown as it mature. The flowers and stems have a smell of peanut butter especially when cut or disturbed. Although *Senna didymobotrya* was not effective against *Candida albicans*; methanolic extracts obtained from its stem bark, root bark, leaves, flowers and immature pods showed very strong activity against *Trichophyton tonsurans* (Jeruto *et al*., 2016).

Figure 2.7. The leaves, flowers and pods of *Senna didymobotrya* growing naturally at Moletjie, Polokwane, Capricorn District, Limpopo Province.

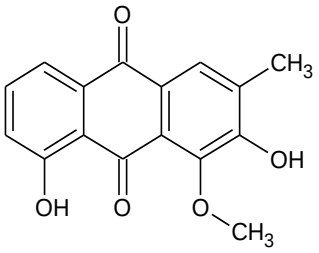
Much work has not been done on the antifungal activity of *Senna didymobotrya* against *Fusarium* pathogens. This plant species was evaluated for activity against human and animal fungal pathogens such as *C. albicans*, *C. parapsilosis*, *C. krusei* and *Cryptococcus neoformans* (Korir *et al*., 2012). The results indicated that all these pathogens were resistant to the application of water, dichloromethane, hexane and methanol extracts obtained from *Senna didymobotrya*. However, these extracts exhibited good activity against some tested bacterial isolates (Korir *et al*., 2012). In another study, acetone and hexane extracts obtained from the roots of *Senna didymobotrya* were both found to show activity with MIC values of 7.5 mg/ml against *C. albicans*, *C. krusei* and *Cryptococcus neoformans* (Samie *et al*., 2010). Ethyl acetate extract obtained from the leaves of *S. didymobotrya* can be used to control *C. albicans* as it showed antifungal activity with diameter zone of inhibition of 18.1 mm (Maobe *et al*., 2013). Chloroform: methanol (1:4) extract from the seeds of this plant demonstrated good antifungal activity with inhibition zone of 22.3 mm against *Aspergillus niger* (Hailu *et al*., 2016). Alemayehu *et al*., (2015) used silica gel chromatography techniques to isolate two compounds from the roots of *S. didymobotrya*. Spectroscopic charecterization of these compounds was found to be closely related to trans-resveratrol and chrysophanol. Other compounds isolated from the roots of this medicinal plant species are obtusifolin, physcion, stigmasterol, physcion-10, 10′-bianthrone and chrysophanol-10,10′-bianthrone (Ochieng *et al*., 2013). The chemical structures of these compounds are illustrated in Figure 2.8.



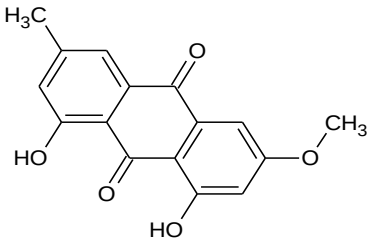
trans-resveratrol



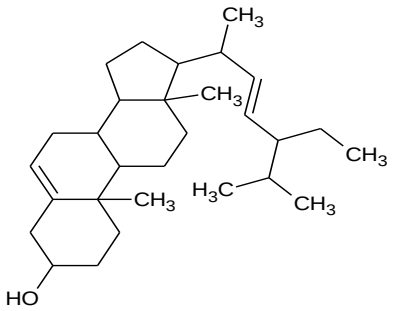
Chrysophanol



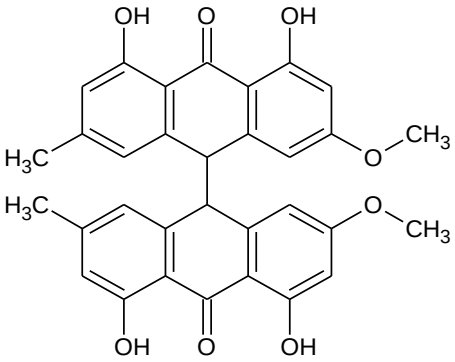
Obtusifolin



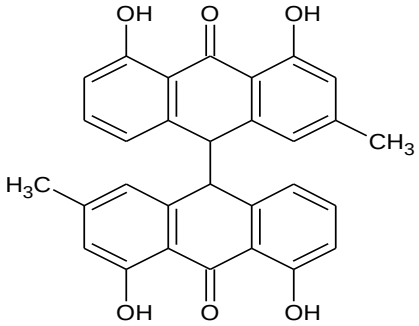
Physcion



Stigmasterol



Physcion-10, 10′-bianthrone



Chrysophanol-10, 10′-bianthrone

Figure 2.8. Chemical structures of the compounds presence in *Senna didymobotrya*.

2.5.3. Combretum erythrophyllum (Burch.) Sond.

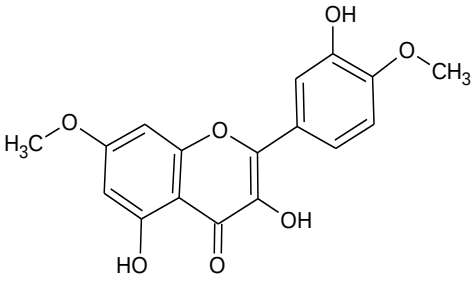
Combretum erythrophyllum belongs to Combretaceae and it is commonly known as river bushwillow or bushveld willow. The tree is native to the southern part of Africa and it is widely distributed in the Eastern Cape, Gauteng, KwaZulu-Natal, Limpopo, Mpumalanga and Northern Cape provinces of South Africa (Mthethwa, 2009). Naturally, it grows along riverbanks where there is enough water. However, nowadays it is grown along the roads and in human settlement as popular shade tree. It is a large deciduous tree, which can reach a height of 12 meters (Gelfand *et al*., 1985). The leaves and yellow-brownish fruits of this plant species are presented in Figure 2.9. Bio-autographic antifungal activity evaluation of acetone, methanol and dichromethane extracts obtained from the leaves of *Combretum erythrophyllum* revealed

the presence of antifungal compounds against *Candida albicans* and *Cryptococcus neoformans* (Masoko and Eloff, 2006). Medicinal plant species with such activity may also display similar or more activity against agricultural crops fungal pathogens, hence it was also included in the current study.

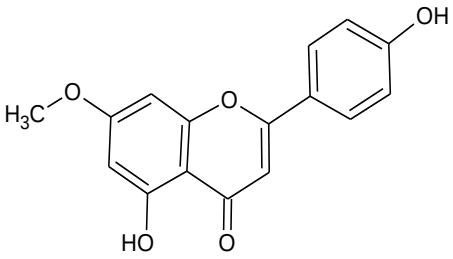
Figure 2.9. The leaves and fruits of *Combretum erythrophyllum* growing naturally at the Agricultural Research Council, Roodeplaat, Pretoria, Gauteng Province.

To the best of our knowledge, there was no literature reports on the antifungal activity of *Combretum erythrophyllum* against *Fusarium* pathogens. However, compounds isolated from this plant species were evaluated for the activity against *Aspergillus niger*, which is also one of crops and post-harvest pathogens. This pathogen is one of the notorious micro-organisms causing devastating crop diseases in agriculture and can cause food spoilage. Among compounds isolated from *C. erythrophyllum* and evaluated for antifungal activity include 5-hydroxy-7, 4'-dimethoxyflavone, genkwanin, apigenin and rhamnazin. These compounds showed no activity against *Aspergillus niger* even at 100 mg/ml. Nonetheless, it is noteworthy

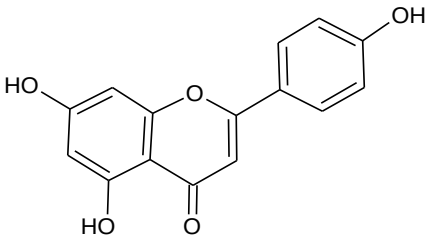
that these compounds exhibited very good antibacterial activity (MIC values ranging from 25 µg/ml-100 µg/ml) against human pathogens such as *Vibrio cholera, Enterococcus faecalis* and *Escherichia coli* (Martini *et al*., 2004). Erythrophyllic acid, 30-carboxy-1α-hydroxycycloartanes and 29-carboxy-1α-hydroxyoleanes of which their chemical structures are presented in Figure 2.10, were also isolated from *Combretum erythrophyllum* (Lawton and Rogers 1991; Rogers 1998).



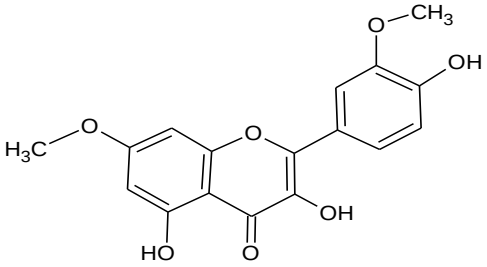
5-hydroxy-7, 4'-dimethoxyflavone



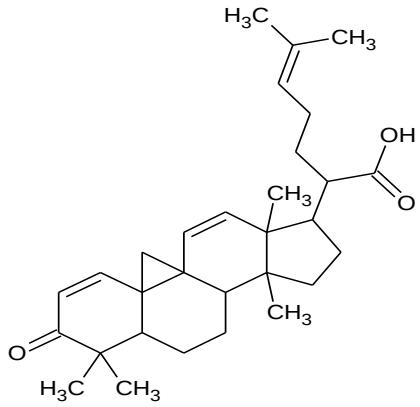
Genkwanin

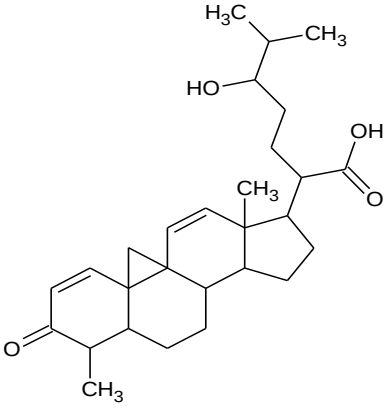


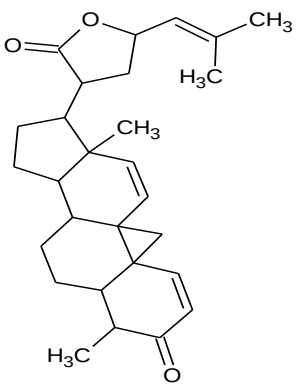
Apigenin



Rhamnazin







Erythrophyllic acid; 30-carboxy-1α-hydroxycycloartanes; 29-carboxy-1α-hydroxyoleanes

Figure 2.10. Chemical structures of compounds isolated from *Combretum erythrophyllum*.

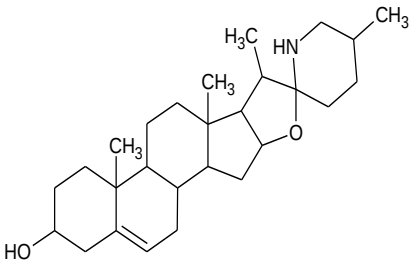
2.5.4. Solanum mauritianum Scop.

Solanum mauritianum is an invasive weedy plant species from the family Solanaceae. Its common names include bugweed, tobacco tree, flannel weed, woolly nightshade and bugtree. This plant is native to South America and it was introduced to Africa, Madagascar, Mauritius, India, New Zealand and Australia through trading (Roe, 1979). It can be found widely distributed in the Western Cape, KwaZulu-Natal, Gauteng and Limpopo provinces of South Africa and is categorized as invasive weedy species (Olckers, 2003). *Solanum mauritianum* is a small tree or shrub that can grow up to 4 metres high. It produces green berries or fruits which turn yellow as it matures (Figure 2.11). Its green stems and leaves are velvety, covered with whitish-felty hairs and its purple flowers are usually clustered on a single terminal stalk (Sankaran and Suresh, 2013).

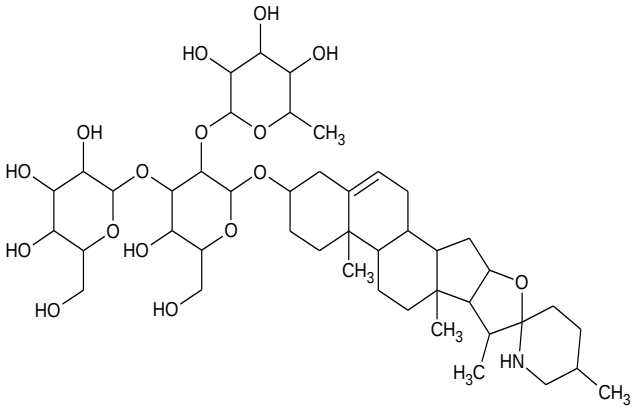
An extract obtained from the stem of *Solanum mauritianum* using a solvent mixture of chloroform: methanol (50:50, v/v) was found to be very active to inactive (2.- 0.0625 mg/ml) against selected pathogenic bacteria (Uche-Okereafor *et al*., 2019). The aquoeus extraction obtained through boiling of *Solanum mauritianum* material was evaluated for the antibacterial activity against *Staphylococcus aureus* and *Salmonella choleraesuis.* The results from that study indicated that *S. mauritianum* was not active against both tested bacterial strains (Avancini *et al*., 2008). Mdee *et al*., 2009, evaluated the antifungal activity of *S. mauritianum* extract against fungal phytopathogens. In that study, acetone extract from the leaves of *S. mauritianum* showed antifungal activity (MIC values: 1.46, 1.46, 0.64, 0.43, 0.53, 1.25, 0.63, 2.50 and 0.84 mg/ml) against *Fusarium oxysporum, Pythium ultimum, Rhizoctonia solani, Aspergillus parasiticus, Aspergillus niger, Phytophthora nicotiana, Colletotrichum gloeosporioides, Trichoderma harzianum* and *Penicillium expansum*, respectively.

Figure 2.11. The leaves and fruits of *Solanum mauritianum* growing naturally at the Agricultural Research Council, Roodeplaat, Pretoria, Gauteng Province.

Phytochemical screening of dried leaves from *Solanum mauritianum* reveals the presence of saponins (2.1 mg/g), alkaloids (4.78 mg/g), phenols (3.3 mg/g), flavanoids (3.5 mg/g) and tannins (1.56 mg/g) (Jayakumar and Murugan, 2016). Figure 2.12 shows the chemical structure of major compounds known to be present in *S. mauritianum*, which include solasodine and solasonine (Everist, 1981; Vieira, 1989; Drewes and van Staden, 1995).



Solasodine



Solasonine

Figure 2.12. Structures of compounds known to be present in *Solanum mauritianum*.

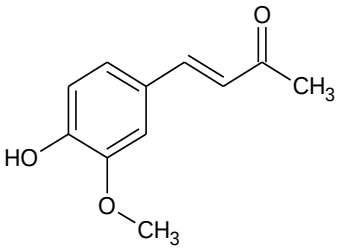
2.5.5. Melia azedarach L.

Melia azedarach belongs to family Meliaceae and it is native to India and China (Nahak and Sahu, 2010). This plant is distributed throughout Mexico, Argentina, Africa, North America and Southern Europe (Chiffelle *et al*., 2009). It is commonly known as chinaberry tree, Cape lilac, white cedar, Persian lilac, bead tree and syringa berry tree. It is often planted as a street tree and ornamental and it can reach a height of more than 20 meters. *Melia azedarach* have a greenish-brown smooth bark and alternate leaves with about 3-11 leaflets. The plant produces fragrant purple flowers and hard, smooth, small yellow fruit or berries (Figure 2.13). In a study conducted by Meziane and Goumri, 2014; antifungal activity evaluation of essential oil obtained from the leaves, flowers and seeds of *Melia azedarach* showed percentage inhibition of 75, 70 and 75% at 100 µl dose against *Candida albicans*, respectively. Ethanolic extract from the leaves of this plant had also showed significant antifungal activity with recorded minimum inhibitory concentration of 0.25 mg/ml against *Candida albicans* and other tested fungal pathogens (Kathireshan *et al*., 2019).

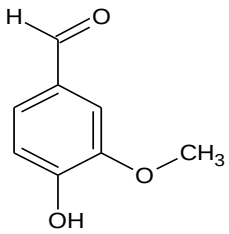
Figure 2.13. The leaves and fruits of *Melia azedarach* growing naturally at the Agricultural Research Council, Roodeplaat, Pretoria, Gauteng Province.

In a study conducted by Meziane and Goumri, (2014); essential oil obtained from the leaves of *Melia azedarach* was evaluated for its antifungal activity against *F. oxysporum*. The oil was found to be active (70 % inhibition) against *F. oxyxporum* at 100 µl dosage. However, water extract from the leaves of this medicinal plant had little or no effect on the growth of *F. oxysporum* (Lovang and Wildt-Persson, 1998). Extracts from different solvents (ethanol, methanol, petroleum ether and aqueous) of the leaves of *M. azedarach* showed antifungal activity (MIC values ranged from 47.3 - 58.3 µg/ml) against *Aspergillus niger*, *Aspergillus flavus* and *F. oxysporum* (Sen and Batra, 2012). Ethanolic extract from the fruits of *M. azedarach* showed antifungal activity with MIC values ranging from 60-500 µg/ml against *Aspergillus flavus* and *Fusarium moniliforme* (Carpinella *et al*., 1999). Ethanolic extract from the leaves showed MIC value of 250 µg/ml against *Aspergillus niger* (Kathireshan *et al*., 2019). Carpinella *et al*., (2003) reported the fungicidal activity of hexane and ethanol extracts obtained

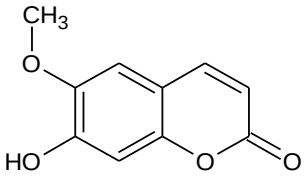
from the fruit, seed kernels and leaves of *Melia azedarach* against *A. flavus*, *F. oxysporum*, *F. solani*, *F. verticilloides* and other pathogens. Chemical compounds responsible for such activity were isolated and characterized as hydroxyl-3-methoxcinnamaldehyde, vanillin, scopoletin and pinoresinol (Carpinella *et al*., 2005). Other compounds found or isolated from *Melia azedarach* include meliartenin, melianol, meliacin or nimbolide and meliacarpin and are shown in Figure 2.14 (Lavie and Jain, 1967; Lee *et al*., 1991; Carpinella *et al*., 2002; Kraus, 1986; Kraus *et al*., 1981).



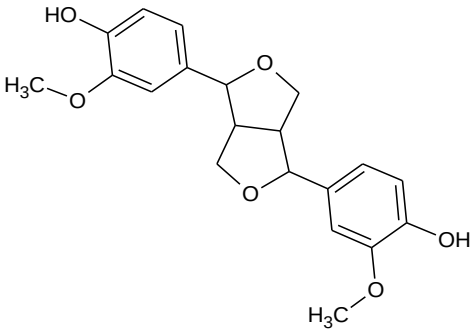
Hydroxyl-3-methoxcinnamaldehyde



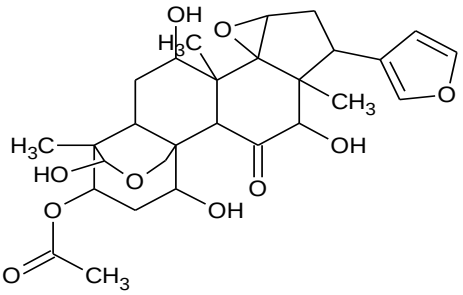
Vanillin



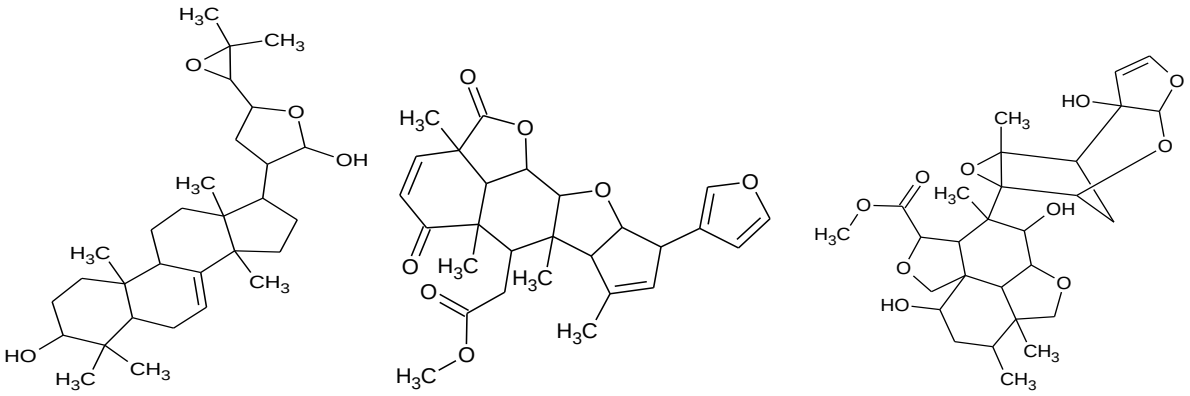
Scopoletin



Pinoresinol



Meliartenin



Melianol Meliacin/Nimbolide Meliacarpin

Figure 2.14. Chemical structures of compounds isolated from *Melia azedarach*.

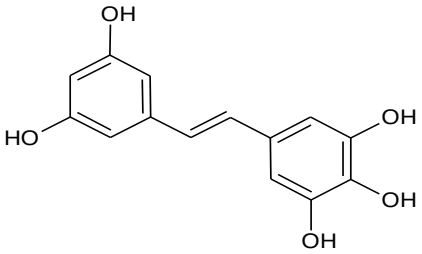
2.5.6. Schotia brachypetala Sond.

Schotia brachypetala belongs to the family Fabaceae. It is a medium-sized deciduous tree commonly known as African walnut or weeping boer-bean. The plant is distributed from the Eastern Cape, through Swaziland, Mpumalanga, Limpopo province and into Mozambique and Zimbabwe. It occurs naturally in various woodlands, along river banks and streams. *Schotia brachypetala* has beautiful flowers with bright red sepals, stamens and flower stalks (Figure 2.15). The flowers are borne in dense masses and produces dripping nectar, especially during spring. The water and dicholoromethane extracts obtained from the barks of *Schotia brachypetala* were found to show noteworthy antifungal activity (0.5 mg/ml) against *Candida albicans* (Nciki *et al*., 2016).

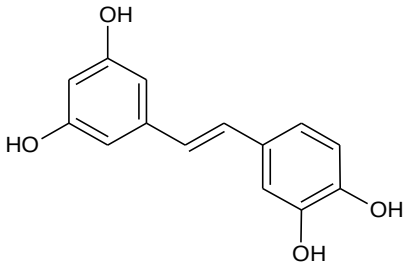
Figure 2.15. The leaves, flowers and pods of *Schotia brachypetala* growing naturally at the Agricultural Research Council, Roodeplaat, Pretoria, Gauteng Province.

There is limited information on the antifungal activity of *Schotia brachypetala* against *Fusarium* pathogens or any phytopathogenic microorganisms. In a study conducted by McGaw *et al*., 2002; ethanolic extract obtained from the leaves of this plant was evaluated for antibacterial activity and was found to show moderate activity with MIC values ranging from 0.16 to 3.13 mg/ml against human pathogens (*Bacillus subtilis*, *Escherichia coli*, *Klebsiella pneumonia* and *Staphylococcus aureus*). The aqueous extract showed activity with MIC values from 4.17 to more than 12.5 mg/ml. Generally, chemical compounds from *Schotia brachypetala* are poorly known (Buckingham, 2008). However, *trans*-3, 3′, 4, 5, 5′-pentahydroxystilbene and *trans*-3, 3′, 4, 5′-tetrahydroxystilbene were isolated from the heartwood of this medicinal plant (Drewes, 1971; Drewes and Fletcher 1974). Other compounds such as 3-*O*-methylquercetin 7-*O*-*β*-glucopyranoside, 3, 4′-di-O-methylquercetin 7-O-β-glucopyranoside and 3, 4′-di-*O*-methylquercetin 7-*O*-[*β*-D-6″ (*E*-*p*-coumaroyl)

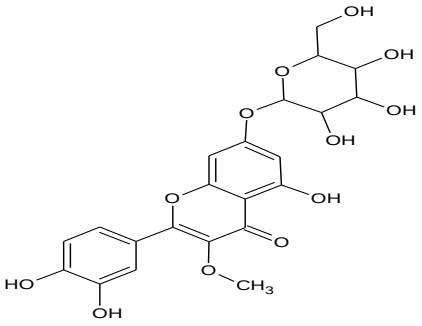
glucopyranoside] were isolated from the seeds of *Schotia brachypetala*. These compounds have demonstrated good antibacterial activity with MIC values ranging 0.13 to 0.63 mg/ml against human pathogens (Du, 2011). The chemical structure of these compounds are presented in Figure 2.16.



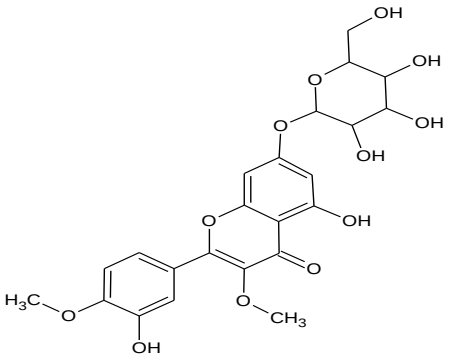
trans-3, 3′, 4, 5, 5′-pentahydroxystilbene



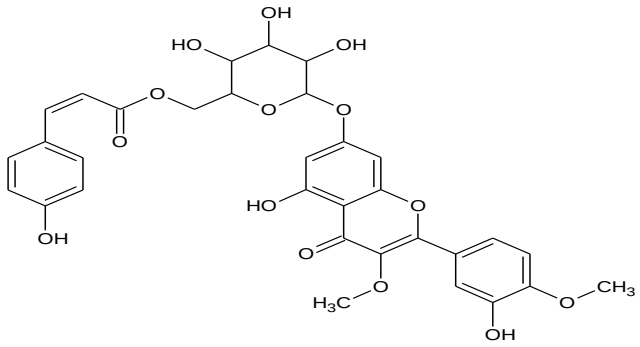
trans-3,3′,4,5′-tetrahydroxystilbene



3-*O*-methylquercetin-7-*O*-*β*-glucopyranoside



3,4′-di-*O*-methylquercetin-7-*O*-*β*-glucopyranoside



3,4′-di-*O*-methylquercetin 7-*O*-[*β*-D-6″(*E*-*p*-coumaroyl)glucopyranoside]

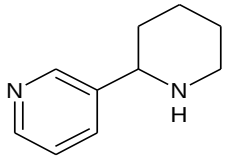
Figure 2.16. Chemical structures of compounds isolated from *Schotia brachypetala*.

2.5.7. Nicotiana glauca Graham

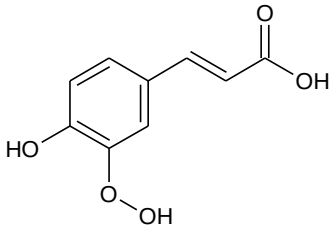
Nicotiana glauca is a small tree or shrub in the family Solanaceae and is native to South America and Argentina. However, it is distributed throughout South Africa, Kenya, Uganda and Tanzania (Henderson, 2001). It is commonly known as Mexican tobacco, mustard tree and tree tobacco. *Nicotina glauca* is evergreen, bluish green, soft-woody shrub as presented in Figure 2.17 and can grow up to 4 meters in height (Halvorson and Guertin, 2003). The leaves are blue-green, leathery with purplish tints and the flowers are yellow which usually appears in cluster at the terminal of the stalk (Hyde *et al*., 2020). Mdee *at al*., 2009 evaluated the antifungal activity of *Nicotina glauca* against 9 phytopathogens which belong to different species or families. Acetone extract obtained from the leaves of this plant showed good activity (0.16-0.64 mg/ml) against *Fusarium oxysporum, Pythium ultimum, Rhizoctonia solani, Aspergillus parasiticus, Aspergillus niger, Phytophthora nicotiana, Colletotrichum gloeosporioides, Trichoderma harzianum* and *Penicillium expansum*. The activity of the extract obtained from the seed and flowers was less (0.16-2.50 mg/ml) when compared to the leaves extract.

Figure 2.17. The leaves and flowers of *Nicotiana glauca* growing naturally at the Agricultural Research Council, Roodeplaat, Pretoria, Gauteng Province.

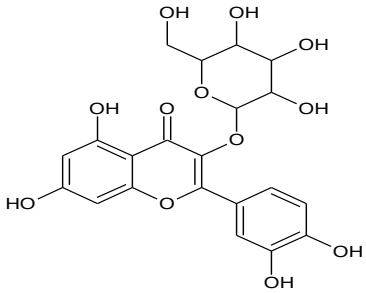
In other studies, *Nicotiana glauca* was reported to be toxic to both humans and animals (Mizrachi *et al.*, 2000; Panter *et al.*, 2000). Its toxicity principle was due to the presence of an alkaloid called anabasine (Furer *et al.*, 2011). HPLC investigation of leafy material from this plant species led to quantification of phytochemical compounds such as ferulic acid, quercetin-3-glucoside, cinnamic acid, quercetin, kaempferol, rutin and kaempferol-3-glucoside (Hassan *et al*., 2014). The structure of few compounds identified in the leaves of *N. glauca* are shown in Figure 2.18.



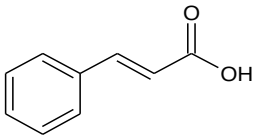
Anabasine



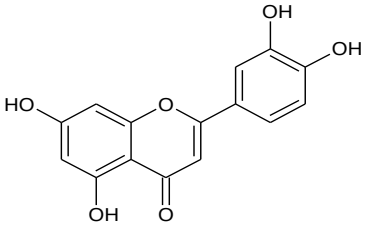
Ferulic acid



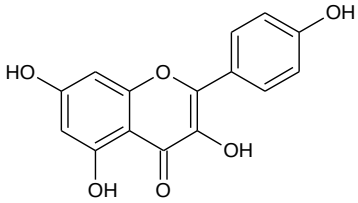
Quercetin-3-glucoside



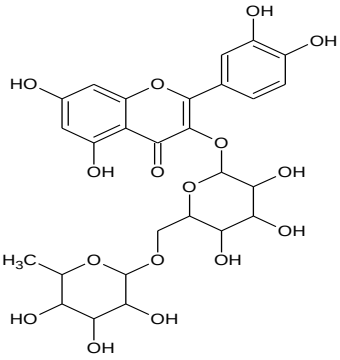
Cinnamic acid



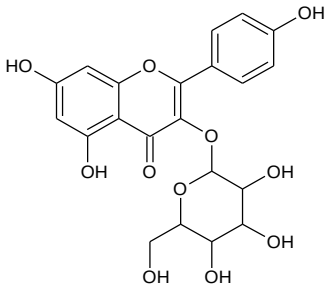
Quercetin



Kaempferol



Rutin



Kaempferol-3-glucoside

Figure 2.18. Chemical structures of compounds present in *Nicotiana glauca*.

2.5.8. Harpephyllum caffrum Bernh.

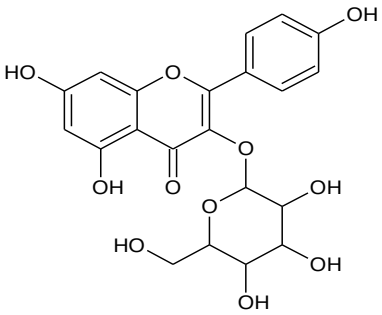
Harpephyllum caffrum is commonly known as wild plum and it belongs to the family Anacardiaceae. The wild plum tree is native to South Africa, distributed in all provinces of South Africa. This tree can also be found in Mozambique and Swaziland. It is evergreen large deciduous tree with an erect trunk (Figure 2.19) and is planted as shade tree in the cities and

townships. The leaves of this plant are shiny dark green, pinnate and are crowded towards the end of the branches (Fern, 2014). Ethanol extract from the barks of *Harpephyllum caffrum* was tested for antifungal activity against *Candida albicans* and it showed strong activity (0.78 mg/ml) compared to other tested medicinal plant species (Buwa and van Staden, 2006).

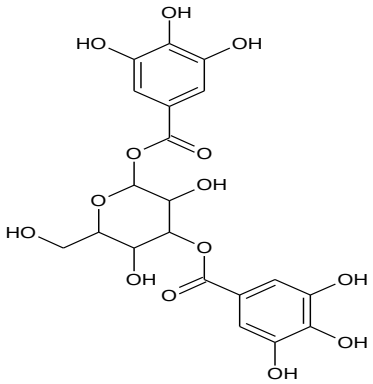
Figure 2.19. The leaves and fruit of *Harpephyllum caffrum* growing naturally at Moletjie, Polokwane, Capricorn District, Limpopo Province.

Different solvent extracts (acetone, hexane, dichlotromethane and methanol) from the leaves of *Harpephyllum caffrum* were evaluated for antifungal activity against phytopathogens (Mahlo *et al*., 2010). *Fusarium oxysporum*, a causative agent of many crop diseases in agriculture was also included as one of the pathogens in that particular study. The results of the study showed that *H. caffrum* had good activity against *F. oxysporum* with MIC values of 0.02, 0.32, 0.16 and 0.04 mg/ml demonstrated by acetone, hexane, dichloromethane and methanol extract, respectively. The total activity reported by methanol extraction against *F. oxysporum*

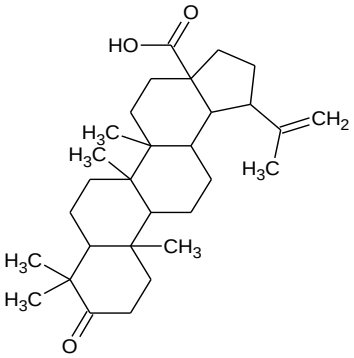
was 11500 ml/g while hexane extraction reported the lowest value (106 ml/g) against the same pathogen (Mahlo *et al*., 2010). Phytochemical composition of *H. caffrum* could be responsible for the observed activity and other pharmacological properties of this medicinal plant species. Several studies have been conducted to isolate and characterize compounds from *H. caffrum* (Nawwar *et al*., 2011; Shabana *et al*., 2011). Compounds such as 3-methoxyellagic acid 4-O-β- galactopyranoside, 1,3-di-O-galloyl glucose, betulonic acid, 3-acetyl methyl betulinate, lupenone and lupeol as presented in Figure 2.20 are among compounds isolated from *H. caffrum*.



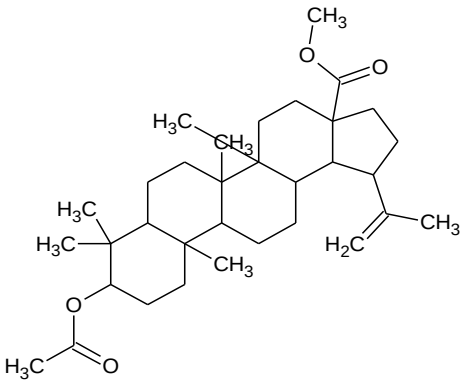
3-methoxyellagic acid 4-O-β- galactopyranoside



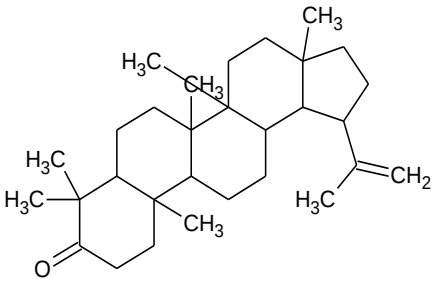
1,3-di-O-galloyl glucose



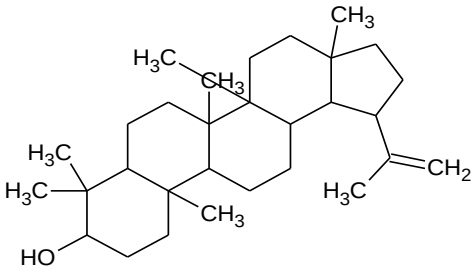
Betulonic acid



3-acetyl methyl betulinate



Lupenone



Lupeol

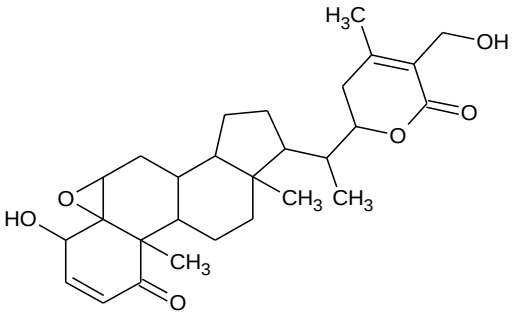
Figure 2. 20. Chemical structures of compounds isolated from *Harpephyllum caffrum*.

2.5.9. Withania somnifera (L.) Dunal

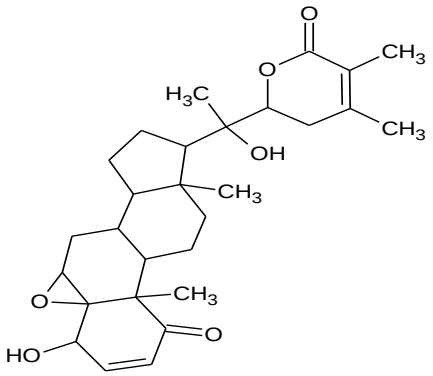
Withania somnifera belongs to the family Solanaceae. It is commonly known as ashwagandha, poison gooseberry, winter cherry and Indian ginseng (Singh *et al*., 2010). It is shrub plant distributed in India, East Asia and in all provinces of South Africa. *Withania somnifera* is a short perennial shrub, with dull green, elliptic leaves and it can grow up to 1-2 meters in height. The plant is evergreen; covered with fine, silver grey hair and it has small green bell-shaped flowers. The fruits are hairless, spherical berries with orange-red to red (Figure 2.21) when ripe and they are covered with brownish, papery, inflated calyx (Hyde *et al*., 2020). Methanolic extract obtained from the roots and leaves of *Withania somnifera* showed good antifungal activity against *Candida albicans* with recorded IC_{50} of 13.07 and 16.67 µg/ml, respectively as compared to 10.30 µg/ml exhibited by standard drug, Amphotericin B (Patel *et al*., 2018).

Figure 2.21. The leaves and berries of *Withania somnifera* growing naturally at Moletjie, Polokwane, Capricorn District, Limpopo Province.

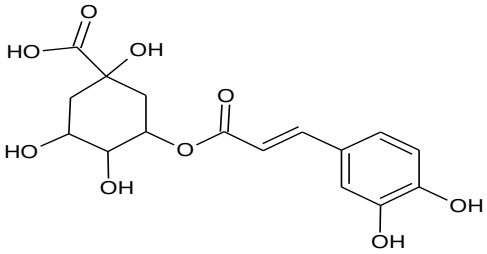
Different solvent extracts (water, chloroform and benzene) from *Withania somnifera* were evaluated for antimicrobial activity and were found to show no activity against *Aspergillus flavus* (Singariya *et al*., 2012). Glycoprotein isolated from the roots of *Withania somnifera* showed promising activity against *A. flavus, A. niger, F.oxysporum, F. verticilloides,* other fungi and bacteria. In the same particular study, *F. solani* was reported to be resistant to the application of this glycoprotein (Girish *et al*., 2006). The antimicrobial and pharmacological properties of this medicinal plant species are attributed to compounds such as withaferin A and withanolide D. Other compounds or alkaloids found in *W. somnifera* are chlorogenic acid, anaferine, ashwagandhanolide, somniferine and beta-sisterol (Lavie *et al*., 1965; Subaraju *et al*., 2006; Gupta and Rana, 2007; Kapoor, 2001; Singh *et al*., 2010). The structures of some of these compounds are presented in Figure 2.22.



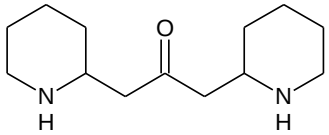
Withaferin A



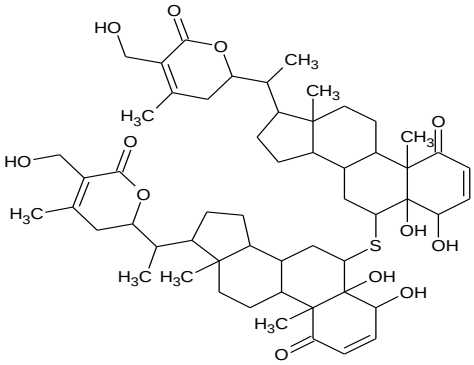
Withanolide D



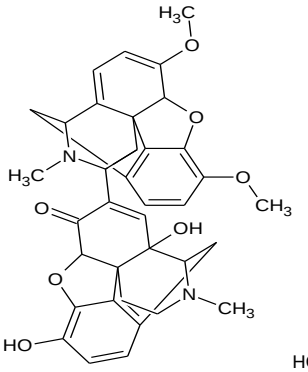
Chlorogenic acid



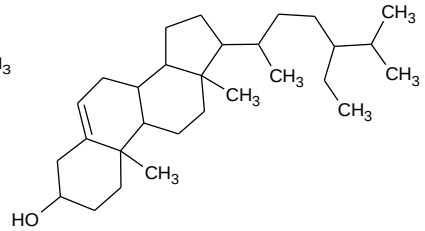
Anaferine



Ashwagandhanolide



Somniferine



Beta-sisterol

Figure 2.22. Structures of bioactive compounds present in *Withania somnifera*.

2.5.10. Combretum molle R. Br. ex G. Don

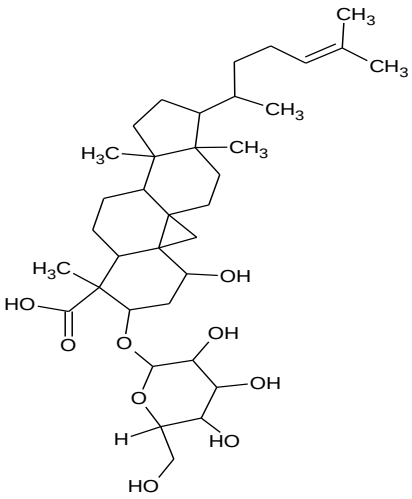
Combretum molle belongs to the family Combretaceae and is known as velvet bushwillow or soft-leaved combretum. The tree is found throughout tropic Africa on river banks and wooded

grasslands where it can reach a height of 7 meters (Drummond and Coates-Palgrave, 1973). It is medium size deciduous tree with grey-brown to almost black barks. The leaves are leathery, velvety and silvery to reddish in colour while the flowers are greenish-yellow and fragrant (Figure 2.23). The fruits are four-winged, light green to reddish when young and turn red-brown or yellow brown when matured (Wickens, 1973). The leaves of *Combretum molle* were evaluated for antifungal activity using bio-autographic assay and the results revealed the presence of antifungal compounds. During that study; this plant species was evaluated for antifungal activity using the most common and important disease-causing fungi of animals (Masoko and Eloff, 2006).

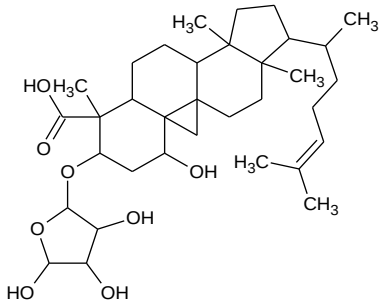
Figure 2.23. The leaves and fruits of *Combretum molle* growing naturally at the Agricultural Research Council, Roodeplaat, Pretoria, Gauteng Province.

There is limited information on the antimicrobial activity of *Combretum molle* against phytopathogenic organisms. Several authors have studied antimicrobial activity of this plant

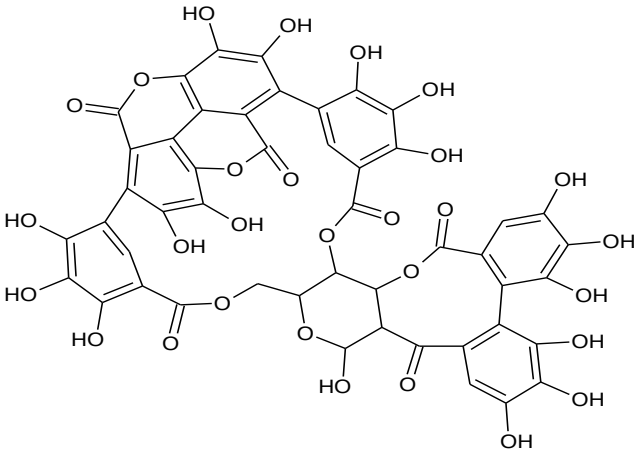
species against human and or animal pathogens (Eloff, 1998; Eloff, 1999; Khan *et al*., 2000; Asres *et al*., 2001; Pegel and Rogers, 1985). The compounds found to contribute to the activity of *C. molle* were mollic acid glucoside, α-arabinoside and punicalagin which were isolated from the leaves and barks, respectively (Pegel and Rogers, 1985; Asres *et al*., 2001). In another unrelated pharmacological study conducted on mice and rats, mollic acid glucoside isolated from this plant was reported to possess analgesic and anti-inflammatory properties (Ojewole, 2008). The structure of these compounds are represented in Figure 2.24.



Mollic acid glucoside



α-arabinoside



Punicalagin

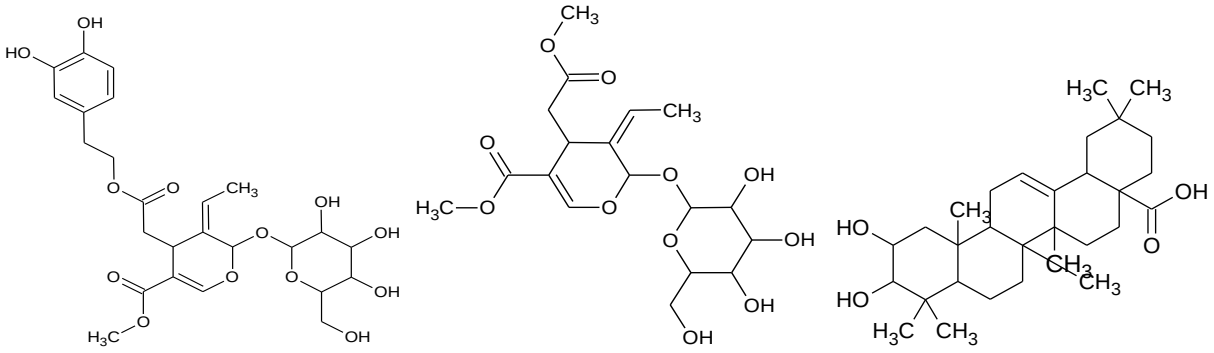
Figure 2.24. Structures of some compounds isolated from *Combretum molle*.

2.5.11. Olea europaea L.

This plant belongs to the family Oleaceae and its common name is wild olive. The plant is native to the Mediterranean, Asia, and Africa. It is best grown in Malaysia, however, it is geographically distributed from the Eastern Cape, KwaZulu-Natal, Swaziland, Mpumalanga, Gauteng, Free State and Limpopo to Northern Cape provinces of South Africa. It is short and thick evergreen tree that can reach 8-15 meters in height depending on the environmental conditions. The trunk of wild olive is typically twisted and has rough grey barks. The leaves are silver or grey green (Figure 2.25) and the fruits are green to purple in colour (Satish and Ansari, 2013). Antifungal activity evaluation of solvent extracts with different polarities obtained from the leaves of *Olea europaea* subspecies *africana* showed strong activity (0.16 - 0.63 mg/ml) against *Candida albicans* and *Cryptococcus neoformans*. Bio-autographic evaluation of these plant extracts also demonstrated the presence of several antifungal compounds against other tested microorganisms (Masoko and Makgapeetja, 2015).

Figure 2.25. The leaves of *Olea europaea* growing naturally at the Agricultural Research Council, Roodeplaat, Pretoria, Gauteng Province.

To the best of our knowledge there is limited data on the antifungal activity of *Olea europaea* against *Fusarium* pathogens. However, this plant species has been evaluated for pharmacological properties and antimicrobial activities in several studies (Cumaoˇglu *et al*., 2011; Casaburi *et al*., 2013; Sudjana *et al*., 2009; Pereira *et al*., 2007; Ko *et al*., 2009). The activity of *O. europaea* was related to the presence of compounds such as oleuropein, oleoside, oleanolic acid, betu1onic acid, ursolic acid, maslinic acid, verbascoside and other compounds isolated from different parts of the plant. Some of which are presented in Figure 2.26 (Bianchi *et al*., 1992; Karioti *et al*., 2006; Peralbo-Molina *et al*., 2012; Long *et al*., 2010).



Oleuropein Oleoside Maslinic acid

Figure 2.26. Chemical structures of compounds isolated from *Olea europaea*.

2.5.12. Quercus acutissima Carruth.

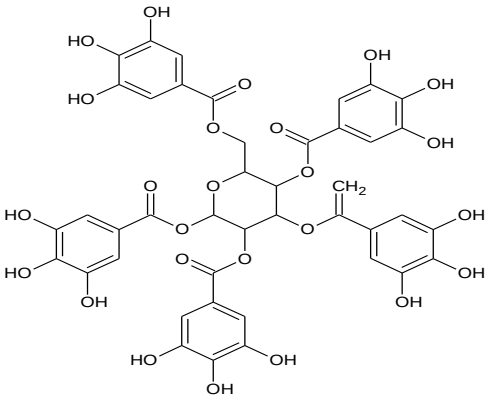
Quercus acutissima is native to China, Korea and Japan; however, it is widely planted across the world including in South Africa. It is an oak tree in the family Fagaceae and is commonly known as sawtooth oak. This is a deciduous tree, which can grow up to 30 meters in height. The barks on the trunk of *Quercus acutissima* are deeply furrowed and the leaves has saw-

tooth like triangular lobes along the margins (Figure 2.27). Different type of oak trees including *Quercus acutissima* were screened for antibacterial activity and their extracts inhibited the growth of *Microcystis aeruginosa* by approximately 50% at 0.02 mg/ml (Park *et al*., 2006).

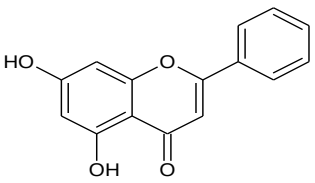
Figure 2.27. The leaves of *Quercus acutissima* growing naturally at the Agricultural Research Council, Roodeplaat, Pretoria, Gauteng Province.

To the best of our knowledge there is no literature data on the antifungal activity of *Quercus acutissima* against *Fusarium* pathogens. A folk medication (*Bokusoku*) manufactured from aqueous extraction of *Q. acutissima* cortex used for the treatment of skin disorder was found to demonstrate good biological and pharmacological activities. Liquid chromatography Mass spectroscopy (LC-MS/MS) analysis of the constituents of this medication revealed the present of pentagalloyl glucose, chrysin, gallic acid, luteolin, quercetin, quercitrin, and genistein (Koseki *et al*., 2015). Other compounds detected in *Q. acutissima* are ellagic acid glucoside)

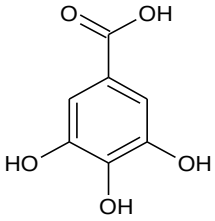
and digalloyl glucoside of which their chemical structures are shown in Figure 2.28 (Vanhessche *et al*., 2007).



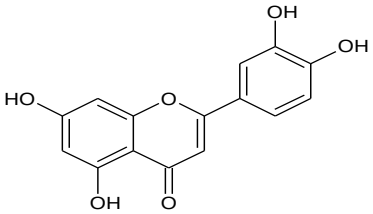
Pentagalloyl glucose



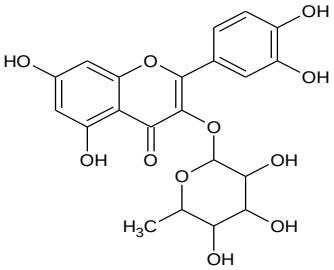
Chrysin



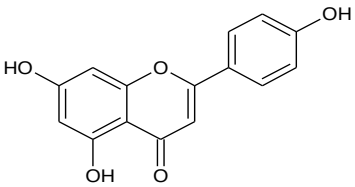
Gallic acid



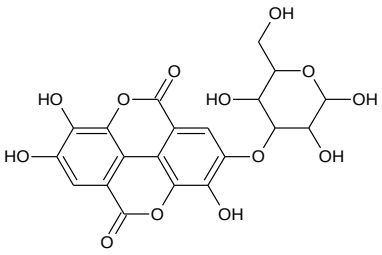
Luteolin



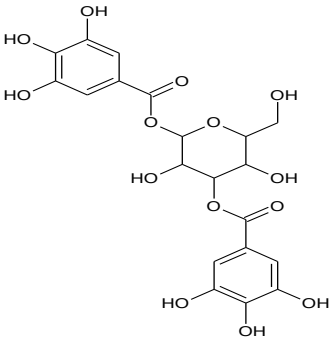
Quercitrin



Genistein



Ellagic acid glucoside



Digalloyl glucoside

Figure 2.28. Chemical structures of compounds found in *Quercus acutissima*

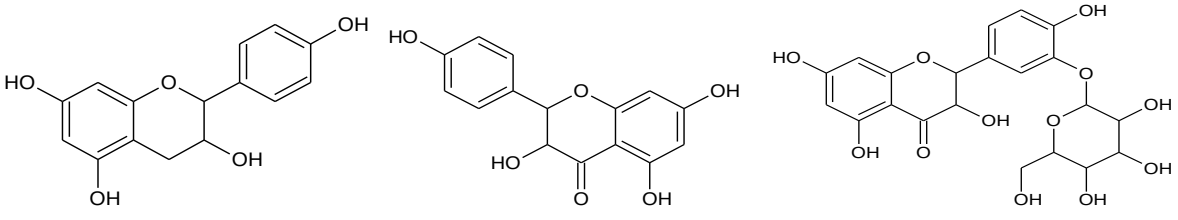
2.5.13. Vangueria infausta Burch

Vangueria infausta belongs to the family Rubiaceae and is commonly called wild medlar. The plant is native to Botswana, Kenya, Namibia, Swaziland, South Africa and other African countries (Bridson, 1998). *Vangueria infausta* is a deciduous small tree that can grow up to 8 m in height with single or short multi-stemmed and hanging branches. The barks are pale grey-brown and the leaves are medium to large in size, light green in colour and covered with velvety short hairs (Figure 2.29). It produces edible fruits that are round, glossy dark green when young and which changes to a light brown colour when ripen and have soft fleshy pulp. Methanolic stem bark and ethanolic leaves extracts of this plant were evaluated for antifungal activity against *Candida mycoderma* using an agar overlay method and they showed good activity with minimum inhibitory concentration ranging from 0.1 to 50 µg (Mbukwa *et al*., 2007).

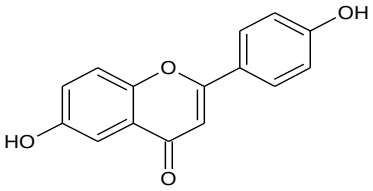
Figure 2.29. The leaves of *Vangueria infausta* growing naturally at Moletjie, Polokwane, Capricorn District, Limpopo Province.

Mahlo *et al*., 2010 evaluated the antifungal activity of acetone, hexane, dichloromethene and methanol extracts from the leaves of *Vangueria infausta* against *Aspergillus parasiticus, Aspergillus niger, Colletotricum gloeosporioides, Penicillium expansum, Penicillium janthinellum, Trichoderma harzianum* and *Fusarium oxysporum.* The extracts showed very good activity against *Fusarium oxysporum*, with MIC value of 0.32 mg/ml demonstrated by acetone, dichloromethane and methanol extracts. Hexane extraction also demonstrated good activity with reported MIC value of 0.63 mg/ml against *F. oxysporum*. In summary, all *V. infausta* extracts showed good to moderate antifungal activity against the tested phytopathogens with MIC values ranging from 0.32 to 2.50 mg/ml.

In another study, ethyl acetate, water and methanol extracts from the leaves of *V. infausta* were evaluated against different pathogens. The results from that study indicated that the extracts were active against *Aspergillus fumigatus*, *Candida albicans* and *Fusarium culmorum* (De Boer *et al*., 2005). Many compounds such as epiafzelechin, dihydrokaempferol, quercetin, luteolin, dihydroquercetin-3'-Oglucoside, daidzein, genistein were isolated from *V. infausta* and evaluated for antimicrobial activity. At least more than one compounds showed activity against *B. subtilis, E. coli, S. aureus, C. mycoderma*. However, other compounds were not active against the tested microorganisms (Mbukwa *et al*., 2007). On the other hand, luteolin-7-O-rutinoside, apigenin-7-O-rutinoside, luteolin-4-O-glucoside, quercetin-3-O-glucoside and quercetin were also isolated from the leaves of this medicinal plant species (Abeer, 2011). Figure 2.30 presence some of the compounds isolated from *V. infausta*.

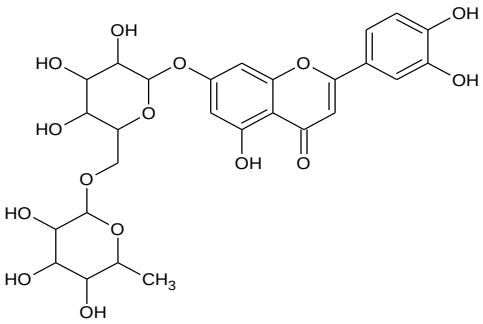


Epiafzelechin

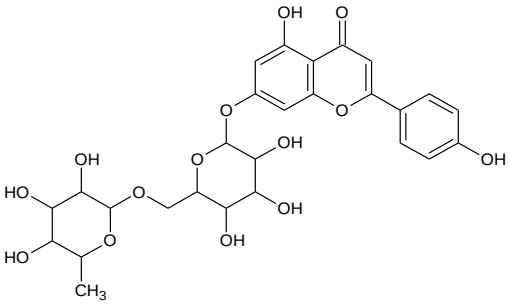


Dihydrokaempferol

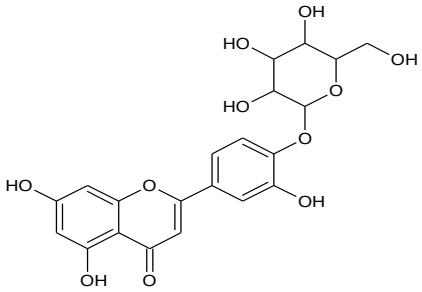
Dihydroquercetin-3'-Oglucoside



Daidzein

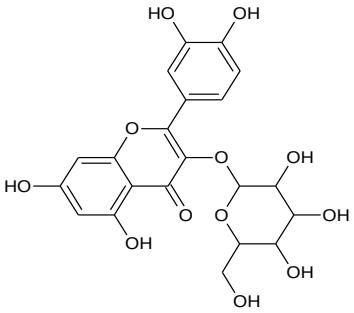


Luteolin-7-O-rutinoside



Apigenin-7-O-rutinoside

Luteolin-4-O-glucoside



Quercetin-3-O-glucoside

Figure 2.30. Chemical structures of compounds isolated from *Vangueria infausta*.

2.6 CONCLUSIONS

Indigenous knowledge system regarding medicinal plants used to treat various diseases or aliments affecting human and livestock demonstrate the important of botanicals in our society. On the other hand, there is desperate need to develop affortable and safe bio-pesticides that can be used in crop protection to manage diseases. As presented in literature review, different parts of the plant contains wide variety of chemical compounds with so many functions and properties. Literature has also shown that extracts from medicinal plants species have biological activity such as antifungal, antibacterial, anti-inflammatory, anti-cancer and many other therapeatic properties. For this reasons, we are studying or investigating these natural resources as potential alternatives bio-pesticides for treating diseases in crop production.

REFERENCES

Abbas, H.K., Cartwright, R.D., Xie, W., Shier, W.T., 2006. Aflatoxin and fumonisin contamination of maize (*Zea mays* L) hybrids in Arkansas. Crop Protection 25, 1–9.

Abeer, T., 2011. Flavonoidal content of *Vangueria infausta* extract grown in Egypt: Investigation of its antioxidant activity. International Research Journal of Pharmacy 2, 157–161.

Adenle, V.O., Cardwell, K.F., 2000. Seed transmission of maize downy mildew (*Peronosclerospora sorghi*) in Nigeria. Plant Pathology 49, 628–634.

Adepoju, A.O., Ogunkunle, A.T.J., Femi-adepoju, A. G., 2014. Antifungal activities of seed oil of neem (*Azadirachta indica* A. Juss.). Global Journal of Biology, Agriculture and Health Sciences 1, 106–109.

Agrios, G.N. Plant Pathology. 5th Edition. San Diego, California: Elsevier Academic Press; 2005.

Ahmad, I., Beg, A.Z., 2001. Antimicrobial and phytochemical studies on 45 Indian medicinal plants against multi-drug resistant human pathogens. Journal of Ethnopharmacology 74, 113–23.

Aktar, Md.W., Sengupta, D., Chowdhury, A., 2009. Impact of pesticides use in agriculture: Their benefits and hazards. Interdisciplinary Toxicology 2, 1–12.

Alemayehu, I., Tadesse, S., Mammo, F., Kibret, B., Endale, M., 2015. Phytochemical analysis of the roots of *Senna didymobotrya.* Journal of Medicinal Plants Research 9, 900–907.

Amadioha, A.C., 2000. Controlling Rice Blast *in vitro* and *in vivo* with extracts of *Azadirachta indica*. Journal of Crop Protection 19, 287–290.

Asres, K., Bucar, F., Edelsbrunner, S., Kartnig, T., Höger, G., Thiel, W., 2001. Investigations on antimycobacterial activity of some Ethiopian Medicinal Plants. Phytotherapy Research 15, 323–326.

Avancini C., Wiest, J.M., Dall'agnol, R., Haas, J.S., von Poser, G.L., 2008. Antimicrobial activity of plants used in the prevention and control of bovine mastitis in southern Brazil. Latin American Journal of Pharmacy 27, 894–899.

Bacon, C.W., Hinton, D.M., 1996. Symptomless endophytic colonisation of maize by *Fusarium moniliforme*. Canadian Journal of Botany 74, 1195–1202.

Bartlett, D. W., Clough, J. M., Godwin, J. R., Hall, A. A., Hamer, M., Parr-Dobrzanski, B. 2002. Review: The Strobilurin Fungicides. Pest Management Science 58, 649–662.

Begum, S., Ayub, A., Zehra, S.Q., Siddiqui, B.S., Choudhary M.I., Samreen, 2014. Leishmanicidal triterpenes from *Lantana camara*. Chemistry and Biodiversity 11, 709–718.

Belewa, V., Baijnath, H., Somai, B.M., 2011. Aqueous extracts from the bulbs of *Tulbaghia violacea* are antifungal against *Aspergillus flavus.* Journal of Food Safety 31, 176–184.

Bianchi, G., Murelli, C., Vlahov, G., 1992. Surface waxes from olive fruits. Phytochemistry 31, 3503–3506.

Bridson, D.M. Rubiaceae. In Flora Zambesiaca. London, United Kingdom: Royal Botanic Gardens, Kew; 1998.

Brinker, F. Herb contraindications and drug interactions. 2nd Edition. Sandy, Eclectic Medical Publications; 1998.

Buckingham, J. Dictionary of Natural Products. Version 16.2. London. New York: Chapman and Hall; 2008.

Buwa, L.V., van Staden, J., 2006. Antibacterial and antifungal activity of traditional medicinal plants used against venereal diseases in South Africa. Journal of Ethnopharmacology 103, 139–142.

Calvo, M.A., Arosemena, E.L., Shiva, C., Adelantado, C., 2011. Antimicrobial activity of plant natural extracts and essential oils. Science against microbial pathogens: communicating current research and technological advances, 1179–1185.

Carpinella, C.M., Ferraroli, C., Valladares, G., Defago, M., Palacios, S., 2002. Potent limnoid insect antifeedant from *Melia azedarach*. Bioscience, Biotechnology, and Biochemistry, 60, 1731–1736.

Carpinella, M.C., Ferrayolic, C.G., Palacios, S.M., 2005. Antifungal synergistic effect of scopoletin, a hydroxycoumarin isolated from *Melia azedarach* L. fruits. Journal of Agricultural and Food Chemistry 53, 2922–2927.

Carpinella, M.C., Giorda, L.M., Ferrayoli, C.G., Palacios, S.M, 2003. Antifungal effects of different organic extracts from *Melia azedarach* L. on phytopathogenic fungi and their isolated active components. Journal of Agricultural and Food Chemistry 51, 2506–2511.

Carpinella, M.C., Herrero, G.G., Alonso, R.A., Palacios, S.M., 1999. Antifungal activity of *Melia azedarach* fruit extract. Fitoterapia 70, 296–298.

Casaburi, I., Puoci, F., Chimento, A., Sirianni, R, Ruggiero, C, Avena, P., Pezzi, V., 2013. Potential of olive oil phenols as chemo preventive and therapeutic agents against cancer: a review of *in vitro* studies. Molecular Nutrition and Food Research 57, 71–83.

Chen, Y., Zhou, M.G., 2009. Characterization of *Fusarium graminearum* isolates resistant to both carbendazim and a new fungicide JS399-19. Phytopathology 99, 441–446.

Chen, Z., Gao, T., Liang, S., Liu, K., Zhou, M., Chen, M., 2014. Molecular mechanism of resistance of *Fusarium fujikuroi* to benzimidazole fungicides. Federation of European Microbiological Societies Letter 357, 77–84.

Cherono, S., Akoo, A. Mursik not too sweet for plant. Magazine-Daily Nation. Kenya: 2011 July 05; page 11.

Chiffelle, I.G., Amanda, H.F., Diego, L.R., 2009. Physical and chemical characterization of *Melia azedarach* L. Fruit and leaf for use as botanical insecticide. Chilean Journal of Agricultural Research 69, 38–45.

Cumaoˇglu, A., Rackova, L., Stefek, M., Kartal, M., Maechler, P., Karasu, C., 2011. Effects of olive leaf polyphenols against H_2O_2 toxicity in insulin secreting β-cells. Acta Biochimica Polonica 58, 45–50.

Daferera, D.J., Ziogas, B.N., Polissiou, M.G., 2003. The effectiveness of plant essential oils on the growth of *Botrytis cinerea*, *Fusarium* sp. and *Clavibacter michiganensis* subsp. *michiganensis*. Crop Protection 22, 39–44.

Das, L., Godbole, S., 2015. Antifungal and phytochemical analysis of *Lantana camara*, *Citrus limonum* (Lemon), *Azadirachta indica* (Neem) and *Hibiscus rosasinensis* (China Rose). Journal of Pharmacy Research 9, 476–479.

De Boer, H.J., Kool, A., Broberg, A., Mziray, W.R., Hedberg, I., Levenfors, J.J., 2005. Antifungal and anti-bacterial activity of some herbal remedies from Tanzania. Journal of Ethnopharmacology 96, 461–469.

Deena, M.J., Thoppil, J.E., 2000. Antimicrobial activity of the essential oil of *Lantana camara.* Fitoterapia 71, 453–455.

Desjardins, A.E., 2006. *Fusarium* mycotoxins: chemistry, genetics and biology. American Phytopathological Society.

Drewes, F. E., van Staden, J., 1995. Initiation of and solasodine production in hairy root cultures of *Solanum mauritianum* Scop. Plant Growth Regulation 17, 27–31.

Drewes, S.E., 1971. Isolation of 3,3',4',5,5'-pentahydroxystilbene from *Scho*tia species. Phytochemistry 10, 2837–2838.

Drewes, S.E., Fletcher, I.P., 1974. Polyhydroxystilbenes from the heartwood of *Schotia brachypetala*. Journal of the Chemical Society, Perkin Transactions 19, 961–962.

Drummond, R. B., Coates-Palgrave, K. Common Trees of the Highweld. 1st Edition. Rhodesia, Longman Rhodesia; 1973.

Du, K. Isolation of bioactive constituents from seeds of *Schotia brachypetala* (Fabaceae) and *Colophospermum mopane* (Fabaceae). Bloemfontein: University of the Free State; 2011.

Eloff, J.N., 1998. A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. Planta Medica 64, 711–713.

Eloff, J.N., 1999. The antibacterial activity of 27 southern African members of the Combretaceae. South African Journal of Science 95, 148–152.

Everist, S.L. Poisonous plants of Australia. Revised edition. London; Sydney: Angus & Robertson; 1981.

Fandohan, P., Gbenou, J.D., Gnonlonfin, B., Hell, K., Marasas, W.F., Wingfield, M.J., 2004. Effect of essential oils on the growth of *Fusarium verticillioides* and fumonisin contamination in corn. Journal of Agricultural and Food Chemistry 52, 6824–6829.

Fayaz, M., Bhat, M.H., Fayaz, M., Kumar, A., Jain, A.K., 2017. Antifungal activity of *Lantana camara* L. leaf extracts in different solvents against some pathogenic fungal strains. Pharmacologia 8, 105–112.

Fern, K., Tropical Plants Database 2014. Updated: 13 June 2019. cited: 19 April 2020. Available from: http://tropical.theferns.info/

Freije, A., Wise, K. A., 2015. Impact of *Fusarium graminearum* inoculum availability and fungicide application timing on *Fusarium* head blight in wheat. Crop Protection 77, 139–147.

Furer, V., Hersch, M., Silvetzki, N., Breuer, G.S., Zevin, S., 2011. *Nicotiana glauca* (Tree Tobacco) intoxication - two cases in one family. Journal of Medical Toxicology, 7, 47–51.

Gelfand, M., Mavi, S., Drummond, R.B., Ndemera, B. The traditional medical practitioner in Zimbabwe: his principles of practice and pharmacopoeia. Gweru: Mambo Press; 1985.

Gilani, A.H., Rahman, A-U., 2005. Trends in Ethnopharmacology. Journal of Ethnopharmacology 100, 43–49.

Girish, K.S., Machiah, K.D., Ushanandini, K.H., Nagaraju, S, Govindappa, M., Vedavathi, M., Kemparaju, K., 2006. Antimicrobial properties of a nontoxic glycoprotein (*WS*G) from *Withania somnifera* (Ashwagandha). Journal of Basic Microbiology 46, 365–374.

Gupta, G.L., Rana, A.C., 2007. *Withania somnifera* (Ashwagandha): A Review. Pharmacognosy Reviews 1, 129–136.

Hailu, T., Bachheti, R.K., Dekebo, A., 2016. Phytochemical analysis and antimicrobial activity of *Senna didymobotrya* seed extracts, Der Pharma Chemica 8, 111–116.

Halvorson, W.L., Guertin, P. *Polpogon monspeliensis* (L.) Desf. USGS weeds in the West Project: Status of introduced plants in southern Arizona Parks. U.S. Geological Survey,Southwest Biological Science Center. Tucson, Arizona, USA; 2003.

Harborne, J.B., Baxter, H., Moss, G.P. Phytochemical dictionary: A handbook of bioactive compounds from plants. 2nd Edition. London: Taylor & Francis Group; 1995.

Harris, C.A., Renfrew, M.J., Woolridge, M.W., 2001. Assessing the risk of pesticide residues to consumers: recent and future developments. Food Additives and Contaminants 18, 1124–1129.

Hart, N.K., Lamberton, J.A., Sioumis, A.A., Saures, H., 1976. New triterpenes of *Lantana camara.* A comparative study of the constituents of several taxa. Australian Journal of Chemistry 29, 655–671.

Hassan, E.H., El-Hameed, T.Z.A., Nasr, E.A., 2014. Ecological and phytochemical studies on *Nicotiana glauca* from Egypt. The Egyptian Society of Experimental Biology 10, 87–95.

Headrick, J., Pataky, J., 1991. Maternal influence on the resistance of sweet corn lines to kernel infection by *Fusarium moniliforme*. Phytopathology 81, 268–274.

Henderson, L. Alien weeds and invasive plants. A complete guide to declared weeds and invaders in South Africa. Pretoria: Agricultural Research Council, Plant Protection Research Institute Handbook No. 12 (South Africa); 2001.

Hubert, J., Mabagala, R.B., Mamiro, D.P., 2015. Efficacy of selected plant extracts against *Pyricularia grisea*, causal agent of Rice Blast Disease. American Journal of Plant Sciences 6, 602–611.

Hyde, M.A., Wursten, B.T., Ballings, P., Coates Palgrave, M., 2020. Flora of Zimbabwe: Cultivated Plants: Species information: *Nicotiana glauca.* Zimbabwe. Updated: 26 March 2018, cited: 19 April 2020. Available from: ttps://www.zimbabweflora.co.zw/cult/species.

Igbedioh, S.O., 1991. Effects of agricultural pesticides on humans, animals and higher plants in developing countries. Archives of Environmental Health 46, 218.

Incremona, M.E., González, M.D.P., Pioli, R.N., Salinas, A.R., 2014. Infection of maize silks by a native *Fusarium* (*Fusarium graminearum*) isolate in Argentina. Chilean Journal of Agricultural and Animal Sciences 30, 203–211.

Jackson-Ziems, T.A., Giesler, L.J., Adesemoye, A.O., Harveson, R.M., Wegulo, S.N., 2017. "Understanding Fungicide Resistance". Papers in Plant Pathology 481.

Jayakumar, K., Murugan, K., 2016. Evaluation of major phytochemicals in the leaves and fruits of *Solanum mauritianum* Scop. A potential herbal drug. International Journal of Research in Ayurveda and Pharmacy 7, 58–60.

Jeruto, P., Arama, P.F., Anyango, B., Akenga, T., Nyunja, R., Khasabuli, D., 2016. *In vitro* antifungal activity of methanolic extracts of different *Senna didymobotrya* (Fresen.) H.S.

Irwin & Barneby plant parts. African Journal of Traditional, Complementary and Alternative Medicines 13, 168–174.

Kalita, S., Kumar, G., Karthik, L., Rao, K.V.B., 2012. A review on medicinal properties of *Lantana camara* Linn. Research Journal of Pharmacy and Technology 5, 711–715.

Kapoor, L.D. Handbook of Ayurvedic Medicinal Plants Herbal Reference Library. 1st Edition. London, United Kingdom: CRC Press, Taylor & Francis Group; 2001.

Karioti, A., Chatzopoulou, A., Bilia, A.R., Liakopoulos, G., Stavrianakou, S., Skaltsa, H., 2006. Novel secoiridoid glucosides in *Olea europaea* leaves suffering from boron deficiency. Bioscience, Biotechnology and Biochemistry 70, 1898–1903.

Karuppusamy, S., Muthuraja, G., Rajasekaran, K.M., 2009. Chemical composition and antimicrobial activity of essential oil from fruits of *Vanasushava pedata* (Apiaceae). Advances in Biological Research 3, 196–200.

Kathireshan, A.K., Priya, T., Jasmine, K.D., Gayathri, G., Kumar, M.R.R., 2019. Assessment of *in vitro* antibacterial and antifungal activities of leaf extracts of *Melia azedarach* Linn. Indian Journal of Pharmaceutical Sciences 81, 380–384.

Khan, M.N., Ngassapa, O., Matee, M. I. N., 2000. Antimicrobial activity of Tanzanian chewing sticks against oral pathogenic microbes. Pharmaceutical Biology 38, 235–240.

Klittich, C.J. 2008. Milestones in fungicide discovery: Chemistry that changed agriculture. Plant Health Progress.

Ko, K.W., Kang, H.J., Lee, B.Y., 2009. Antioxidant, antimicrobial, and antiproliferative activities of olive (*Olea europaea* L.) leaf extracts. Food Science and Biotechnology 18, 818–821.

Korir, R.K., Mutai, C., Kiiyukia, C., Bii, C., 2012. Antimicrobial activity and safety of two medicinal plants traditionally used in Bomet District of Kenya. Research Journal of Medicinal Plant 6, 370–382.

Koseki, J., Matsumoto, T., Matsubara, Y., Tsuchiya, K., Mizuhara, Y., Sekiguchi, K., Nishimura, H., Watanabe, J., Kaneko, A., Hattori, T., Maemura, K., Kase, Y., 2015. Inhibition of rat 5α-reductase activity and testosterone-induced sebum synthesis in hamster sebocytes by an extract of *Quercus acutissima* cortex. Evidence-Based Complementary and Alternative Medicine.

Kraus, W., 1986. Constituents of neem and related species. A revised structure of azadirachtin. Studies in Organic Chemistry 26, 237–256.

Kraus, W., Cramer, R., Sawitzki, G., 1981. Tetranotriterpenoids from seeds of the neem tree, *Azadirachta indica*. Phytochemistry 20, 117–120.

Lavie, D., Glotter, E., Shvo, Y., 1965. Constituents of *Withania somnifera* Dun. Part IV The structure of withaferin-A. Journal of the American Chemical Society 30, 7517–7531.

Lavie, D., Jain, M.K., 1967. Tetranortriterpenoids from *Melia azedarach* L. Journal of the Chemical Society, Chemical Communications, 278–280.

Lee, S.M., Klocke, J.A., Barnby, M.A., Yamasaki, R.B., Balandrin, M.F., 1991. Insecticidal constituents of *Azadirachta indica* and *Melia azedarach*. (Meliaceae). In: Hedin, P.H. Editor. Naturally occurring pest bio-regulators, American Chemical Society Symposium. Series. Washington DC, USA, 449, 293–304.

Leslie, J.F., Summerell, B.A. The *Fusarium* laboratory manual. 1st Edition. Ames, Iowa, USA: Blackwell Publishing Professional; 2006.

Lew, H., Adler, A., Ediner, W., 1991. Moniliformin and European corn borer (*Ostrnia nubilalis*) Mycotixin Research, mycotoxins in Italy. Plant Disease 79, 727–731.

Lewandowski, S.M., Bushnell, W.R. Evans, C.K., 2006. Distribution of mycelial colonies and lesions in field-grown barley inoculated with *Fusarium graminearum*. Phytopathology 96, 567–581.

Logrico, A., Mule, G., Moretti, A., Bottalico., A., 2002. Toxigenic *Fusarium* species and mycotoxins associated with maize ear rot in Europe. European Journal of Plant Pathology 108, 597–609.

Lonare, M.K., Sharma, M., Hajare, S.W., Borekar, V.I., 2012. *Lantana camara*: overview on toxic to potent medicinal properties. International Journal of Pharmaceutical Sciences and Research 3, 3031–3035.

Long, H.S., Tilney, P.M., Van Wyk, B.E. 2010. The ethnobotany and pharmacognosy of *Olea europaea subsp. africana* (Oleaceae). South African Journal of Botany 76, 324–331.

Lovang, U.T., Wildt, P., 1998. The effect of aqueous extracts of *Melia azedarach* and *Trichilia emetic* on selected pathogens of tomato, bean and maize. Botanical pesticides Minor-Field-Studies International Office. Swedish University of Agricultural Sciences 52, 23–60.

Luseba, D., Tshisikhawe, M.P., 2013. Medicinal plants used in the treatment of livestock diseases in Vhembe region, Limpopo province, South Africa. Journal of Medicinal Plants Research 7, 593–601.

Magan, N., Hope, R., Colleate, A., Baxter, E.S., 2002. Relationship between growth and mycotoxin production by *Fusarium* species, biocides and environment. European Journal of Plant Pathology 108, 685–690.

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, 1529–1533.

Mahmoudi, R., 2017. Application of medicinal plants: from past to present. MedCrave Online Journal of Biology and Medicine 1, 80.

Maobe, M.A.G., Gitu, L., Gatebe, E., Rotich, H., Karanja, P.N., Votha, D.M., Nderitu, I.W., Kungu, W. 2013. Antifungal activity of eight selected medicinal herbs used for the

treatment of diabetes, malaria and pneumonia in Kisii Region, southwest Kenya. World Journal of Medical Sciences 8, 74-78.

Marasas, W.F.O., Nelson, P.E., Toussoun, T.A. Toxigenic *Fusarium* species: identity and mycotoxicology. Pennsylvania. The Pennsylvania State University Press; 1984.

Maregesi, S.M., Ngassapa, O.D., Pieters. L., Vlietinck, A.J., 2007. Ethnopharmacological survey of the Bunda district, Tanzania: Plants used to treat infectious diseases, Journal of Ethnopharmacology 113, 457–470.

Martínez, J.A. Natural Fungicides Obtained From Plants. In: Dhanasekaran, D., Thajuddin, N., Panneerselvam, A., Editors. Fungicides for Plant and Animal Diseases. London, United Kingdom: IntechOpen; 2012.

Martini, N.D., Katerere, D.R.P., Eloff, J.N., 2004. Biological activity of five antibacterial flavonoids from *Combretum erythrophyllum* (Combretaceae). Journal of Ethnopharmacology 93, 207–212.

Masika, P.J., Afolayan, A.J. 2002. Antimicrobial activity of some plants used for the treatment of livestock disease in the Eastern Cape, South Africa. Journal of Ethnopharmacology 83, 129-134.

Masoko, P., Eloff, J.N., 2006. Bioautography indicates the multiplicity of antifungal compounds from twenty-four southern African *Combretum* species (Combretaceae). African Journal of Biotechnology 18, 1625–1647.

Masoko, P., Makgapeetja, D.M., 2015. Antibacterial, antifungal and antioxidant activity of *Olea africana* against pathogenic yeast and nosocomial pathogens. BMC Complementary and Alternative Medicine 15, 409.

Mbukwa, E., Chacha, M., Majinda, R.R.T., 2007. Phytochemical constituents of *Vangueria infausta*: their radical scavenging and antimicrobial activities. Archive for Organic Chemistry 9, 104–112.

McGaw, L.J., Jäger, A.K., van Staden, J., 2002. Variation in antibacterial activity of *Schotia* species. South African Journal of Botany 68, 41–46.

Mdee, L.K., Masoko, P., Eloff, J.N., 2009. The activity of extracts of seven common invasive plant species on fungal phytopathogens. South African Journal of Botany 75, 375–379.

Meziane, M., Goumri, H., 2014. The antimicrobial effect of extracts of *Melia azedarach* on some pathogenic microorganisms. International Journal of Applied and Natural Sciences 3, 173–180.

Mishra, R., Lone, R., Manzoor, D., Shuab, R., 2018. Imbalance due to pesticide contamination in different ecosystems. International Journal of Theoretical and Applied Sciences 1, 239–246.

Mizrachi, N., Levy, S., Goren, Z.Q., 2000. Fatal poisoning from *Nicotiana glauca* leaves: identification of anabasine by Gas Chromatography/Mass Spectrometry. Journal of Forensic Sciences, 45, 736–741.

Morton, V., Staub, T. A short history of fungicides. Saint Paul, Minnesota: The American Phytopathological Society (APS). Updated: 03 August 2008; cited: 17 April 2020. Available from: https://www.apsnet.org/edcenter/apsnetfeatures/Pages/Fungicides.

Mthethwa, N.S. Antimicrobial activity testing of traditionally used plants for treating wounds and sores at Ongoye area KwaZulu-Natal. Empangeni, South Africa: University of Zululand; 2009.

Munkvold, G. P., Carlton, M.W., 1997. Influence of inoculation method on systemic *Fusarium moniliforme* infection of maize plants grown from infected seeds. Plant Disease 81, 211–216.

Munkvold, G.P., Desjardins, A.E., 1997. Fumonisins in maize: Can we reduce their occurrence? Plant Disease 81, 556–565.

Nahak, G., Sahu, R.K., 2010. *In vitro* antioxidative acitivity of *Azadirachta indica* and *Melia azedarach* leaves by DPPH scavenging assay. Journal of American Science 6, 6.

Nawwar, M., Hussein, S., Ayoub, N., Hashim, A., El-Sharawy, R., Lindequist, U., Wende, K., 2011. Constitutive phenolics of *Harpephyllum caffrum* (Anacardiaceae) and their biological effects on human keratinocytes. Fitoterapia 82, 1265–1271.

Nciki, S., Vuuren, S., van Eyk, A., de Wet, H., 2016. Plants used to treat skin diseases in Northern Maputaland, South Africa: antimicrobial activity and *in vitro* permeability studies. Pharmaceutical Biology 54, 2420–2436.

Nelson, P.E., Desjardins, A.E., Plattner, R.D., 1993. Fumonisins, mycotoxins produced by *Fusarium* species: biology, chemistry and significance. Annual Review of Phytopathology 31, 233–252.

Nelson, P.E., Dignani, M.C., Anaissie, E.J., 1994. Taxonomy, biology, and clinical aspects of *Fusarium* species. Clinical Microbiology Reviews 7, 479–504.

Newman, D.J., Cragg, G.M., Snader, K.M., 2000. The influence of natural products upon drug discovery. Natural Products Reports 17, 175–285.

Ochieng, C.O., Shrivastava, A., Chaturvedi, U., Sonkar, R., Khanna, A.K., Bhatia, G., Asthana, R.K., Owuor, P.O., Manguro, L.A., Saxena, A.K. Effects of *Senna didymobotrya* root extract and compounds on tritoninduced hyperlipidaemic rats and differentiation of 3T3-Li preadipocytes. The Natural Products Journal 3.

Ojewole, J.A.O., 2008. Analgesic and anti-inflammatory effects of mollic acid glucoside, a 1 alpha-hydroxycycloartenoid saponin extractive from *Combretum molle* R. Br. ex G. Don (Combretaceae) leaf. Phytotherapy Research 22, 30–35.

Olckers, T., 2003. Assessing the risks associated with the release of a flower bud weevil, *Anthonomus santacruzi*, against the invasive tree *Solanum mauritianum* in South Africa. Biological Control 28, 302–312.

Omidpanah, S., Haseli, A., Manayi, A., 2015. Evaluation of antifungal properties of some medicinal plants against *Aspergillus flavus* isolated from contaminated corn *in vitro.* Mediterranean Journal of Chemistry 3, 1093–1099.

Padvi, S.A., Hingole, D.G., Khaire, P.B., 2018. *In vitro* efficacy of fungicides against *Fusarium solani* incited by dry root rot of sweet orange. Journal of Pharmacognosy and Phytochemistry 7, 3270–3273.

Panter, K.E., Weinzweig, J., Gardner, D.R., Stegelmeier, B.L., James, L.F., 2000. Comparison of cleft palate induction by *Nicotiana glauca* in goats and sheep. Teratology 61, 203–210.

Panth, M., Hassler, S.C., Baysal-Gurel, F., 2020. Methods for management of soilborne diseases in crop production. Agriculture 10, 16.

Parajapati, N.D., Purohit, S.S., Sharma A.K., Kumar, T. A Handbook of Medicinal Plants, A Complete Source Book. India: Agrobios; 2003.

Park, M.H., Hwang, S.J., Ahn, C.Y., Kim, B.H., Oh, H.M., 2006. Screening of seventeen oak extracts for the growth inhibition of the cyanobacterium *Microcystis aeruginosa* Kütz em. Elenkin. Bulletin of Environmental Contamination and Toxicology 1, 9–14.

Patel, V., Suthar, M., Pande, V., Arote, S., 2018. *In-vitro* screening of *Withania somnifera* leaf and root extracts for antifungal activities using NCCLS assay. Inventi Rapid: Pharmaceutical Biotechnology and Microbiology 1, 1–4.

Pegel, K.A., Rogers, C.B., 1985. The characterization of mollic acid-3β-D-xyloside and its genuine aglycon mollic acid, two novel 1α-hydroxycycloartenoids from *Combretum molle*. Journal of the Chemical Society of Perkin Transactions 1, 1711–1715.

Peralbo-Molina, A., Priego-Capote, F., de Castro, M. D. L. 2012. Tentative identification of phenolic compounds in olive pomace extracts using liquid chromatography-tandem mass

spectrometry with a quadrupole-quadrupole-time-of-flight mass detector. Journal of Agricultural and Food Chemistry 60, 11542–11550.

Pereira, A.P., Ferreira, I.C.F.R., Marcelino, F., Valentão, P., Andrade, P.B., Seabra, R., Estevinho, L., Bento, A., Pereira, J.A., 2007. Phenolic compounds and antimicrobial activity of olive (*Olea europaea* L. *Cv*. Cobranc¸osa) leaves. Molecules 12, 1153–1162.

Popiel, D., Dawidziuk, A., Koczyk, G., Mackowiak, A., Marcinkowska, K., 2017. Multiple facets of response to fungicides – the influence of azole treatment on expression of key mycotoxin biosynthetic genes and candidate resistance factors in the control of resistant *Fusarium* strains. European Journal of Plant Pathology 147, 773–785.

Portillo, A., Vila, R., Freixa, B., Adzet, T., Canigueral, S., 2001. Antifungal activity of Paraguayan plants used in traditional medicine. Journal of Ethnopharmacology 76, 93–98.

Ramaiah, K.A., Garampalli, R.K.H., 2015. *In vitro* antifungal activity of some plant extracts against *Fusarium oxysporum f. sp. lycopersici*. Asian Journal of Plant Science and Research 5, 22–27.

Ribeiro, A., Romeiras, M.M., Tavares, J., Faria, M.T., 2010. Ethnobotanical survey in Canhane village, district of Massingir, Mozambique: medicinal plants and traditional knowledge. Journal of Ethnobiology and Ethnomedicine 6, 33.

Roe, K.E. Dispersal and speciation in *Solanum* section Brevantherum. In: Hawkes, J.G., Lester, R.N., Skelding, A.D., Editors. The biology and taxonomy of the Solanaceae. London: Academic Press; 1979. p. 563–567.

Rogers, C.B., 1998. Cycloartenoid dienone acids and lactones from *Combretum erythrophyllum*. Phytochemistry 49, 2069–2076.

Russell, P. E., 2005. A century of fungicide evolution. Journal of Agricultural Science 143, 11–25.

Rwangabo, P.C. 1988. Umuhengerin. a new antimicrobially active flavonoid from *Lantana trifolia*. Journal of Natural Products 51, 966-968.

Sailaja, I., 2014. Antifungal activity of some wild plant extracts against fungal pathogens. International Journal Integrative Medical. Sciences 1, 41–44.

Salami, A., Kamara, A.B., Brixiova, Z. Smallholder agriculture in East Africa: trends, constraints and opportunities. Working Papers Series No. 105. Tunisia: African Development Bank Group; 2010.

Samie, A., Tambani, T., Harshfield, E., Green, E., Ramalivhana, J.N., Bessong, P.O., 2010. Antifungal activities of selected Venda medicinal plants against *Candida albicans*, *Candida krusei* and *Cryptococcus neoformans* isolated from South African AIDS patients, African Journal of Biotechnology 9, 2965–2976.

Samuelsson, G. Drugs of Natural Origin: A Textbook of Pharmacognosy. 5th Edition. Stockholm**,** Sweden: Swedish Pharmaceutical Press; 2004.

Sankaran, K.V., Suresh, T.A. Invasive alien plants in the forests of Asia and the Pacific. Bangkok, Thailand: Food and Agriculture Organization of the United Nations Regional Office for the Asia and the Pacific; 2013.

Sathish, R., Vyawahare, B., Natarajan, K., 2011. Antiulcerogenic activity of *Lantana camara* leaves on gastric and duodenal ulcers in experimental rats. Journal of Ethonopharmacology 134, 195–197.

Satish, T., Ansari, Z., 2013. Ethno Botanical Medicinal Importance of *Olea europea* (zaytun) - A Review, International Journal of Pure and Applied Bioscience 1, 15–18.

Sen, A., Batra, A., 2012. Evaluation of antimicrobial activity of different solvent extracts of medicinal plant: *Melia azedarach* L., International Journal of Current Pharmaceutical Research 4.

Shabana, M.M., Sayed, A.M.El, Yousif, M.F., Sayed, A.M.El, Sleem, A.A., 2011. Bioactive constituents from *Harpephyllum caffrum* Bernh. and *Rhus coriaria* L, Pharmacognosy Magazine 7, 298–306.

Singariya, P., Mourya, K.K., Kumar, P., 2012. Antimicrobial activity of the crude extracts of *Withania somnifera* and *Cenchrus setigerus In-vitro.* Pharmacognosy Journal 4, 27.

Singh, G., Sharma, P.K., Dudhe, R., Singh, S. 2010. Biological activities of Withania somnifera. Annals of Biological Research 1, 56-63.

Smith, I.M. Editor. Fungicides for Crop Protection: 100 Years of Progress. Portland: International Specialized Book Services.1986.

Spencer, D. The future of agriculture in sub-Saharan Africa and South Asia. In: Sustainable Food Security for All by 2020. Proceedings of an International Conference, September 4-6, 2001, Bonn, Germany: International Food Policy Research Institute, Washington; 2002.

Subaraju, G.V., Vanisree, M., Rao, C.V., Sivaramakrishna, C., Sridhar, P., Jayaprakasam, B., Sudjana, A.N., D'Orazio, C., Ryan, C., Rasool, N., Ag, J., Islam, N., Riley, T.V., Hammer, K.A., 2009. Antimicrobial activity of commercial *Olea europaea* (olive) leaf extract. International Journal of Antimicrobial Agents 33, 461–463.

Sudjana, A.N., D'Orazio, C., Ryan, V., Rasool, N., Ng, J., Islam, N., Riley, T.V., Hammer, K.A. 2009. Antimicrobial activity of commercial *Olea europaea* (olive) leaf extract. International Journal of Antimicrobial Agents, 33, 461–463.

Summerell, B.A., Laurence, M.H., Liew, E.C.Y., Leslie, J.F., 2010. Biogeography and phylogeography of *Fusarium*: a review. Fungal Diversity 44, 3–13.

Sumner, D. A. 1990. Imperfect information and intervention in farm credit: discussion. American Journal of Agricultural Economics 72, 780–781.

Thamotharan, G., Sekar, G., Ganesh, T., Chakraborty, S.R., Senthil.kumar, N., 2010. Antiulcerogenic effect of *Lantana camara* leaves on *in vivo* test models in Rats. Asian Journal of Pharmaceutical and Clinical Research 3, 57–60.

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, 1479–1486.

Uche-Okereafor, N., Sebola, T., Tapfuma, K., Mekuto, L., Green, E., Mavumengwana, V., 2019. Antibacterial activities of crude secondary metabolite extracts from *Pantoea* Species obtained from the stem of *Solanum mauritianum* and their effects on two cancer cell lines. International Journal of Environmental Research and Public Health 16, 602.

Umaerus, V.R., Scholte, K., Turkensteen, L.J. Crop rotation and the occurrence of fungal diseases in potatoes. In: Vos, J., Loon, C.D., van Bollen, G.J. Editors. Effects of Crop Rotation on Potato Production in the Temperate Zones. Dordrecht, Kluwer Academic Publishers, Springer; 1989. p. 171–189.

Vanhessche, B.A., Vandermillion, A.M., Scott, D.E., Scholes, C.M., Chapman, J.M. Distribution, quantification and identification of tannins in acorns from blackjack, sawtooth and Texas live oak trees. In: Proceedings of the Midwest Regional Meeting, November 7–10, Kansas City, Mo, U.S.A. 2007.

Varaprasad, B., Prasanth, K.K., Chandrasekhar, K.N., Somasekhar, P., 2009. Antifungal activity of selected plant extracts against phytopathogenic fungi *Aspergillus niger* F2723.,Indian Journal of Science and Technology 2, 4.

Vieira, R.F. Quantitative evaluation of Solasodina in fruits of *Solanum mauritianum* Scopoli on two soils in the state of Parana, Brazil. Curitiba, Brasil: Federal University of Parana; 1989.

Voss, K.A., Plattner, R.D., Riley, R.T., Meredith, F.I., Norred, W.P., 1998. *In vivo* effects of fumonisin B1-producing and fumonisin B1 non producing isolates are similar: Fumonisins B2 and B3 cause hepato- and nephrotoxicity in rats. Mycopathologia 141, 45–58.

Wickens, G. E. Flora of Tropical East Africa. Combretaceae. East African community. Tanzania: Royal Botanic Gardens, Kew; 1973.

Wilson, C.L., Solar, J.M., Ghaouth, A. 1997. Rapid evaluation of plant extracts and essential oils for antifungal activity against *Botrytis cinerea*. Plant disease 81, 204–210.

Wisniewski, M., Droby, S., Norelli, J., Liu, J., Schena, L., 2016. Alternative management technologies for postharvest disease control: The journey from simplicity to complexity. Postharvest Biology and Technology 122.

Wu, Q., Li, Y., Wang, C., Liu, Z., Zang, X., Zhou, X., Wang, Z., 2009. Dispersive liquid-liquid micro extraction combined with High Performance Liquid Chromatography Fluorescence detection for the determination of carbendazim and thiabendazole in environmental samples. Analytica Chimica Acta 638, 139-145.

Xu, X., Nicholson, P. 2009. Community ecology of fungal pathogens causing wheat head blight. Annual Review of Phytopathology 47, 83–103.

Yan, K., Dickman, M.B., 1996. Isolation of a β-tubulin gene from *Fusarium moniliforme* that confers cold-sensitive benomyl resistance. Applied and Environmental Microbiology 62, 3053–3056.

Yang, C., Hamel, C., Vujanovic, V., Gan, Y., 2011. Fungicide: Modes of action and possible impact on non-target microorganisms. International Scholarly Research Network.

Zhang, M., Jeyakumar, J.M.J. *Fusarium* species complex causing pokkah boeng in China. In: Askun, Tulin . Editor. *Fusarium* - Plant Diseases, Pathogen Diversity, Genetic Diversity, Resistance and Molecular Markers. Intech Open; 2018.

CHAPTER 3

IN VITRO ANTIFUNGAL ACTIVITY OF INDIVIDUAL MEDICINAL PLANT EXTRACTS

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Antifungal activity of medicinal plant extracts for potential management of *Fusarium* pathogens

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ABSTRACT

Yield losses due to fungal attacks, post-harvest losses and food spoilage, amongst others present a challenge to food security. There is an ongoing need and search for accessible, affordable and environmentally-friendly alternatives to the use of synthetic pesticides in food production. The aim of this study was to search for affordable potent plant extracts that can be used in smallholder farming system to manage *Fusarium* related diseases. Extracts from 11 medicinal plant species previously screened against human and animal pathogens in the literature were selected and investigated for their *in vitro* antifungal activity against five economically important phytopathogenic *Fusarium* species. Dried leaf powders were extracted with solvents of different polarity and evaluated for antifungal activity using a microdilution method. At least, one of the solvent extracts obtained from a minimum of three plant species demonstrated very strong activity with minimum inhibitory concentration (MIC) less than 0.1 mg/ml against *F. equisite*, *F. oxysporum*, *F. semitectum*, *F. chlamydosporum* and *F. subglutinans*. Acetone and ethyl acetate solvent extracts were found in most cases to exhibit stronger antifungal activity compared to water and petroleum ether extracts. However, water extract of *Combretum molle* was particularly noteworthy as it demonstrated antifungal activity against the tested five *Fusarium* species. The use of medicinal plant extracts as an antifungal agent presented a cheap, accessible and sustainable source of eco-friendly pesticides useful for crop protection in organic cultivation and small-holder farming.

Key words : *Fusarium*, organic farming, plant diseases, plant extracts, synthetic fungicides

INTRODUCTION

Smallholder farming remains a source of food and income generation for many households in rural communities. Maize, cowpea, sweet potatoes, soybeans and tomatoes are among major crops cultivated by smallholder farmers. These crops provide carbohydrates, proteins and vitamins required for human well-being. A successful production of these crops can be limited by a number of plant diseases such as vascular wilting, head blight, damping-off, ear and root rots caused by *Fusarium* pathogens. Plant disease is problematic in farming as it negatively impacts on and results in poor crop quality or commodities. It may result in massive yield losses both in the field and during storage (Oren *et al.*, 2003).

Fusarium species are soil-borne fungal pathogens; however, when conditions are favourable they can cause spoilage of crops, kernels, grains and fruits during post-harvest processes and storage (Smith, 1986). Some *Fusarium* species are capable of producing mycotoxins and allergens which contaminate food and invariably pose health risks to consumers of contaminated products (Gelderblom *et al.*, 1988; Marasas, 1995; Shephard *et al.*, 1996; Fandohan *et al.*, 2005; Omidpanah *et al.*, 2015). Synthetic fungicides have been beneficial to the agricultural sector for many decades and their importance in the reduction of plant diseases cannot be overlooked. Although they remain an important part of efficient plant disease management practice, synthetic fungicides are not easily accessible to, or affordable for smallholder or

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ABSTRACT

Yield losses due to fungal attacks, post-harvest losses and food spoilage, amongst others present a challenge to food security. There is an ongoing need and search for accessible, affordable and environmentally-friendly alternatives to the use of synthetic pesticides in food production. The aim of this chapter or section was to search for affordable potent plant extracts that can be used in smallholder farming system to manage *Fusarium* related diseases. Extracts from thirteen (13) medicinal plant species previously screened against human and animal pathogens in the literature were selected and investigated for their *in vitro* antifungal activity against nine (9) economically important phytopathogenic *Fusarium* species. Dried leaf powders were extracted with solvents of different polarity (water, petroleum ether, ethyl acetate and acetone) and evaluated for antifungal activity using a micro dilution method.

At least, one of the solvent extracts obtained from a minimum of three plant species demonstrated very strong activity with minimum inhibitory concentration (MIC) less than 0.1 mg/ml against *F. chlamydosporum, F. equiseti, F. subglutinans, F. verticilloides, F. oxysporum, F. proliferatum, F. semitectum, F. solani* and *F. gramineraum.* Acetone and ethyl acetate solvent extracts were found in most cases to exhibit stronger antifungal activity compared to water and petroleum ether extracts. However, water extract of *Combretum molle* and *Olea europaea* were particularly noteworthy as they demonstrated antifungal activity against eight (8) of the tested *Fusarium* species. The use of medicinal plant extracts as an antifungal agent presented a cheap, accessible and sustainable source of eco-friendly pesticides useful for crop protection in organic cultivation and smallholder farming.

Keywords: *Fusarium*, organic farming, plant diseases, plant extracts, synthetic fungicides

3.1. INTRODUCTION

Smallholder farming remains a source of food and income generation for many households in rural communities. Maize, cowpea, sweet potatoes, soybeans and tomatoes are among major crops cultivated by smallholder farmers. These crops provide carbohydrates, proteins and vitamins required for human well-being. A successful production of these crops can be limited by a number of plant diseases such as vascular wilting, head blight, damping-off, ear and root rots caused by *Fusarium* pathogens. Plant disease is problematic in farming as it negatively impacts on and results in poor crop quality or commodities. It may result in massive yield losses both in the field and during storage (Oren *et al*., 2003).

Fusarium species are soil-borne fungal pathogens; however, when conditions are favourable they can cause spoilage of crops, kernels, grains and fruits during post-harvest processes and storage (Smith, 1986). Some *Fusarium* species are capable of producing mycotoxins and allergens which contaminate food and invariably pose health risks to consumers of contaminated products (Gelderblom *et al*., 1988; Marasas, 1995; Shephard *et al*., 1996; Fandohan *et al*., 2005; Omidpanah *et al*., 2015).

Synthetic fungicides have been beneficial to the agricultural sector for many decades and their importance in the reduction of plant diseases cannot be overlooked. Although they remain an important part of efficient plant disease management practice, synthetic fungicides are not easily accessible to, or affordable for smallholder or subsistence farmers. This kind of farming in particular is more often practised in poor rural communities (Stephens *et al*., 1989; Thembo *et al*., 2010).

The use of synthetic fungicides in agricultural production is being increasingly discouraged due to their harmful effects on non-target organisms in conjunction with the contamination of aquatic systems and the development of pathogen-resistant strains (Lalitha *et al*., 2010; Thippeswamy *et al*., 2011; Adepoju *et al*., 2014; Silva *et al*., 2014; Dube and Maleka, 2017).

The problems associated with food security and the applications of synthetic fungicides necessitate the need to search for affordable and environmental friendly fungicides. Therefore, this chapter was aimed at investigating the *in vitro* antifungal activity of individual extracts from thirteen selected medicinal plants against nine *Fusarium* pathogens. The medicinal plant species used in this study were selected from available literature based on their documented strong antimicrobial activity when tested against human and/or animal fungal pathogens, as discussed in Chapter 2.

3.2. MATERIALS AND METHODS

3.2.1. Selection and collection of medicinal plant species

Thirteen medicinal plant species distributed among 8 different families were selected to be used in this study. The plant species were selected from the literature, because of their good antimicrobial activity against either human or animal pathogens. These plant species are available in South Africa and there is a paucity of information available on their antifungal activity against *Fusarium* pathogens causing variety of crop diseases in agricultural sector. The following thirteen plant species were selected: *Combretum erythrophyllum, Melia azedarach, Solanum mauritianum, Vanguria infausta, Nicotiana glauca, Schotia brachypetala, Lantana camara, Withania somnifera, Harpephyllum caffrum, Combretum molle, Quercus acutissima, Olea europaea* and *Senna didymobotrya*.

The leaves of *Combretum erythrophyllum, Melia azedarach, Solanum mauritianum, Nicotiana glauca, Schotia brachypetala, Lantana camara, Combretum molle, Quercus acutissima* and *Olea europaea* were collected at the Agricultural Research Council Campus, Roodeplaat located at Pretoria, South Africa. Other medicinal plant species including *Vanguria infausta, Withania somnifera, Harpephyllum caffrum* and *Senna didymobotrya* were collected from Capricorn district municipality at Limpopo province, South Africa. The leaves of these plants

species were collected from naturally growing trees during October/Novenber 2016. The species were identified and authenticated by Dr Bronwyn Egan and voucher specimens as detailed in Table 3.1 were deposited at the University of Limpopo herbarium.

Table 3.1. Thirteen medicinal plant species selected for the investigation of antifungal activity against nine phytopathogenic *Fusarium* fungi.

Plant species	Voucher specimen
Combretum erythrophyllum (Burch.) Sond.	UNIN 121005
Combretum molle R.Br. ex G.Don	UNIN 121013
Harpephyllum caffrum Bernh.	UNIN 121002
Lantana camara L.	UNIN 121003
Melia azedarach L.	UNIN 121007
Nicotiana glauca Graham	UNIN 121009
Olea europaea L.	UNIN 121014
Quercus acutissima Carruth.	UNIN 121015
Schotia brachypetala Sond.	UNIN 121008
Senna didymobotrya (Frozen.) H.S.Irwin & Barneby	UNIN 121004
Solanum mauritianum Scop.	UNIN 121006
Vangueria infausta Burch.	UNIN 121016
Withania somnifera (L.) Dunal	UNIN 121010

3.2.2. Drying, grinding of plant materials and preparation of the extracts

Fresh and clean leaves of the thirteen selected medicinal plant species were collected into brown paper bags and shade dried at room temperature (25 ± 2.0 °C). The dried materials were ground into fine powder using milling machine (Fritsch Pulverisette 14, Labotec, SA). The powdered materials were stored in dark in brown bags until extraction. During extraction process, 100 g of powder plant material was added to an Erlenmeyer flask, followed by 1000

ml of the solvent. The plant materials were extracted non-sequentially with solvents of different polarities (water, petroleum ether, ethyl acetate and acetone).

The mixture was shaken or sonicated on a shaking machine (Shaker LS500, Gerhardt, Analytical Systems, Germany) for an hour and afterwards the extraction solvent was decanted into another flask. The remaining residue was re-extracted with 1000 ml solvent by shaking on a machine for an hour. The extraction solvent was decanted and combined with the one already in the flask. It was then filtered through Whatman No. 1 filter paper. After filtration, plant material rich organic solvent was concentrated under reduced pressure using a rotary evaporator (Stuart, RE300DB, Lasec, SA) at a temperature below 45 °C. The extract was further dried completely under the stream of flowing air in the fume wood. However, water extracts were freeze-dried or lyophilized (Sentry 2.0 VirTis SP, United Scientific). All the extracts were quantified and stored in airtight containers until required for antifungal evaluations assays.

3.2.3 Sub-culturing and preparation of *Fusarium* fungal pathogens

The following nine *Fusarium* fungal species: *Fusarium verticillioides* (PPRI 9278), *F. proliferatum* (PPRI 18679), *F. subglutinans* (PPRI 6740), *F. graminearum* (PPRI 10728), *F. solani* (PPRI 19147), *F. oxysporum* (PPRI 10175), *F. semitectum* (PPRI 6739), *F. chlamydosporum* (PPRI 5116) and *F. equiseti* (PPRI 19029) were obtained from the Agricultural Reserach Council-Plant Health and Protection, at Roodeplaat, Pretoria. The pathogens were sub-cultured by innoculation in sterillzed water in small vials, which were correctly labelled and stored at -4 °C. The other set was innoculated in 10% aqueous sterilized glycerol and stored at -80 °C. The remaining portion of pathogen was left on potato dextrose agar and stored in the fridge at -4 °C. The pathogens were maintained at these conditions until required during antifungal activity evaluation assays.

During antifungal activity evaluation assays, the fungal strains maintained in water were allowed to thaw and sub-cultured on sterillized potato dextrose agar (PDA) in a petri dish. It was incubated at 27 ℃ and allowed to grow for at least four to seven days. The fungal suspension was scrapped-off (1% inoculum), sub-cultured into sterillized potato dextrose broth (PDB) and incubated for three to four days. Thereafter, fungal spores were collected by straining cultured broth through double layer cheesecloth and the number of spores were determined using microscope and haemocytometer (Aberkane *et al*., 2002). Appropriate dilutions were made and the final spore concentration was adjusted to approximately 1.0 x 10^6 spores/ml to be used in the antifungal assays (Mahlo *et al*., 2010).

3.2.4. Micro-plate dilution assay

A modified serial dilution method Masoko *et al*., (2005), was used to evaluate the antifungal activity of thirteen medicinal plant extracts against nine *Fusarium* pathogens. In brief, 100 µl of sterilized potato dextrose broth was dispensed into all wells of 96 well micro-plate. Petroleum ether, ethyl acetate and acetone plant extracts were re-dissolved in 100% acetone while water extracts were re-dissolved in 50% aqueous acetone to make a final concentration of 10 mg/ml. One hundred micro-liters (100 µl) of plant extract at this specified concentration was added to the first well and was two-fold serially diluted to the last well of the plate. Amphotericin B (Phytotek Lab, South Africa) was used as positive control and acetone at various concentration levels was included as negative control. One hundred micro-liters (100 µl) of fungal broth standardized or adjusted to 1.0 x 10^6 spores/ml was added to all experimental or treatment wells. The plate was covered and incubated at 27 °C for three to four days. After incubation, 50 µl of 0.5 mg/ml of p-iodonitro-tetrazolium violet (INT) was added to all wells and the plate was incubated further for overnight. The experiment was repeated twice and each time it was conducted in triplicate.

3.2.5. Determination of minimum inhibitory concentration (MIC) and minimum inhibitory dilution (MID)

Incubation of fungal pathogen in the presence of growth indicator (INT) results in colour change from colourless to pink-red, which indicates that the pathogen is actively growing. However, if the growth indicator remains colourless it indicates that there is no growth and the plant extract or positive control has successfully inhibit or slow down the growth of the pathogen for a specific period of time. The minimum inhibitory concentration value was determined and recorded as the lowest plant extract concentration that inhibited the growth of fungal pathogen. The results were recorded as the mean from triplicates of independent experiments and were expressed in milligram/milliliters (mg/ml).

Minimum inhibitory dilution was calculated by dividing the quantity (milligram) of crude obtained from extraction of 1 gram of fine powdered leave material by the MIC value recorded for the pathogen (Eloff, 2004). MID is also referred to as total activity; it is used to determine or indicate the volume an extract obtained from 1 gram of plant material can be diluted and still inhibit the growth of the pathogen (Eloff, 1999; Amoo *et al*., 2012).

3.3. RESULTS AND DISCUSIONS

Subsistence or smallholder farming has been a source of food and income generation for households especially in many rural communities. However, yield losses due to *Fusarium* pathogens, amongst others, have been a problem through the years. Application of synthetic fungicides, which is generally considered as not eco-friendly, as control measures in this kind of farming system proved to be challenging as most households could not afford the chemicals. The increasing global preference and demand for organic production in organic farming system

required crop protection measures employing organic practices. In this chapter, medicinal plant extracts were evaluated *in vitro* for possible control of *Fusarium* pathogens such as *F. chlamydosporum, F. equisite, F. oxysporum, F. semitectum, F. graminearum, F. solani, F. proliferatum, F. semitectum* and *F. subglutinans*. A total of 52 extracts from 13 medicinal plant species were investigated for antifungal activity.

As shown in Figure 2.1, extraction with water as a solvent gave more yield compared to the use of other solvents. The more polar the solvent used for extraction, the higher the yield recorded. For example, the yield of water extract obtained from *Nicotiana glauca* was 9.97-fold of what was recorded with its petroleum ether extract. Similar results were observed with water extract from *Senna didymobotrya* and was 11.5-fold compared to its petroleum ether extract (Figure 3.1). Of the four solvents used, extraction with water gave the highest yield of crude extract. It was demonstrated in several studies that extraction of plant material with polar solvents resulted in greater yield compared to non-polar solvents (Eloff, 1998; Lekganyane *et al*., 2012; Masoko and Makgapeetja, 2015).

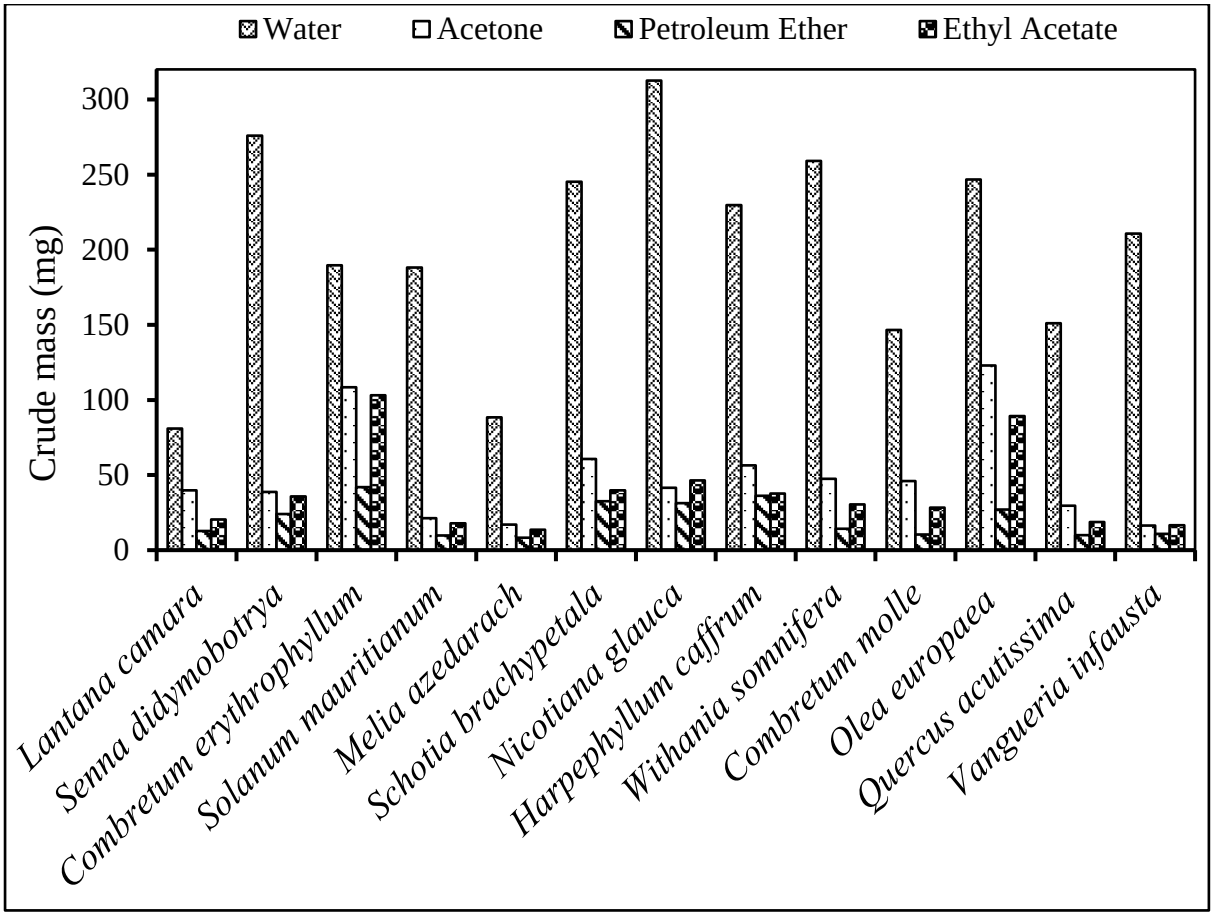

Figure 3.1. The amount (mg) of crude obtained from extraction of 1 gram of fine powdered leaf material using solvents of different polarities.

Table 3.2 shows the antifungal activity based on minimum inhibitory concentrations of different medicinal plant species extracts evaluated against nine *Fusarium* species. At least, one of the solvent extracts obtained from eight, nine, eleven, twelve and thirteen plant species demonstrated very strong activity with minimum inhibitory concentration (MIC) less than 0.1 mg/ml against *F. chlamydosporum*, *F. subglutinans*, *F. verticilloides* and *F. graminearum*, *F. solani* and *F. proliferatum*, respectively. Of the 52 plant extracts tested against the nine pathogens; 60, 55, 35 and 14 extracts obtained from acetone, ethyl acetate, petroleum ether and water, respectively, showed very strong activity with MIC < 0.1 mg/ml. In general, acetone and ethyl acetate solvent extracts were found in most cases to exhibit stronger antifungal

activity compared to water and petroleum ether extracts. Although crude extract obtained through water extraction was the highest in all the investigated species, its antifungal activity on average was not as strong as that of the other solvents used. This observation correlated with the findings from several authors who reported that aqueous extract generally exhibited little or no antimicrobial activity compared to non-polar extract (Parekh and Chanda, 2007; Van Vuuren and Naidoo, 2010; Kitonde *et al*., 2014). This might be due to lower solubility of antifungal compounds in polar solvent as compared to non-polar solvents in such medicinal plant species (Bhattacharjee *et al*., 2011).

Nevertheless, *Combretum molle* and *Olea europaea* water extracts showed very strong activity against *F. chlamydosporum* and *F. proliferatum*. Water extract of *Combretum molle* was particularly noteworthy as it demonstrated clear antifungal activity (MIC $\leq$ 2.5 mg/ml) against eight tested *Fusarium* species. Water is readily available; therefore, smallholder farmers can prepare crude plant extracts themselves and apply on their fields, gardens or plots to manage *Fusarium* diseases. The use of water extract is also applicable for organic farming. Furthermore, all the extracts were prepared from the leaves of these species. The use of leaves is sustainable from a conservation point of view as leaves are a renewable part that can be safely harvested without threatening plant growth and survival.

Table 3.2. Minimum inhibitory concentration (MIC in mg/ml) values of thirteen different medicinal plant species against nine pathogenic *Fusarium* species.

Plant species	Extracts	*Fusarium* fungal pathogens								
		F. chlamydosporum	*F. equiseti*	*F. subglutinans*	*F. verticilloides*	*F. oxysporum*	*F. proliferetum*	*F. semitectum*	*F. solani*	*F. graminearum*
Combretum erythrophyllum	WA	0.63	0.63	1.25	> 2.5	> 2.5	0.31	1.25	0.16	2.5
	PE	0.63	0.31	**0.04**	**0.04**	**0.04**	**0.04**	0.63	0.16	0.16
	EA	**0.04**	0.16	**0.04**	**0.04**	0.16	**0.04**	0.31	**0.08**	0.16
	AC	**0.04**	**0.08**	**0.08**	**0.04**	**0.04**	**0.04**	0.31	**0.04**	**0.08**
Combretum molle	WA	**0.04**	0.63	1.25	> 2.5	1.25	**0.04**	2.5	**0.04**	1.25
	PE	0.63	0.31	0.63	0.16	0.31	**0.04**	0.63	0.16	0.31
	EA	**0.04**	0.16	0.16	0.63	0.16	**0.04**	**0.04**	**0.04**	0.63
	AC	**0.04**	0.31	0.63	1.25	0.16	**0.04**	**0.08**	**0.04**	0.63
Harpephyllum caffrum	WA	> 2.5	0.31	1.25	2.5	> 2.5	> 2.5	> 2.5	0.31	0.63
	PE	0.16	0.31	0.31	0.31	0.31	**0.04**	> 2.5	0.16	**0.08**
	EA	1.25	0.16	**0.08**	**0.08**	0.16	**0.04**	> 2.5	**0.08**	0.16
	AC	0.16	0.16	0.31	**0.08**	0.31	**0.04**	1.25	**0.04**	**0.08**
Lantana camara	WA	> 2.5	0.63	0.16	**0.04**	2.5	> 2.5	> 2.5	1.25	0.63
	PE	0.16	0.63	**0.04**	0.16	1.25	**0.04**	1.25	0.31	**0.08**
	EA	2.5	0.31	**0.04**	0.16	0.31	**0.04**	**0.08**	0.16	**0.08**
	AC	2.5	0.16	**0.04**	0.16	0.63	**0.04**	**0.04**	**0.04**	0.63
Melia azedarach	WA	> 2.5	0.63	**0.08**	1.25	2.5	> 2.5	> 2.5	> 2.5	0.63
	PE	0.31	0.31	0.16	0.31	0.16	**0.04**	2.5	**0.08**	**0.08**
	EA	0.63	0.16	0.16	0.16	**0.08**	**0.04**	0.31	**0.08**	**0.08**

Plant species	Extracts	*Fusarium* fungal pathogens								
		F. chlamydosporum	*F. equiseti*	*F. subglutinans*	*F. verticilloides*	*F. oxysporum*	*F. proliferetum*	*F. semitectum*	*F. solani*	*F. graminearum*
	AC	**0.04**	0.16	**0.08**	**0.08**	0.16	**0.08**	0.63	**0.04**	0.16
Nicotiana glauca	WA	> 2.5	0.31	**0.08**	0.16	2.5	> 2.5	> 2.5	> 2.5	0.63
	PE	**0.04**	0.63	**0.04**	0.31	0.31	**0.04**	1.25	> 2.5	0.16
	EA	**0.08**	0.31	**0.04**	**0.04**	0.16	**0.04**	0.63	0.16	0.16
	AC	**0.08**	0.16	**0.04**	0.16	0.16	**0.04**	**0.08**	**0.08**	0.16
Olea europaea	WA	**0.04**	0.63	0.31	**0.08**	1.25	**0.04**	> 2.5	2.5	2.5
	PE	**0.04**	1.25	0.31	0.63	0.63	**0.04**	2.5	**0.08**	**0.04**
	EA	1.25	0.31	0.31	0.16	0.31	**0.04**	1.25	**0.04**	**0.02**
	AC	> 2.5	0.31	0.31	0.16	0.31	**0.04**	1.25	**0.04**	**0.02**
Quercus acutissima	WA	0.16	1.25	1.25	> 2.5	> 2.5	1.25	> 2.5	1.25	0.31
	PE	**0.04**	0.31	0.16	0.04	0.16	0.16	0.63	**0.08**	**0.04**
	EA	0.16	0.16	**0.08**	**0.08**	**0.08**	**0.04**	0.31	**0.04**	**0.02**
	AC	**0.04**	**0.08**	0.63	**0.08**	0.16	**0.04**	0.31	**0.04**	**0.02**
Schotia brachypetala	WA	> 2.5	1.25	1.25	2.5	> 2.5	> 2.5	> 2.5	> 2.5	**0.04**
	PE	**0.04**	0.31	0.31	0.63	0.31	**0.04**	2.5	>2.5	0.16
	EA	**0.04**	0.16	0.31	0.31	0.16	**0.04**	1.25	1.25	0.16
	AC	**0.04**	0.31	0.31	0.16	0.16	**0.04**	0.31	0.63	0.16
Senna didymobotrya	WA	> 2.5	0.16	**0.04**	0.31	1.25	1.25	> 2.5	> 2.5	0.63
	PE	0.63	0.16	**0.08**	0.63	0.31	**0.04**	2.5	0.31	**0.08**
	EA	1.25	0.31	**0.04**	0.16	0.16	**0.04**	1.25	**0.08**	0.16
	AC	> 2.5	0.31	**0.08**	**0.08**	0.16	**0.04**	1.25	**0.08**	0.63
Solanum mauritianum	WA	> 2.5	0.16	**0.08**	1.25	2.5	> 2.5	> 2.5	> 2.5	0.63

Plant species	Extracts	*Fusarium* fungal pathogens								
		F. chlamydosporum	*F. equiseti*	*F. subglutinans*	*F. verticilloides*	*F. oxysporum*	*F. proliferetum*	*F. semitectum*	*F. solani*	*F. graminearum*
	PE	0.31	0.31	0.16	0.31	0.31	**0.04**	0.63	**0.04**	**0.08**
	EA	0.31	**0.08**	**0.04**	**0.04**	**0.08**	**0.04**	1.25	**0.04**	0.16
	AC	0.31	0.31	**0.04**	0.16	**0.04**	**0.04**	1.25	**0.04**	**0.04**
Vanguria infausta	WA	**0.04**	1.25	0.16	2.5	0.63	> 2.5	> 2.5	0.63	0.31
	PE	0.63	0.63	0.31	**0.04**	0.31	**0.04**	0.63	0.16	**0.08**
	EA	0.31	0.31	0.31	**0.08**	0.16	**0.04**	**0.08**	**0.04**	0.31
	AC	**0.08**	0.16	0.31	**0.04**	0.16	**0.04**	0.16	**0.04**	0.16
Withania somnifera	WA	> 2.5	1.25	0.16	0.63	1.25	**0.04**	> 2.5	0.63	2.5
	PE	0.63	0.63	**0.08**	0.16	0.16	**0.04**	0.63	0.63	0.16
	EA	1.25	0.16	0.63	**0.08**	**0.08**	**0.04**	**0.04**	**0.08**	1.25
	AC	0.63	0.31	0.31	**0.08**	**0.08**	**0.04**	**0.08**	**0.04**	1.25
Amphotericin B (µg/ml)		23.438	187.50	93.75	2.93	11.72	0.37	23.44	0.37	187.50

Highlighted values indicate strong antifungal activity with MIC value less than 0.1 mg/ml. WA: Water, PE: Petroleum ether, EA: Ethyl acetate and AC: Acetone.

Of the thirteen medicinal plants investigated, *Combretum erythrophyllum* acetone extract was particularly the most active as it demonstrated very strong activity (MIC < 0.1 mg/ml) against eight pathogens (*F. equisite, F. oxysporum, F. chlamydosporum, F. verticilloides, F. solani, F. subglutinans, F. proliferatum, F. graminearum*) and a strong activity (MIC < 1 mg/ml) against *F. semitectum.* The antimicrobial activity of isolated compounds such as apigenin, genkwanin and 5-hydroxy-7, 4′-dimethoxyflavone from *C. erythrophyllum* was reported (Alexandra *et al.,* 1992; Martini and Eloff, 1998; Martini *et al.,* 2004). Strong antifungal activity of *C. erythrophyllum* extracts reported in the current study may be due to the presence of these compounds and other flavonoids. Compared to all the medicinal plants evaluated, *Schotia brachypetala* appear to be less active with petroleum ether, ethyl acetate and acetone extracts demonstrating very strong activity against *F. chlamydosporum* and *F. proliferatum.* Water extract from this plant extract was very active against *F. graminearum.*

Of all the pathogens used in this study, *F. proliferatum* was the most susceptible pathogen with 41 extracts exhibiting very strong inhibitory activity against it. On the other hand, and in comparison to other pathogens, only three extracts (acetone extracts from *C. erythrophyllum, Quercus acutissima* and ethyl acetate extract from *Solanum mauritianum*) demonstrated strong inhibitory activity against *F. equisite.* It was of particular interest that the inhibitory activity demonstrated by the aforementioned extracts was higher than that of the positive control used in this study against *F. equisite*. Minimum inhibitory dilution (referred to as 'total activity' by some authors) is also an important factor to be considered when evaluating the antifungal activity of medicinal plant extracts, especially if they are intended for practical application. Minimum inhibitory dilution indicates the volume (ml) of solvent which can be added to crude extract obtained from one gram of ground, dried plant material and still inhibits the growth of the pathogen (Eloff, 2004; Amoo *et al*., 2012).

Table 3.3 presents the minimum inhibitory dilution (MID) of the medicinal plant extracts evaluated against the nine pathogenic *Fusarium* species. In most cases, water and acetone extracts gave the highest MID, followed by ethyl acetate extract, while petroleum ether extract gave a low MID. Fifty-nine out of the 468 extracts evaluated had a MID > 1000 ml/g. These extracts are highlighted in bold font in Table 3.3. Of this number, 23 extracts with MID > 1000 ml/g are either water or acetone extracts. Both solvents, but especially water, are accessible and affordable to smallholder farmers to prepare crude extracts for application. The volume of the extract to be prepared will depend on the size of cultivated plot or field and fungal infestation level. In any case, the high MID indicates that small amount of the leaf material will be needed to achieve the desired effect.

Overall, *Senna didymobotrya* water extract gave the highest MID against *F. subglutinans* with a recorded value of 6896 ml/g dried powder plant material, followed by *Withania somnifera* and *Olea europaea* water extracts against *F. proliferatum* with recorded MID of 6476 and 6168 ml/g, respectively. The lowest MID was found in petroleum ether extract from *Melia azedarach* against *F. semitectum*. It is noteworthy that the acetone extract of *C. erythrophyllum* which gave a very strong activity (Table 3.2) against eight pathogens (*F. chlamydosporum, F. equiseti, F. subglutinans, F. verticilloides, F. oxysporum, F. proliferetum, F. solani* and *F. graminearum*) also gave a very high MID (> 1000 ml/g) against the same organisms. Extracts with potent activity (low MIC value) and high MID such as *C. erythrophyllum* acetone extract also had potential to be developed into commercially affordable plant based fungicides.

Table 3.3. Minimum inhibitory dilution (ml/g) of thirteen medicinal plant extracts screened against nine pathogenic *Fusarium* species.

Plant species	Extracts	*Fusarium* fungal pathogens								
		F. chlamydosporum	*F. equiseti*	*F. subglutinans*	*F. verticilloides*	*F. oxysporum*	*F. proliferetum*	*F. semitectum*	*F. solani*	*F. graminearum*
Combretum erythrophyllum	WA	301	301	152	na	na	612	152	**1185**	76
	PE	67	135	**1048**	**1048**	**1048**	**1048**	67	262	262
	EA	**2580**	645	**2580**	**2580**	645	**2580**	333	**1290**	645
	AC	**2710**	**1355**	**1355**	**2710**	**2710**	**2710**	350	**2710**	**1355**
Combretum molle	WA	**3663**	233	117	na	117	**3663**	59	**3663**	117
	PE	16	33	16	65	33	260	16	65	33
	EA	709	177	177	45	177	709	709	709	45
	AC	**1152**	149	73	37	288	**1152**	576	**1152**	73
Harpephyllum caffrum	WA	na	741	184	92	na	na	na	741	365
	PE	227	117	117	117	117	907	na	227	453
	EA	30	236	472	472	236	944	na	472	236
	AC	353	353	182	706	182	**1412**	45	**1412**	706
Lantana camara	WA	na	128	505	**2021**	32	na	na	65	128
	PE	80	20	320	80	10	320	10	41	160
	EA	8	66	510	128	66	510	255	128	255
	AC	16	249	996	249	63	996	996	996	63
Melia azedarach	WA	na	140	**1105**	71	35	na	na	na	140
	PE	27	27	52	27	52	208	3	104	104
	EA	21	84	84	84	169	338	44	169	169
	AC	423	106	212	212	106	212	27	423	106

Plant species	Extracts	*Fusarium* fungal pathogens								
		F. chlamydosporum	*F. equiseti*	*F. subglutinans*	*F. verticilloides*	*F. oxysporum*	*F. proliferetum*	*F. semitectum*	*F. solani*	*F. graminearum*
Nicotiana glauca	WA	na	**1008**	**3906**	**1953**	125	na	na	na	496
	PE	783	50	783	101	101	783	25	na	196
	EA	580	150	**1160**	**1160**	290	**1160**	74	290	290
	AC	519	259	**1037**	259	259	**1037**	519	519	259
Olea europaea	WA	**6168**	392	796	**3084**	197	**6168**	na	99	99
	PE	675	22	87	43	43	675	11	338	675
	EA	71	288	288	558	288	**2230**	71	**2230**	**4461**
	AC	na	397	397	769	397	**3075**	98	**3075**	**6150**
Quercus acutissima	WA	944	121	121	na	na	121	na	121	487
	PE	252	32	63	252	63	63	16	126	252
	EA	117	117	235	235	235	469	61	469	938
	AC	741	370	47	370	185	741	96	741	**1482**
Schotia brachypetala	WA	na	196	196	98	na	na	na	na	**6133**
	PE	812	105	105	52	105	812	13	na	203
	EA	995	249	128	128	249	995	32	32	249
	AC	**1515**	196	196	379	379	**1515**	196	96	379
Senna didymobotrya	WA	na	**1724**	**6896**	890	221	221	na	na	438
	PE	38	150	299	38	77	599	10	77	299
	EA	29	116	896	224	224	896	29	448	224
	AC	na	125	483	483	242	967	31	483	61
Solanum mauritianum	WA	na	**1176**	**2353**	151	75	na	na	na	299
	PE	32	32	61	32	32	244	16	244	122

Plant species	Extracts	*Fusarium* fungal pathogens								
		F. chlamydosporum	*F. equiseti*	*F. subglutinans*	*F. verticilloides*	*F. oxysporum*	*F. proliferetum*	*F. semitectum*	*F. solani*	*F. graminearum*
	EA	58	223	447	447	223	447	14	447	112
	AC	69	69	532	133	532	532	17	532	532
Vanguria infausta	WA	**5268**	169	**1317**	84	334	na	na	334	680
	PE	17	17	35	269	35	269	17	67	134
	EA	54	54	54	208	104	416	208	416	54
	AC	205	102	53	409	102	409	102	409	102
Withania somnifera	WA	na	207	**1619**	411	207	**6476**	na	411	104
	PE	23	23	179	89	89	358	23	23	89
	EA	24	190	48	380	380	760	760	380	24
	AC	75	153	153	593	593	**1187**	593	**1187**	38

Highlighted values show remarkable minimum inhibitory dilution value of more than 1000 ml/g of dried plant material. WA: Water, PE: Petroleum ether, EA: Ethyl acetate and AC: Acetone. na: Not applicable since minimum inhibitory concentration was > 2.5 mg/ml.

3.4. CONCLUSION

In the current study, 36, 47, 47, 44, 39, 43, 23, 41 and 46 individual extracts (which equate to 69, 90, 90, 85, 75, 83, 44, 79 and 88% of the extracts, respectively) demonstrated strong activity (with at least MIC < 1 mg/ml) against *F. chlamydosporum*, *F. eqiusite*, *F. subglutinans*, *F. verticilloides*, *F. oxysporum*, *F. proliferatum*, *F. semitectum*, *F. solani* and *F. graminearum*, respectively. Some of these extracts manifested antifungal activity that is higher than that of the positive control used in this study. The results indicate that organic extracts displayed stronger antifungal activity than the aqueous extracts. However, aqueous extracts of medicinal plants such as *Withania somnifera*, *Combretum molle*, *Olea europaea* and *Lantana camara* were very active and demonstrate the ability of medicinal plant extracts as alternative agents to fight *Fusarium* pathogens in agricultural sector. The use of renewable leafy part of medicinal plants is affordable, accessible, sustainable and eco-friendly. It also lends itself as a cost-effective option for smallholder farmers.

REFERENCES

Aberkane, A., Cuenca-Estrella M., Gomez-Lopez, A., Petrikkou, E., Mellado, E., Monzón, A., Rodriguez Tudela, J.L., 2002. Comparative evaluation of two different methods of inoculums preparation for antifungal susceptibility testing of filamentous fungi. Journal of Antimicrobial Chemotherapy 50, 719–722.

Adepoju, A.O., Ogunkunle, A.T.J., Femi-Adepoju, A.G., 2014. Antifungal activities of seed oil of neem (*azadirachta indica*A. *juss*). Global Journal of Biology, Agriculture and Health Sciences 3, 106–109.

Alexandra, D.M., Bhana, N., Bhika, K.H., Rogers, C.B., 1992. Antimicrobial testing of selected plant extracts from *Combretum* species. South African Journal of Science 88, 342–344.

Amoo, S.O., Finnie, J.F., Van Staden, J., 2012. Acetylcholinesterase inhibition, antioxidant, anti-inflammatory, antimicrobial and phytochemical properties of *Huernia hystrix*. Phytotherapy Research 26, 639–645.

Bhattacharjee, I., Chatterjee, S.K., Ghosh, A., Chandra, G., 2011. Antibacterial activities of some plant extracts used in Indian traditional folk medicine. Asian Pacific Journal of Tropical Biomedicine 1, 165–169.

Elmer, W.H., LaMondia, J.A., Taylor G.S., 1999. Asparagus cultivar trials in Connecticut. Acta Horticulturae 479, 189–198.

Eloff, J.N., 1998. Which extractant should be used for the screening and isolation of antimicrobial components from plants?. Journal of Ethnopharmacology 60, 1–8.

Eloff, J.N., 2004. Quantification of the bioactivity of plant extracts during screening and bioassay guided fractionation. Phytomedicine 11, 370–371.

Fandohan, P., Gnonlonfin, B., Hell, K., Marasas, W.F.O., Wingfield, M.J., 2005. Natural occurrence of *Fusarium* and subsequent fumonisin contamination in preharvest and

stored maize in Benin, West Africa. International Journal of Food Microbiology 99, 173-–183.

Gelderblom, W.C.A., Jaskiewickz, K., Marasas, W.F.O., Thiel, P.G., Horak, R.M., Vleggaar, R., Kriek, N.P.J., 1988. Fumonisins-novel mycotoxins with cancer promoting activity produced by *Fusarium moniliforme*. Applied and Environmental Microbiology 54, 1806–1811.

Kitonde, C.K., Fidahusein, D.S., Lukhoba, C.W., Jumba, M.M., 2014. Antimicrobial activity and phytochemical screening of *Senna didymobotrya* used to treat bacterial and fungal infections in Kenya. International Journal of Education and Research 2, 1–12.

Lalitha, V., Raveesha, K.A., Kiran, B., 2010. Antimicrobial activity of *Solanum torvum Swart* against important seed borne pathogens of paddy. Iranica Journal of Energy and Environment 1, 160–164.

Lekganyane, M. A., Matsebatlela, M. T., Howard, R. L., Shai, L. J., Masoko, P., 2012. The phytochemical, antibacterial and antioxidant activity of five medicinal plants against the wound infecting bacteria. African Journal of Biotechnology 11, 13210–13219.

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, 1529–1533.

Marasas, W.F.O., 1995. Fumonisins: their implications for human and animal health. Natural Toxins 3, 193–198.

Martínez, J.A., 2012. Natural fungicides obtained from plants, fungicides for plant and animal diseases, D. Dhanasekaran, N. Thajuddin and A. Panneerselvam, Intech Open, DOI: 10.5772/26336. Available from: https://www.intechopen.com/books/fungicides-for-plant-and-animal-diseases/natural-fungicides-obtained-from-plants.

Martini, N., Eloff, J.N., 1998. The preliminary isolation of several antibacterial compounds from *Combretum erythrophyllum* (Combretaceae). Journal of Ethnopharmacology 62, 255–263.

Martini, N.D., Katerere, D.R.P., Eloff, J.N., 2004. Biological activity of five antibacterial flavonoids from *Combretum erythrophyllum* (Combretaceae). Journal of Ethnopharmacology 93, 207–212.

Masoko, P., Makgapeetja, D.M., 2015. Antibacterial, antifungal and antioxidant activity of *Olea africana* against pathogenic yeast and nosocomial pathogens. BMC Complementary and Alternative Medicine 15, 409.

Masoko, P., Picard, J., Eloff, J.N., 2005. Antifungal activities of six South African *Terminali*a species (Combretaceae). Journal of Ethnopharmacology 99, 301–308.

Omidpanah, S., Haseli, A., Manayi, A., 2015. Evaluation of antifungal properties of some medicinal plants against *Aspergillus flavus* isolated from contaminated corn *in vitro.* Mediterranean Journal of Chemistry 3, 1093–1099.

Oren, L., Smadar, E., David, C., Amir, S., 2003. Early events in the *Fusarium verticillioides*-maize interaction characterized by using a green fluorescent protein-expressing transgenic isolate. Applied and Environmental Microbiology 69, 1695–1701.

Parekh, J., Chanda, S.V., 2007. *In vitro* antimicrobial activity and phytochemical analysis of some Indian medicinal plants. Turkish Journal of Biology 3, 53–58.

Shephard, G.S., Thiel, P.G., Stockenstrom, S., Sydenham, E.W., 1996. Worldwide survey of fumonisin contamination of corn and corn-based products. Journal of Association of Analytical Communities International 79, 671–687.

Silva, J.L., Souza, P.E., Monteiro, F.P., Freitas, M.L., Silva, M.B., Belan, L.L., 2014. Antifungal activity using medicinal plant extracts against pathogens of coffee tree. Revista Brasileira de Plantas Medicinais 16, 539–544.

Smith, I.M., 1986. Fungicides for Crop Protection: 100 Years of Progress. International Specialized Book Services. Portland, updated 2016.

Stephens, C.T., Vries, R.M.de., Sink, K.C., 1989. Evaluation of Asparagus species for resistance to *Fusarium oxysporum f.sp. asparagi* and *Fusarium moniliforme*. American Society for Horticultural Science 24, 365–368.

Sydenham, S., De Villiers, C., 2014. Reducing the risk of *Fusarium* head blight disease for irrigated wheat. ARC-Small Grain Institute. Pest Control on farm level.

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, 1479–1486.

Thippeswamy, S., Praveen, P., Mohana, D.C., Manjunath, K., 2011. Antimicrobial evaluation and phytochemical analysis of a known medicinal plant *Samanea saman* (jacq) merr. against some human and plant pathogenic bacteria and fungi. International Journal of Pharma and Bio Sciences 2, 443–451.

Van Vuuren, S.F., Naidoo, D., 2010. An antibacterial investigation of plants used traditionally in South Africa to treat sexually transmitted infections. Journal of Ethnopharmaocology 130, 552–558.

CHAPTER 4

ANTIFUNGAL ACTIVITY OF COMBINED EXTRACTS FROM DIFFERENT MEDICINAL PLANT SPECIES

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Sustainable use of thirteen South African medicinal plants for the management of crop diseases caused by *Fusarium* species – An *in vitro* study

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ABSTRACT

Crop fungal infections such as those caused by *Fusarium* species often result in yield loss pre- and post-harvest and during storage, contaminate food and exacerbate food insecurity. Synthetic fungicides are becoming less attractive as they pose health risks to consumers and are not environmentally friendly. As an alternative to synthetic fungicides, medicinal plant species may present sustainable and affordable sources of environmentally friendly fungicides for controlling plant diseases. This study was aimed at evaluating the *in vitro* antifungal activity of different medicinal plant extracts individually or in combination against *Fusarium proliferatum, F. solani, F. verticillioides* and *F. graminearum*. Different solvent (water, acetone and ethyl acetate) extracts obtained from the leaves of thirteen medicinal plant species were evaluated for antifungal activity using the micro-plate dilution assay. Their combined effect was evaluated by determining their fractional inhibitory concentration index (FICI). When evaluated individually, acetone extracts obtained from *Combretum erythrophyllum, Harpephyllum caffrum* and *Quercus acutissima* were the most active extracts inhibiting the growth of all the four pathogens with minimum inhibitory concentration (MIC) values less than 0.1 mg/ml. Out of 204 extract combinations evaluated, 150 extract combinations demonstrated either synergistic or additive antifungal activity. among the best combinations, combined acetone extracts of *Harpephyllum caffrum* and *Combretum erythrophyllum* showed strong synergistic antifungal activity against *F. graminearum, F. proliferatum* and *F. verticillioides* (MIC values of 0.02 mg/ml, 0.002 mg/ml and 0.001 mg/ml, respectively), and strong additive antifungal activity against *F. solani* (MIC = 0.02 mg/ml). The approach of using medicinal plant extracts from renewable plant parts either individually or in combination is sustainable, affordable, environmentally friendly and may be more beneficial in the fight against crop pathogenic diseases, particularly in organic farming.

1. Introduction

Food and nutrition security remains a global challenge. A recent report by the Food and Agriculture Organization (FAO, 2018) indicated that there has been a global increase in the number of people suffering from hunger particularly in the past three years, with climate variability and extremes as a major driver. While the ultimate goal behind agricultural practices is to produce adequate and high quality food or commodities in a sustainable manner, climate variability and extremes negatively impact agricultural productivity, food production and cropping patterns, resulting in reduced food availability, quality and safety (FAO, 2018). For instance, climate variability can result in the preponderance of phytopathogenic fungi infecting crops pre- and post-harvest, leading to, or exacerbating yield loss and food contamination.

Synthetic fungicides are a good weapon in fighting plant pathogens and there is no doubt that they have benefited conventional agricultural system for many years. Nowadays, the use of synthetic fungicide to control plant pathogens has been restricted due to their harmful effect on humans and environments (Harris et al., 2001; Aktar et al., 2009). These chemicals are liable to remain in food, vegetables and fruits following their application, and pose health risks to the consumers (Martinez, 2012). On the other hand, because of prolonged and/or misuse of these agrochemicals, cases of plant pathogens developing resistance have been reported (Wilson et al., 1997; Daferera et al., 2003; Ramaiah and Garampalli, 2015). Different fungicides such as carbendazim, tebuconazole, mancozeb and chlorothalonil are available in the market to control fungal crop diseases including those caused by *Fusarium* species (Freije and Wise, 2015; Karuppaiyan et al., 2015; Zhang and Jeyakumar, 2018).

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ABSTRACT

Crop fungal infections such as those caused by *Fusarium* species often result in yield loss pre- and post-harvest and during storage, contaminate food and exacerbate food insecurity. Synthetic fungicides are becoming less attractive as they pose health risks to consumers and are not environmentally friendly. As an alternative to synthetic fungicides, medicinal plant species may present sustainable and affordable sources of environmentally-friendly fungicides for controlling plant diseases. This chapter was aimed at evaluating the *in vitro* antifungal activity of combined extracts from different medicinal plant species against *F. chlamydosporum, F. eqiusite, F. subglutinans, F. verticilloides, F. oxysporum, F. proliferatum, F. semitectum, F. solani* and *F. graminearum*. The combined effect of these plant extracts was evaluated by determining their fractional inhibitory concentration index (FICI). Out of 348 extract combinations evaluated, 116 and 87 extract combinations demonstrated synergistic and additive antifungal activity, respectively. The strongest antifungal activity recorded for the combined plant extracts resulted from synergistic interaction with MIC value of 0.001 mg/ml against *Fusarium proliferatum* and *F. verticilloides*.

Keywords: Antifungal activity, combined extracts, *Fusarium*, plant fungal diseases

4.1. INTRODUCTION

Food and nutrition security remains a global challenge. A recent report by Food and Agriculture Organization (FAO, 2018) indicated that there has been a global increase in the number of people suffering from hunger particularly in the past three years, with climate variability and extremes as a major driver. While the ultimate goal behind agricultural practices is to produce adequate and high quality food or commodities in a sustainable manner, climate variability and extremes negatively impact agricultural productivity, food production and cropping patterns, resulting in reduced food availability, quality and safety (FAO, 2018). For instance, climate

variability can result in the preponderance of phytopathogenic fungi infecting crops pre- and post-harvest, leading to, or exacerbating yield loss and food contamination.

Synthetic fungicides are a good weapon in fighting plant pathogens and there is no doubt that they have benefited conventional agricultural system for many years. Nowadays, the use of synthetic fungicide to control plant pathogens has been restricted due to their harmful effect on humans and environments (Harris *et al*., 2001; Aktar *et al*., 2009). These chemicals are liable to remain in food, vegetables and fruits following their application, and pose health risks to the consumers (Martínez, 2012). On the other hand, because of prolonged and/or misuse of these agrochemicals, cases of plant pathogens developing resistance have been reported (Wilson *et al*., 1997; Daferera *et al*., 2003; Ramaiah and Garampalli, 2015).

Different fungicides such as prothioconazole, benomyl, carbendazim, tebuconazole, benzimidazole, mancozeb, captan and chlorothalonil are available in the market to control crop diseases including those caused by *Fusarium* species (Freije and Wise, 2015; Karuppaiyan *et al*., 2015; Zhang and Jeyakumar, 2018). However, there are reports about possible existence of resistance to carbendazim by *F. proliferatum*, *F. graminearum* and *F. verticilloides* (Chen *et al*., 2008; Chen and Zhou, 2009; Xu *et al*., 2019). This resistance has been related to mutation in the β1-tubulin gene particilarly in *F. verticilloides* (Yan and Dickman, 1996; Chen *et al*., 2014). *F. graminearum* isolates were also found to exhibit resistance towards tebuconazole, benzimidazole and flusilazole (Zhang *et al*., 2009; Jackson-Ziems *et al*., 2017; Popiel *et al*., 2017). Moreover, efficiency evaluation of different fungicides against *F. solani* revealed that mancozeb, captan and benomyl were less effective and incapable to achieve more than 50% minimum mycelia inhibition; although good results were observed with carbendazim, chlorothalonil and other tested fungicides (Padvi *et al*., 2018). Despite the emergence of fungicide-resistant pathogens, conventional farmers may still have a range of synthetic fungicides to control plant diseases while the options available for organic farmers are very

limited. These crop protection challenges necessitate a need to search for, and evaluate alternative strategies that are environmentally safe, accessible, and affordable even for smallholder farmers.

Bioactive compounds extracted from plants are considered as potential alternative fungicides in the fight against agricultural crop diseases (Prithiviraj *et al*., 1996; Amadioha and Obi, 1999; Alkhail, 2005). Although there are reports on the antimicrobial activity of traditional medicinal plants against phytopathogenic fungi (Singh *et al*., 1994; Pizana *et al*., 2010; Hadian *et al*., 2011), there is a paucity of information especially in comparison to documented study that focus on human pathogens. The use of medicinal plant products against crop pathogens may inhibit the development of fungal resistance due to the presence of different constituent antifungal compounds and their synergisms (Fandohan *et al*., 2004; Calvo *et al*., 2011).

The treatment of pathogenic diseases with a combination of extracts from different plant species may be more effective and possibly diminish negative side effects (Nwankwo *et al*., 2010). Combination of different antibiotics or antibiotic with certain medicinal plants has been successful for many years in the treatment of multi-drug resistant micro-organisms infecting both human and animals (Olajuyigbe and Afolayan, 2012; Stefanović and Comic, 2012; Moussaoui and Alaoui, 2016). This combination therapy approach also presents an opportunity in crop protection against fungicide-resistant pathogens. A study conducted by Nedelcu and Alexandri (1995) has long demonstrated that a combination of fungicides such as metamidoxime and copper oxychloride showed very strong synergistic effect against *F. oxysporum f. sp. lycopersici*. A similar positive effect was observed with a mixture of carbendazim and mancozeb, which showed complete inhibition of *F. solani* (Padvi, *et al*., 2018). Furthermore, Etebarian (1992) also reported a complete inhibition of fungal growth using a combination of iprodione and carbendazim. The mode of action of combined fungicides

or in this case plant extracts against specific pathogen can be completely different from that of individual or single extract.

However, not all combinations can be expected to act synergistically and amplify the antifungal activity of medicinal plant extracts against pathogen (s) in question. Therefore, an evaluation and calculation of fractional inhibitory concentration index (FICI) of combined plant extract becomes necessary to define or establish the nature of interactions (synergistic, additive, indifference or antagonistic) of extracts against tested pathogen (s). In this chapter, thirteen medicinal plant species (as presented in Chapter 3, Table 3.1) were selected for evaluation based on their previously reported activity against animal and/or human fungal pathogens. The aim was to investigate the *in vitro* antifungal activity of combined extracts from different plant species against nine *Fusarium* pathogens known to cause massive yield reduction in agriculture.

4.2. MATERIALS AND METHODS

4.2.1. Preparation of combined plant extracts

Similar solvent extracts from different medicinal plants that showed very strong antifungal activity (MIC < 0.1 mg/ml) when tested or evaluated individually against the same fungal pathogen were selected. Active medicinal plant extracts were selected as guided by MIC values obtained in Chapter 3, Table 3.2. The same medicinal plant extracts obtained and stored as described in Chapter 3**,** Section 3.2.2 were used to evaluate antifungal activity of combined plant extracts. During the antifungal assay, the plant extracts were weighed and re-dissolved separately. Organic extracts (petroleum ether, ethyl acetate and acetone) were re-dissolved in 100% acetone and water extracts were re-dissolved in 50% aqueous acetone. Similar solvent extracts were prepared and combined in equal proportion (v/v) such that the final concentration of each plant extract in the mixture or combination is maintained at 10 mg/ml.

4.2.2. Preparation of fungal pathogens

F. verticillioides, F. proliferatum, F. subglutinans, F. graminearum, F. solani, F. oxysporum, F. semitectum, F. chlamydosporum and *F. equiseti* were sub-cultured and fungal spores were determined as described in Chapter 3, Section 3.2.3.

4.2.3. Micro-plate dilution assay and determination of MIC values

Antifungal activity of combined extracts from different medicinal plant species was evaluated using modified serial dilution method (Masoko *et al*., 2005) as described in Chapter 3, Section 3.2.4. Minimum inhibitory concentration values of combined plant extracts was determined and recorded as described in Section 3.2.5. They were recorded as the lowest concentration of combined plant extracts that inhibited the growth of fungal pathogen. The results were recorded as the mean from triplicates of independent experiments and were expressed in milligram/milliliters (mg/ml).

4.2.4. Determination of fractional inhibitory concentration index (FICI)

The antifungal interaction (synergistic, additive, indifference and antagonistic interation) of combined medicnal plant extracts against *Fusarium* fungal pathogen was determined by calculating the fractional inhibitory concentration (FIC) and fractional inhibitory concentration index. FIC was calculated by dividing the MIC value recorded for the combined plant extract by the MIC value recorded for the individual plant extracts that makes up the mixture. For both combined and individual plant extracts antifungal assays, the MIC values were obtained against

the same fungal pathogen. The FICI was calculated by simply adding up the two FIC values obtained for each medicinal plant extracts as described in the following equation:

$$\text{FICI} = \frac{\text{MIC of plant extracts in combination}}{\text{MIC of plant extract (1)}} + \frac{\text{MIC value of plant extracts in combination}}{\text{MIC value of plant extract(2)}}$$

Antifungal interaction or effect of combined medicinal plant extracts against the tested pathogen was classified as synergistic (FICI $\leq$ 0.5), additive (FICI > 0.5 $\leq$ 1.0), indifference (FICI > 1.0 $\leq$ 4.0), or antagonistic (FICI > 4.0) (Van Vuuren and Viljoen, 2011). The recorded MIC values were mean results obtained from triplicates repeated independent experiments.

4.3. RESULTS AND DISCUSIONS

Application of single fungicide or drug towards the treatment of crop, human or animal pathogenic diseases may sometimes not produce effective antifungal activity compared to when the drugs are combined (Nedelcu and Alexandri, 1995; Barhate *et al*., 2015; Padvi *et al*., 2018). The same can happen with individual plant extract; hence, application of combined plant extracts should be evaluated and recommended where possible. Combining plant extracts may increase efficacy, surpass individual constituent antifungal activity and possibly prevent the emergence of resistance. In this chapter, fractional inhibitory concentration index (FICI) values of combined plant extracts were calculated in order to define the nature of their interactions (synergistic, additive, indifference or antagonistic interaction) against nine *Fusarium* pathogens causing crop diseases. Tables 4.1 – 4.9 showed the FICI values of the different combined extracts against *F. chlamydosporum*, *F. equisite*, *F. graminearum*, *F. oxysporum*, *F. proliferatum*, *F. semitectum*, *F. solani*, *F. subglutinans* and *F. verticillioides*, respectively.

In some cases, the choice of extraction solvent makes a difference when combining plant species. For example, a combination of ethyl acetate extracts of *O. europea* and *Q. acutissima* resulted in an indifferent interaction wheareas a combination of acetone extracts of the same plant materials resulted in a synergistic interaction against *F. graminearum* (Table 4.3). On the other hand, all combinations made up of the two *Combretum* species showed antagonistic interaction against *F. proliferatum* (Table 4.5), but additive interaction against *F. solani* (Table 4.7), irrespective of the extraction solvent used. Thus, it would appear that the choice of the extraction solvent and pathogen are of vital consideration in determining the antifungal potency of plant extract combinations.

Of the 99 combinations evaluated against *F. proliferatum*, 46, 26, 21 and 6 extract combinations showed synergistic, additive, indifferent and antagonistic antifungal activities, respectively (Table 4.5). Sixty combinations were evaluated against *F. solani*, of which 16, 34, 9 and 1 combinations showed synergistic, additive, indifference and antagonistic activities, respectively (Table 4.7). Thus, in general, the majority of the combined extracts showed synergistic or additive antifungal activity.

The plant extract combinations that showed synergistic and additive antifungal activity are of particular interest. Synergistic or additive antifungal effect within medicinal plant extracts implies that their combined effect is stronger than the activity of the individual extract. This effect may be due to complex formation and/or increased concentration of antifungal compounds at the inhibition sites. Another possibility is that the combined plant extracts may have different modes of action, which enhance their antifungal activity against the pathogen. Some plant extract combinations were found to show very strong antifungal activity against certain pathogens but less active when evaluated against other pathogen (s). The antifungal activity of medicinal plant extracts is pathogen-specific and, to some extent, depends on the polarity of the solvent used for extraction.

Table 4.1. Antifungal activity of combined medicinal plant extracts against *Fusarium chlamydosporum*.

Extracts	MIC Plant 1	MIC Plant 2	MIC	FIC1	FIC2	FICI	Remarks
Water	*C .molle*	*O. europaea*	0.63	15.63	15.63	31.25	Antagonistic
	C. molle	*V. infausta*	0.63	15.63	15.63	31.25	Antagonistic
	O. europaea	*V. infausta*	0.31	7.81	7.81	15.63	Antagonistic
Petroleum ether	*S. brachypetala*	*N. glauca*	0.16	3.91	3.91	7.82	Antagonistic
	S. brachypetala	*O. europaea*	0.16	3.91	3.91	7.82	Antagonistic
	S. brachypetala	*Q. acutissima*	0.16	3.91	3.91	7.82	Antagonistic
	N. glauca	*O. europaea*	0.08	1.95	1.95	3.91	Indifference
	N. glauca	*Q. acutissima*	0.08	1.95	1.95	3.91	Indifference
	O. europaea	*Q. acutissima*	0.08	1.95	1.95	3.91	Indifference
Ethyl acetate	*C. erythrophyllum*	*S. brachypetala*	0.16	3.91	1.95	5.86	Antagonistic
	C. erythrophyllum	*N. glauca*	0.16	3.91	1.95	5.86	Antagonistic
	C. erythrophyllum	*C. molle*	0.08	1.95	1.95	3.91	Indifference
	S. brachypetala	*N. glauca*	0.16	3.91	1.95	5.86	Antagonistic
	S. brachypetala	*C. molle*	0.16	3.91	3.91	7.82	Antagonistic
	N. glauca	*C. molle*	0.16	1.95	3.91	5.86	Antagonistic
Acetone	*C. erythrophyllum*	*M. azedarach*	0.16	3.91	3.91	7.82	Antagonistic
	C. erythrophyllum	*S. brachypetala*	0.31	7.81	7.81	15.63	Antagonistic
	C. erythrophyllum	*N. glauca*	0.16	3.91	1.95	5.86	Antagonistic
	C. erythrophyllum	*C. molle*	0.08	1.95	1.95	3.91	Indifference
	C. erythrophyllum	*Q. acutissima*	0.04	0.98	0.98	1.96	Indifference
	C. erythrophyllum	*V. infausta*	0.31	7.81	3.91	11.72	Antagonistic
	M. azedarach	*S. brachypetala*	0.31	7.81	7.81	15.63	Antagonistic
	M. azedarach	*N. glauca*	0.16	3.91	1.95	5.86	Antagonistic
	M. azedarach	*C. molle*	0.16	3.91	3.91	7.82	Antagonistic
	M. azedarach	*Q. acutissima*	0.04	0.98	0.98	1.96	Indifference
	M. azedarach	*V. infausta*	0.16	3.91	1.95	5.86	Antagonistic
	S. brachypetala	*N. glauca*	0.16	3.91	1.95	5.86	Antagonistic
	S. brachypetala	*C. molle*	0.31	7.81	7.81	15.63	Antagonistic
	S. brachypetala	*Q. acutissima*	0.31	7.81	7.81	15.63	Antagonistic
	S. brachypetala	*V. infausta*	0.04	0.98	0.49	1.47	Indifference
	N. glauca	*C. molle*	0.08	0.98	1.95	2.93	Indifference
	N. glauca	*Q. acutissima*	0.02	0.24	0.49	0.73	Additive
	N. glauca	*V. infausta*	0.08	0.98	0.98	1.95	Indifference
	C. molle	*Q. acutissima*	0.16	3.91	3.91	7.82	Antagonistic

Extracts	MIC Plant 1	MIC Plant 2	MIC	FIC1	FIC2	FICI	Remarks
	C. molle	*V. infausta*	0.16	3.91	1.95	5.86	Antagonistic
	Q. acutissima	*V. infausta*	0.08	1.95	0.98	2.93	Indifference

Table 4.2. Antifungal activity of combined medicinal plant extracts against *Fusarium equiseti*

Extract	MIC Plant 1	MIC Plant 2	MIC	FIC1	FIC2	FICI	Remarks
Acetone	*C. erythrophyllum*	*Q. acutissima*	0.02	0.24	0.24	**0.49**	Synergistic

Values highlighted in bold indicate strong synergistic interaction between extracts from different species with FICI value of less or equals to 0.5

Table 4.3. Antifungal activity of combined medicinal plant extracts against *Fusarium graminerum*

Extract	MIC Plant 1	MIC Plant 2	MIC	FIC1	FIC2	FICI	Remarks
Petroleum ether	*L. camara*	*S. didymobotrya*	0.005	0.06	0.06	**0.12**	Synergistic
	L. camara	*S. mauritianum*	0.005	0.06	0.06	**0.12**	Synergistic
	L. camara	*M. azedarach*	0.005	0.06	0.06	**0.12**	Synergistic
	L. camara	*H. caffrum*	0.005	0.06	0.06	**0.12**	Synergistic
	L. camara	*O. europaea*	0.005	0.06	0.12	**0.18**	Synergistic
	L. camara	*Q. acutissima*	0.005	0.06	0.12	**0.18**	Synergistic
	L. camara	*V. infausta*	0.005	0.06	0.06	**0.12**	Synergistic
	S. didymobotrya	*S. mauritianum*	0.005	0.06	0.06	**0.12**	Synergistic
	S. didymobotrya	*M. azedarach*	0.005	0.06	0.06	**0.12**	Synergistic
	S. didymobotrya	*H. caffrum*	0.005	0.06	0.06	**0.12**	Synergistic
	S. didymobotrya	*O. europaea*	0.005	0.06	0.12	**0.18**	Synergistic
	S. didymobotrya	*Q. acutissima*	0.005	0.06	0.12	**0.18**	Synergistic
	S. didymobotrya	*V. infausta*	0.005	0.06	0.06	**0.12**	Synergistic
	S. mauritianum	*M. azedarach*	0.005	0.06	0.06	**0.12**	Synergistic
	S. mauritianum	*H. caffrum*	0.078	0.98	0.98	1.95	Indifference
	S. mauritianum	*O. europaea*	0.078	0.98	1.95	2.93	Indifference
	S. mauritianum	*Q. acutissima*	0.010	0.12	0.25	**0.37**	Synergistic
	S. mauritianum	*V. infausta*	0.005	0.06	0.06	**0.12**	Synergistic
	M. azedarach	*H. caffrum*	1.250	15.63	15.63	31.25	Antagonistic
	M. azedarach	*O. europaea*	1.250	15.63	31.25	46.88	Antagonistic
	M. azedarach	*Q. acutissima*	0.313	3.91	7.81	11.72	Antagonistic
	M. azedarach	*V. infausta*	0.313	3.91	3.91	7.81	Antagonistic
	H. caffrum	*O. europaea*	0.078	0.98	1.95	2.93	Indifference

Extract	MIC Plant 1	MIC Plant 2	MIC	FIC1	FIC2	FICI	Remarks
	H. caffrum	*Q. acutissima*	0.005	0.06	0.12	**0.18**	Synergistic
	H. caffrum	*V. infausta*	1.250	15.63	15.63	31.25	Antagonistic
	O. europaea	*Q. acutissima*	1.250	31.25	31.25	62.50	Antagonistic
	O. europaea	*V. infausta*	0.156	3.91	1.95	5.86	Antagonistic
	Q. acutissima	*V. infausta*	0.039	0.98	0.49	1.47	Indifference
Ethyl acetate	*L. camara*	*M. azedarach*	0.002	0.03	0.03	**0.06**	Synergistic
	L. camara	*O. europaea*	0.002	0.03	0.12	**0.15**	Synergistic
	L. camara	*Q. acutissima*	0.010	0.12	0.49	0.61	Additive
	M. azedarach	*O. europaea*	0.010	0.12	0.49	0.61	Additive
	M. azedarach	*Q. acutissima*	0.020	0.24	0.98	1.22	Indifference
	O. europaea	*Q. acutissima*	0.039	1.96	1.96	3.91	Indifference
Acetone	*C. erythrophyllum*	*S. mauritianum*	0.010	0.12	0.25	**0.37**	Synergistic
	C. erythrophyllum	*H. caffrum*	0.020	0.24	0.24	**0.49**	Synergistic
	C. erythrophyllum	*O. europaea*	0.020	0.24	0.98	1.22	Indifference
	C. erythrophyllum	*Q. acutissima*	0.010	0.12	0.49	0.61	Additive
	S. mauritianum	*H. caffrum*	0.005	0.12	0.06	**0.18**	Synergistic
	S. mauritianum	*O. europaea*	0.002	0.06	0.12	**0.18**	Synergistic
	S. mauritianum	*Q. acutissima*	0.002	0.06	0.12	**0.18**	Synergistic
	H. caffrum	*O. europaea*	0.010	0.12	0.49	0.61	Additive
	H. caffrum	*Q. acutissima*	0.002	0.03	0.12	**0.15**	Synergistic
	O. europaea	*Q. acutissima*	0.005	0.25	0.25	**0.49**	Synergistic

Values highlighted in bold indicate strong synergistic interaction between extracts from different species with FICI value of less or equals to 0.5

Table 4.4. Antifungal activity of combined medicinal plant extracts against *Fusarium oxysporum*

Extracts	MIC Plant 1	MIC Plant 2	MIC	FIC1	FIC2	FICI	Remarks
Ethyl acetate	*S. mauritianum*	*M. azedarach*	0.04	0.49	0.49	0.98	Additive
	S. mauritianum	*W. somnifera*	0.08	0.98	0.98	1.95	Indifference
	S. mauritianum	*Q. acutissima*	0.04	0.49	0.49	0.98	Additive
	M. azedarach	*W. somnifera*	0.04	0.49	0.49	0.98	Additive
	M. azedarach	*Q. acutissima*	0.08	0.98	0.98	1.95	Indifference
	W. somnifera	*Q. acutissima*	0.08	0.98	0.98	1.95	Indifference
Acetone	*C. erythrophyllum*	*S. mauritianum*	0.04	0.98	0.98	1.96	Indifference
	C. erythrophyllum	*W. somnifera*	0.04	0.98	0.98	1.96	Indifference
	S. mauritianum	*W. somnifera*	0.08	1.95	0.98	2.93	Indifference

Table 4.5. Antifungal activity of combined medicinal plant extracts against *Fusarium proliferetum.*

Extracts	MIC Plant 1	MIC Plant 2	MIC	FIC1	FIC2	FICI	Remarks
Water	*W. somnifera*	*C. molle*	0.020	0.49	0.49	0.98	Additive
	W. somnifera	*O. europaea*	0.039	0.98	0.98	1.95	Indifference
	C. molle	*O. europaea*	0.039	0.98	0.98	1.95	Indifference
Petroleum ether	*S. didymobotrya*	*C. erythrophyllum*	0.039	0.98	0.98	1.95	Indifference
	S. didymobotrya	*N. glauca*	0.039	0.98	0.98	1.95	Indifference
	S. didymobotrya	*W. somnifera*	0.020	0.49	0.49	0.98	Additive
	C. erythrophyllum	*S. brachypetala*	0.078	1.95	1.95	3.91	Indifference
	C. erythrophyllum	*N. glauca*	0.020	0.49	0.49	0.98	Additive
	C. erythrophyllum	*W. somnifera*	0.039	0.98	0.98	1.95	Indifference
	C. erythrophyllum	*C. molle*	0.020	0.49	0.49	0.98	Additive
	C. erythrophyllum	*O. europaea*	0.020	0.49	0.49	0.98	Additive
	S. mauritianum	*M. azedarach*	0.020	0.49	0.49	0.98	Additive
	S. mauritianum	*C. molle*	0.020	0.49	0.49	0.98	Additive
	S. brachypetala	*N. glauca*	0.039	0.98	0.98	1.95	Indifference
	S. brachypetala	*O. europaea*	0.020	0.49	0.49	0.98	Additive
	N. glauca	*W. somnifera*	0.020	0.49	0.49	0.98	Additive
	N. glauca	*O. europaea*	0.020	0.49	0.49	0.98	Additive
Ethyl Acetate	*L. camara*	*S. didymobotrya*	0.010	0.24	0.24	**0.49**	Synergistic
	L. camara	*C. erythrophyllum*	0.039	0.98	0.98	1.95	Indifference
	L. camara	*S. mauritianum*	0.039	0.98	0.98	1.95	Indifference
	L. camara	*N. glauca*	0.010	0.24	0.24	**0.49**	Synergistic
	L. camara	*H. caffrum*	0.020	0.49	0.49	0.98	Additive
	L. camara	*W. somnifera*	0.020	0.49	0.49	0.98	Additive
	L. camara	*C. molle*	0.039	0.98	0.98	1.95	Indifference
	L. camara	*Q. acutissima*	0.020	0.49	0.49	0.98	Additive
	L. camara	*V. infausta*	0.020	0.49	0.49	0.98	Additive
	S. didymobotrya	*C. erythrophyllum*	0.020	0.49	0.49	0.98	Additive
	S. didymobotrya	*S. mauritianum*	0.039	0.98	0.98	1.95	Indifference
	S. didymobotrya	*N. glauca*	0.039	0.98	0.98	1.95	Indifference
	S. didymobotrya	*H. caffrum*	0.020	0.49	0.49	0.98	Additive
	S. didymobotrya	*Q. acutissima*	0.039	0.98	0.98	1.95	Indifference
	C. erythrophyllum	*S. mauritianum*	0.020	0.49	0.49	0.98	Additive
	C. erythrophyllum	*S. brachypetala*	0.078	1.95	1.95	3.91	Indifference
	C. erythrophyllum	*N. glauca*	0.020	0.49	0.49	0.98	Additive

Extracts	MIC Plant 1	MIC Plant 2	MIC	FIC1	FIC2	FICI	Remarks
	C. erythrophyllum	*H. caffrum*	0.020	0.49	0.49	0.98	Additive
	C. erythrophyllum	*W. somnifera*	0.010	0.24	0.24	**0.49**	Synergistic
	C. erythrophyllum	*C. molle*	1.250	31.25	31.25	62.50	Antagonistic
	C. erythrophyllum	*Q. acutissima*	0.020	0.49	0.49	0.98	Additive
	C. erythrophyllum	*V. infausta*	0.039	0.98	0.98	1.95	Indifference
	S. mauritianum	*M. azedarach*	0.020	0.49	0.49	0.98	Additive
	S. mauritianum	*N. glauca*	0.020	0.49	0.49	0.98	Additive
	S. mauritianum	*H. caffrum*	0.156	3.91	3.91	7.81	Antagonistic
	S. mauritianum	*W. somnifera*	0.078	1.95	1.95	3.91	Indifference
	S. mauritianum	*Q. acutissima*	0.156	3.91	3.91	7.81	Antagonistic
	S. mauritianum	*V. infausta*	0.078	1.95	1.95	3.91	Indifference
	M. azedarach	*W. somnifera*	0.039	0.98	0.98	1.95	Indifference
	M. azedarach	*Q. acutissima*	0.039	0.98	0.98	1.95	Indifference
	S. brachypetala	*N. glauca*	0.020	0.49	0.49	0.98	Additive
	S. brachypetala	*C. molle*	1.250	31.25	31.25	62.50	Antagonistic
	N. glauca	*H. caffrum*	0.078	1.95	1.95	3.91	Indifference
	N. glauca	*W. somnifera*	0.001	0.03	0.03	**0.06**	Synergistic
	N. glauca	*C. molle*	0.001	0.03	0.03	**0.06**	Synergistic
	N. glauca	*Q. acutissima*	0.001	0.03	0.03	**0.06**	Synergistic
	N. glauca	*V. infausta*	0.001	0.03	0.03	**0.06**	Synergistic
	H. caffrum	*W. somnifera*	0.005	0.12	0.12	**0.24**	Synergistic
	H. caffrum	*Q. acutissima*	0.005	0.12	0.12	**0.24**	Synergistic
	H. caffrum	*V. infausta*	0.020	0.49	0.49	0.98	Additive
	W. somnifera	*C. molle*	0.039	0.98	0.98	1.95	Indifference
	W. somnifera	*Q. acutissima*	0.001	0.03	0.03	**0.06**	Synergistic
	W. somnifera	*V. infausta*	0.002	0.06	0.06	**0.12**	Synergistic
	C. molle	*V. infausta*	0.020	0.49	0.49	0.98	Additive
	Q. acutissima	*V. infausta*	0.020	0.49	0.49	0.98	Additive
Acetone	*L. camara*	*S. didymobotrya*	0.002	0.06	0.06	**0.12**	Synergistic
	L. camara	*C. erythrophyllum*	0.002	0.06	0.06	**0.12**	Synergistic
	L. camara	*S. mauritianum*	0.005	0.12	0.12	**0.24**	Synergistic
	L. camara	*W. somnifera*	0.005	0.12	0.12	**0.24**	Synergistic
	L. camara	*C. molle*	0.010	0.24	0.24	**0.49**	Synergistic
	S. didymobotrya	*C. erythrophyllum*	0.005	0.12	0.12	**0.24**	Synergistic
	S. didymobotrya	*S. mauritianum*	0.010	0.24	0.24	**0.49**	Synergistic
	S. didymobotrya	*M. azedarach*	0.010	0.24	0.12	**0.37**	Synergistic
	S. didymobotrya	*H. caffrum*	0.010	0.24	0.24	**0.49**	Synergistic
	S. didymobotrya	*W. somnifera*	0.010	0.24	0.24	**0.49**	Synergistic
	S. didymobotrya	*Q. acutissima*	0.005	0.12	0.12	**0.24**	Synergistic

Extracts	MIC Plant 1	MIC Plant 2	MIC	FIC1	FIC2	FICI	Remarks
	S. didymobotrya	*V. infausta*	0.005	0.12	0.12	**0.24**	Synergistic
	C. erythrophyllum	*S. mauritianum*	0.005	0.12	0.12	**0.24**	Synergistic
	C. erythrophyllum	*M. azedarach*	0.005	0.12	0.06	**0.18**	Synergistic
	C. erythrophyllum	*S. brachypetala*	0.001	0.03	0.03	**0.06**	Synergistic
	C. erythrophyllum	*H. caffrum*	0.002	0.06	0.06	**0.12**	Synergistic
	C. erythrophyllum	*W. somnifera*	0.002	0.06	0.06	**0.12**	Synergistic
	C. erythrophyllum	*C. molle*	0.625	15.63	15.63	31.25	Antagonistic
	C. erythrophyllum	*Q. acutissima*	0.002	0.06	0.06	**0.12**	Synergistic
	C. erythrophyllum	*V. infausta*	0.005	0.12	0.12	**0.24**	Synergistic
	S. mauritianum	*W. somnifera*	0.001	0.03	0.03	**0.06**	Synergistic
	M. azedarach	*S. brachypetala*	0.001	0.02	0.03	**0.05**	Synergistic
	M. azedarach	*H. caffrum*	0.001	0.02	0.03	**0.05**	Synergistic
	M. azedarach	*W. somnifera*	0.002	0.03	0.06	**0.09**	Synergistic
	M. azedarach	*C. molle*	1.250	15.63	31.25	46.88	Antagonistic
	M. azedarach	*Q. acutissima*	0.010	0.12	0.24	**0.37**	Synergistic
	S. brachypetala	*Q. acutissima*	0.002	0.06	0.06	**0.12**	Synergistic
	S. brachypetala	*V. infausta*	0.001	0.03	0.03	**0.06**	Synergistic
	H. caffrum	*W. somnifera*	0.001	0.03	0.03	**0.06**	Synergistic
	H. caffrum	*Q. acutissima*	0.002	0.06	0.06	**0.12**	Synergistic
	H. caffrum	*V. infausta*	0.002	0.06	0.06	**0.12**	Synergistic
	W. somnifera	*C. molle*	0.002	0.06	0.06	**0.12**	Synergistic
	W. somnifera	*Q. acutissima*	0.002	0.06	0.06	**0.12**	Synergistic
	W. somnifera	*V. infausta*	0.001	0.03	0.03	**0.06**	Synergistic
	C. molle	*Q. acutissima*	0.001	0.03	0.03	**0.06**	Synergistic
	C. molle	*V. infausta*	0.002	0.06	0.06	**0.12**	Synergistic
	Q. acutissima	*V. infausta*	0.002	0.06	0.06	**0.12**	Synergistic

Values highlighted in bold indicate strong synergistic interaction between extracts from different species with FICI value of less or equals to 0.5.

Table 4.6. Antifungal activity of combined medicinal plant extracts against *Fusarium semitectum*

Extracts	MIC Plant 1	MIC Plant 2	MIC	FIC1	FIC2	FICI	Remarks
Ethyl acetate	*L. camara*	*W. somnifera*	0.08	0.98	1.95	2.93	Indifference
	L. camara	*C. molle*	0.02	0.24	0.49	0.73	Additive
	L. camara	*V. infausta*	0.16	1.95	1.95	3.91	Indifference
	W. somnifera	*C. molle*	0.31	7.81	7.81	15.63	Antagonistic

Extracts	MIC Plant 1	MIC Plant 2	MIC	FIC1	FIC2	FICI	Remarks
	W. somnifera	*V. infausta*	0.04	0.98	0.49	1.46	Indifference
	C. molle	*V. infausta*	0.02	0.49	0.24	0.73	Additive
Acetone	*L. camara*	*N. glauca*	0.04	0.98	0.98	1.95	Indifference
	L. camara	*W. somnifera*	0.02	0.49	0.24	0.73	Additive
	L. camara	*C. molle*	0.08	1.95	0.98	2.93	Indifference
	N. glauca	*W. somnifera*	0.04	0.49	0.49	0.98	Additive
	N. glauca	*C. molle*	0.08	0.98	0.98	1.95	Indifference
	W. somnifera	*C. molle*	0.08	0.98	0.98	1.95	Indifference

Table 4.7. Antifungal activity of combined medicinal plant extracts against *Fusarium solani*

Extracts	Plant 1	Plant 2	MIC	FIC1	FIC2	FICI	Remarks
Ethyl acetate	*C. erythrophyllum*	*S. mauritianum*	0.02	0.24	0.49	0.73	Additive
	C. erythrophyllum	*H. caffrum*	0.08	0.98	0.98	1.95	Indifference
	C. erythrophyllum	*W. somnifera*	0.08	0.98	0.98	1.95	Indifference
	C. erythrophyllum	*Q. acutissima*	0.02	0.24	0.49	0.73	Additive
	C. erythrophyllum	*V. infausta*	0.02	0.24	0.49	0.73	Additive
	S. mauritianum	*H. caffrum*	0.02	0.49	0.24	0.73	Additive
	S. mauritianum	*W. somnifera*	0.01	0.25	0.12	**0.37**	Synergistic
	S. mauritianum	*Q. acutissima*	0.01	0.25	0.25	**0.49**	Synergistic
	S. mauritianum	*V. infausta*	0.01	0.25	0.25	**0.49**	Synergistic
	H. caffrum	*W. somnifera*	0.02	0.24	0.24	**0.49**	Synergistic
	H. caffrum	*Q. acutissima*	0.02	0.24	0.49	0.73	Additive
	H. caffrum	*V. infausta*	0.02	0.24	0.49	0.73	Additive
	W. somnifera	*Q. acutissima*	0.02	0.24	0.49	0.73	Additive
	W. somnifera	*V. infausta*	0.02	0.24	0.49	0.73	Additive
	Q. acutissima	*V. infausta*	0.02	0.49	0.49	0.98	Additive
	Q. acutissima	*M. azedarach*	0.01	0.25	0.12	**0.37**	Synergistic
	C. erythrophyllum	*C. molle*	0.02	0.24	0.49	0.73	Additive
	S. didymobotrya	*S. mauritianum*	0.31	3.91	7.81	11.72	Antagonistic
	S. didymobotrya	*H. caffrum*	0.08	0.98	0.98	1.95	Indifference
	S. didymobotrya	*Q. acutissima*	0.08	0.98	1.95	2.93	Indifference
	S. mauritianum	*C. molle*	0.02	0.49	0.49	0.98	Additive
Acetone	*S. didymobotrya*	*C. erythrophyllum*	0.02	0.24	0.49	0.73	Additive
	S. didymobotrya	*M. azedarach*	0.02	0.24	0.49	0.73	Additive
	S. didymobotrya	*N. glauca*	0.02	0.24	0.24	**0.49**	Synergistic
	S. didymobotrya	*H. caffrum*	0.02	0.24	0.49	0.73	Additive
	S. didymobotrya	*W. somnifera*	0.02	0.24	0.49	0.73	Additive

Extracts	Plant 1	Plant 2	MIC	FIC1	FIC2	FICI	Remarks
	S. didymobotrya	*Q. acutissima*	0.02	0.24	0.49	0.73	Additive
	S. didymobotrya	*V. infausta*	0.02	0.24	0.49	0.73	Additive
	C. erythrophyllum	*M. azedarach*	0.02	0.49	0.49	0.98	Additive
	C. erythrophyllum	*N. glauca*	0.02	0.49	0.24	0.73	Additive
	C. erythrophyllum	*H. caffrum*	0.02	0.49	0.49	0.98	Additive
	C. erythrophyllum	*W. somnifera*	0.02	0.49	0.49	0.98	Additive
	C. erythrophyllum	*Q. acutissima*	0.02	0.49	0.49	0.98	Additive
	C. erythrophyllum	*V. infausta*	0.02	0.49	0.49	0.98	Additive
	M. azedarach	*N. glauca*	0.02	0.49	0.24	0.73	Additive
	M. azedarach	*H. caffrum*	0.02	0.49	0.49	0.98	Additive
	M. azedarach	*W. somnifera*	0.02	0.49	0.49	0.98	Additive
	M. azedarach	*Q. acutissima*	0.02	0.49	0.49	0.98	Additive
	M. azedarach	*V. infausta*	0.01	0.25	0.25	**0.49**	Synergistic
	N. glauca	*H. caffrum*	0.02	0.24	0.49	0.73	Additive
	N. glauca	*W. somnifera*	0.01	0.12	0.25	**0.37**	Synergistic
	N. glauca	*Q. acutissima*	0.01	0.12	0.25	**0.37**	Synergistic
	N. glauca	*V. infausta*	0.01	0.12	0.25	**0.37**	Synergistic
	H. caffrum	*W. somnifera*	0.01	0.25	0.25	**0.49**	Synergistic
	H. caffrum	*Q. acutissima*	0.01	0.25	0.25	**0.49**	Synergistic
	H. caffrum	*V. infausta*	0.01	0.25	0.25	**0.49**	Synergistic
	W. somnifera	*Q. acutissima*	0.005	0.12	0.12	**0.25**	Synergistic
	W. somnifera	*V. infausta*	0.005	0.12	0.12	**0.25**	Synergistic
	Q. acutissima	*V. infausta*	0.01	0.25	0.25	**0.49**	Synergistic
	M. azedarach	*S. mauritianum*	0.04	0.98	0.98	1.96	Indifference
	C. erythrophyllum	*C. molle*	0.02	0.49	0.49	0.98	Additive
	M. azedarach	*C. molle*	0.04	0.98	0.98	1.96	Indifference
	C. molle	*V. infausta*	0.04	0.98	0.98	1.96	Indifference
	L. camara	*S. didymobotrya*	0.02	0.49	0.24	0.73	Additive
	L. camara	*C. erythrophyllum*	0.04	0.98	0.98	1.96	Indifference
	L. camara	*S. mauritianum*	0.02	0.49	0.49	0.98	Additive
	L. camara	*N. glauca*	0.02	0.49	0.24	0.73	Additive
	L. camara	*W. somnifera*	0.02	0.49	0.49	0.98	Additive
	L. camara	*C. molle*	0.08	1.95	1.95	3.91	Indifference
	W. somnifera	*C. molle*	0.02	0.49	0.49	0.98	Additive

Values highlighted in bold indicate strong synergistic interaction between extracts from different species with FICI value of less or equals to 0.5

Table 4.8. Antifungal activity of combined medicinal plant extracts against *Fusarium subglutinans*

Extracts	MIC Plant 1	MIC Plant 2	MIC	FIC1	FIC2	FICI	Remarks
Water	*S. didymobotrya*	*M. azedarach*	1.25	31.25	15.63	46.88	Antagonistic
	S. didymobotrya	*N. glauca*	1.25	31.25	15.63	46.88	Antagonistic
	S. mauritianum	*N. glauca*	1.25	15.63	15.63	31.25	Antagonistic
Petroleum Ether	*C. erythrophyllum*	*N. glauca*	0.31	7.81	7.81	15.63	Antagonistic
	L. camara	*N. glauca*	0.63	15.63	15.63	31.25	Antagonistic
	L. camara	*W. somnifera*	0.63	15.63	7.81	23.44	Antagonistic
	L. camara	*C. erythrophyllum*	0.16	3.91	3.91	7.82	Antagonistic
	S. didymobotrya	*N. glauca*	0.16	1.95	3.91	5.86	Antagonistic
	S. didymobotrya	*W. somnifera*	0.16	1.95	1.95	3.91	Indifference
	C. erythrophyllum	*W. somnifera*	0.16	3.91	1.95	5.86	Antagonistic
Ethyl Acetate	*C. erythrophyllum*	*S. mauritianum*	0.04	0.98	0.98	1.96	Indifference
	C. erythrophyllum	*H. caffrum*	0.08	1.95	0.98	2.93	Indifference
	C. erythrophyllum	*Q. acutissima*	0.01	0.25	0.12	**0.37**	Synergistic
	S. mauritianum	*N. glauca*	0.01	0.25	0.25	**0.49**	Synergistic
	S. mauritianum	*H. caffrum*	0.01	0.25	0.12	**0.37**	Synergistic
	S. mauritianum	*Q. acutissima*	0.01	0.25	0.12	**0.37**	Synergistic
	N. glauca	*H. caffrum*	0.01	0.25	0.12	**0.37**	Synergistic
	N. glauca	*Q. acutissima*	0.01	0.25	0.12	**0.37**	Synergistic
	H. caffrum	*Q. acutissima*	0.02	0.24	0.24	**0.49**	Synergistic
	C. erythrophyllum	*N. glauca*	0.02	0.49	0.49	0.98	Additive
	N. glauca	*S. didymobotrya*	0.16	3.91	3.91	7.82	Antagonistic
	L. camara	*S. mauritianum*	0.63	15.63	15.63	31.25	Antagonistic
	L. camara	*H. caffrum*	0.31	7.81	3.91	11.72	Antagonistic
	L. camara	*Q. acutissima*	0.31	7.81	3.91	11.72	Antagonistic
	L. camara	*C. erythrophyllum*	0.04	0.98	0.98	1.96	Indifference
	S. didymobotrya	*S. mauritianum*	0.04	0.98	0.98	1.96	Indifference
	S. didymobotrya	*H. caffrum*	0.16	3.91	1.95	5.86	Antagonistic
	S. didymobotrya	*Q. acutissima*	0.04	0.98	0.49	1.47	Indifference
Acetone	*S. didymobotrya*	*C. erythrophyllum*	0.02	0.24	0.24	**0.49**	Synergistic
	S. didymobotrya	*M. azedarach*	0.02	0.24	0.24	**0.49**	Synergistic
	S. didymobotrya	*N. glauca*	0.02	0.24	0.49	0.73	Additive
	C. erythrophyllum	*M. azedarach*	0.04	0.49	0.49	0.98	Additive
	C. erythrophyllum	*N. glauca*	0.02	0.24	0.49	0.73	Additive
	M. azedarach	*N. glauca*	0.02	0.24	0.49	0.73	Additive
	M. azedarach	*S. mauritianum*	0.08	0.98	1.95	2.93	Indifference
	L. camara	*S. didymobotrya*	0.04	0.98	0.49	1.47	Indifference

Extracts	MIC Plant 1	MIC Plant 2	MIC	FIC1	FIC2	FICI	Remarks
	L. camara	*C. erythrophyllum*	0.02	0.49	0.24	0.73	Additive
	L. camara	*S. mauritianum*	0.02	0.49	0.49	0.98	Additive
	L. camara	*N. glauca*	0.02	0.49	0.49	0.98	Additive

Values highlighted in bold indicate strong synergistic interaction between extracts from different species with FICI value of less or equals to 0.5

Table 4.9. Antifungal activity of combined medicinal plant extracts against *Fusarium verticilloides*

Extract	MIC Plant 1	MIC Plant 2	MIC	FIC 1	FIC 2	FICI	Remarks
Water	*L. camara*	*O. europaea*	1.250	31.25	15.63	46.88	Antagonistic
Petroleum Ether	*C. erythrophyllum*	*Q. acutissima*	0.156	3.91	1.95	5.86	Antagonistic
	C. erythrophyllum	*V. infausta*	0.156	3.91	3.91	7.81	Antagonistic
	Q. acutissima	*V. infausta*	0.078	0.98	1.95	2.93	Indifference
Ethyl Acetate	*C. erythrophyllum*	*S. mauritianum*	0.005	0.12	0.12	**0.25**	Synergistic
	C. erythrophyllum	*N. glauca*	0.010	0.25	0.25	**0.49**	Synergistic
	C. erythrophyllum	*H. caffrum*	0.001	0.03	0.02	**0.05**	Synergistic
	C. erythrophyllum	*W. somnifera*	0.001	0.03	0.02	**0.05**	Synergistic
	C. erythrophyllum	*Q. acutissima*	0.001	0.03	0.02	**0.05**	Synergistic
	C. erythrophyllum	*V. infausta*	0.001	0.03	0.02	**0.05**	Synergistic
	S. mauritianum	*N. glauca*	0.039	0.98	0.98	1.96	Indifference
	S. mauritianum	*H. caffrum*	0.020	0.49	0.24	0.73	Additive
	S. mauritianum	*W. somnifera*	0.039	0.98	0.49	1.47	Indifference
	S. mauritianum	*Q. acutissima*	0.078	1.95	0.98	2.93	Indifference
	S. mauritianum	*V. infausta*	0.039	0.98	0.49	1.47	Indifference
	N. glauca	*H. caffrum*	0.313	7.81	3.91	11.72	Antagonistic
	N. glauca	*W. somnifera*	0.156	3.91	1.95	5.86	Antagonistic
	N. glauca	*Q. acutissima*	0.020	0.49	0.24	0.73	Additive
	N. glauca	*V. infausta*	0.039	0.98	0.49	1.47	Indifference
	H. caffrum	*W. somnifera*	0.078	0.98	0.98	1.95	Indifference
	H. caffrum	*Q. acutissima*	0.156	1.95	1.95	3.91	Indifference
	H. caffrum	*V. infausta*	0.156	1.95	1.95	3.91	Indifference

Extract	MIC Plant 1	MIC Plant 2	MIC	FIC 1	FIC 2	FICI	Remarks
	W. somnifera	*Q. acutissima*	0.001	0.02	0.02	**0.03**	Synergistic
	W. somnifera	*V. infausta*	0.001	0.02	0.02	**0.03**	Synergistic
	Q. acutissima	*V. infausta*	0.039	0.49	0.49	0.98	Additive
Acetone	*S. didymobotrya*	*C. erythrophyllum*	0.001	0.02	0.03	**0.05**	Synergistic
	S. didymobotrya	*M. azedarach*	0.001	0.02	0.02	**0.03**	Synergistic
	S. didymobotrya	*H. caffrum*	0.039	0.49	0.49	0.98	Additive
	S. didymobotrya	*W. somnifera*	0.001	0.02	0.02	**0.03**	Synergistic
	S. didymobotrya	*Q. acutissima*	0.002	0.03	0.03	**0.06**	Synergistic
	S. didymobotrya	*V. infausta*	0.005	0.06	0.12	**0.18**	Synergistic
	C. erythrophyllum	*M. azedarach*	0.001	0.03	0.02	**0.05**	Synergistic
	C. erythrophyllum	*H. caffrum*	0.001	0.03	0.02	**0.05**	Synergistic
	C. erythrophyllum	*W. somnifera*	0.001	0.03	0.02	**0.05**	Synergistic
	C. erythrophyllum	*Q. acutissima*	0.002	0.06	0.03	**0.09**	Synergistic
	C. erythrophyllum	*V. infausta*	0.001	0.03	0.03	**0.06**	Synergistic
	M. azedarach	*H. caffrum*	1.250	15.63	15.63	31.25	Antagonistic
	M. azedarach	*W. somnifera*	0.039	0.49	0.49	0.98	Additive
	M. azedarach	*Q. acutissima*	0.156	1.95	1.95	3.91	Antagonistic
	M. azedarach	*V. infausta*	0.039	0.49	0.98	1.47	Indifference
	H. caffrum	*W. somnifera*	0.039	0.49	0.49	0.98	Additive
	H. caffrum	*Q. acutissima*	1.250	15.63	15.63	31.25	Antagonistic
	H. caffrum	*V. infausta*	1.250	15.63	31.25	46.88	Antagonistic
	W. somnifera	*Q. acutissima*	0.156	1.95	1.95	3.91	Indifference
	W. somnifera	*V. infausta*	0.039	0.49	0.98	1.47	Indifference
	Q. acutissima	*V. infausta*	1.250	15.63	31.25	46.88	Antagonistic

Values highlighted in bold indicate strong synergistic interaction between extracts from different species with FICI value of less or equals to 0.5

4.4. CONCLUSIONS

The results of the study demonstrated that combining different extracts can improve antifungal potency. A combination of *C. erythrophyllum* and *Q. acutissima* acetone extract showed

synergistic antifungal activity against *F. proliferatum, F. solani*, *F. equisite*, *F. verticilloides* and *F. graminearum*. A combination of ethyl acetate extract from similar medicinal plant species also demonstrated sygergistic antifungal effect against *F. proliferatum, F. solani, F. subglutinans* and *F. verticilloides*. The above *Fusarium* species (*F. proliferatum, F. solani, F. subglutinans* and *F. verticilloides*) were also inhibited due to synergistic interation of combined acetone extract obtained from *C. erythrophyllum* and *M. azedarach*. The plant extract combinations showing additive and/or synergistic antifungal effect have potential to be developed into plant-based antifungal products for agricultural application, particularly in organic food production and smallholder farming. Plant extracts combination that showed very good activity against more *Fusarium* pathogens are of particular interest since they may be used to control several crop diseases in single treatment. The mixture of antifungal constituents or compounds in combined plant extracts may target different sites of actions, decrease the likelihood of the pathogens developing resistance, and may provide potential lead of novel compounds with different mode(s) of action. Application of medicinal plant extracts either as individual or in combination holds a great potential as an alternative source of affordable, accessible, sustainable, and environmentally-friendly bio-pesticides for controlling crop diseases in agricultural sector.

REFERENCES

Aberkane, A., Cuenca-Estrella, M., Gomez-Lopez, A., Petrikkou, E., Mellado, E., Monzón, A., Rodriguez Tudela, J.L., 2002. Comparative evaluation of two different methods of inoculums preparation for antifungal susceptibility testing of filamentous fungi. Journal of Antimicrobial Chemotherapy 50, 719–722.

Aktar, Md.W., Sengupta, D., Chowdhury, A., 2009. Impact of pesticides use in agriculture: their benefits and hazards. Interdisciplinary Toxicology 2, 1–12.

Alkhail, A.A.A., 2005. Antifungal activity of some extracts against some plant pathogenic fungi. Pakistan Journal of Biological Sciences 8, 413–41.

Amadioha, A.C., Obi, V.I., 1999. Control of anthranose disease of cowpea by *Cymbopogono citratus* and *Ocimum gratissimum*. Acta Phytopathologica et Entomologica Hungarica 34, 83–89.

Amoo, S.O., Finnie, J.F., Van Staden, J., 2009. *In vitro* pharmacological evaluation of three Barleria species. Journal of Ethnopharmacology 121, 274–277.

Bhattacharjee, I., Chatterjee, S.K., Ghosh, A., Chandra, G., 2011. Antibacterial activities of some plant extracts used in Indian traditional folk medicine. Asian Pacific Journal of Tropical Biomedicine 1, 165–169.

Bisignano, G., Tomaino, A., Cascio, R., Crisafi, G., Uccella, N., Saija, A., 1999. On the in vitro antimicrobial activity of oleuropein and hydroxytyrosol. Journal of Pharmacy and Pharmacology 51, 971–974.

Buwa, L.V., van Staden, J., 2006. Antibacterial and antifungal activity of traditional medicinal plants used against venereal diseases in South Africa. Journal of Ethnopharmacology 103, 139–142.

Calvo, M.A., Arosemena, E.L., Shiva, C., Adelantado, C., 2011. Antimicrobial activity of plant natural extracts and essential oils. In: A. Méndez-Vilas (Ed.), Science against microbial

pathogens: Communicating Current Research and Technological Advances pp. 1179–1185.

Chen, Y., Li, H., Chen, C.J., Zhou, M.G., 2008. Sensitivity of Fusarium graminearum to fungicide JS399-19: in vitro determination of baseline sensitivity and the risk of developing fungicide resistance. Phytoparasitica 36, 326–337.

Chen, Y., Zhou, M. G., 2009. Characterization of *Fusarium graminearum* isolates resistant to both carbendazim and anew fungicide JS399-19. Phytopathology, 99, 441–446.

Chen, Z., Gao, T., Liang, S., Liu, K., Zhou, M., Chen, M., 2014. Molecular mechanism of resistance of *Fusarium fujikuroi* tobenzimidazole fungicides. Federation of European Microbiological Societies Letter 357, 77–84.

Daferera, D.J., Ziogas, B.N., Polissiou, M.G., 2003. The effectiveness of plant essential oils on the growth of *Botrytis cinerea, Fusarium* sp. and *Clavibacter michiganensis* subsp. *michiganensis*. Crop Protection 22, 39–44.

Das, L., Godbole, S., 2015. Antifungal and phytochemical analysis of *Lantana camara*, *Citrus limonum* (Lemon), *Azadirachta indica* (Neem) and *Hibiscus rosasinensis* (China Rose). Journal of Pharmacy Research 9, 476–479.

Eloff, J.N., 1998. Which extractant should be used for the screening and isolation of antimicrobial components from plants? Journal of Ethnopharmacology 60, 1–8.

Etebarian, H.R., 1992. Studies of *Fusarium* wilt of tomato and its chemical control in Varamin area. Iranian Journal of Agriculture Science 23, 1–14.

Fandohan, P., Gbenou, J.D., Gnonlonfin, B., Hell, K., Marasas, W.F., Wingfield, M.J., 2004. Effect of essential oils on the growth of *Fusarium verticillioides* and fumonisin contamination in corn. Journal of Agricultural and Food Chemistry 52, 6824–6829.

Fandohan, P., Gnonlonfin, B., Hell, K., Marasas, W.F.O., Wingfield, M.J., 2005. Natural occurrence of *Fusarium* and subsequent fumonisin contamination in preharvest and

stored maize in Benin, West Africa. International Journal of Food Microbiology 99, 173–183.

FAO, IFAD, UNICEF, WFP, WHO., 2018. The State of Food Security and Nutrition in the World 2018. Building Climate Resilience for Food Security and Nutrition. Rome, FAO. Licence: CC BY-NC-SA 3.0 IGO.

Freije, A. N., Wise, K. A., 2015. Impact of *Fusarium graminearum* inoculums availability and fungicides application timing on *Fusarium* head blight in wheat. Crop Protection 77, 139-–147.

Hadian, S., Rahnama, K., Jamali, J., Eskandari, A., 2011. Comparing neem extract with chemical control on *Fusarium oxysporum* and *Meloidogyne incognita* complex of tomato. Advances in Environmental Biology 5, 2052–2057.

Harris, C.A., Renfrew, M.J., Woolridge, M.W., 2001. Assessing the risk of pesticide residues to consumers: recent and future developments. Food Additives and Contaminants 18, 1124–1129.

Jackson-Ziems, T.A., Giesler, L.J., Adesemoye, A.O., Harveson, R.M., Wegulo, S.N., 2017. "Understanding Fungicide Resistance". Papers in Plant Pathology 481.

Jeruto, P., Arama, P.F., Anyango, B., Akenga, T., Nyunja, R., Khasabuli, D., 2016. In vitro antifungal activity of methanolic extracts of different *Senna didymobotrya* (Fresen.) H.S. Irwin & Barneby plant parts. African Journal of Traditional, Complementary and Alternative Medicines 13, 168–174.

Kamali, H.H.E.L., Amir, M.Y.E.L., 2010. Antibacterial activity and phytochemical screening of ethanolic extracts obtained from selected Sudanese medicinal plants. Current Research Journal of Biological Science 2, 143–146.

Karuppaiyan, R., Ram, B., Ramdiya, S., Ali, M., Meena, M., 2015. The incidence of Pokkah boeng in indigenous and exotic sugarcane (*Saccharum officinarum*) clones. Indian Journal of Agricultural Sciences 85, 596–601.

Kathireshan, A.K., Priya, T., Jasmine, K.D., Gayathri, G., Kumar, M.R.R., 2019. Assessment of in vitro antibacterial and antifungal activities of leaf extracts of *Melia azedarach* Linn. Indian Journal of Pharmaceutical Sciences 81, 380–384.

Kumar, P., Kumaravel, S., Lalitha, C., 2010. Screening of antioxidant activity, total phenolics and GC-MS study of Vitex negundo. African Journal of Biochemistry Research 4, 191–195.

Le Toutour, B., Guedon, D., 1992. Antioxidative activities of *Olea europaea* leaves and related phenolic compounds. Phytochemistry 31, 1173–1178.

Leslie, J.F., Summerell, B.A., 2006. The Fusarium laboratory manual, Blackwell, Ames.

Liu, Y., McKeever, C.L., Malik, N.S.A., 2017. Assessment of the antimicrobial activity of olive leaf extract against foodborne bacterial pathogens. Frontiers in Microbiology 8, 113.

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, 1529–1533.

Marasas, W.F.O., 1995. Fumonisins: their implications for human and animal health. Natural Toxins 3, 193-198.

Maroyi, A., 2018. Nutraceutical and ethnopharmacological properties of *Vangueria infausta* subsp. *infausta*. Molecules 23, 1089.

Martínez, J.A., 2012. Natural Fungicides Obtained from Plants, Fungicides for Plant and Animal Diseases, Dr. Dharumadurai Dhanasekaran (Ed.), ISBN: 978-953-307-804-5, InTech.

Masoko, P., Eloff, J.N., 2006. Bioautography indicates the multiplicity of antifungal compounds from twenty-four southern African *Combretum* species (Combretaceae). African Journal of Biotechnology 18, 1625–1647.

Masoko, P., Makgapeetja, D.M., 2015. Antibacterial, antifungal and antioxidant activity of *Olea africana* against pathogenic yeast and nosocomial pathogens. BMC Complementary and Alternative Medicine 15, 409.

Masoko, P., Picard, J., Eloff, J.N., 2005. Antifungal activities of six South African *Terminalia* species (Combretaceae). Journal of Ethnopharmacology 99, 301–308.

Mbukwa, E., Chacha, M., Majinda, R.R.T., 2007. Phytochemical constituents of *Vangueria infausta*: their radical scavenging and antimicrobial activities. Archive for Organic Chemistry 9, 104–112.

Mdee, L.K., Masoko, P., Eloff, J.N., 2009. The activity of extracts of seven common invasive plant species on fungal phytopathogens. South African Journal of Botany 75, 375–379.

Meziane, M., Goumri, H., 2014. The antimicrobial effect of extracts of *Melia azedarach* on some pathogenic microorganisms. International Journal of Applied and Natural Sciences 4, 173–180.

Moussaoui, F., Alaoui, T., 2016. Evaluation of antibacterial activity and synergistic effect between antibiotic and the essential oils of some medicinal plants. Asian Pacific Journal of Tropical Biomedicine 6, 32–37.

Nciki, S., Vuuren, S., van Eyk, A., de Wet, H., 2016. Plants used to treat skin diseases in Northern Maputaland, South Africa: antimicrobial activity and in vitro permeability studies. Pharmaceutical Biology 54, 2420–2436.

Nedelcu, L., Alexandri, A.A., 1995. Synergistic effect between metamidoxime and copper oxychloride. Probleme de Protectia Plantelor 23, 13–21.

Nwankwo, B.O.K., Abdulhadi, S., Magagi, A., Gabriel, I., 2010. Methicillin resistant *S. aureus* (MRSA) and their antibiotic sensitivity pattern in Kano Nigeria. African Journal of Clinical and Experimental Microbiology 1, 129–136.

Olajuyigbe, O.O., Afolayan, A.J., 2012. Synergistic interactions of methanolic extract of *Acacia mearnsii* De Wild. with antibiotics against bacteria of clinical relevance. International Journal of Molecular Sciences 13, 8915–8932.

Padvi, S.A., Hingole, D.G., Khaire, P.B., 2018. In vitro efficacy of fungicides against *Fusarium solani* incited by dry root rot of sweet orange. Journal of Pharmacognosy and Phytochemistry 7, 3270–3273.

Park, M.H., Hwang, S.J., Ahn, C.Y., Kim, B.H., Oh, H.M., 2006. Screening of seventeen oak extracts for the growth inhibition of the cyanobacterium *Microcystis aeruginosa* Kütz em. Elenkin. Bulletin of Environmental Contamination and Toxicology 1, 9–14.

Patel, V., Suthar, M., Pande, V., Arote, S., 2018. In-vitro screening of *Withania somnifera* leaf and root extracts for antifungal activities using NCCLS assay. Inventi Rapid: Pharmaceutical Biotechnology and Microbiology 1, 1–4.

Pavarini, D.P., Pavarini, S.P., Niehues, M., Lopes, N.P., 2012. Exogenous influences on plant secondary metabolite levels. Animal Feed Science and Technology 176, 5–16.

Pizana, C.G., Necha, L.L.B., Gomez, M.Y.R., 2010. Evaluation of the fungicidal activity of leaves powders and extracts of fifteen Mexican plants against *Fusarium oxysporum f. sp. gadioli* (Massey) Snyder and Hansen. Journal of Plant Pathology 9, 103–111.

Popiel, D., Dawidziuk, A., Koczyk, G., Mackowiak, A., Marcinkowska, K., 2017. Multiple facets of response to fungicides – the influence of azole treatment on expression of key mycotoxin biosynthetic genes and candidate resistance factors in the control of resistant *Fusarium* strains. European Journal of Plant Pathology 147, 773–785.

Prithiviraj, B., Singh, U.P., Khiste, S., Ram, D., 1996. Effect of methanol extract of *Aegle marmelos* leaves on *Sclerotiumrolfsii*. International Journal of Pharmacognosy 34, 148–150.

Ramaiah, K.A., Garampalli, R.K.H., 2015. *In vitro* antifungal activity of some plant extracts against *Fusarium oxysporum f. sp. Lycopersici*. Asian Journal of Plant Science and Research 5, 22–27.

Singh, J., Dubeyd, A.K., Tripathi, N.N., 1994. Antifungal activity of *Mentha spicata*. Pharmaceutical Biology 32, 314–319.

Stefanović, O., Comic, L., 2012. Synergistic antibacterial interaction between *Melissa officinalis* extracts and antibiotics. Journal of Applied Pharmaceutical Science 02, 01–05.

Uddin, Q., Samiulla, L., Singh, V.K., Jamil, S.S., 2012. Phytochemical andpharmacological profile of *Withania somnifera* Dunal: A review. Journal of Applied Pharmaceutical Science 2, 170–175.

Van Vuuren, S.F, Viljoen, A.M., 2011. Plant-based antimicrobial studies-methods and approaches to study the interaction between natural products. Planta Medica 77, 1168–1182.

Wilson, C.L., Solar, J.M., Ghaouth, A. 1997. Rapid evaluation of plant extracts and essential oils for antifungal activity against *Botrytis cinerea*. Plant Disease 81, 204–210.

Xu, S., Wang, J., Wang, H., Bao, Y., Li, Y., Govindaraju, M., Yao, Y., Chen, B., Zhang, M., 2019. Molecular characterization of Carbendazim resistance of *Fusarium* species complex that causes sugarcane Pokkah boeng disease. BMC Genomics 20, 115.

Yan, K., Dickman, M.B., 1996. Isolation of a β-tubulin gene from *Fusarium moniliforme* that confers cold-sensitive Benomyl resistance. Applied and Environmental Microbiology 62, 3053–3056.

Yildiz, G., Uylaser, V., 2011. A natural antimicrobial: Oleuropein. Journal of Agricultural Faculty of Uludag University 25, 131–142.

Zhang, M., Jeyakumar, J.M.J., 2018. *Fusarium* Species Complex Causing Pokkah Boeng in China, *Fusarium* - Plant Diseases, Pathogen Diversity, Genetic Diversity, Resistance and Molecular Markers, Tulin Askun, Intech Open, DOI: 10.5772/intechopen.73133.

CHAPTER 5

IN VIVO ANTIFUNGAL ACTIVITY OF MEDICINAL PLANT EXTRACTS AGAINST *FUSARIUM* PATHOGENS AND THEIR PHYTOTOXICITY ON MAIZE SEEDS GERMINATION AND SEEDLINGS GROWTH

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In Vivo Antifungal Activity of South African Medicinal Plant Extracts against *Fusarium* pathogens and Their Phytotoxicity Evaluation

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Abstract: Smallholder farmers play a major role in crop production towards household food security, particularly in resource-poor communities. Maize is a common crop produced in smallholder farming and it is cultivated from seeds that has been stored and re-used for years. Spoilage of stored grains is a major challenge, which leads to yield loss and poor seed quality. The objectives of this study were to evaluate in vivo antifungal activity of selected plant extracts against *Fusarium* pathogens on maize seeds, and to evaluate their phytotoxicity on seed germination and seedling growth. Fresh leaves collected from eight medicinal plants were dried and selectively extracted with water, ethyl acetate or acetone. The dried extracts were evaluated for antifungal activity against *Fusarium* pathogens (*F. proliferatum*, *F. oxysporum*, *F. subglutinans*, *F. verticilloides*, *F. semitectum*, *F. chlamydosporum*, *F. solani*, *F. equisite* and *F. graminearum*) inoculated on maize seeds. *Melia azedarach* acetone extract showed strong antifungal activity (97% inhibition) against *F. proliferatum* while combined acetone extracts from *Combretum erythrophyllum* and *Quercus acutissima* exhibited 96%, 67% and 56% inhibition against *F. verticilloides*, *F. proliferatum* and *F. solani*, respectively. With the exception of *Quercus acutissima* ethyl acetate, none of the extracts significantly inhibited seed germination when compared to untreated seeds. This study showed that plant extracts could control *Fusarium* diseases without any adverse effects on maize seed germination or plant growth.

Keywords: *Fusarium* species; maize seeds; medicinal plant extracts; seed germination; smallholder farmers

ABSTRACT

Smallholder farmers play a major role in crop production towards household food security, particularly in resource-poor communities. Maize is a common crop produced in this farming system and is cultivated from seeds stored from year to year. Spoilage of stored grains is a major challenge, which leads to yield loss and poor seed quality. The objectives of this study were to evaluate *in vivo* antifungal activity of selected plant extracts against *Fusarium* pathogens on maize seeds, and to evaluate their phytotoxicity on seed germination and seedling growth. Fresh leaves collected from eight medicinal plants were dried and selectively extracted with water, ethyl acetate or acetone. The dried extracts were evaluated for antifungal activity against *Fusarium* pathogens (*F. proliferatum, F. oxysporum, F. subglutinans, F. verticilloides, F. semitectum, F. chlamydosporum, F. solani, F. equisite* and *F. graminearum*) inoculated on maize seeds. *Melia azedarach* acetone extract showed strong antifungal activity (97% inhibition) against *F. proliferatum* while combined acetone extracts from *Combretum erythrophyllum* and *Quercus acutissima* exhibited 96, 67 and 56% inhibition against *F. verticilloides, F. proliferatum* and *F. solani*, respectively. With the exception of *Quercus acutissima* ethyl acetate, none of the extracts significantly inhibited seed germination when compared to untreated seeds. This study shows the potential for developing plant extract-based products that could be used during maize seed storage for their protection against *Fusarium* pathogens. The use of leaf extracts is relatively inexpensive and a sustainable source of natural agents to control post-harvest diseases.

Keywords: *Fusarium* species, maize seeds, medicinal plant extracts, seed germination, smallholder farmers

5.1. INTRODUCTION

Maize (*Zea mays L*) is the most important grain crop and dietary staple food in the world (Smale *et al*., 2001; Chulze, 2010). It has potential to alleviate poverty and plays a key role in food security and economic well-being particularly in sub-Saharan Africa. In most developing countries, maize is consumed primarily as a porridge and sometimes as freshly boiled or roasted grains. It is also consumed in processed form such as snacks and cereals. Naturally, it is a source of carbohydrate, fat, fibre, vitamins, macro- and micronutrient elements (Chaudhary, 1983). In addition to its utilization as feed for livestock and poultry, maize is an excellent source of raw material for many industrial products such as starch, oil, organic liquids and alcoholic beverages (Pimentel and Patzek, 2005; Muimba-Kankolongo, 2018). Maize is cultivated in over 48 African countries, with more than 208 million people in sub-Saharan Africa depending on it as a source of food and income (Abate *et al*., 2017). In South Africa, both commercial and smallholder-farming systems are responsible for the production of maize, although this production is dominated by commercial farming. In addition to the availability of cultivated land, commercial farmers rely on irrigation systems, fertilization, pesticides, improved maize cultivars or hybrids and modern machineries to optimize production. In contrast, maize production in smallholder farming is labour-intensive and almost fully rain-fed. Smallholder farmers depend on indigenous knowledge and traditional rudimentary methods to control crop diseases both in the field and during storage.

Smallholder farming system is faced with challenges including climate change, crop diseases, spoilage of grains during storage and poor seed germination (Mungai *et al*., 2016). These challenges, among others, may result in drastic yield loss and an increase in food insecurity and food prices. Nevertheless, smallholder farming remains an important source of food and income generation, particularly in poor rural communities (Singh *et al*., 2002; Baiphethi and Jacobs, 2009; Salami *et al*., 2010; Crush *et al*., 2011). Successful maize production in

smallholder farming is dependent on local seed system. After harvest, the seeds are stored, exchanged or sold to other community members for consumption or for cultivation during the next planting season. This household-based seed system approach is self-sustainable (Sperling *et al*., 2004; McGuire and Sperling, 2016). However, it is threatened by microbial infections that often occur during cultivation, transportation and post-harvest storage (Kusena *et al*., 2017). Microbial infections can reduce yield, nutritional value of the seeds and negatively affect seed germination (Govender *et al*., 2008).

Fusarium species including *F. verticillioides, F. subglutinans* and *F. proliferatum* are among spoilage pathogens associated with yield loss in maize production (Munkvold and Desjardins, 1997; Munkvold and Carlton, 1997). Another major concern is health complications associated with consumption of grains contaminated with mycotoxins produced by these species (Marasas *et al*., 1984; Nelson *et al*., 1993; Desjardin, 2006). Mycotoxins may cause fungal keratitis, kidney disorders, oesophageal and liver cancer (Marasas *et al*., 1984; Rocha *et al*., 2014; Desjardin, 2006; Sun *et al*., 2007; Cumagun *et al*., 2009). The use of conventional synthetic fungicides as a strategy to control fungi during storage may not be ideal in smallholder farming. These chemicals are largely inaccessible and unaffordable to smallholder farmers. Smallholder farming is practiced in poor-resource communities where stored grains are consumed during storage period. Therefore, seeds or grains treated with synthetic fungicides may cause complications or food poisoning. There is a need to develop relatively cheap and sustainable strategies that can be used to control or reduce grain spoilage particularly during storage in poor rural communities.

The use of botanicals as an alternative source of bio-pesticides in crop protection has gained momentum because plants are biodegradable and readily available (Cherry *et al*., 2005; Isman, 2006; Sukanya *et al*., 2011). Medicinal plant species synthesize different secondary metabolites that perform important biological functions and defend plants against microbes and insects

(Chopra *et al*., 1992; Harborne *et al*., 1995; Ahmad and Beg, 2001). Plants have been traditionally used for many years to treat different ailments in both human and domesticated animals, and they are considered to be relatively safe and environmental-friendly (Masika and Afolayan, 2002; Khan and Nasreen, 2010; Sukanya *et al*., 2011). Therefore, the aim of this study was to evaluate *in vivo* antifungal activity of extracts obtained from the leaves of eight selected medicinal plants against different maize seed *Fusarium* pathogens. Extract selection was based on previous *in vitro* study against the same pathogens (Seepe *et al*., 2019; Seepe *et al*., 2020). Promising extracts were evaluated for their phytotoxicty during seed germination and seedling growth.

5.2. MATERIALS AND METHODS

5.2.1. Collection and disinfection of maize Seeds

White maize seeds were obtained from smallholder farmer at Moletjie, ga-Maleka, in Limpopo Province of South Africa. The seeds were collected two weeks after harvest and were visually examined for spoilage, mechanical and weevil damages. Disinfection of the seeds was achieved by soaking them in bleach (3.5% sodium hypochlorite) for 10 minutes, rinsed several times with sterilized water and then sprayed with 70% ethanol. Thereafter, they were air-dried aseptically in a bio-safety cabinet.

5.2.2. Collection of plant materials

Eight medicinal plant species (*Withania somnifera*, *Combretum molle*, *Combretum erythrophyllum*, *Quercus acutissima*, *Solanum mauritianum*, *Melia azedarach*, *Lantana camara* and *Nicotiana glauca*) were selected based on their *in vitro* antifungal activities against different *Fusarium* species (Seepe *et al*., 2019; Seepe *et al*., 2020). The leaves of these plants

were collected in October and November 2016 from naturally growing plants at the Agricultural Research Council, Roodeplaat, Pretoria, Gauteng Province and from Capricorn district in Limpopo Province, South Africa. Their identification was confirmed through consultation with Dr. Bronwyn Egan (Larry Leach Herbarium Curator, University of Limpopo) and their voucher specimens were prepared and deposited as previously described by Seepe *et al*., (2019 and 2020).

5.2.3. Preparation of plant extracts

Fresh green leaves (about 5.0 kg) were collected into brown paper bags and shade dried immediately at room temperature (25 ± 2 °C). Dried material was grinded into fine powder using a pulveriser (Fritsch Pulverisette 14, Labotec, South Africa) and stored in brown paper bags until extraction. Extraction was initiated by adding 100 g of dried powder into an Erlenmeyer flask, followed by addition of 1000 ml solvent. The material was extracted non-sequentially with water, ethyl acetate or acetone in an ultrasonic bath (Branson, 5510E-MT, Lasec, South Africa) for an hour. The mixture was filtered through Whatman No.1 filter paper. The residue was re-extracted with equivalent volume of solvent. Ethyl acetate and acetone extracts were concentrated using rotary evaporators (Stuart, RE300DB, Lasec, South Africa) and air-dried further in a fume hood. Water extracts were freeze-dried (Sentry 2.0 VirTis SP scientific). All the dried extracts were kept in airtight containers in the dark.

5.2.4. Preparation of *Fusarium* athogens

Mycology Laboratory at the Agricultural Research Council - Plant Health and Protection, Roodeplaat in Pretoria provided *F. proliferatum* (PPRI 18679), *F. semitectum* (PPRI 6739), *F. oxysporum* (PPRI 10175), *F. subglutinans* (PPRI 6740), *F. chlamydosporum* (PPRI 5116), *F.*

equiseti (PPRI 19029), *F. verticillioides* (PPRI 9278), F. *solani* (PPRI 19,147) and *F. graminearum* (PPRI 10,728) used in this study. Fungal strains were sub-cultured on potato dextrose agar (Merck, South Africa) and incubated at 27 °C for four to seven days. Thereafter, pathogen suspensions were prepared in potato dextrose broth (Merck, South Africa), which were incubated for three to four days. Fungal spores were collected by straining cultured broth through sterilized cheesecloth. The number of spores was determined using a microscope and haemocytometer, and appropriate dilutions were made to adjust final spore concentration to 1.0 x 10^6 spores/ml (Aberkane *et al*., 2002; Mahlo *et al*., 2010).

5.2.5. *In vivo* activity against maize seed inoculated with pathogens

Approximately 7.5 grams of disinfected maize seeds was added to a sterilized jar, and inoculated with *Fusarium* pathogen (1000 µl) adjusted to 1.0 x 10^6 spores/ml. The seeds were shaken for few seconds and air-dried aseptically in a bio-safety cabinet for an hour. Thereafter, 1000 µl of medicinal plant extract at 2.5 mg/ml (combined or individual) was added, shaken well and dried for an additional hour. The selection of combined plant extracts was based on their additive and synergistic antifungal activity against tested *Fusarium* pathogens as was reported in the previous study (Seepe *et al*., 2020).

The experiment was repeated twice and each treatment was replicated thrice for every fungal pathogen. Amphotericin B (Phytotek Lab, South Africa) and 10% aqueous acetone (used to dissolve the dried plant extracts) were included as positive and negative controls, respectively. A mixture of seeds and plant extract was included for each treatment and was used for colour or turbidity correction. The jars were sealed and incubated at 27 °C for four days. After incubation period, 20 ml of sterile water was added to each jar and shaken for one min. The treatments were filtered through cheesecloths and their absorbance measured at 700 nm using

Specord 210 spectrophotometer (Analytik Jena, Germany). Antifungal activity of the extracts was recorded as percentage inhibition of fungal growth, calculated from the absorbance readings of the control and treatment using the following equation:

$$\text{Antifungal activity (\%)} = \left(\frac{\text{C} - (\text{T} - \text{B})}{\text{C}}\right) \text{X } 100$$

where C is the absorbance reading of the negative control (seeds treated with 10% aqueous acetone and inoculated with fungal pathogen); T is the absorbance reading of the treatment (seeds treated with plant extract or amphotericin B, and inoculated with fungal pathogen); and B is the absorbance reading of the blank (seeds treated with plant extract or amphotericin B only).

5.2.6. *In vivo* activity against pathogen inoculated on maize seedlings

A combination of *Combretum erythrophyllum* and *Quercus acutissima* acetone extract was evaluated against *F. verticilloides* inoculated on maize seedlings. The extract was selected based on its remarkable antifungal activity exhibited against *F. verticilloides*. Moreover, it showed very strong synergistic antifungal activity against more *Fusarium* pathogens as compared to other combinations as was reported in the previous study (Seepe *et al*., 2020). Maize seeds were treated with a combination of *C. erythrophyllum* and *Q. acutissima* acetone extract (2.5 mg/ml) and air-dried in a bio-safety cabinet. Each seed was planted in 25 cm pot filled with moistened sterilized red soil. The experiment or trial was laid out randomly with ten replications and it consist of untreated seed, plant extract treated seed and commercial fungicide (Efekto-Virikop®) treated seed. After planting, 200 ml of *F. verticilloides* at 1.0 x 10^6 fungal spore/ml was added to pots with treated seeds and untreated seeds. The second set of untreated seed received 200 ml water and served as negative control.

The experiment was conducted at the Agricultural Research Council, Roodeplaat Campus, Pretoria, South Africa (latitude 17º 49'S, longitude 31º 04'E), during November/December 2019 and was kept under greenhouse conditions (27.5 ± 2.5 °C and 90.0 ± 5% relative humidity). Four days after planting, 200 ml of combined *C. erythrophyllum* and *Q. acutissima* acetone extract was applied to each of the treatment pots and subsequently every seventh day, while water was added to negative control pots. Commercial fungicide (Efekto-Virikop®) at 2.5 mg/ml was also applied to positive control pots at the same interval as plant extract. All the pots were irrigated every second day.

Plant growth parameters (number of leaves, chlorophyll content, stem diameter and plant height) were recorded 20 days after planting and thereafter, were recorded every week. The number of leaves were counted and plant height was measured with tape from soil level to the terminal of the developing leaf. Chlorophyll content was measured from three mature leaves per plant using chlorophyll meter (Minolta, Spad-502) and stem diameter was measured with electronic digital vernier caliper (Calibre MILIM, Digital, Linberts).

After 90 days of planting, the plants were harvested and placed in brown paper bags. Pathogenicity test was also conducted during harvesting period. The crop was cut above soil surface and visually examined for discolouration. Discolouration symptom or disease severity was rated and recorded based on the following scale: 0 = no discolouration, 1 = trace to 25% diseased area, 2 = 25 to 50% diseased area, 3 = 50 to 80% diseased area and 4 = 100% diseased area (Ledingham *et al*., 1973; Saseetharan and Zakaria, 1978). The root system was carefully removed and washed off soil particles. The harvested materials were oven-dried at 45 °C. At the end of the experiment, data recorded from 20 days after planting to harvesting period were averaged and treated statistically.

5.2.7. Phytotoxicity evaluation of plant extracts maize seed germination

Medicinal plant extracts that showed antifungal activity (≥ 50% inhibition) were evaluated for potential phytotoxicity on maize seed germination. The seeds were soaked overnight in a plant extract at pre-determined concentration (2.5 mg/ml) and air-dried in a bio-safety cabinet for an hour. Water was used as a control treatment. Twenty seeds were placed per petri dish lined with a moistened double layer filter paper. Each treatment was replicated five times. The experiment was set up in an incubator at constant 25 °C and alternating cycle of 12 h light and 12 h darkness. The filter papers were kept wet throughout the experimental period. The number of germinated seeds was recorded 14 days after sowing. The experiment was repeated twice with five replicates per treatment. Percentage seed germination was calculated using the following equation:

$$\text{Percentage seed germination} = \left(\frac{\text{Number of germinated seeds}}{\text{Total number of seeds}}\right) \text{X } 100$$

5.2.8. Phytotoxicity evaluation of plant extracts maize seedling growth

Maize seeds were treated with a combined *C. erythrophyllum* and *Q. acutissima* acetone extract and air-dried in a bio-safety cabinet. Each treated seed was planted in 25 cm pots filled with moistened sterilized red soil. Untreated seeds were included as negative control. The experiment was conducted at the same greenhouse conditions as detailed in section 5.2.7 above and it was replicated ten times. Four days after planting, 200 ml of a combined *C. erythrophyllum* and *Q. acutissima* acetone extract (2.5 mg/ml) was applied to each of the treatment pots and subsequently every seventh day, while water was added to control pots. All the pots were irrigated every second day. Plant growth parameters were recorded, averaged and treated as described in section 4.5.2.

5.2.9. Statistical analysis

The difference between the treatments for each parameter was evaluated using one-way analysis of variance (ANOVA). Data were expressed as mean ± standard error. Where statistical significance ($p = 0.05$) was established, mean separation was done using Duncan's Multiple Range Test (DMRT). The difference in growth parameter between negative control and plant extract in the seedling phytotoxicity experiment was analysed using Student's *t*-test ($p \leq 0.05$).

5.3. RESULTS

5.3.1. Antifungal activity against maize seeds inoculated with *Fusarium* pathogens

An individual application of *Combretum erythrophyllum* ethyl acetate, *Quercus acutissima* ethyl acetate and *Melia azedarach* acetone extracts showed antifungal activity of more than 50% inhibition against *F. proliferatum* (Figure 5.1). However, their corresponding different solvent extractions were less active (≤ 50% inhibition) against the same pathogen. The combined application of *C. erythrophyllum* and *Q. acutissima* acetone extract showed a somewhat synergistic antifungal activity (66.9% inhibition), against *F. proliferatum*. There was no significant difference in terms of antifungal activity observed between positive control (97.1% inhibition) and *M. azedarach* acetone extract (97.1% inhibition) against *F. proliferatum* (Figure 5.1).

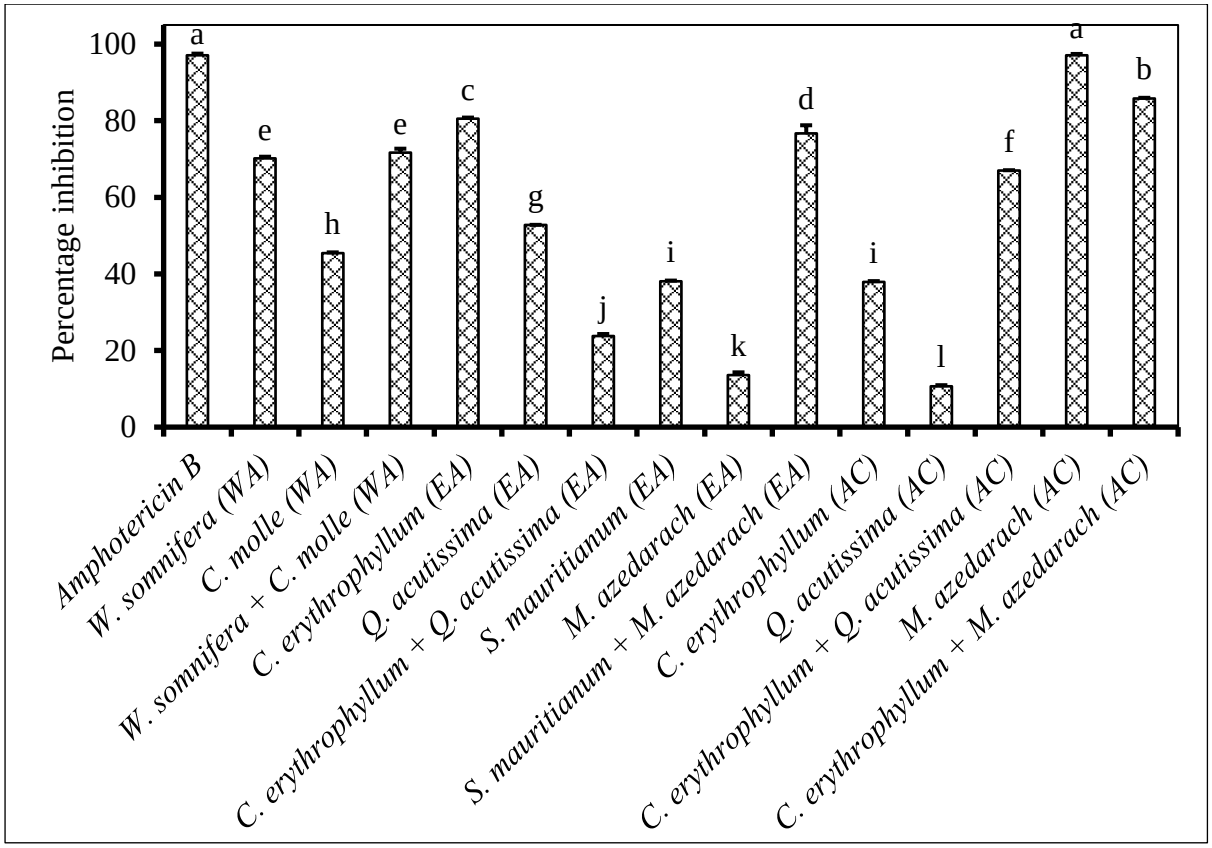


Figure 5.1. Percentage inhibition by plant extracts against *Fusarium proliferatum* inoculated on maize seeds. Bars bearing different letters indicate significant difference (p = 0.05). WA: Water, EA: Ethyl acetate and AC: Acetone. The extracts were produced from *Withania somnifera*, *Combretum molle*, *Combretum erythrophyllum*, *Quercus acutissima*, *Solanum mauritianum*, *Melia azedarach* and used at a concentration of 2.5 mg dried extract/mL of 10% acetone.

Application of individual extracts from *C. erythrophyllum* and *Q. acutissima* demonstrated poor (<50% inhibition) antifungal activity against both *F. subglutinans* and *F. verticilloides* (Figures 5.2 and 5.3). The combination of *C. erythrophyllum* and *Q. acutissima* ethyl acetate extract appeared to show a synergistic activity with 67% inhibition against *F. subglutinans* (Figure 5.2). Similarly, a combination of *C. erythrophyllum* and *Q. acutissima* acetone extracts

showed strong, synergistic antifungal activity (96% inhibition) against *F. verticilloides*. Notably, this activity was significantly higher than that of amphotericin B used as a positive control (Figure 5.3).

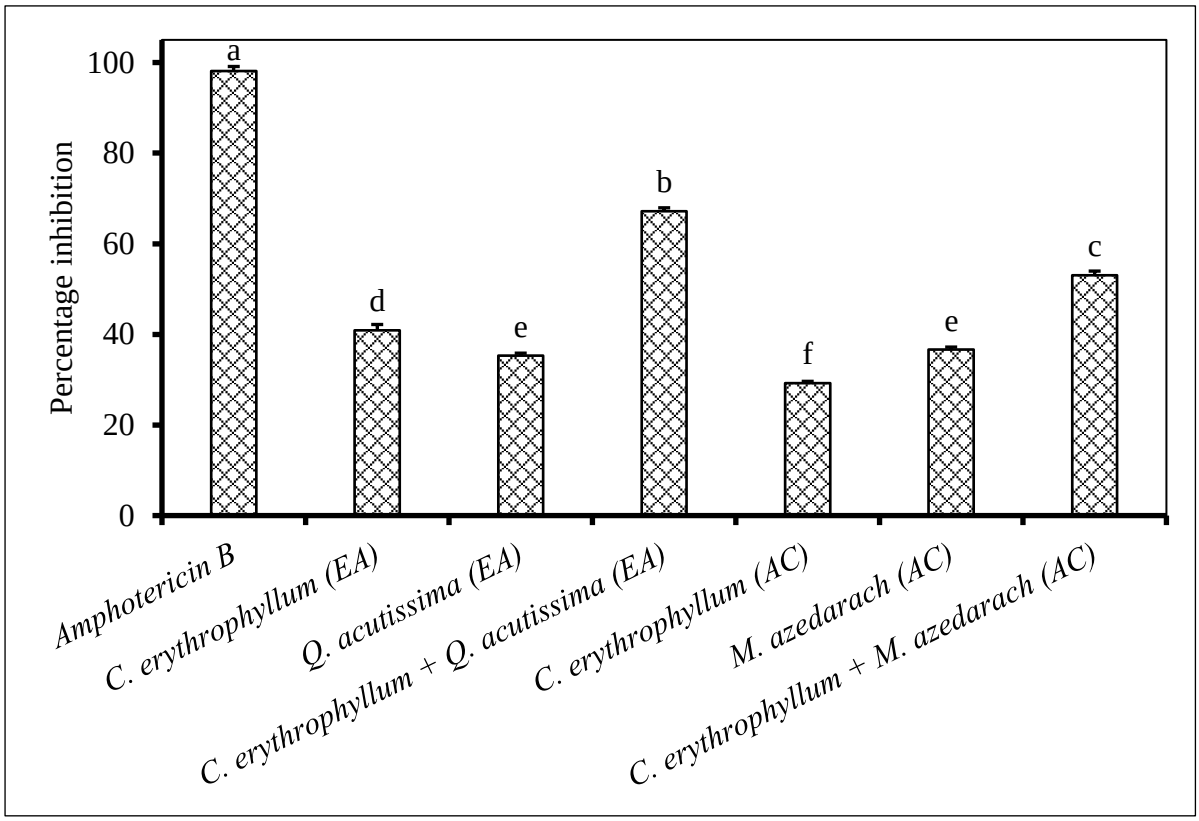


Figure 5.2. Percentage inhibition by plant extracts against *Fusarium subglutinans* inoculated on maize seeds. Bars bearing different letters indicate significant difference (p = 0.05). EA: Ethyl acetate and AC: Acetone. The extracts were produced from *Combretum erythrophyllum, Quercus acutissima, Melia azedarach* and used at a concentration of 2.5 mg dried extract/mL of 10% acetone.

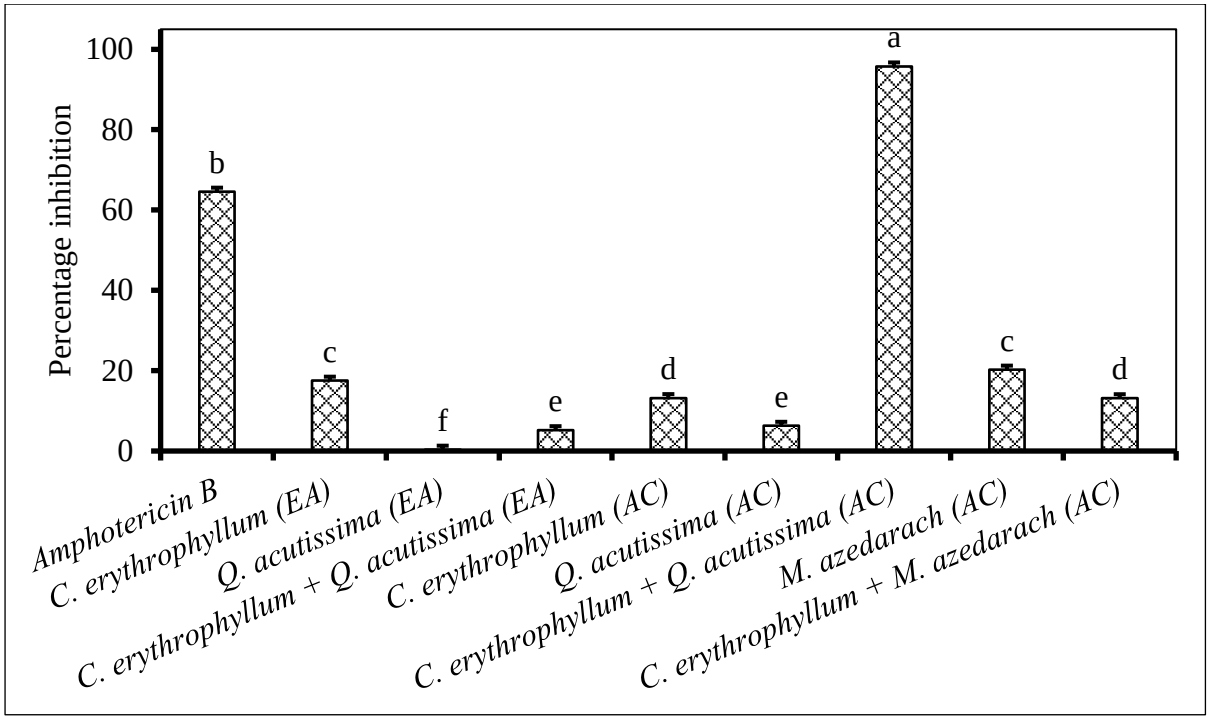


Figure 5.3. Percentage inhibition by plant extracts against *Fusarium verticilloides* inoculated on maize seeds. Bars bearing different letters indicate significant difference (p = 0.05). EA: Ethyl acetate and AC: Acetone. The extracts were produced from *Combretum erythrophyllum, Quercus acutissima, Melia azedarach* and used at a concentration of 2.5 mg dried extract/mL of 10% acetone.

Both individual application of acetone and ethyl acetate extracts from *C. erythrophyllum* showed poor antifungal activity against *F. solani* (Figure 5.4). The combination of acetone or ethyl acetate extracts of *C. erythrophyllum* and *Q. acutissima* resulted in a significantly improved antifungal activity when compared to their individual activity against *F. solani*. Similarly, a combination of *C. erythrophyllum* and *M. azedarach* acetone extracts resulted in a significantly improved antifungal activity (Figure 5.4).

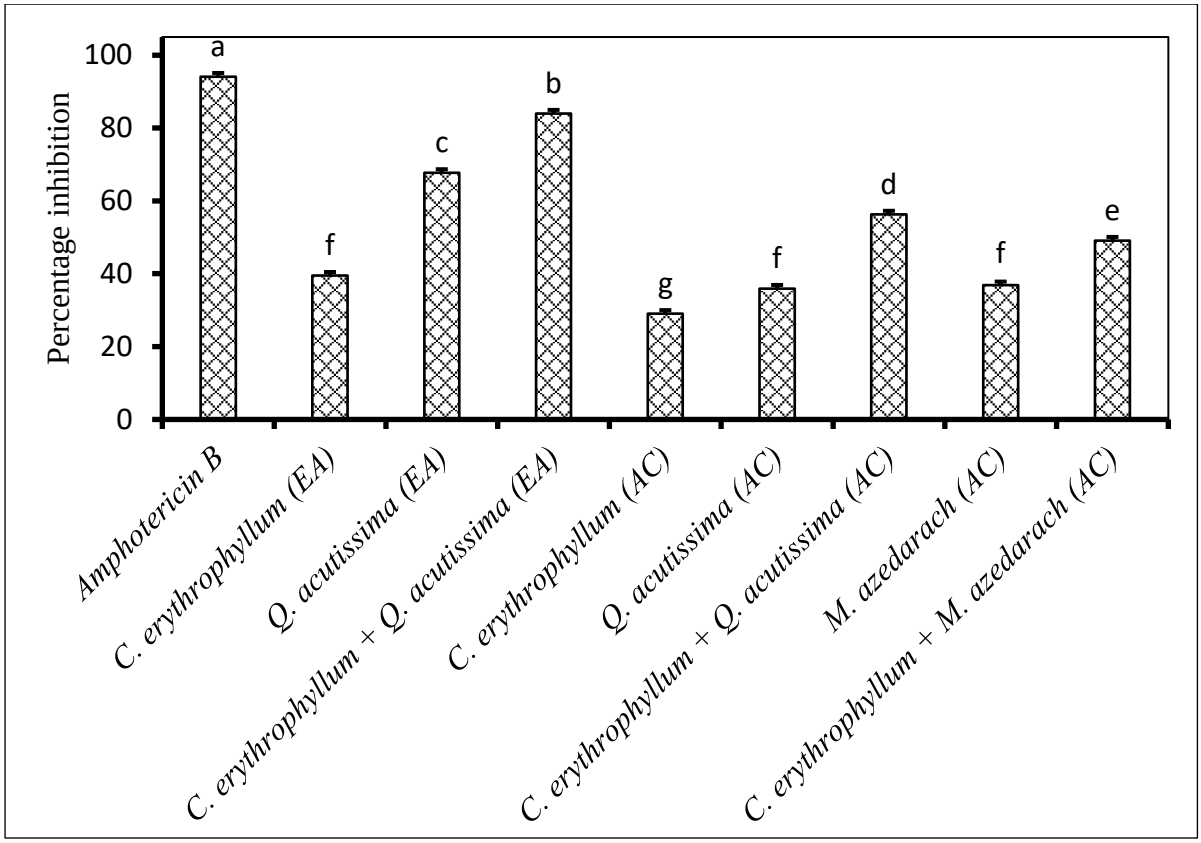


Figure 5.4. Percentage inhibition by plant extracts against *Fusarium solani* inoculated on maize seeds. Bars bearing different letters indicate significant difference (p = 0.05). EA: Ethyl acetate and AC: Acetone. The extracts were produced from *Combretum erythrophyllum*, *Quercus acutissima*, *Melia azedarach* and used at a concentration of 2.5 mg dried extract/mL of 10% acetone.

The combination of *Solanum mauritianum* and *Melia azedarach* ethyl acetate extracts appeared to be antagonistic as the antifungal activity observed against *F. oxysporum* was much less when compared to the individual application of *S. mauritianum* and *M. azedarach* (Figure 5.5). The same plant extract combination exhibited a synergistic antifungal activity against *F. proliferatum* (Figure 5.1). The antifungal activity exhibited by *M. azedarach* was equivalent to what was recorded for the positive control (Figure 5.5). The combination of *Lantana camara*

and *Combretum molle* ethyl acetate extract did not improve the antifungal activity against *F. semitectum* when compared to individual application of *C. molle* ethyl acetate, which showed a very strong antifungal activity (Figure 5.6).

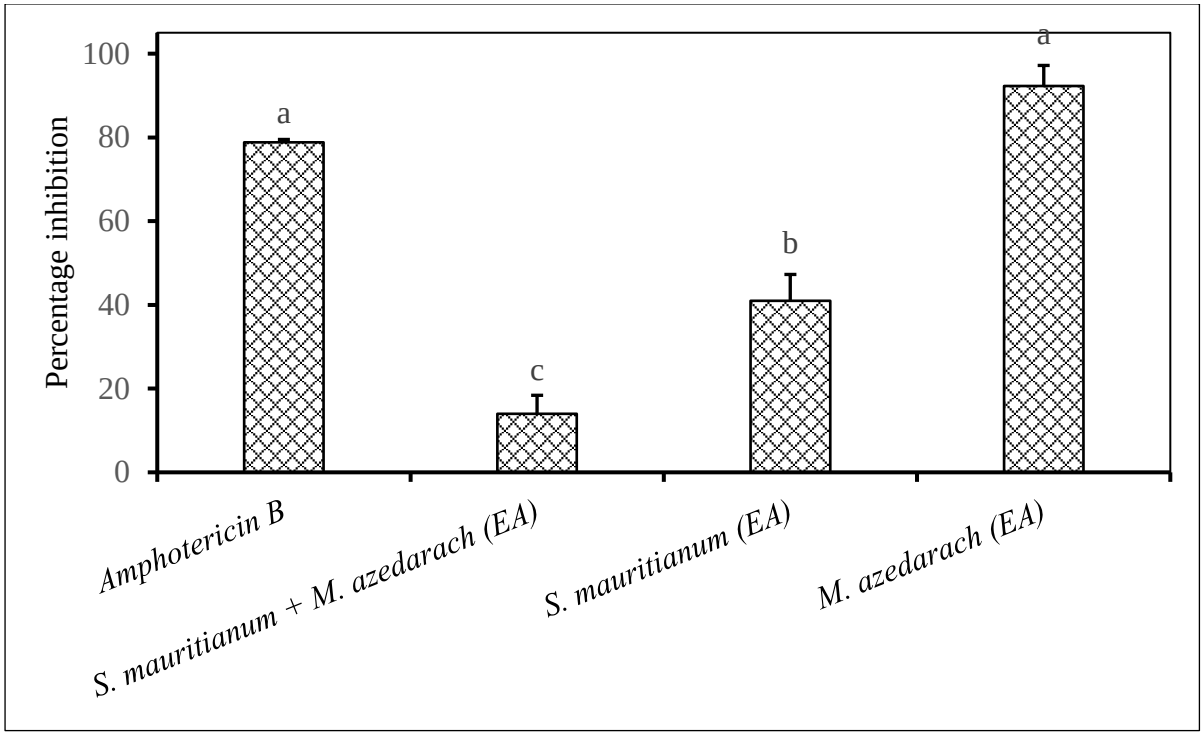


Figure 5.5. Percentage inhibition by plant extracts against *Fusarium oxysporum* inoculated on maize seeds. Bars bearing different letters indicate significant difference (p = 0.05). EA: Ethyl acetate. The extracts were produced from *Solanum mauritianum, Melia azedarach* and used at a concentration of 2.5 mg dried extract/mL of 10% acetone.

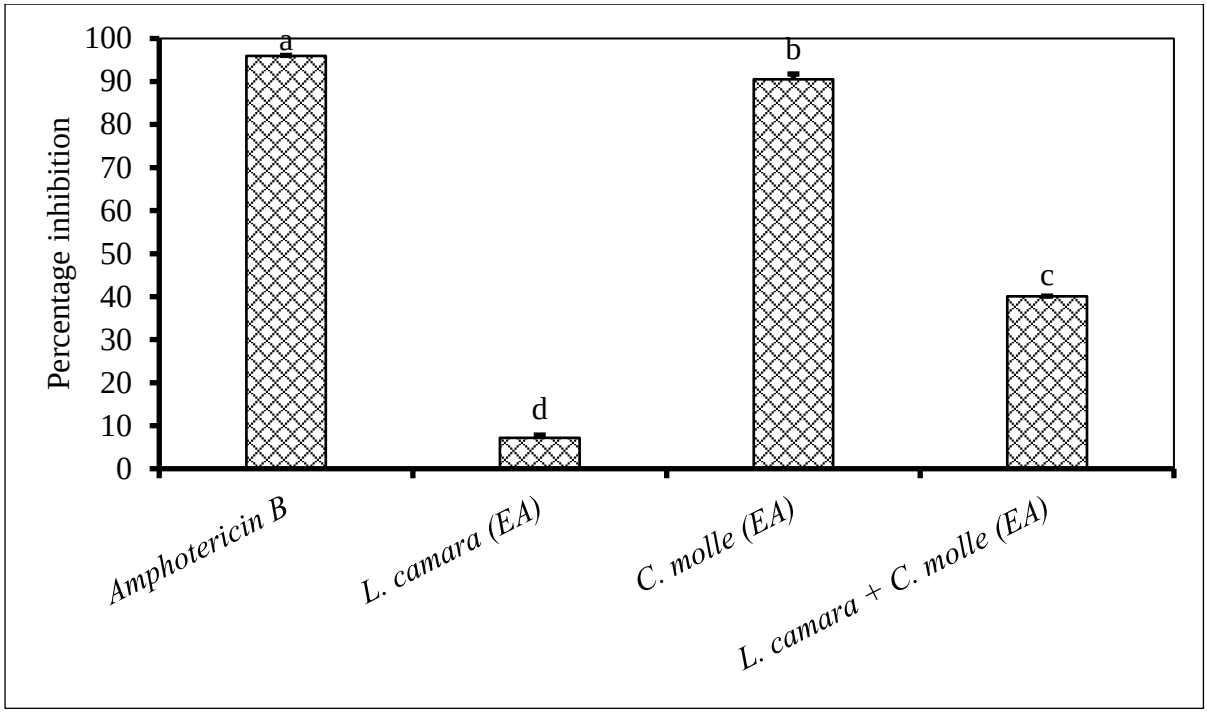


Figure 5.6. Percentage inhibition by plant extracts against *Fusarium semitectum* inoculated on maize seeds. Bars bearing different letters indicate significant difference (p = 0.05). EA: Ethyl acetate. The extracts were produced from *Lantana camara, Combretum molle* and used at a concentration of 2.5 mg dried extract/mL of 10% acetone.

On the other hand, a combination of *Nicotiana glauca* and *Quercus acutissima* acetone extracts showed synergistic, very strong antifungal activity against *F. chlamydosporum*. This activity was significantly higher compared to that of the positive control (Figure 5.7). Similarly, the combination of *C. erythrophyllum* and *Q. acutissima* acetone extracts exhibited an improved antifungal activity against *F. equisite* when compared to the antifungal activity of individual extract (Figure 5.8). The antifungal activity of combined acetone extracts from *C. erythrophyllum* and *Q. acutissima* against *F. graminearum* was 3.5 times weaker compared to the individual application of *C. erythrophyllum* acetone extract against the same pathogen (Figure 5.9).

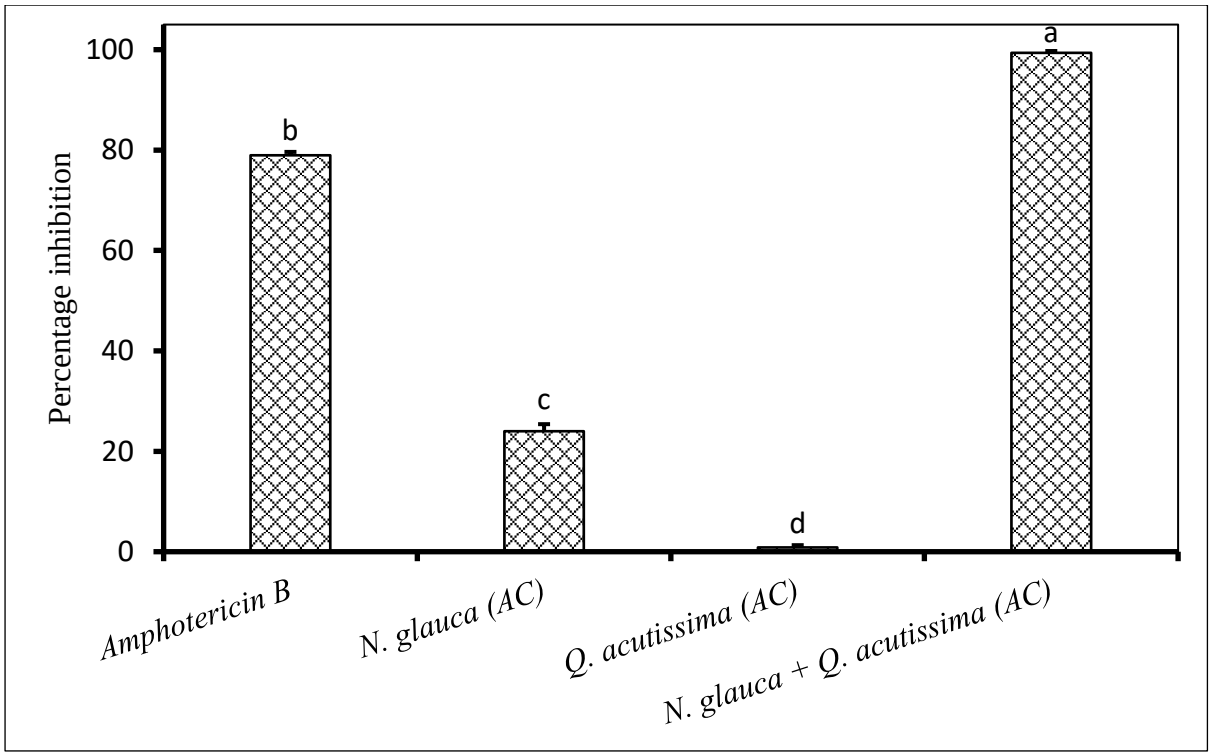


Figure 5.7. Percentage inhibition by plant extracts against *Fusarium chlamydosporum* inoculated on maize seeds. Bars bearing different letters indicate significant difference (p = 0.05). AC: Acetone. The extracts were produced from *Nicotiana glauca*, *Quercus acutissima*, and used at a concentration of 2.5 mg dried extract/mL of 10% acetone.

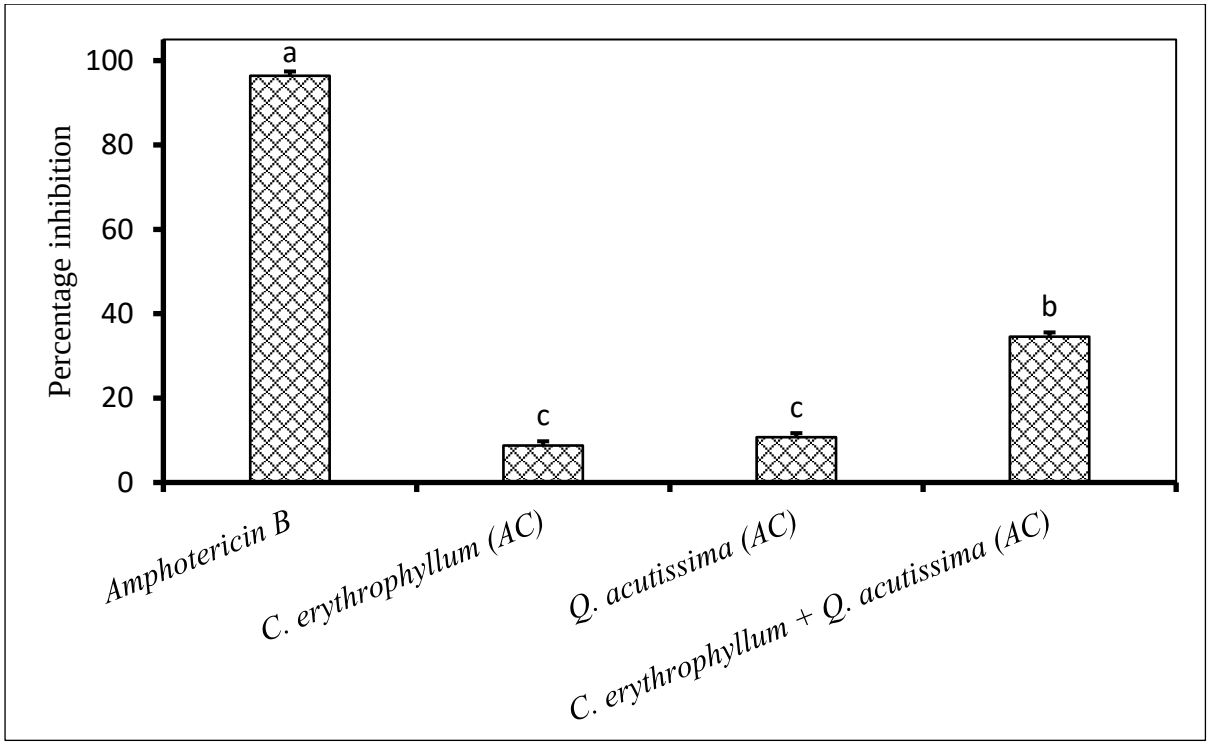


Figure 5.8. Percentage inhibition by plant extracts against *Fusarium equisite* inoculated on maize seeds. Bars bearing different letters indicate significant difference (p = 0.05). AC: Acetone. The extracts were produced from *Combretum erythrophyllum, Quercus acutissima* and used at a concentration of 2.5 mg dried extract/mL of 10% acetone.

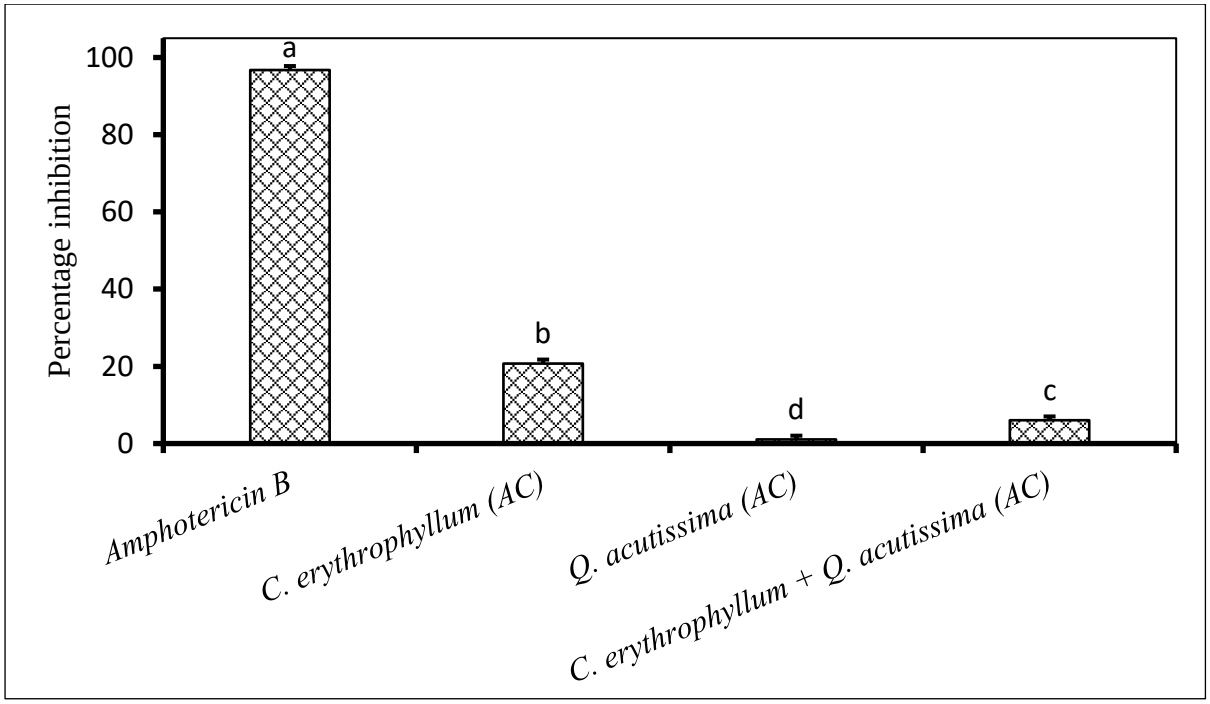


Figure 5.9. Percentage inhibition by plant extracts against *Fusarium graminearum* inoculated on maize seeds. Bars bearing different letters indicate significant difference (p = 0.05). AC: Acetone. The extracts were produced from *Combretum erythrophyllum*, *Quercus acutissima* and used at a concentration of 2.5 mg dried extract/mL of 10% acetone.

5.3.2. Phytotoxicity on maize seed germination

Maize seeds treated with *Quercus acutissima* ethyl acetate extract had the lowest percentage germination (86%) as compared to other treatments. There is no significant difference in seed germination of untreated seeds in comparison to all other extract treatments (Figure 5.10).

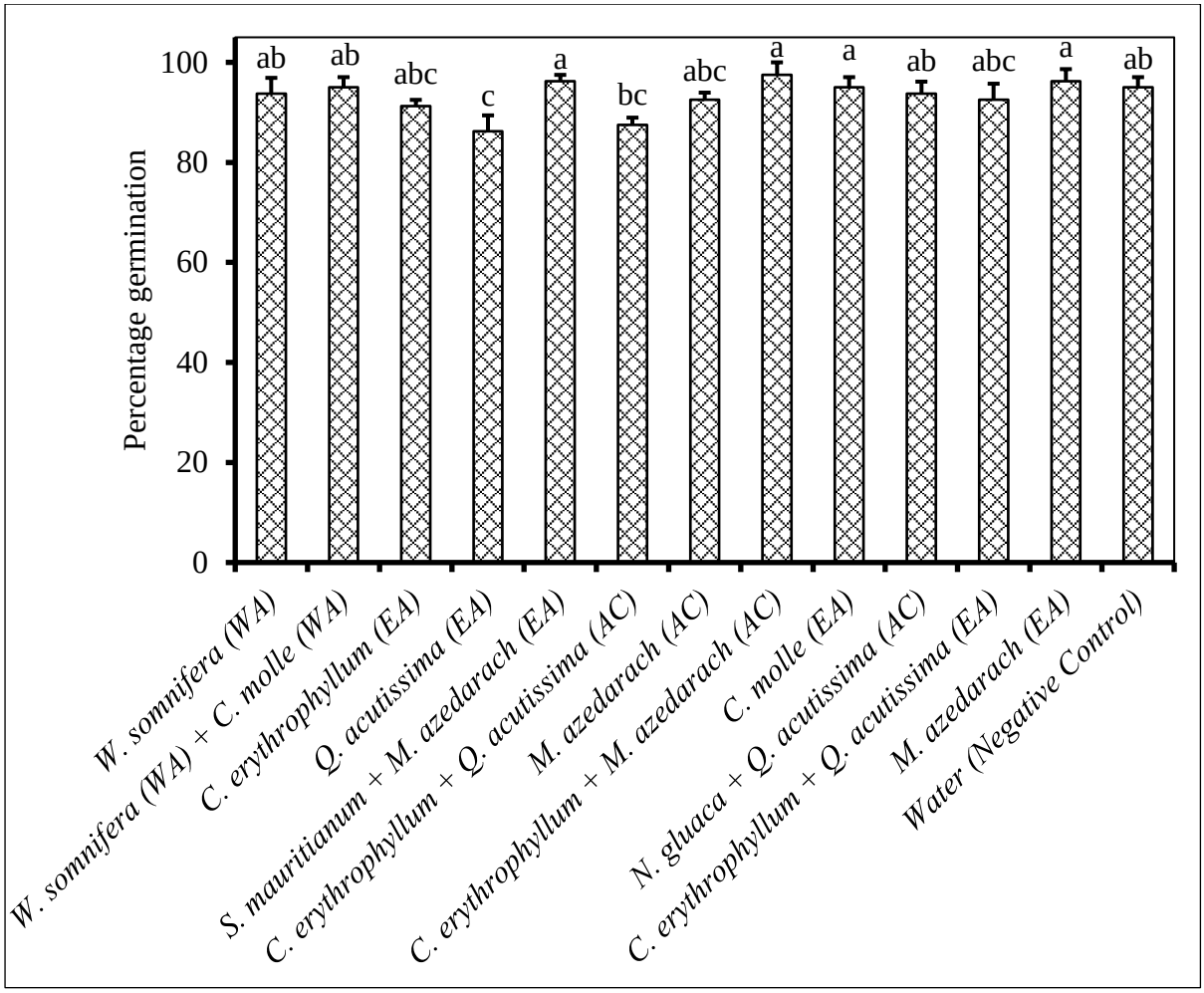


Figure 5.10. Percentage germination of maize seeds treated with extracts of different plants. WA: Water, EA: Ethyl acetate and AC: Acetone. The extracts were produced from *Withania somnifera, Combretum molle, Combretum erythrophyllum, Quercus acutissima, Solanum mauritianum, Melia azedarach, Nicotiana glauca* and used at a concentration of 2.5 mg dried extract/mL of 10% acetone.

5.3.3. Antifungal activity of plant extracts against pathogen inoculated on maize seedlings

Untreated maize seedlings inoculated with *F. verticilloides* exhibited maximum disease symptom as compared to other treatments (Figure 5.11 and Figure 5.12). Inoculated seedlings

treated with commercial fungicide and plant extract had significantly reduced disease discolouration symptom.

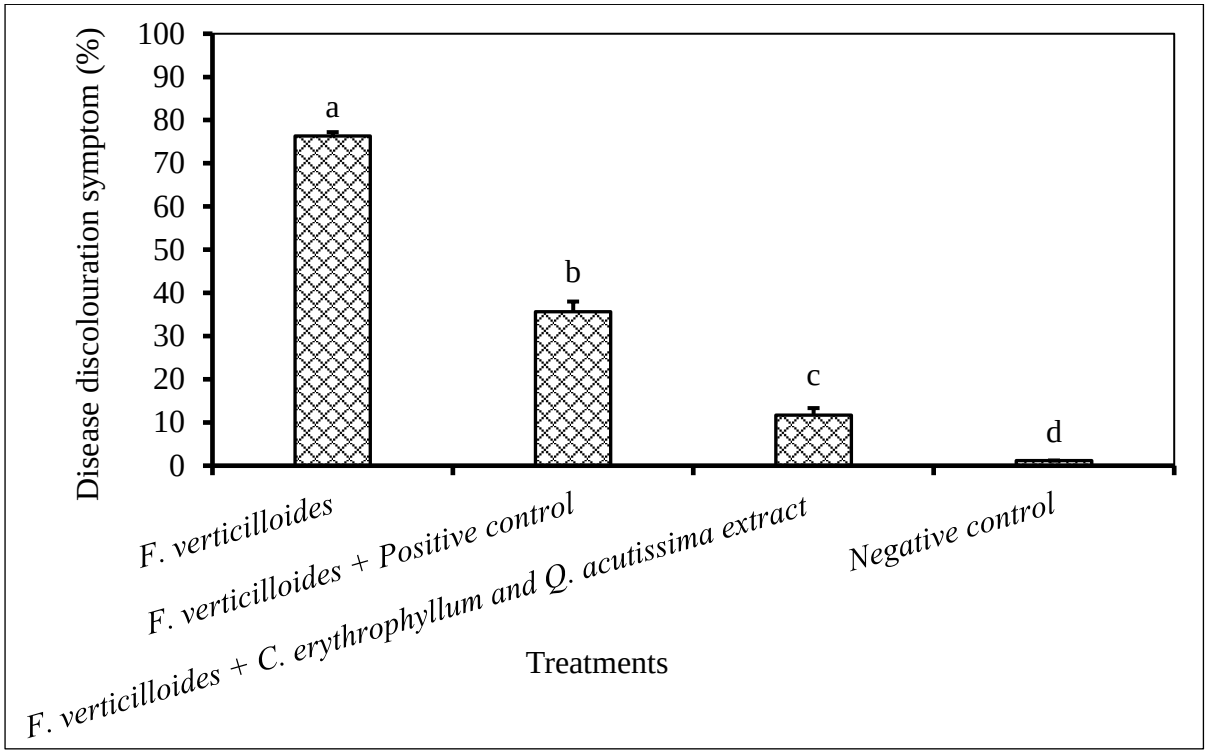


Figure 5.11. Percentage disease discolouration caused by *F. verticilloides* inoculated on maize seedlings grown in a greenhouse. The extracts were produced from *Combretum erythrophyllum, Quercus acutissima* and used as combined at a concentration of 2.5 mg dried extract/mL of 10% aqueous acetone. Bars bearing different letters indicate significant difference (p = 0.05) according to Duncan's Multiple Range Test (DMRT).

Maize crops treated with water (Negative control)

Maize crops inoculated with *F. verticilloides*

Maize crops inoculated with *F. verticilloides* and treated with plant extract

Maize crops inoculated with *F. verticilloides* and treated with positive control

Figure 5.12. Disease discolouration symptom on maize crop cultivated in a greenhouse. The crop was inoculated with *F. verticilloides* and treated with a combination of *Combretum erythrophyllum and Quercus acutissima* acetone extract at a concentration of 2.5 mg dried extract/mL of 10% aqueous acetone. Commercial fungicide (Efekto-Virikop®) at a concentration of 2.5 mg/mL was used as a positive control and water was used as a negative control.

5.3.4. Phytotoxicity of plant extract on maize seedling growth

Application of *C. erythrophyllum* and *Q. acutissima* acetone extract had no significant effect on the growth of maize seedlings (Table 5.1). Relative to the control, the extract increased chlorophyll content and dry root mass while stem diameter and number of leaves were reduced.

Table 5. 1. Phytotoxicity of combined *Combretum erythrophyllum* and *Quercus acutissima* acetone extract on maize seedling growth in a greenhouse. Mean values within rows were not significantly different ($p \leq 0.05$) according to t-test. R.I: relative impact.

Growth parameters	Negative control	*C. erythrophyllum* and *Q. acutissima* acetone extract	R.I (%)
Plant height (mm)	320.3 ± 34.80 ^{ns}	319.8 ± 19.82 ^{ns}	-0,14
Chlorophyll content (SPAD)	36.5 ± 2.30 ^{ns}	38.0 ± 1.49 ^{ns}	4,14
Number of leaves	6.6 ± 0.63 ^{ns}	6.2 ± 0,32 ^{ns}	-5,85
Dry shoot mass (g)	9.3 ± 1.12 ^{ns}	9.2 ± 1,03 ^{ns}	-0,19
Stem diameter (mm)	9.85 ± 1.14 ^{ns}	8.82 ± 0,58 ^{ns}	-10,41
Dry root mass (g)	4,32 ± 1,54 ^{ns}	4,55 ± 0,47 ^{ns}	5,48

ns: not significant

5.4. DISCUSSION

Management of *Fusarium* pathogens in maize seed using medicinal plant extracts presents an affordable disease control strategy for smallholder or subsistence farmers. Synthetic chemical control measures poses serious threats or concerns because in this farming system, some farmers fall back on stored seeds for food during off-season.

The present study showed that leaf ethyl acetate extract of *Q. acutissima* was effective (≥ 50% inhibition) in inhibiting the growth of *F. proliferatum* and *F. solani*. In contrast, acetone extract from *Q. acutissima* was not active against both *F. proliferatum* and *F. solani*. Similarly, the leaf acetone extract of *M. azedarach* was very effective in inhibiting the growth of *F. proliferatum* while its ethyl acetate extract was ineffective against the same pathogen. Notably, solvent polarity plays an important role in the antifungal activity of medicinal plant extracts. Acetone is slightly more polar than ethyl acetate (Snyder, 1978). Slight variation in solvent parameter may determine the amount and type of antifungals or metabolites extracted. The difference in the antifungal activity of acetone and ethyl acetate extracts from the same plant species was also observed with *C. erythrophyllum* against *F. proliferatum, F. subglutinans, F. verticilloides* and *F. solani*. Medicinal plants contain a wide variety of secondary metabolites including tannins, terpenoids, flavonoids, phenols, saponins and other chemical constituents (Snyder, 1978; Rishi *et al*., 2003; Sultana *et al*., 2013). The polarity of the constituent metabolites differs significantly and has influence on their solubility during extraction and thereafter the antifungal activity of the extracts.

Apart from polarities of extraction solvents, the difference in cellular structure and defence mechanisms of the pathogens may also influence antifungal efficacy of medicinal plant extracts. It was observed that acetone extract of *M. azedarach* was very effective in inhibiting the growth of *F. proliferatum*, but ineffective against *F. subglutinans, F. verticilloides* and *F. solani*. A similar trend was also observed with individual application of ethyl acetate extracts from *S. mauritianum* and *M. azedarach* against both *F. oxysporum* and *F. proliferatum*. *Quercus acutissima* showed large variation in its antifungal activity against different *Fusarium* species (*F. proliferatum, F. verticilloides, F. chlamydosporum, F. solani, F. equisite* and *F. graminearum*). Generally, the antifungal activity of the extract appears to depend on both the polarity of extracting solvents and the pathogens.

In vivo antifungal evaluation of individual plant extracts such as *N. glauca*, *L. camara* and *S. mauritianum* did not produce good activity as compared to the results reported in the *in vitro* study (Seepe *et al*., 2019). It is speculated that the interaction between the tested pathogens and maize seeds might have created a favourable condition for the pathogen such that these plant extracts were not able to deliver the desired effects. However, in spite of the polarity of the solvents used for extraction and the pathogens, species such as *Withania somnifera*, *C. erythrophyllum*, *Q. acutissima*, *M. azedarach* and *C. molle* showed remarkable *in vivo* antifungal activity with values ranging from 68 to 98% inhibition.

Extracts with activity greater than 50% inhibition have potential to be developed into affordable and accessible seed fungicides to control *Fusarium* diseases in crop farming. These plant species are widely distributed in many African countries and are readily available. As an example, *M. azedarach* and *C. erythrophyllum* are often planted as shade or ornamental trees in many human settlements (Khan *et al*., 2008). Therefore, utilization of such plants as bio-pesticides can be achieved at minimum cost. Management of *Fusarium* species is an important agricultural practice to ensure minimum yield loss during post-harvest storage. Many *Fusarium* species are common causes of maize ear rot diseases and can lead into serious yield reduction in maize farming (Gonzalez *et al*., 1995; Munkvold and Desjardins, 1997; Desjardins, 2003; Summerell and Leslie, 2011). They have been frequently isolated in maize production areas both at the field and during post-harvest storage (Kossou and Aho, 1993; Chulze *et al*., 1996; Kedera *et al*., 1999; Aguín *et al*., 2014). Fungal pathogens including *Fusarium* species can cause about 50-80% damage on maize grains during storage (Chulze *et al*., 1996). In addition, several *Fusarium* species produce mycotoxins that contaminate grains and render them unsuitable for human consumption (Nelson and Desjardins, 1993; Marasas *et al*., 1984; Mokoena *et al*., 2005).

Combining extracts from different plant species improved antifungal potency *in vitro* through synergistic and additive interactions, as compared to their individual application (Seepe *et al*., 2020). In the current study, a combined application of *C. erythrophyllum* and *Q. acutissima* acetone extract also showed improved antifungal activity against *F. verticilloides*, which was 15 and 7 times stronger when compared to the individual application of *Q. acutissima* and *C. erythrophyllum*, respectively. This combined extract also showed improved activity against *F. solani* and *F. proliferatum*. Nonetheless, a combined ethyl acetate extract from the same plant species (*Q. acutissima* and *C. erythrophyllum*) demonstrated reduced activity against *F. proliferatum* and *F. verticilloides* whereas an improved activity was demonstrated against *F. solani*. The combined acetone extract of *N. glauca* and *Q. acutissima* exhibited an improved antifungal activity against *F. chlamydosporum*. An improvement in the antifungal activity of combined extracts may be due to an increased concentration of antifungal compounds at the inhibition sites on the pathogen. It may also be due to the formation of different chemical constituents with different modes of action. However, not all combined extracts demonstrated an improved activity. Thus, similar to the individual extracts, the activity of the combined extracts also depends on the polarity of extraction solvents and is pathogen-specific.

The antifungal activity of plants demonstrated in the current study could be due to the presence of different chemical constituents in their leaves. As an example, mollic acid glucoside isolated from acetone leaf extract of *C. molle* was found to be a major terpenoid contributing to the antifungal activity of this plant (Pegel and Rogers, 1985). Although water and ethyl acetate extracts were used in our study, this terpenoid and other chemicals such as α-arabinoside and punicalagin known to be present in *C. molle* might have contributed to the activity demonstrated by this plant (Asres *et al*., 2001). *Combretum erythrophyllum,* which also showed very good activity, is known to contain antimicrobial compounds such as apigenin, rhamnazin, genkwanin and 5-hydroxy-7,4'- dimethoxyflavane (Martini *et al*., 2004). Hydroxyl-3-

methoxcinnamaldehyde, vanillin, scopoletin and pinoresinol isolated from different parts of *M. azedarach* showed good activity against different pathogens including some *Fusarium* species (Carpinella *et al*., 2003; Carpinella *et al*., 2005). The activity of *W. somnifera* water extract against *F. proliferatum* could be due to withaferin A and withanolide D present in the leaves of this shrub (Devi *et al*., 1993; Kulkarni *et al*., 1998).

Safety or toxicity of these plant extracts is also an important factor to be considered because in smallholder farming, stored maize seeds and other grains are sometimes consumed as food during off-season. Therefore, the plant species intended for use as seed protecting agents must be safe or at least be known to be used in the traditional medicine without toxicity effect. Medicinal plant species have been used in many developing countries to treat aliments affecting both humans and animals, and they are arguably safe, biodegradable and environmental friendly (Masika and Afolayan, 2002; Khan and Nasreen, 2010; Sukanya *et al*., 2011). As an example, *W. somnifera* is considered one of the valuable plants in the Indian traditional medicine. It is used to treat various neurological disorders, diarrhea, gastrointestinal disorders, arthritis, stress and behaviour-related problems (Kulkarni *et al*., 1998; Acharyya *et al*., 2009). *W. somnifera* is used as nutrient and health restorative decoction by pregnant women and elderly peaple (Alam *et al*., 2012). An infusion made from the leaves of this plant is used for fever (Mirjalili *et al*., 2009). Additionally, plant species such as *M. azedarach* and *C. molle* are used in traditional medicine for the treatment of diarrhea and as an anthelmintic (Warrier *et al*., 1995; Fyhrquist, 2007; Sen *et al*., 2010). Oak species including *Q. acutissima* are used topically to treat burns, wounds and are applied orally for gastritis or diarrhoea (Committee on Herbal Medicinal Products, 2009). The infusion from *Quercus* family are known to be used as gargle for sore throat and for the treatment of ulcers and toothache (Committee on Herbal Medicinal Products, 2009; Koseki *et al*., 2015). There are reports about possible toxicity of *N. glauca* due to the presence of anabasine (Mizrachi *et al*., 2000; Panter *et al*., 2000; Furer *et al*., 2011).

Plants contain different constituents, so drug-drug interaction can play an important role in the safety of the extracts. Besides improving activity through additive and synergistic interactions, the practice of combining extracts from different plant species may also reduce toxicity effect or the opposite.

Effective botanical insecticide products such as NeemPro® and NeemAzal® had no negative effect on maize seed germination (Nukenine *et al*., 2011; Danga *et al*., 2015). The medicinal plant extracts investigated in the current study did not have a pronounced negative effect on maize seed germination. In smallholder farming, surplus maize seeds and other grains are used for planting and can be recycled for years or generations (Danga *et al*., 2015; Muimba-Kankolongo, 2018). Therefore, it is essential that plant-based products are not only effective in controlling post-harvest storage pathogens but they must also not affect seed germination negatively.

Furthermore, the current study revealed that a combination of *C. erythrophyllum* and *Q. acutissima* acetone extract showed no toxicity on maize seedling growth. The combination had no significant effect on all seedling growth variables (plant height, number of leaves, chlorophyll content, dry shoot mass, stem diameter and dry root mass) when compared to the negative control. To some extent, this combined extract exhibited *in vivo* antifungal activity against *F. verticilloides* inoculated on maize seedlings, evident by lower disease percentage area on seedlings inoculated and treated with this combined extract. A combination of medicinal plant extracts such as *C. erythrophyllum* and *Q. acutissima* acetone leaf extract has potential to be developed into seed treatment agent and can also be applied in chemigation.

5.5. CONCLUSIONS

The potential application of medicinal plant extracts as alternative bio-pesticides to protect maize seeds against *Fusarium* pathogens was established. Different solvent extracts from the same medicinal plant species demonstrated different activities against tested organisms. Although some plant extracts can be used individually, the combinations of some extracts exhibited stronger activity than their individual extracts against *Fusarium* pathogens. Almost all the tested extracts did not have any negative effect on maize seed germination. A combination of *C. erythrophyllum* and *Q. acutissima* acetone extract had no negative effect on maize seedling growth. The use of plant leaves is renewable, affordable and accessible to poor-resourced smallholder farmers for controlling post-harvest storage diseases in agriculture. The stability of the extracts during grain storage treatment and the frequency of applications are aspects that warrant further research.

REFERENCES

Abate, T.; Fisher, M.; Abdoulaye, T.; Kassie, G.T.; Lunduka, R.; Marenya, P.; Asnake, W., 2017. Characteristics of maize cultivars in Africa: How modern are they and how many do smallholder farmers grow?. Agriculture and Food Security 6, 30.

Aberkane, A.; Cuenca-Estrella, M.; Gomez-Lopez, A.; Petrikkou, E.; Mellado, E.; Monzon, A.; Rodriguez Tudela, J.L., 2002. Comparative evaluation of two different methods of inoculums preparation for antifungal susceptibility testing of filamentous fungi. Journal of Antimicrobial Chemotherapy 50, 719–722.

Acharyya, S.; Patra, A.; Bag, P.K., 2009. Evaluation of the antimicrobial activity of some medicinal plants against enteric bacteria with particular reference to multi-drug resistant *Vibrio cholerae*. Tropical Journal of Pharmaceutical Research 8, 231–237.

Aguín, O.; Cao, A.; Pintos, C.; Santiago, R.; Mansilla, P.; Butrón, A., 2014. Occurrence of *Fusarium* species in maize kernels grown in northwestern Spain. Plant Pathology 63, 946–951.

Ahmad, I.; Beg, A.Z., 2001. Antimicrobial and phytochemical studies on 45 Indian medicinal plants against multi-drug resistant human pathogens. Journal of Ethnopharmacology 74, 113-123.

Alam, N.; Hossain, M.; Mottalib, M.A.; Sulaiman, S.A.; Gan, S.H.; Khalil, M.I., 2012. Methanolic extracts of *Withania somnifera* leaves, fruits and roots possess antioxidant properties and antibacterial activities. BMC Complementary and Alternative Medicine 12, 175.

Asres, K.; Bucar, F.; Edelsbrunner, S.; Kartnig, T.; Höger, G.; Thiel, W., 2001. Investigations on antimycobacterial activity of some Ethiopian Medicinal Plants. Phytotherapy Research 15, 323-326.

Baiphethi, M.N.; Jacobs, P.T., 2009. The contribution of subsistence farming to food security in South Africa. Agrekon 48, 459–482.

Carpinella, M.C.; Giorda, L.M.; Ferrayoli, C.G.; Palacios, S.M., 2003. Antifungal effects of different organic extracts from *Melia azedarach* L. on phytopathogenic fungi and their isolated active components. Journal of Agricultural and Food Chemistry 51, 2506-2511.

Carpinella, M.C.; Ferrayolic, C.G.; Palacios, S.M., 2005. Antifungal synergistic effect of scopoletin, a hydroxycoumarin isolated from *Melia azedarach* L. fruits. Journal of Agricultural and Food Chemistry 53, 2922-2927.

Chaudhary, A.R. Maize in Pakistan. 1st Edition. Faisalabad: *Punjab Agricultural Research Coordination Board, University of Agriculture*; 1983.

Cherry, A.J.; Bantino, A.; Djegui, D.; Lomers, C., 2005. Suppression of the stem borer *Sesamia calamistis (Lepidoptera: Noctuidae*) in maize following seed dressing, topical application and stem injection with African isolates of *Beauveria bassiana*. International Journal of Pest Management 50, 67–73.

Chopra, R.N.; Nayer, S.L.; Chopra, I.C.; Glossary of Indian Medicinal Plants. 3rd Edition. New Delhi: Council of Scientific and Industrial Research; 1992.

Chulze, S.N., 2010. Strategies to reduce mycotoxin levels in maize during storage: a review. Food additives & contaminants. Part A, Chemistry, analysis, control, exposure & risk assessment 27, 651-657.

Chulze, S.; Ramirez, M.L.; Farnochi, M.C.; Pascale, M.; Visconti, A.; March, G., 1996. *Fusarium* and fumonisins occurrence in Argentinean corn at different ear maturity stages. Journal of Agricultural and Food Chemistry 44, 2797– 2801.

Committee on Herbal Medicinal Products (HMPC), Assessment report on *Quercus robur* L., *Quercus petraea* (Matt.) Liebl., *Quercus pubescens* Willd., cortex. EMA/HMPC/3206/2009.

Crush, J.; Hovorka, A.; Tevera, D., 2011. Food security in southern African cities: the place of urban agriculture. Progress in Development Studies 11, 285–305.

Cumagun, C.J.R.; Ramos, J.S.; Dimaano, A.O.; Munaut, F.; Van Hove, F., 2009. Genetic characteristics of *Fusarium verticilloides* from corn in the Philippines. Journal of General Plant Pathology 75, 405-412.

Danga, S.P.Y.; Nukenine, E.N.; Fotso, G.T.; Adler, C., 2015. Use of NeemPro®, a neem product to control maize weevil *Sitophilus zeamais* (Motsch.) (Coleoptera: Curculionidae) on three maize varieties in Cameroon. Agriculture and Food Security 4, 18.

Desjardin, A.E., 2006. *Fusarium* mycotoxins: chemistry, genetics and biology. American Phytopathological Society, 335-336.

Desjardins, A.E., 2003 Gibberella from A (venaceae) to Z (eae). Annual Review of Phytopathology, 41, 177–198.

Devi, P.U.; Sharada, A.C.; Solomon, F.E., 1993. Antitumor and radiosensitizing effects of *Withania somnifera* (Ashwagandha) on a transplantable mouse tumor, Sarcoma-180. Indian Journal of Experimental Biology 31, 607-611.

Furer, V.; Hersch, M.; Silvetzki, N.; Breuer, G.S.; Zevin, S., 2011. *Nicotiana glauca* (Tree Tobacco) intoxication - two cases in one family. Journal of Medical Toxicology 7, 47–51.

Fyhrquist, P. Traditional medicinal uses and biological activities of some plant extracts of African *Combretum* Loefl., *Terminalia* L. and *Pteleopsis* Engl. species (Combretaceae). Helsinki: University of Helsinki; 2007.

Glotter, E.; Kirson, I.; Abraham, A.; Lavie, D., 1973. Constituents of *Withania somnifera* Dun—XIII : The withanolides of chemotype II. Tetrahedron 29, 1353-1364.

Gonzalez, H.H.L.; Resnik, S.L.; Boca, R.T.; Marasas, W.F.O., 1995. Mycoflora of Argentinean corn harvested in the main production area in 1990. Mycopathologia 130, 29–36.

Govender, V.; Aveling, T.A.S.; Kritzinger, Q., 2008. The effect of traditional storage methods on germination and vigour of maize (*Zea mays* L.) from northern KwaZulu-Natal and southern Mozambique. South African Journal of Botany 74, 190-196.

Harborne, J.B.; Baxter, H.; Moss, G.P. Phytochemical dictionary: A handbook of bioactive compounds from plants. London: Taylor & Francis; 1995.

Isman, M.B., 2006. Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world. Annual Review of Entomology 51, 45–66.

Kedera, C.J.; Plattner, R.D.; Desjardins, A.E., 1999. Incidence of *Fusarium* spp and levels of fumonisins in maize in western Kenya. Applied and Environmental Microbiology 65, 41–44.

Khan, Z.S.; Nasreen, S., 2010. Phytochemical analysis, antifungal activity and mode of action of methanol extracts from plants against pathogens. Journal of Agricultural Science and Technology 6, 793-805.

Khan, A.V.; Khan, A.A.; Shukla, I., 2008. *In vitro* antibacterial potential of *Melia azedarach* crude leaf extracts against some human pathogenic bacterial strains. Ethnobotanical Leaflets 12, 39-45.

Koseki, J.; Matsumoto, T.; Matsubara, Y.; Tsuchiya, K.; Mizuhara, Y.; Sekiguchi, K.; Nishimura, H.; Watanabe, J.; Kaneko, A.; Hattori, T.; Maemura, K.; Kase, Y., 2015. Inhibition of Rat 5α-Reductase Activity and Testosterone-Induced Sebum Synthesis in Hamster Sebocytes by an Extract of *Quercus acutissima* Cortex, Evidence-Based Complementary and Alternative Medicine.

Kossou, D.K.; Aho, N. Stockage et conservation des grains alimentaires tropicaux: principes et pratiques. Benin: Les Editions du Flamboyant; 1993.

Kulkarni, S.K.; George, B.; Mathur, R. 1998. Protective effect of *Withania somnifera* root extract on electrographic activity in a lithiumpilocarpine model of status epilepticus. Phytotherapy Research 12, 451–453.

Kusena, K.; Wynberg, R.; Mujaju, C., 2017. Do smallholder farmer-led seed systems have the capacity to supply good-quality, fungal-free sorghum seed?, Agriculture and Food Security 6, 52.

Ledingham, R.J.; Atkinson, T.G.; Horricks, J.S.; Mills, J.T.; Piening, L.J.; Tinline, R.D., 1973. Wheat losses due to common root rot in the prairie provinces of Canada. Canadian Plant Disease Survey 53, 113–122.

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, 1529–1533.

Marasas, W.F.O.; Nelson, P.E.; Toussoun, T.A. Toxigenic *Fusarium* species: identity and mycotoxicology. Pennsylvania: The Pennsylvania State University Press; 1984.

Marasas, W.F.O.; Wehner, F.C.; Van Schlkwyk, D.J., 1981. Mycoflora of corn produced in human oesophageal cancer areas in Transkei. Physiopathology 71, 792-796.

Martini, N.D.; Katerere, D.R.P.; Eloff, J.N., 2004. Biological activity of five antibacterial flavonoids from *Combretum erythrophyllum* (Combretaceae). Journal of Ethnopharmacology 93, 207-212.

Masika, P.J.; Afolayan, A.J., 2002. Antimicrobial activity of some plants used for the treatment of livestock disease in the Eastern Cape, South Africa. Journal of Ethnopharmacology 83, 129-134.

McGuire, S.; Sperling, L., 2016. Seed system smallholder farmers use. Food Security 8, 179–195.

Mirjalili, M.H.; Moyano, E.; Bonfill, M.; Cusido, R.M.; Palazón, J., 2009. Steroidal Lactones from *Withania somnifera*, an Ancient Plant for Novel Medicine. Molecules 14, 2373-2393.

Mizrachi, N.; Levy, S.; Goren, Z.Q., 2000. Fatal poisoning from *Nicotiana glauca* leaves: identification of anabasine by Gas Chromatography/Mass Spectrometry. Journal of Forensic Sciences 45, 736–741.

Mokoena, M.P.; Chelule, P.K.; Gqaleni, N., 2005. Reduction of fumonisin B1 and zearalenone by lactic acid bacteria in fermented maize meal. Journal of Food Protection 68, 2095–2099.

Muimba-Kankolongo, A. Food crop production by smallholder farmers in southern Africa: challenges and opportunities for improvement. 1st Edition. *Elsevier Science Publishing Company*; 2018.

Mungai, L.M.; Snapp, S.; Messina, J.P.; Chikowo, R.; Smith, A.; Anders, E.; Richardson, R.B.; Li, G., 2016. Smallholder farms and the potential for sustainable intensification. Frontiers in Plant Science 7, 1720.

Munkvold, G.P.; Desjardins, A.E., 1997. Fumonisins in maize: Can we reduce their occurrence?. Plant Disease 81, 556-565.

Munkvold, G.P.; Carlton, M.W., 1997. Influence of inoculation method on systemic *Fusarium moniliforme* infection of maize plants grown from infected seeds. Plant Disease 81, 211–216.

Nelson, P.E.; Desjardins, A.E.; Plattner, R.D., 1993. Fumonisins, mycotoxins produced by Fusarium species: biology, chemistry and significance. Annual Review of Phytopathology 31, 233–252.

Nukenine, E.N.; Tofel, H.K.; Adler, C., 2011. Comparative efficacy of NeemAzal® and local botanicals derived from *Azadirachta indica* and *Plectranthus glandulosus* against *Sitophilus zeamais* on maize. Journal of Pest Science 84, 479–486.

Panter, K.E.; Weinzweig, J.; Gardner, D.R.; Stegelmeier, B.L.; James, L.F., 2000. Comparison of cleft palate induction by *Nicotiana glauca* in goats and sheep. Teratology 61, 203–210.

Pegel, K.A.; Rogers, C.B., 1985. The characterization of mollic acid-3β-D-xyloside and its genuine aglycon mollic acid, two novel 1α-hydroxycycloartenoids from *Combretum molle*. Journal of the Chemical Society, Perkin Transactions 1, 1711-1715.

Pimentel, D.; Patzek, T.W., 2005. Ethanol production using corn, switchgrass, and wood; Biodiesel production using soybean and sunflower. Natural Resources Research 14, 65-76.

Rishi, K.; Singh, R., 2003. Chemical components and insecticidal properties of Bakain (*Melia azedarach* L.) - A Review. Agricultural Revolution 24, 101-115.

Rocha, M.E.; Freire, F.D.; Maia, F.E.; Guedes, M.I.; Rondina, D., 2014. Mycotoxins and their effects on human and animal health. Food Control 36, 159–165.

Salami, A.; Kamara, A.B.; Brixiova, Z. Smallholder agriculture in East Africa: trends, constraints and opportunities. Working Papers Series No. 105. Tunisia: African Development Bank Group; 2010.

Saseetharan, N.H.M.; Zakaria, L., 2014. Occurrence of *Fusarium* spp. on vegetable crops and assessment of their pathogenicity. Pertanika Journal of Tropical Agricultural Science 37, 445–455.

Seepe, H.A.; Amoo, S.O.; Nxumalo, W.; Adeleke, R.A., 2019. Antifungal activity of medicinal plant extracts for potential management of *Fusarium* pathogens, Reserach on Crops, 399-406.

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

Sen, A., Batra, A., Rao, D.V., 2010. Pivotal role of plant growth regulators in clonal propogation of *Melia azedarach* L. International Journal of Pharmaceutical Sciences Review and Research 5, 43-49.

Singh, R.; Kumar, P.; Woodhead, T. Smallholder farmers in India: Food Security and Agricultural Policy. 1st Edition. Bangkok: FAO Regional Office for Asia and the Pacific; 2002.

Smale, M.; Bellon, M.R.; Gomez, J.A.A., 2001. Maize diversity, variety attributes, and farmers' choices in South eastern Guanajuato, Mexico. Economic Development and Cultural Change 50, 201–225.

Snyder, L.R., 1978. Classification of the solvent properties of common liquids. Journal of Chromatography A 16, 223–234.

Sperling, L.; Remington, T.; Haugen, J.M.; Nagoda, S. Addressing seed security in disaster response: linking relief with development, Overview. 1st Edition. Cal: International Center for Tropical Agriculture *(CIAT)*. 2004.

Sukanya, S.L.; Yamini, D.; Fathima, S.K., 2011. Eco-friendly management of *Pyricularia oryzae*, the causal agent of blast of paddy. Current Botany 2, 46-49.

Sultana, S.; Akhtar, N.; Asif, H.M., 2013. Phytochemical screening and antipyretic effects of hydro-methanol extract of *Melia azedarach* leaves in rabbits. Bangladesh Journal of Pharmacology 8, 214-217.

Summerell, B.A.; Leslie, J.F., 2011. Fifty years of *Fusarium*: How could nine species have ever been enough? Fungal Diversity 50, 135–144.

Sun, G.; Wang, S.; Hu, X.; Su, J.; Huang, T.; Yu, J., 2007. Fumonisin B1. Contamination of home-grown corn in high-risk areas for oesophageal and liver cancer in china. Food Additives & Contaminants: Part A 24, 181-185.

Suresh, K.; Deepa, P.; Harisaranraj, R.; Vaira, A.V., 2008. Antimicrobial and phytochemical investigation of the leaves of *Carica papaya* L., *Cynodon dactylon* L. Pers., *Euphorbia Hirta* L., *Melia azedarach* L. and *Psidium guajava* L. Ethnobotanical Leaflets 12, 1184-1191.

Warrier, P. K. (Eds); Nambiar, V. P. K. (Ed); Ramankutty, C (Ed). Indian medicinal plants: A compendium of 500 species. Hyderabad, India: Orient Longman Ltd. 1995, 10-12.

CHAPTER 6

TLC-BIOAUTOGRAPHIC EVALUATION OF PLANT EXTRACTS, ANTIFUNGAL ACTIVITY AND STRUCTURAL DETERMINATION OF ISOLATED COMPOUNDS

Part of information from this chapter has been prepared for submision to Molecules for publication. **Title**: Antifungal activity of isolated compounds from the leaves of *Combretum erythrophyllum* (Burch.) Sond and *Withania somnifera* (L.) Dunal against *Fusarium* pathogens. The other part has been prepared for submission to Fitoterapia for publication. **Title**: Characterization and antifungal activity of lantadene A and boswellic acid isolated from the leaves of *Lantana camara*.

ABSTRACT

Medicinal plants are considered as sources of many important chemicals, ingredients and drugs, which have broad applications in different industries such as agriculture, food and pharmaceuticals. The beneficial effect of medicinal plants is due to the presence of secondary metabolites which possess various chemotherapeutic activities. In this study, we report on antifungal activity and chemical structure of compounds isolated from the leaves of *Combretum erythrophyllum*, *Lantana camara* and *Withania somnifera* as potential scaffold fungicides for application in agricultural sector. Leaf materials was collected, extracted with either acetone or ethyl acetate and profiled for the presence of antifungal compounds using thin layer chromatography (TLC) bio-autographic technique against *Fusarium* pathogens.

Active extracts were subjected to open silica column and TLC chromatography for purification of the fractions. The fractions were evaluated for antifungal activity against selected *Fusarium* pathogens using micro-plate dilution assay. Compounds purified from fractions that showed moderate to good antifungal activity (MIC $\leq$ 0.63 mg/ml) were analyzed using nuclear magnetic resonance (NMR) and mass spectrospocic (MS) techniques for structural

determination. The structure of compounds obtained from fraction **A** obtained from the leaves of *Combretum erythrophyllum* were characterised as a mixture of salvigenin (*5-hydroxy-6,7-dimethoxy-2-(4-methoxyphenyl)-4H-1-benzopyran-4-one*) and apigenin (*5,7-dihydroxy-2-(4-hydroxyphenyl)-4H-1-benzopyran-4-one*). Derivative of maslinic acid (*21-hydroxy-3-oxo-olean-12-en-28-oic acid*) was obtained from fraction **B** that was also isolated from the leaves of *Combretum erythrophyllum*. Fraction **A** showed minimum inhibitory concentration (MIC) of 0.01 and 0.31 mg/ml against *F. proliferatum* and *F. verticilloides*, respectively. Antifungal compound purified from fraction **L** which was isolated from the leaves of *L. camara* was characterised as lantadene A (*22- angeloyloxy-9-hydroxy-3-oxo-olean-12-en-28-oic acid*) and it demonstrated strong antifungal activity (MIC = 0.04 mg/ml) against *F. proliferatum*. Others compounds isolated in this study include physagulin or withanolide glycosides isolated from the leaves of *Withania somnifera*. This study demonstrates that medicinal plant species may be used as potential source of fungicides. The isolated antifungals may serve as lead compounds or active derivatives during large-scale production of agricultural fungicides against *Fusarium* pathogens.

Keywords: Antifungal activity, *Fusarium* pathogens, isolated compounds, apeginin, maslinic acid, lantadene A, withaferin A, column fractionation.

6.1. INTRODUCTION

Drug resistance is a well-known phenomenon in the treatment of infections in both human and animals. This is also a challenge in the agricultural sector, given that some of the pathogens are developing resistance towards available pesticides. As an example, efficacy evaluation study, showed that the strains of *F. solani* were resistant to the applications of mancozeb, captan and benomyl Padvi *et al*., 2018. Although carbendazim was very effective against *F. solani*, other

studies have reported that *F. verticilloides*, *F. proliferatum* and *F. graminearum* were resistant to this fungicide (Chen *et al*., 2008; Chen and Zhou, 2009; Xu *et al*., 2019). In addition to fungal resistance, there is also public concern about pesticide residues in fruits and vegetables and their impact on the environment (Fandohan *et al*., 2004; Adepoju *et al*, 2014).

Because of these challenges, researchers have focused on medicinal plants as an alternative source of compounds with potnetial to be developed as new classes of fungicides. Plant based fungicides are less likely to affect the environment, livestock and human health than randomly synthesised conventional fungicides (Martínez, 2012). Medicinal plants are a sources or reservoirs of secondary metabolites such as flavonoids, alkaloids, steroids, terpenoids, tannins and other organic compounds with complex chemical structures. These secondary metabolites have various pharmacolocical properties (Rishi and Singh, 2003; Suresh *et al*., 2008). As part of our search for antifungal compounds against agricultural crop pathogens, in this study we reported on the activity of isolated compounds against *Fusarium* species. Different compounds were isolated from three medicinal plant species (*Combretum erythrophyllum, Lantana camara* and *Withania somnifera*) and their chemical structures were determined.

6.2. MATERIALS AND METHODS

6.2.1. Antifungal activity evaluation of plant extracts using TLC-bioautographic assay

Medicinal plant extracts that showed very strong antifungal activity (MIC < 0.1 mg/ml) when evaluated using micro-plate dilution assay as presented in Chapter 3**,** Table 3.2; were selected and re-evaluated using TLC-bioautography assay. The idea was to find or establish the number of compounds or bands responsible for such activity. Due to small quantity of petroleum ether extracts obtained in all medicinal plant species, we decided not to include it in bio-autographic

assay and hence in isolation of active antifungals procedure or process. Water extracts were also excluded due to their inability to evaporate quickly after been spotted on TLC plate.

Medicnal plant extracts obtained and stored as described in Chapter 3**,** Section 3.2.2; were weighed and dissolved in 100% acetone to make concentration of 10 mg/ml. Twenty microlitre of each extract was spotted on aluminium-backed TLC plate (Merck, silica gel 60 F254) and developed in a tank using Toluene: Methanol: Acetonitrile: Acetic acid (80:10:5:5) as mobile phase. The solvent front was allowed to travel at least 1.5 cm from the end of the plate and thereafter, the plate was removed and dried for a week under a stream of air in the fume hood. The developed TLC plates or chromatograms were sprayed with finely suspension of *Fusarium* pathogen spores adjusted to the concentration of 1.0×10^6 spore/ml, which was sub-cultured in potato dextrose broth as described in Chapter 3, Section 3.2.3. They were incubated at 27 °C for two days and thereafter were sprayed with 2.0 mg/ml p-iodonitrotetrazolium (INT) solution. After spraying, they were then incubated for overnight or longer and observation was carried out. The white spots observed indicate the presence of antifungal compounds or fractions that inhibited the growth of the test pathogen. The number of white spots were recorded on each chromatogram. Retention factor (RF) values were calculated for the white spots by dividing the distance travelled by the spot of interest to the solvent front (distance travelled by the mobile phase). The pictures of the chromatograms were recorded; thereafter the plates were autoclaved and disposed accordingly.

6.2.2. Isolation of compounds from plant extracts using silica gel column and TLC chromatography

Based on results obtained in Section 6.2.1, *Combretum erythrophyllum, Lantana camara* and *Withania somnifera* were selected for isolation and purification of antifungal compounds. These plant species had shown good inhibition or antifungal activity against four and more

Fusarium pathogens. Bulk extraction (300 g plant material: 3000 ml solvent) of these plant species was carried out as described in Chapter 3**,** Section 3.2.2. *Withania somnifera* and *Lantana camara* were extracted with ethyl acetate while *Combretum erythrophyllum* was extracted with acetone. A schematic diagram of purification and isolation is presented in Figure 6.1 below.

Isolation procedure was initiated by packing 100 g of silica gel (Grade 7734, pore size 60 Å, 70-230 mesh, Sigma Aldrich) into a glass column to a height of 35 cm and 3.5 cm internal diameter. The column was equilibrated and kept wet with about 200 ml petroleum ether. Medicinal plant extract (3.50 g) was dissolved completely in 10-20 ml acetone and mixed with 15 g silica gel. It as allowed to dry in a fume hood under a stream of air and crushed with morter and pestle into fine powder. The fine silica extract mixture was spread flat on top of the equilibrated column; covered with cotton wool and filter paper cut to the size of the column to minimaze surface distrubance during addition of mobile solvents.

The column was first eluted with 150 ml of 100% petroleum ether, followed by 50 ml of the following mobile phase mixtures (v/v): petroleum ether: ethyl acetate (45:5), (35:15), (25:25),(15:35), (5:45) and ethyl acetate: methanol (50:0), (45:5), (35:15), (25:25), (15:35), (5:45), (0:50) . It was allowed to run under gravity and the fractions were collected into 20 ml peel vials. It was further eluted with 100 ml absolute methanol and then followed by equivalent volume of dichloromethane to clean up the column. In order to increase the amount of fractions collected; the column was prepared and run twice (re-run) with similar plant extract. The collected fractions were concentrated using rotary evaporators and further dried in the fume hood.

The dried fractions collected from the column were weighed, dissolved in acetone (20 mg/ml) and 20 µl was spotted on TLC plate. The chromatograms were developed as described in Section 6.2.1, allowed to dry and were observed under UV-visible light (254 and 364 nm).

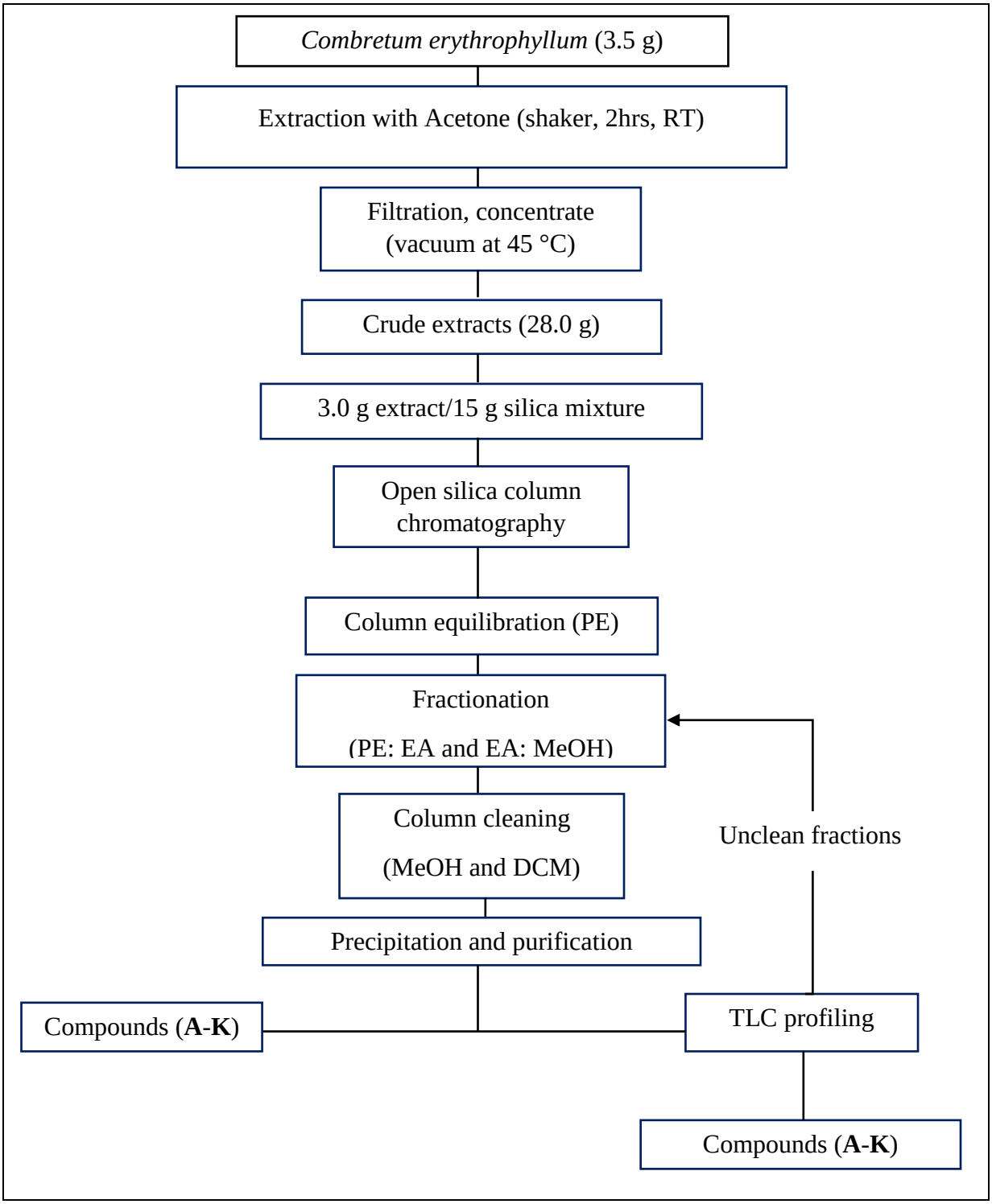


Figure 6.1. Extraction, fractionation, and isolation of compounds from the leave of *Combretum erythrophyllum* (RT: room temperature, PE: petroleum ether, EA: ethyl acetate, The fractions or compounds were labelled alphabetically.

The separated spots were identified and highlighted with a pencil marks. Thereafter, the plate was sprayed with vanillin-sulphuric acid-methanol reagent (0.1 g vanillin: 28 ml methanol: 1 ml sulphuric acid) and heated at 110 °C until colour develops. Retention factor values were calculated for all visible spots (marked with pencil) and compared with the values recorded for active spots observed in section 6.2.1. The fractions with RF values comparable to active spots or white areas and with similar TLC profile were combined for further fractionation or purification. The fractions with RF values not matching the active spots were discarded and not included in the isolation or purification procedures. Moreover, isolated compounds or fractions found to be pure were weighed in pre-weighed vials and kept in the dark until evaluated for antifungal activity and send for nuclear magnetic reasonance (NMR) structural analysis.

6.2.3. *In vitro* antifungal activity of isolated fractions

The fraction was dissolved in acetone to make a final concentration of 10 mg/ml. Antifungal activity of all isolated fractions was evaluated and their minimum inhibitory concentration values were determined using modified serial dilution method (Masoko *et al*., 2005) as described in Chapter 3, section 3.2.4 and 3.2.5.

6.2.4. Phytotoxicity evaluation of isolated antifungal compounds against maize seed germination and their effect on shoots and roots length

Characterised antifungal compounds were evaluated for possible phytotoxicity on maize seed germination using a method described by Seepe *et al*., (2020). In brief, seeds were soaked in a mixture of apigenin and salvigenin as while as in maslinic acid at pre-determined concentration of 0.63 mg/ml in 10% acetone. An other set of seeds were soaked in withaferin A glycoside

prepared at a concentration of 0.16 mg/ml and air-dried in a bio-safety cabinet for an hour. Water and 10% acetone were used as controls. Twenty-five seeds were placed per petri dish lined with a moistened double layer filter papers. Each treatment was replicated five times. The experiment was kept in an incubator at constant 25 °C and alternating cycle of 12 h light and 12 h darkness. The filter papers were kept wet throughout the experiment. The number of germinated seeds was recorded 3 days after sowing. Shoots and roots length was measured using a ruler. The experiment was repeated twice with five replicates per treatment. Percentage seed germination was calculated using the following equation:

$$\text{Percentage seed germination} = \left(\frac{\text{Number of germinated seeds}}{\text{Total number of seeds}}\right) \text{X } 100$$

6.2.5. Statistical analysis of phytotoxicity data

The data was analysed using STATISTICA-8 software. The difference between the treatments was evaluated using one-way analysis of variance (ANOVA). Data were expressed as mean ± standard error. Where a statistical significance ($p = 0.05$) was established, means separation was done using Duncan's Multiple Range Test (DMRT).

6.3. Structural determination of isolated antifungal compounds

6.3.1. Melting point and UV-Vis spectrophotometric analysis

The melting points of the compounds were determined using melting point apparatus (Stuart SMP3) and were uncorrected. Ultraviolet-visible spectra were scanned and recorded using spectrophotometer (Specord 210, Analytic Jena, Germany) and liquid chromatography-

photodiode array detector (LC-PDA, Shimadzu, Scientific Instruments, Japan). The data were compared with available reported literature.

6.3.2. Mass spectrophotometric (MS) analysis

Mass spectral characterization were recorded using liquid chromatography-mass spectrometer (LC-MS-2020, Shimadzu, Scientific Instruments, Japan) connected with an electrospray ionization (ESI) source operating in negative (m/z 250 to 1000) and positive (m/z 250 to 1000) ionization mode. Mass spectrometer ionization conditions were as follows: nebulizing gas (1.5 ℓ/min); DL temperature (250 °C); heat block temperature (200 °C); detector voltage (0.19 Kv). The fraction was dissolved in HPLC grade acetonitrile (Lab-scan analytical sciences) and approximately 2 µl was injected into the chromatography system equipped with reverse phase shim-pack C18 column (5 µm, 250 mm x 2.1 internal diameter). Mobile phase comprises of mixture A (10 mM ammonium formate in 90% acetonitrile: water, v/v) and mixture B (0.1% formic acid in acetonitrile, v/v). Water used was LC and UV grade obtained from Macron, fine chemicals. Isocratic elution was achieved with 30% mixture A and 70% mixture B and was delivered at a flow rate of 200 µl/minute. Data acquisition and spectral analysis were executed on Lab Solution software (Shimadzu) and data was recorded as absolute intensity and *m/z* values. Mass spectroscopy data was loaded into m/z Cloud software to search for suspected compounds (HighChem LLC, 2013-2020).

6.3.3. Nuclear magnetic resonance (NMR) analysis

^{1}H NMR, ^{13}C NMR spectra and all two dimensional experiments [Correlation Spectroscopy (COSY), Distortionless Enhancement by Polarization Transfer (DEPT), Heteronuclear Multiple Quantum Coherence (HMQC) and Heteronuclear Multiple Bond Correlation (HMBC)] were recorded using nuclear magnetic resonance (NMR) Bruker Ascend 400 MHz Topspin 3.2 spectrometer operating at 400 MHz (^{1}H) and 100 MHz (^{13}C). The sample or

compound was weighed (20-30 mg) into clean NMR tubes and dissolved with either deuterated chloroform ($CDCl_3$) or deuterated dimethyl sulfoxide (DMSO-d6). The residual deuterated solvent resonances or signals was used as internal reference. Chemical shifts were recorded in parts per million (ppm): (δ_H 7.25 and δ_C 77.0 ppm for $CDCl_3$ or δ_H 2.50 and δ_C 40.0 ppm for DMSO-d6). Where applicable, the coupling constants were given in Hertz (Hz) and multiplicity of the signals was abbreviated as follows: s (singlet), d (doublet), dd (doublet of doublet), t (triplet), q (quartet), bs (broad singlet) and m (multiplet). The system used for numbering the atoms on the compounds was for convenience only and may not reflect IUPAC nomenclature numbering system.

6.3.4. Screening of phytochemical constituents of the fractions or isolated compounds

Phytochemical screening or analysis of active fractions or compounds were carried out using qualitative methods described by Rashmi *et al*., 2016; Harbone, 1973. The fractions or compounds were screened for the presence of alkaloids, glycoside, terpenoids or triterpenes, flavonoids and steroids using Dragendroff's test, Kellar Killani's test, Salkowski test, Alkaline reagent test and Salkowski test; respectively. Such information is very important during structural elucidation since it gives ideal structural indication or skeleton of the compound.

6.4. RESULTS AND DISCUSIONS

6.4.1. TLC-bioautographic antifungal activity of plant extracts and retention factor of antifungal compounds

TLC-bioautography is one of the most important techniques used in bioassay guided methods during isolation of antimicrobial compounds from medicinal plant extracts. It is simply used to determine the number of active compounds or active fractions in the extracts. The results

(chromatograms) of bioautography assay of selected plant extracts against *Fusarium* pathogens evaluated in this study are presented in Figure 6.2 and in Appendixes 1 to 8. The chromatograms sprayed with pathogen and incubated in the presence of p-iodonitrotetrazolium violet, resulted in white areas or spots.

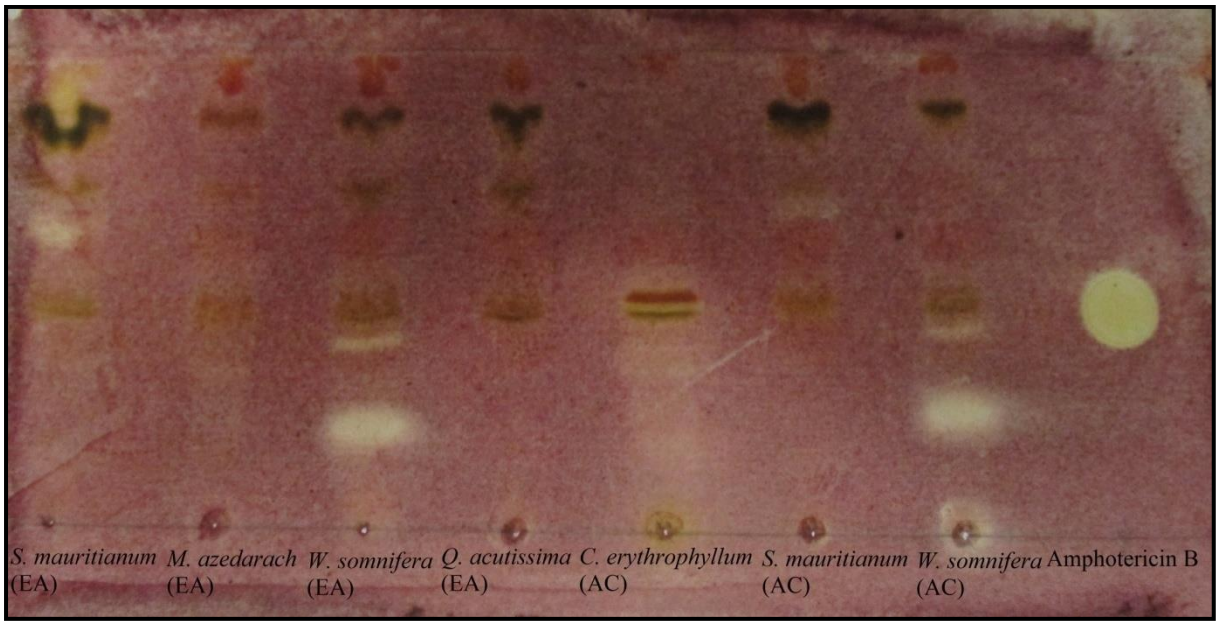


Figure 6.2. Bio-autographic chromatogram of medicinal plant extracts against *F. oxysporum*.

The presence of white area or clear zone on the chromatograms is an indication that the plant constituents has inhibited the growth of the pathogen. However, red colour on the chromatogram indicated that the pathogen was not inhibited and has reduced p-iodonitrotetrazolim violet into red formazan (Begue and Kline, 1972). The corresponding retention factor values of profiled plant constituents are presented in Appendix 9. The extracts obtained from *C. erythrophyllum*, *L. camara* and *W. somnifera* showed higher number of antifungal compounds or the presence of antifungal compounds in these plant species were able to inhibit at least 7, 5 and 5 pathogens, as presented in Appendix 9.

However, chemical compounds profiled from acetone extracts of plant species such as *C. molle*, *Q. acutissima*, *S. brachpetala* and *H. caffrum* were not active against tested *Fusarium*

pathogens. Ethyl acetate extracts obtained from *S. didymobotrya* and *S. brachpetala* were also found to have no active compounds or spots. These plant extracts had strong antifungal activity (MIC ≤ 0.1 mg/ml) when evaluated using microplate assay in Chapter 3. The absence of active bands or spots from these plant extracts as observed on TLC-bioautography chromatograms could be explained by disruption of synergistic effect of the compounds or plant constituents. The other possibility is that the active compounds were volatile and had evaporated when TLC chromatograms were dried for a week in the fume hood (Masoko and Eloff, 2006).

6.4.2. Phytochemical analysis and yield of isolated fractions or compounds

Open silica column and TLC chromatography techniques were used for purification of selected plant extracts. The fractions were collected into clean pre-weighed vials and quantified prior evaluation for antifungal activity. The quantity or total yield of active fractions isolated from extracts obtained from *C. erythrophyllum*, *L. camara* and *W. somnifera* is presented in Table 6.14. These fractions have demonstrated different levels of phytochemicals such as glycosides, flavanoids, terpenoids and steroids (Table 6.1). Flavanoids were found to be abundant in fraction **A** and **X** while fraction **I** and **AI** where found to contain moderate amount of this phytochemicals. On the other hand, terpenoids and or steroids were the most abundant phytochemicals while alkaloids were not detected in most isolated fractions.

Table 6.1. Yield of active fractions obtained from 7.0 grams of crude medicinal plant extract and their phytochemical constituents

Plant species	Fractions	Yield (mg)	Phytochemical analysis				
			Alkaloids	Glycosides	Flavanoids	Terpenoids	Steroids
C.erythrophyllum	**A**	156.30	-	-	+ + +	-	-
	B	97.50	-	-	-	+ ++	+
	D	167.90	-	+	-	+ + +	+

Plant species	Fractions	Yield (mg)	Phytochemical analysis				
			Alkaloids	Glycosides	Flavanoids	Terpenoids	Steroids
	I	384.50	-	+ + +	+ +	+ + +	+ + +
	J	380.10	-	+	-	+ + +	+
L. camara	**L**	192.80	-	+	-	+ + +	+ + +
	N	530.70	-	+++	+	+ + +	+ +
	O	427.20	na	na	na	na	na
	P	74.80	na	na	na	na	na
	Q	557.90	na	na	na	na	na
	R	598.50	-	+	+	+++	+++
	S	469.40	+	+	+	+	+
	T	207.80	na	na	na	na	na
	U	419.00	-	+ + +	+	+ + +	+ + +
	V	203.30	na	na	na	na	na
	W	393.30	na	na	na	na	na
	X	108.20	-	+	+ + +	+	+
W. somnifera	**AB**	309.60	-	+	+	+ + +	+ + +
	AF	164.00	-	+ + +	-	+ + +	+ + +
	AI	158.90	-	+ + +	-	+ + +	+ + +

(+++) abundantly present, (++) moderately present, (+) low presence, (−) absent, na; Not analyzed

6.4.4. *In vitro* antifungal activity of isolated fractions or compounds

A total of 20 active fractions were collected; of which three (3), five (5) and twelve (12) of them were obtained from *W. somnifera*, *C. erythrophyllum* and *L. camara*, respectively. The antifungal activity of these fractions against selected *Fusarium* species is presented based on their minimum inhibition concentrations as shown in Table 6.2 to 6.4. At least, one of the fractions isolated from these three (*C. erythrophyllum*, *L. camara* and *W. somnifera*) medicinal plant species showed antifungal activity with minimum inhibitory concentration value of less than 1.0 mg/ml. Of the five fractions isolated from *C. erythrophyllum*, fraction **A** demonstrated the strongest antifungal activity (MIC = 0.01 mg/ml) against *F. proliferatum*. As shown in table 6.2, this fraction had also demonstrated moderate activity with MIC values of 0.31, 0.31 and

0.63, 0.63 mg/ml against *F. verticilloides*, *F. solani* and *F. graminearum*, *F. chlamydosporum*, respectively. Out of seven pathogens tested, fraction **I** showed activity of 0.63 mg/ml against only two pathogens (*F. oxysporum* and *F. subglutinans*).

On the other hand, fraction **D** demonstrated moderate activity with recorded MIC values ranging from 0.31 to 0.63 mg/ml against all seven tested *Fusarium* pathogens. Of the twelve (12) fractions isolated from *L. camara*, eight (8) fractions showed minimum inhibitory concentration values ranging from 0.04 to 0.63 mg/ml against all five tested pathogens. The strongest activity recorded from this plant was demonstrated from fraction **L** with MIC value of 0.04 mg/ml against *F. proliferatum* (Table 6.3). On the other hand, out of twelve (12) fractions isolated from *W. somnifera* only three fractions demonstrated activity (MIC values of 0.16 to 0.63 mg/ml) against at least one of the tested *Fusarium* pathogens. Fraction **AI** demonstrated the strongest antifungal activity with recorded MIC value of 0.16 mg/ml against *F. verticilloides* (Table 6.4).

With exception to the activity of fraction **A**, **B**, **L** and **U** demonstrated against *F. proliferatum*, *F. verticilloides*, *F. proliferatum* and *F. proliferatum*, *F. semitectum*, respectively; the observed antifungal activity was weaker than what was reported for the corresponding medicinal plant extracts in Chapter 3. It was speculated that disruption of possible synergistic effect of the compounds could have occurred during isolation or purification process and led to reduction in antifungal compounds composition of the extracts. However, fraction **A** isolated from the leaves of *C. erythrophyllum* was particularly noteworthy as it demonstrated antifungal activity of 0.01 mg/ml against *F. proliferatum*. This recorded antifungal activity is at least 4 times stronger than the strongest activity (MIC = 0.04 mg/ml) demonstrated by extracts obtained from *C. erythrophyllum* as shown in Chapter 3 (Table 3.2). The extracts might have chemical constituents or compounds which acted antigonistically with fraction **A** and affected the antifungal activity of the extract.

Table 6.2. Minimum inhibitory concentration (MIC) values of fractions isolated from acetone leaves extract of *C. erythrophyllum* investigated for antifungal activity against phytopathogenic fungi.

Fractions	MIC value (mg/ml)						
	F. oxysporum	*F. verticilloides*	*F. subglutinans*	*F. proliferatum*	*F. solani*	*F. graminearum*	*F. chlamydosporum*
A	1.25	**0.31**	1.3	**0.01**	**0.31**	**0.63**	**0.63**
B	**0.31**	**0.08**	**0.63**	**0.31**	**0.63**	**0.63**	1.3
C	> 2.5	2.5	1.25	> 2.5	> 2.5	1.3	2.5
D	**0.63**	**0.63**	**0.63**	**0.63**	**0.63**	**0.63**	**0.31**
E	> 2.5	> 2.5	> 2.5	> 2.5	> 2.5	> 2.5	> 2.5
F	2.5	> 2.5	> 2.5	> 2.5	> 2.5	2.5	1.3
G	2.5	> 2.5	> 2.5	> 2.5	> 2.5	2.5	>2.5
H	1.25	> 2.5	1.25	> 2.5	1.3	1.3	1.3
I	**0.63**	> 2.5	**0.63**	> 2.5	1.3	2.5	> 2.5
J	**0.63**	**0.63**	**0.63**	1.3	1.3	1.3	**0.63**
K	1.3	1.3	2.5	1.3	1.3	1.3	1.3
Amphotericin B (µg/ml)	11.72	2.93	93.75	0.37	11.72	23.44	23.44

Values highlighted in bold indicate antifungal activity with MIC value less than 1.0 mg/ml.

Table 6.3. Minimum inhibitory concentration (MIC) values of fractions isolated from ethyl acetate leaves extract of *L. camara* investigated for antifungal activity against phytopathogenic fungi.

Fractions	MIC value (mg/ml)				
	F. subglutinans	*F. proliferatum*	*F. solani*	*F. graminearum*	*F. semitectum*
L	**0.63**	**0.04**	**0.63**	**0.63**	**0.63**
M	> 2.5	2.5	1.3	> 2.5	2.5
N	1.3	**0.63**	**0.63**	1.3	**0.16**
O	1.3	**0.16**	**0.63**	**0.63**	**0.63**
P	**0.31**	**0.31**	**0.31**	**0.63**	**0.31**
Q	**0.63**	**0.63**	**0.63**	**0.63**	**0.31**
R	**0.63**	1.3	2.5	2.5	**0.63**

Fractions	MIC value (mg/ml)				
	F. subglutinans	*F. proliferatum*	*F. solani*	*F. graminearum*	*F. semitectum*
S	**0.31**	**0.16**	**0.31**	**0.31**	**0.16**
T	**0.31**	**0.16**	**0.31**	**0.63**	**0.31**
U	**0.16**	**0.08**	**0.31**	**0.63**	**0.08**
V	**0.31**	**0.16**	**0.31**	**0.63**	**0.31**
W	**0.63**	**0.63**	**0.31**	**0.63**	**0.31**
X	**0.63**	**0.63**	1.3	1.3	1.3
Amphotericin B (µg/ml)	93.75	0.37	11.72	23.44	23.44

Values highlighted in bold indicate antifungal activity with MIC value less than 1.0 mg/ml.

Table 6.4. Minimum inhibitory concentration (MIC) values of fractions isolated from ethyl acetate leaves extract of *W. somnifera* investigated for antifungal activity against phytopathogenic fungi.

Fractions	MIC value (mg/ml)				
	F. oxysporum	*F. verticilloides*	*F. proliferatum*	*F. semitectum*	*F. solani*
Y	> 2.5	> 2.5	> 2.5	> 2.5	> 2.5
Z	> 2.5	> 2.5	> 2.5	> 2.5	> 2.5
AA	> 2.5	> 2.5	> 2.5	> 2.5	> 2.5
AB	**0.63**	**0.31**	**0.31**	1.25	1.25
AC	2.5	> 2.5	2.5	2.5	2.5
AD	2.5	1.25	2.5	> 2.5	2.5
AE	2.5	1.25	2.5	2.5	1.25
AF	> 2.5	**0.63**	> 2.5	**0.63**	**0.63**
AG	> 2.5	> 2.5	1.25	> 2.5	> 2.5
AH	> 2.5	> 2.5	2.5	> 2.5	> 2.5
AI	1.25	**0.16**	1.25	1.25	2.5
AJ	> 2.5	> 2.5	2.5	> 2.5	> 2.5
Amphotericin B (µg/ml)	11.72	2.93	0.37	23.44	11.72

Values highlighted in bold indicate antifungal activity with MIC value less than 1.0 mg/ml.

6.4.4. Structural determination of isolated antifungal compounds or fractions

Fraction **A** was isolated from the leaves of *C. erythrophyllum* as yellow amorphous powder. Its maximum absorbance readings recorded on LC-PDA was 270 nm and 330 nm, which indicated the presence of flavones skeleton or derivative. Its recorded melting point was approximately 340-343 °C. The positive electrospray ionization-mass spectrum (ESI-MS) of this compound shows peaks at *m/z* 284.95, 286.00, 327.00, 328.00 and molecular ion base peak $[M+H]^+$ at *m/z* 326.00. The ^{13}C-NMR spectral of fraction **A** is comparable with reported literature data in Table 6.5.

Table 6.5. ^{13}C-NMR chemical shifts of fraction **A** showing similarities with reported literature data (Mohamed *et al*., 2015).

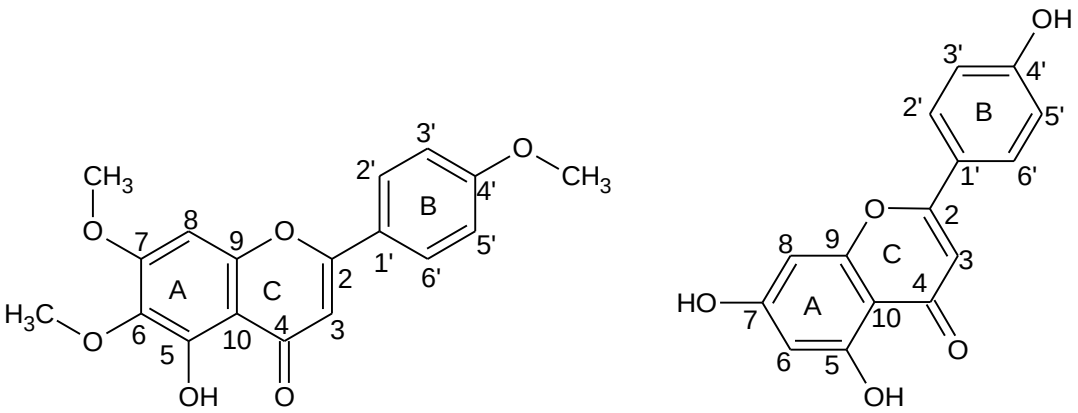
Signals	Nucleus	Multiplicity	δC (DMSO-*d6*)		
			Fraction **A**	Apigenin	Salvigenin
1	4-OCH_3	s	55.578	-	55.54
2	7-OCH_3	s	56.073	-	56.30
3	C-8	d	94.061	93.99	90.57
4	C-6	s	98.908	98.87	-
5	C-3	d	103.538	102.82	104.15
6	C-10	s	103.761	103.65	106.16
7	-	s	114.592	-	-
8	C-3', C-5'	d	115.980	115.97	114.53
9	C-1'	s	122.824	121.17	123.59
10	-	s	128.356	-	-
11	C-2', C-6'	d	128.596	128.48	128.01
-	C-6	-	-	-	132.67
-	C-5	-	-	-	153.09
-	C-9	-	-	-	153.24
-	C-7	-	-	-	158.74

Signals	Nucleus	Multiplicity	δC (DMSO-*d6*)		
			Fraction **A**	Apigenin	Salvigenin
12	C-9	s	157.357	157.33	-
13	C-4'	s	161.463	161.20	162.63
14	C-5	s	162.319	161.46	-
15	C-7	s	163.299	163.72	-
16	C-2	s	164.256	164.29	164.03
17	C-4	s	181.819	181.75	182.69

The ^{1}H-NMR spectrum of fraction **A** displayed the presence of about four aromatic protons at δ_H 7.95 (2H, d, H-2', H-6') and at δ_H 6.92 (2H, d, H-3', H-5'). This indicates substituted B ring of flavones skeleton. HSQC spectrum also confirmed a direct connection between protons at δ_H 6.92 and 7.95 with carbons at δ_C 115.98 and 128.59 ppm, respectively. This was further supported by two doublet signals at 6.92 and 7.95 ppm on ^{1}H-NMR spectrum. ^{1}H-^{1}H COSY spectrum indicates that these neighboring protons split each other (H-2′ is coupling with H-3′ and H-5′ with H-6′). There is also single bond correlation of aromatic protons at (δ_H 6.88, 6.86 and 6.37) with carbons at δ_C 103.76, 94.49 and 98.91; and they can be assigned to carbon 3, 8 and 6, respectively. These proton signals appear as singlets with no protons in the neighboring carbon atoms. The ^{1}H-NMR spectrum showed singlet peaks at δ_H 3.36 and δ_H 3.85 ppm, which can be assigned to 3 protons of two methoxy groups. The position of methoxy protons at δ_H 3.36 was assigned to (3H, s, 4'-OMe) and the ones at δ_H 3.85 was considered to be downfield and was assigned to (3H, s, 7-OMe). This was confirmed by ^{13}C-NMR signals at 55.58 (C-4') and 56.07 (C-7) and by HSQC experiment, which showed direct correlation of protons at (δ_H 3.36 and 3.85) with carbons at (δ_C 56.01 and 56.51), respectively.

Examination of both ^{13}C-NMR and DEPT (135 °C) spectra revealed the presence of eight (8) quaternary carbons at δ_C 103.54, 122.82, 157.36, 161.46, 162.32, 163.29, 164.26 and 181.82, which can be assigned to carbons 10, 1', 9, 4', 5, 7, 2 and 4, respectively. A characteristic of

fused rings in flavones derivatives or compounds. The position of hydroxyl group at carbon-5, was assigned based on HMBC long correlation of δ_H 12.94 (-OH) with carbons at δ_C 98.91 (C-6), 103.76 (C-3), 161.46 (C-4'), 162.32 (C-5), 163.29 (C-7) and 164.26 (C-2). Based on melting point, MS fragmentation, NMR and comparison with literature data, the structure of fraction **A** was tentatively elucidated as a mixture of compound **A** and compound **B**. The structures of these compounds are presented in Figure 6.3, and are denoted as salvigenin (*5-hydroxy-6,7,4'-trimethoxyflavone*) and apigenin (*4',5,7-trihydroxyflavone*), respectively.



Compound **A** Compound **B**

Figure 6.3. The structure of compounds obtained from fraction **A** isolated from acetone leave extract of *Combretum erythrophyllum*.

In a study conducted by Martini *et al*., 2004, several flavonoid compounds including apigenin (*4',5,7-trihydroxyflavone*) were isolated from the leaves of *Combretum erythrophyllum*. The isolated apigenin have also showed antimicrobial activity against *Vibrio cholerae* and *Enterococcus faecalis*, with MIC values ranging from 25-50 µg/ml. Apigenin isolated from *Chromolaena hirsuta* was further reported to show very good antimicrobial activity (MIC 100-500 µg/ml) against different bacterial strains, particularly *Staphylococcus aureus* and *Escherichia coli* (Taleb-Contini *et al*., 2003). In a different study, flavonoid compound was

isolated from the leaves of *Macaranga gigantifolia* and it was characterised as apigenin. This compound was evaluated for anticancer activity and it showed IC_{50} value of 14.13 μg/ml against murine leukimia P-388 cell line (Sofa *et al*., 2016). Shibata *et al*., 2014, reported that apigenin displayed antiviral activity by inhibiting hepatitis C virus replication. This compound is one of the most widely distributed flavonoids in the plant kingdom, and the most studied phenolics. Its various nutritional and organoleptic properties could lead to its inclusion in difference nutracetical products or formulations (Salehi *et al*., 2009). Nonetheless, there is little or no information with regard to possible application of apigenin to control crop diseases in the agricultural sector.

In an experiment involving tumor bearing mice, salvigenin (*5-hydroxy-6,7,4'-trimethoxyflavone*) isolated from *Tanacetum canescens* demonstrated possible reduction of tumor cell growth and it also showed inhibition of tumor tissue *in vivo* assay (Noori *et al*., 2013). This compound was also isolated from water extract obtained from the roots of *Astragalus propinquus*; however, in that study neither pharmacological properties nor antimicrobial activity of the compound was investigated (Chaturvedula and Prakash, 2013). Salvigenin isolated from *Salvia officinalis* was reported to show dose-dependent analgesic effect and it was concluded that it could be used to control acute and chronic pain (Mansourabadi *et al*., 2015). To the best of our knowledge, there is no literature study focused on the antifungal activity of salvigenin isolated from the leaves of *Combretum erythrophyllum*. However, a closely related compound (*5-hydroxy-7,4'-dimethoxyflavone*) isolated from the leaves of *Combretum zeyheri* showed a very good antifungal activity against *Candida albicans* (Mangoyi *et al*., 2015). Our study is one of its kind to investigate the antifungal activity of salvigenin and apigenin against fungal pathogens, particulary phytopathogenic *Fusarium* species.

Fraction **B** was isolated from the leaves of *C. erythrophyllum* as orange oily substance, which crystallized into colourless compound during evaporation in the fume hood. The melting point of this fraction was 260-268 °C and its maximum absorbance recorded on LC-PDA was 245 nm. The ^{13}C-NMR spectral of fraction **B** is comparable with reported literature data in Table 6.6.

Table 6.6. ^{13}C-NMR chemical shifts of fraction **B** showing similarities with reported literature data (Moghaddam *et al*., 2007).

Signals	Nucleus	^{13}C, ppm in $CDCl_3$		Signals	Nucleus	^{13}C, ppm in $CDCl_3$	
		Fraction **B**	Reference			Fraction **B**	Reference
1	-	14.09	-	16	C-14	44.41	42.5
2	C-24	15.94-15.98	16.1	17	C-4	44.76	-
3	C-25	16.26	16.9	18	C-6	51.79	47.9
4	C-26	17.63	17.8	19	C-5	54.25	55.6
5	-	18.63	18.9	20	-	114.02	-
6	C-30	22.64	23.7	21	-	124.18	-
7	-	25.66	-	22	-	124.22	-
8	-	26.58	-	23	C-12	124.32	125.4
9	C-11	26.68	-	24	-	131.21	-
10	C-9	28.19	27.8	25	-	134.83	-
11	C-23	29.31	29.1	26	C-13	135.04	137.9
12	C-15	29.64-29.60	28.4	27	-	176.91	-
13	C-5	30.91	31.1	28	-	177.81	-
14	C-7	31.87	33.6	29	C-28	178.08	179.1
15	C-1	39.68-39.66	39.2	30	C-3	207.07	ketone

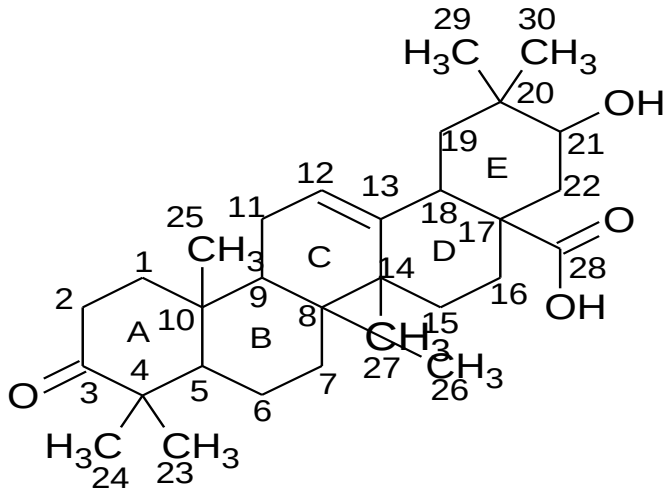
The spectra of fraction **B** displayed about 30 carbon signals and almost 12 proton signals. From the HSQC spectrum, the olefinic carbon signal at 124.32 ppm was found to be connected directly to a proton resonating at 5.13 ppm. This proton signal (5.13 ppm) is splitted into triplet,

probably by two neighboring protons. There is another olefinic carbon signal at 135.05 ppm, which was displayed by ^{13}C-NMR and DEPT as quaternary carbon. From this assumption or observation, carbons resonating at 124.32 ppm and 135.04 ppm could be assigned to C-12 and C-13, respectively. Examination of ^{1}H-^{1}H COSY spectrum showed that protons at 1.96 ppm are coupling with both proton signals at 2.06 and 5.13 ppm. Additionally, all these proton signals (1.96, 2.06 and 5.13 ppm) are triplets and this could suggest that protons resonating at 1.96 ppm might be connected to C-11 while the one at 2.06 ppm is attached to C-9.

The DEPT spectrum displayed about nine (9) methylene carbon signals at 54.30, 39.75, 31.94, 29.71, 29.38, 28.27, 26.74, 26.63 and 22.71 ppm, which may be assigned to either carbons at C-1, 2, 6, 7, 15, 16, 22, 19 and 11. The number of methylene carbon signals could also suggest that methyl carbons numbered 29 and 30 are attached to the same carbon. The HMBC experiment showed long-range connection between the proton at 2.17 ppm with carbon signals at 205.07 and 44.76 ppm. This proton was found to be connected to carbon signal at 30.91 ppm as evidenced by HSQC spectrum. Based on this information, carbon resonating at 30.91 and 44.76 ppm could be assigned to carbon 5 and 4, respectively. This assignment was also supported by ^{13}C-NMR and DEPT spectra that displayed carbon signal at 44.76 as quaternary carbon. The ^{13}C-NMR spectrum showed carbonyl signals at 207.07 and 178.08 ppm assignable to ketone and carboxylic acid carbons numbered C-3 and C-28, respectively. The ^{1}H-NMR spectrum of fraction **B** shows proton signals at chemical shift ranging from 0.83 to 5.13 ppm and there is similarities with data reported in literature suggesting the presence of 12-oleanene type of triterpene with a secondary hydroxyl and keto group (Mena-Rejón *et al*., 2007).

The MS fragmentation spectrum of this fraction showed peaks at *m/z* 306.15, 400.25, 452.35, 482.35, 498.35, 516.35, 530.35 and molecular ion base peak $[M+H]^+$ at *m/z* 468.35 which corresponds to molecular formula $C_{30}H_{44}O_4$. This molecular ion peak is comparable to data

reported for synthesized 3-oxo-olean-12-en-28-oic acid (Wicht, 2007). Based on spectroscopic data and literature information, fraction **B** could be characterised as compound **C**, maslinic acid (*2α,3β)-2,3-dihydroxyolean-12-en-28-oic acid*) or a derivative of oleanolic acid (*21-hydroxy-3-oxo-olean-12-en-28-oic acid*) as shown in Figure 6.4.



Compound **C**

Figure 6.4. The structure of compound obtained from fraction **B** isolated from acetone leave extract of *Combretum erythrophyllum*.

In a study devoted to the evaluation of antibacterial activity of compounds derived from *Olea europaea*, maslinic acid isolated from the leaves of this plant was reported to be the most active (4.9–312 µg/ml) compound against oral bacterial species such as *Porphyromonas gingivalis*, *Fusobacterium nucleatum*, *Parvimonas micra* (Karygianni *et al*., 2019). Maslinic acid is naturally occurring triterpene. It was first detected in *Crataegus oxyacantha* (L) and later in several plant species, vegetables, herbs and fruits. It was studied for health promoting properties such as antioxidant, antidiabetic, antitumoral, antiviral and anti-inflammatory (Lozano-Mena *et al*., 2014; Hashmi *et al*., 2015). Animal model experiments revealed that

maslinic acid had no adverse side effects; hence, it was suggested as potential nutraceutical and therapeutic agent for the treatment of various disorders. In the current study, compound **C** isolated from the leaves of *Combretum erythrophyllum* and characterized as maslinic acid was evaluated for antifungal activity against agricultural crop diseases. To be best of our knowledge, this is the first study dealing with isolation of maslinic acid from the leaves of *Combretum erythrophyllum* and its antifungal activity against *Fusarium* pathogens.

Fraction **L** was isolated as brown-orange powder from ethyl acetate extract obtained from the leave of *Lantana camara.* The melting point of this fraction ranged from 275 to 285 °C and its maximum ultraviolet absorbance in methanol or ethanol was 448 nm (UV-Vis spectrum). The positive electrospray ionization-mass spectrum (ESI-MS) displayed peaks at *m/z* 563.20, 507.72, 551.35, 593.20, 639.35, 683.35, 727.45, 905.5, 963.56 and molecular ion base peak at *m/z* 569 $[M+H]^+$ which is consistent with molecular formula $C_{35}H_{52}O_6$. The ^{13}C-NMR spectral data of fraction **L** is comparable with reported literature data in Table 6.7.

Table 6.7. ^{13}C-NMR chemical shifts of fraction **L** showing similarities with reported literature data (Fatope *et al*., 2002; Kartika *et al*., 2018).

Signals	Nucleus	^{13}C, ppm in $CDCl_3$		Signals	Nucleus	^{13}C, ppm in $CDCl_3$	
		Fraction **L**	Reference			Fraction **L**	Reference
1	C-25	15.58	15.86	24	C-4	45.70	46.51
2	C-26	17.24	16.83	25	C-17	50.09	50.29
3	-	18.26	-	26	C-9	50.68	50.62
4	C-6	19.62	19.27	27	C-22	67.66	65.82
5	C-34	20.48	20.78	28	-	98.75	-
6	C-11	23.66	23.86	29	-	115.89	-
7	C-16	24.12	24.25	30	C-12	122.36	122.33
8	-	25.31	-	31	-	122.44	-
9	C-23	26.04	26.29	32	-	122.51	-

Signals	Nucleus	^{13}C, ppm in $CDCl_3$		Signals	Nucleus	^{13}C, ppm in $CDCl_3$	
		Fraction **L**	Reference			Fraction **L**	Reference
10	-	27.16	-	33	-	125.59	-
11	C-15	27.68	27.86	34	C-32	127.73	127.95
12	-	29.29	-	35	-	138.09	-
13	-	30.01	-	36	C-33	138.41	138.93
14	-	30.04	-	37	-	142.98	-
15	C-20	30.62	30.29	38	C-13	143.49	143.51
16	-	30.87	-	39	-	157.17	-
17	C-29	33.65	33.94	40	-	165.32	-
18	C-2	34.52	34.38	41	C-31	166.46	166.66
19	C-10	35.01	36.66	42	C-28	178.89	177.72
20	C-1	38.21	38.58	43	-	179.23	-
21	C-8	39.00	39.27	44	-	182.70	-
22	-	40.17	-	45	C-3	182.87	-
23	C-14	41.90	42.21				

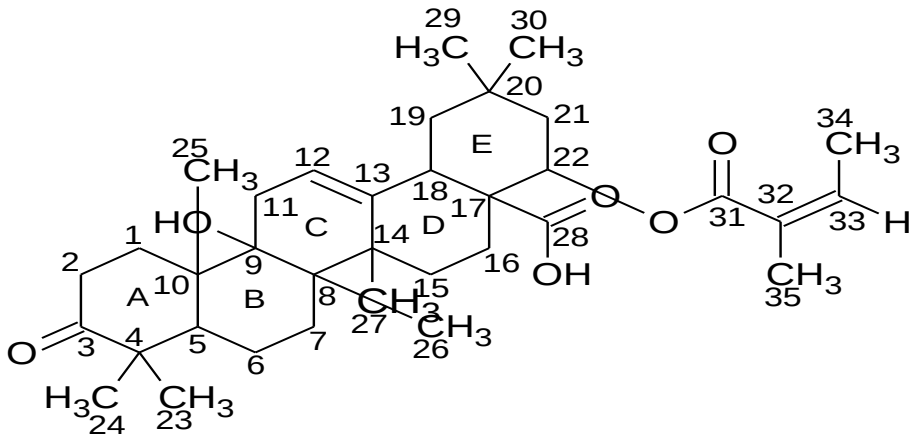
The ^{13}C-NMR spectrum of fraction **L** revealed the presence of 45 carbon signals and from DEPT spectrum, 17 of those were characterised as quaternary carbon signals. The quaternary carbon signal at chemical shift δ_C 50.68 ppm can be assigned to C-9, which is attached to hydroxyl group. This assignment is supported by the proton signal at δ_H 5.05 ppm; since it corresponds to the chemical shift of hydroxyl proton connected to a quaternary carbon. The ^1H-NMR spectrum of fraction **L** shows signals ranging from 0.73 to 6.00 ppm assignable to methyl (-CH_3), methylene (-CH_2) and methine (-CH) protons. The ^1H-^1H COSY spectrum shows split coupling of protons at δ_H 5.05 ppm and 1.81 ppm, assuming that these protons (δ_H 1.81 ppm) are attached to C-11 (secondary carbon). In fact, the protons at δ_H 1.81 ppm are also coupling with protons at 5.39 ppm that appears as triplet, confirming the attachment of two (2) hydrogen atoms to the neighboring carbon (C-11).

HSQC experiment shows the direct correlation of protons at 5.39 ppm with carbon signal at 122.36 ppm and this carbon was assigned C-12. This assignment assume that carbon-13 is quaternary; hence, proton COSY spectrum shows the coupling between H-11 (δ_H 1.81 ppm) and H-12 (δ_H 5.39 ppm) only. The quaternary carbon signal at 143.49 ppm was assigned to C-13 and was further supported by already assigned proton and carbon at position 12 at chemical shifts 5.39 and 122.36 ppm, respectively. These chemical shifts are characteristics of proton and carbon attached to the double bond. The ^{1}H-NMR spectrum shows multiplet or quartet signal at 5.98 ppm, which is also a characteristic of chemical shift of proton attached to the double bond and it corresponds to H-33. This was supported by HSQC experiment, which shows correlation of this proton (δ_H 5.98 ppm) with carbon signal at 138.42 ppm.

Examination of ^{13}C-NMR and DEPT spectra further confirmed the assignment of carbon signal at 138.42 ppm to C-33. The quaternary carbon signal that appeared at chemical shift δ_C 182.87 ppm corresponds very well with ketone carbonyl resonance and was assigned to C-3. The peak at chemical shift δ_C 178.89 ppm is a characteristic of carbonyl atom corresponding to carboxylic acid group, hence was assigned to C-28. The signal at 166.49 ppm appeared as quaternary carbon and since is within chemical shift characteristics of carbonyl esters, it was assigned to C-31. The carbon signal at 67.66 ppm correspond to R_3**C**-OR and was assigned to C-22, which was further supported by DEPT spectrum. The proton connected to this carbon (C-22) resonance at chemical shift δ_H 3.87-4.25 ppm as evidenced by HSQC experiment.

There are almost eight (8) signals that appear as singlets at chemical shifts δ_H 0.73, 0.76, 0.85, 0.97, 1.00, 1.06, 1.12 and 1.15 ppm in the ^{1}H-NMR spectrum and these signals do not split couple with any proton on the COSY spectrum. The combination of ^{13}C-NMR and DEPT spectra could make the assignment of methyl and methylene carbons possible as are assignable at chemical shift ranging from 50.00 to 14.00 ppm. Based on the spectroscopic information and

literature data, fraction **L** was characterized as compound **D** as shown in Figure 6.5 and was denoted lantadene A (*22- angeloyloxy-9-hydroxy-3-oxo-olean-12-en-28-oic acid*).



Compound **D**

Figure 6. 5. The structure of compound obtained from fraction **L** isolated from ethyl acetate leaves extract of *Lantana camara*.

Lantadene A is one of the most important pentacylic triterpenoids presence in the leaves of *Lantana camara*. Although it was reported to be toxic to the brine shrimp larvae, this compound was not lethal to *Spodoptera littoralis*, *Clavigralla tomentosicollis* and *Aphis craccivora*. In the same study, lantadene A was however, reported to suppressed the fecundity of *Clavigralla tomentosicollis* at a concentration of 5000 µg/ml (Fatore *et al*., 2002). An antifeedant activity of lantadene compounds obtained from *Lantana camara* has also been reported (Dong *et al*., 2005). In another study, different lantadene compounds including lantadene A were reported to show weak to moderate cytotoxic activity against different cancer cell lines (Litaudon *et al*., 2009). Lantadene A and derivative compounds from lantadene were also reported to show cytotoxicity activity against human cancer cell lines and antitumor activity against squamous cell in mice (Sharma *et al*., 2008). There is limited information on the antimicrobial activity of

compounds isolated from *Lantana camara* as compared to the activity of the crude extracts from this plant. In the present study, lantadene A was evaluated for antifungal activity against phytopathogenic species and it showed very good activity (MIC ≤ 0.65) against *Fusarium subglutinans, F. proliferatum, F. solani, F. graminearum* and *F. semitectum*.

Fraction **R** was isolated as yellow to whitish substance from ethyl acetate extract obtained from the leave of *Lantana camara.* Negative electrospray ionization-mass spectrum (ESI-MS) displayed peaks at *m/z* 485.2, 515.3, 555.3, 583.3, 599.3, 939.6 and molecular ion base peak at 469.3. This peak is consistent with molecular formula $C_{30}H_{46}O_4$. ^{13}C-NMR data of this fraction is compared with literature information in Table 6.8. Phytochemical analysis of this fraction indicated the presence of terpenoid structure or skeleton. Melting point was found to be 190 to 195 °C.

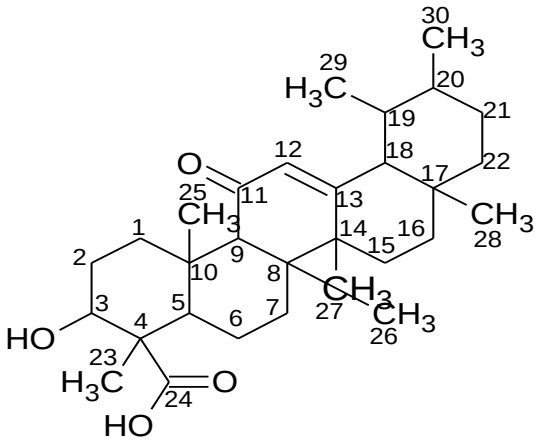
Table 6.8. ^{13}C-NMR data of fraction **R** isolated from ethyl acetate leaves extract obtained from *Lantana camara* is comparable with literature data (Al-Harrasi *et al*., 2018; Al-Harrisi, 2015).

Signals	Nucleus	Fraction **R**	References
		$\delta_{13C,}$ ppm	
1	C-29	15.6	15.6
2	C-26	16.93	16.6
3		17.22	
4		17.50	
5	C-27	17.78	18.1
6	C-6	18.27	18.7
7		19.54	
8		19.64	
9	C-27	20.48	20.5
10	C-30	21.07	21.2
11		23.14	

Signals	Nucleus	Fraction **R**	References
		δ_{13C}, ppm	
12	C-2	23.66	23.5
13		25.31	
14	C-16	27.10	26.7
15	C-15	27.17	27.2
16	C-28	29.29	28.9
17		30.03	
18		30.63	
19	C-21	30.95	30.9
20	C-7	31.17	32.8
21	C-1	34.85	34.6
22	C-10	35.02	37.3
23		38.26	
24		38.42	
25	C-20	38.75	39.3
26	C-19	39.05	39.3
27	C-8	40.18	39.9
28	C-22	41.68	40.9
29		41.86	
30		41.91	
31	C-14	41.99	43.7
32	C-4	48.06	46.4
33	C-5	50.12	50.4
34		53.05	
35	C-9	67.73-67.89	60.3
36	C-3	75.0	73.0
37		100.23	
38		122.54	
39		125.60	
40	C-12	138.11	130.5
41	C-13	143.00	146.3
42	C-11	173.0	

Signals	Nucleus	Fraction **R**	References
		$\delta_{13C,}$ ppm	
43	C-24	182.55	181.2

Based on available literature information, melting point and spectroscopic data, fraction **R** was tentatively characterized as *11-keto-β-boswellic acid* or *3-O-acetyl-11-keto-β-boswellic acid*. The structure of this compound is illustrated in Figure 6.6.



Compound **E**

Figure 6.6. The structure of compound obtained from fraction **R** isolated from ethyl acetate leaves extract of *Lantana camara*.

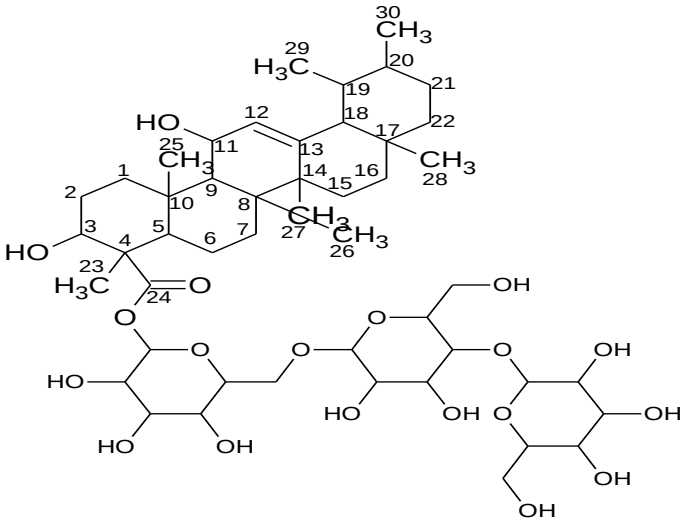
Fraction **U** was isolated as sticky brown material from ethyl acetate extract obtained from the leave of *Lantana camara.* Phytochemical analysis of fraction **U** showed the presence of terpenoid glycosides. The positive electrospray ionization-mass spectrum (ESI-MS) displayed peaks at *m/z* 432.2, 512.0, 586.4, 632.4, 730.5, 788.6, 871.4, 871.4, 941.7, 963.7, 1056.8 and molecular ion base peak 958.8. The ion based peak is consistent with molecular formula $C48H_{78}O_{19}$. The ^{1}H-NMR and ^{13}C-NMR spectral data of fraction **U** is presented in Table 6.9.

To some extend NMR data of fraction **U** is similar to that of fraction **R**, however, fraction **U** does not show carbon atoms signals at chemical shift, 182.5 and 173.0 ppm, which, are characteristics of ketone and carboxylic acid carbonyl, respectively. Phytochemical analysis of fraction **U** revealed the presence of glucosides group or moiety. This was further supported by information obtained from m/z Cloud compounds search. Therefore, fraction **U** was tentatively characterized as boswellic acid glycosides and is represented in Figure 6.7 as compound **F.**

Table 6.9. ^{1}H-NMR and ^{13}C-NMR data of fraction **U** isolated from ethyl acetate leaves extract obtained from *Lantana camara* (Al-Harrasi *et al*., 2018; Al-Harrisi, 2015).

Fraction **U**					Nucleus	Reference
Signals	($\delta_{H,}$ ppm)	Multiplicity	Type of proton	$\delta_{13C,}$ ppm		
1	0.74	d	CH_3	16.8	C-26	16.6
2	0.85	s	CH_3	17.5		
3	0.95	d	CH	18.2	C-27	18.7
4	1.02	s	CH_3	19.5		
5	1.05	s	CH_3	20.7	C-27	20.5
6	1.1	s	CH_3	21.2	C-30	21.2
7	1.25	m	CH_2	22.9		
8	1.35	m	CH_2	23.7	C-2	23.5
9	1.49	m	CH_2	24.8		
10	1.5-1.8	m	CH_2	25.3		
11	2.0	m	CH	27.1	C-16	26.7
12	2.08	s	CH_3	27.8	C-15	27.2
13	2.15	m	CH_2	29.3	C-28	28.9
14	2.25	m	CH	29.7		
15	2.4	s	CH	30.6		
16	2.6			30.9	C-21	30.9
17	3.45	dd	CH	31.2	C-7	32.8
18	3.51-3.61			32.2		
19	3.8			34.8	C-1	34.6

Fraction **U**					Nucleus	Reference
Signals	($\delta_{H,}$ ppm)	Multiplicity	Type of proton	$\delta_{13C,}$ ppm		
20	3.95			38.2		
21	4.2	dd	CH	38.4	C-20	39.3
22	5.0			39.2	C-19	39.3
23	5.35	d	CH	40.2	C-8	39.9
24	5.9			41.9	C-22	40.9
25	6.47			42.0		
26	6.9			42.4	C-14	43.7
27				48.1	C-4	46.4
28				49.3		
29				50.2	C-5	50.4
30				50.7		
31				51.7		
32				67.8	C-9	60.3
33				75.0	C-3	73.0
34				125.0		
35				126.0		
36				137.2	C-12	130.5



Compound **F**

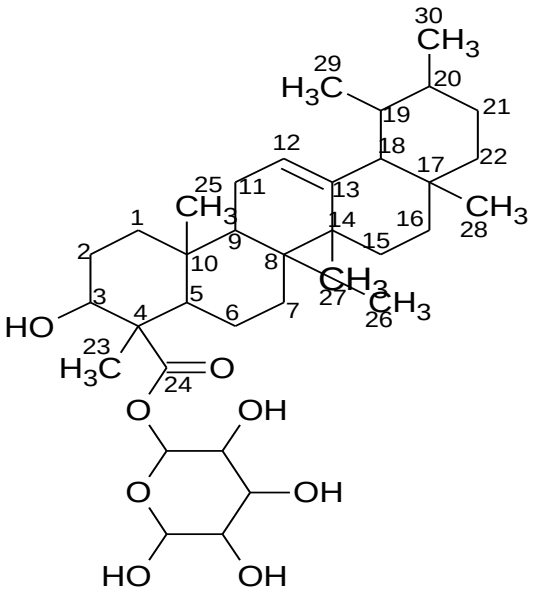
Figure 6.7. The structure of compound obtained from fraction **U** isolated from ethyl acetate leaves extract of *Lantana camara*

Fraction **N** was isolated as sticky whitish material from ethyl acetate extract obtained from the leave of *Lantana camara*. Negative electrospray ionization-mass spectrum (ESI-MS) displayed peaks at *m/z* 469.2, 485.2, 515.25, 555.3, 583.3, 939.7 and molecular ion base peak at 599.3. Proton NMR and ^{13}C-NMR data of this fraction is presented in Table 6.10. Phytochemical analysis of this fraction revealed the presence of terpenoid glycosides structure. m/z Cloud compound search using MS data of fraction **N** lead to tentative characterisation of compound **G** as presented in Figure 6.8. This compound seems to have formed due to the breaking down of compound **E** and **F**.

Table 6.10. ^{1}H-NMR and ^{13}C-NMR data of fraction **N** isolated from ethyl acetate leaves extract obtained from *Lantana camara* (Al-Harrasi *et al*., 2018; Al-Harrisi, 2015).

Signals	($\delta_{H,}$ ppm)	Multiplicity	Type of proton	$\delta_{13C,}$ ppm	Reference
1	0.49			15.61	
2	0.63			17.23	
3	0.94-0.98	d	CH	18.25	18.7
4	1.01-1.04	s	CH_3	19.3	
5	1.07-1.13	s	CH_3	20.49	20.5
6	1.23	m	CH_2	25.31	
7	1.41			27.31	27.2
8	1.49	m	CH_2	29.26	28.9
9	1.69	m	CH_2	30.02	30.9
10	1.77			30.63	
11	1.83			30.95	
12	1.93			35.02	34.6
13	1.93-1.95			38.22	39.3
14	2.12	m	CH_2	39.07	39.3

Signals	($\delta_{H,}$ ppm)	Multiplicity	Type of proton	$\delta_{13C,}$ ppm	Reference
15	2.16		CH_2	40.19	39.9
16	2.99-3.04			41.91	40.9
17	3.87			50.69	50.4
18	4.20	dd	CH		
19	5.04				
20	5.25				
21	5.36	d	CH		
22	5.6				
23	5.95-5.99				



Compound **G**

Figure 6.8. The structure of compound obtained from fraction **N** isolated from ethyl acetate leaves extract of *Lantana camara*.

Boswellic acids are pentacyclic triterpenes usually exudated by the plant species belonging to the *Boswellia* family. A gum resin obtained from these plants is well known in traditional

medicine for the treatment of inflammation (Chatterjee and Pal, 1984). The chemical constituents of the gum were reported to be active against cancer, arthritis, ulcerative colitis and bronchial asthma (Reddy and Dhar, 1987; Gupta *et al*., 1997; Krieglstein *et al*., 2001; Kimmatkar *et al*., 2003). There is little or no information about antimicrobial activity of boswellic acid compounds against crop pathogens. However, a study on the antibacterial activity of boswellic acid compounds demonstrated that acetyl-11-keto-β-boswellic acid obtained from *Boswellia serrata* was the most active (2-8 μg/ml) compound against tested Gram-positive pathogens (Raja *et al*., 2011). In the current study, keto-β-boswellic acid and two boswellic acid glycoside compounds were isolated from the leaves of *Lantana camara* and investigated for the antifungal activity against *Fusarium* pathogens. To our knowledge, this is the first report about isolation of boswellic acid compounds from the leaves of *Lantana camara* and its antifungal activity against phytopathogenic species, particulary *Fusarium* pathogens.

Fraction **AI** was isolated as sticky yellowish substance from ethyl acetate extract obtained from the leave of *Withania somnifera*. The positive electrospray ionization-mass spectrum (ESI-MS) of this fraction displayed peaks at *m/z* 262.9, 399.1, 417.1, 435.1, 453.2, 488.0, 534.2, 942.6, 958.5 and molecular ion base peak at 963.3. Phytochemical analysis showed the presence of terpenoids and or steroid glycosides structure. ^{13}C-NMR and ^{1}H-NMR spectral information of fraction **AI** is comparable with reported literature data in Table 6.11 and 6.12, respectively.

Table 6.11. ^{13}C-NMR chemical shifts of fraction **AI** showing similarities with reported literature data (Zhao *et al*., 2002; Chaurasiya *et al*., 2012).

Signals	Nucleus	^{13}C, ppm		
		Fraction **AI**	Reference	Withaferin A
1	C-19	9.45	-	

Signals	Nucleus	^{13}C, ppm		
		Fraction **AI**	Reference	Withaferin A
2	C-18	12.34	11.7	11.6
		-	13.5	13.3
3	C-21	14.68	-	-
4		15.05	-	-
		-	-	17.4
		-	19.5	19.9
5	C-28	20.53	20.5	-
6		21.55	-	-
7	C-11	22.87	-	22.2
		-	24.5	24.3
		-	27.2	27.3
		-	29.8	29.8
8	C-23	32.39	32.1	31.2
9		32.69	32.2	-
10	C-8	35.15	-	-
11		35.92	-	-
12		36.54	-	-
13	C-12	36.69	37.9	38.8
		-	39.1	39.4
		-	41.4	-
14	C-13	42.84	42.8	42.6
15	C-9	45.81	-	44.2
16	C-13	48.63	-	-
17	C-17	50.90	52.0	51.9
18	C-14	56.23	56.3	56.1
19	C-10	57.17	-	57.5
		-	63.4	63.8
		-	72.3	69.9
20	C-5	73.23	74.4	76.6
		-	-	77.4
21	C-22	78.70	78.2	78.8

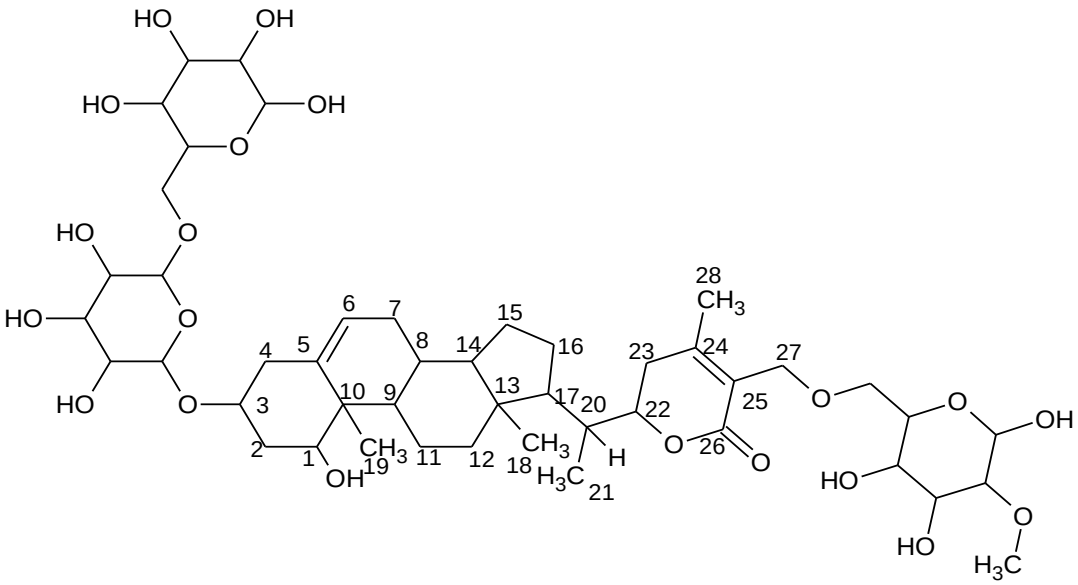
Signals	Nucleus	^{13}C, ppm		
		Fraction **AI**	Reference	Withaferin A
22	C-17	84.59	-	-
23	C-25	121.34	123.9	125.1
24	C-2	128.92	-	131.6
25	C-3	139.73	139.3	137.5
		-	-	141.1
26	C-24	150.56	157.0	152.6
		-	-	161.6
27	C-26	167.20	166.0	166.9
28	C-1	203.21	-	202.2

Table 6.12. ^{1}H-NMR chemical shifts of fraction **AI** showing similarities with reported literature data (Zhao *et al*., 2002).

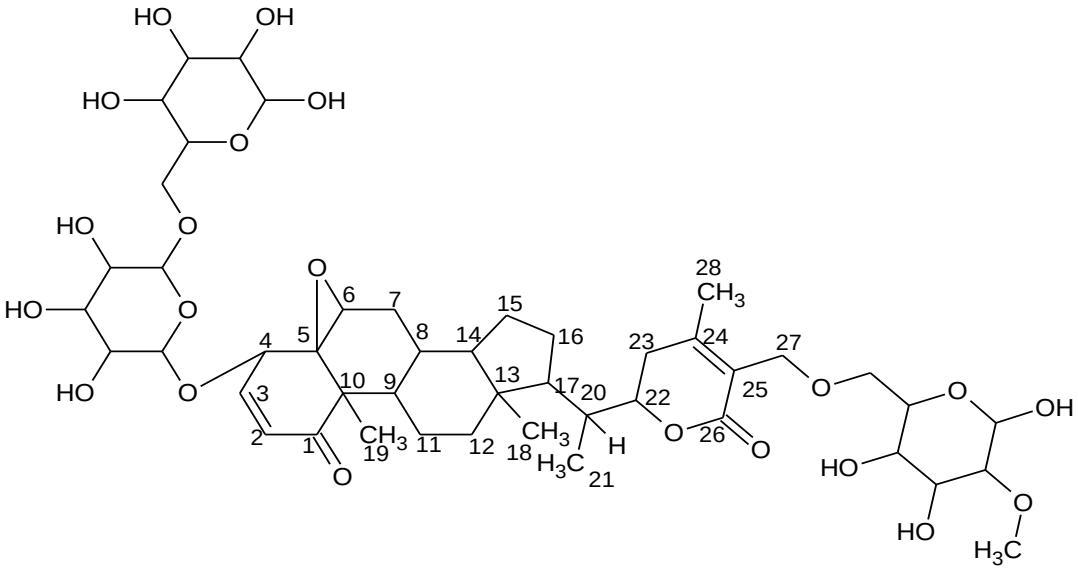
Signals	Fraction **AI**		Reference	
	$\delta_{H,}$ ppm	Multiplicity	$\delta_{H,}$ ppm	Multiplicity
1	0.84	1H, m	0.88	1H, m
2	0.94	3H, d	0.94	3H, d
3	0.97	3H, s	0.98	3H, s
4	1.01	1H, m	1.01	1H, m
5	1.14	1H, m	-	
6	1.20	1H, m	-	
7	1.22	1H, m	-	
8	1.28	1H, t	1.34	1H, t
9	1.53	1H, m	-	
10	1.69	1H, m	1.66	1H, m
11	1.93	1H, m	-	
12	1.95	1H, m	-	
13	1.99	1H, dd	1.98	1H, dd
14	2.2	1H, dd	2.17	1H, dd
15	2.45	1H, m	-	

Signals	Fraction **AI**		Reference	
	$\delta_{H,}$ ppm	Multiplicity	$\delta_{H,}$ ppm	Multiplicity
16	2.46	1H, m	-	
17	2.50	1H, t	-	
18	2.56	1H, t	2.62	1H, t
19	2.70	1H, d	2.78	1H, d
20	2.8	1H, d	2.81	1H, d
21	3.04	1H, d	-	
22	3.15	1H, d	-	
23	3.3	1H, m	-	
24	4.2	1H, m	4.22	1H, m
25	4.6	1H, d	4.68	1H, d
26	5.8	1H, d	5.56	1H, d
27	6.5	1H, m	-	

The ^{13}C-NMR analysis of fraction **AI** showed the presence of 28 carbon signals. From DEPT analysis, seven (7) of those signals were characterized as quaternary carbons. These quaternary carbons resonance at δ_C 203.2, 73.2, 57.2, 48.6, 150.6, 121.3, 167.2 ppm and they can be assigned to C-1, C-5, C-10, C-13, C-24, C-25, C-26, respectively. Assignment of these carbons was also supported by HSQC experiment or analysis, which, showed that there is no protons attached to these carbons. The carbon peak at chemical shift δ_C 203.2 ppm correspond with ketone carbonyl resonance hence it was assigned to C-1. The peak at 167.2 ppm is at chemical shift characteristics of carbonyl esters and it was assigned to C-26. MS spectral analysis of fraction **AI** using m/z Cloud compounds search showed the presence of withanolide and withaferin A glycoside compounds. To some extend this was supported by phytochemical analysis data as presented in Table 6.1. Based on available literature and spectroscopic data, fraction **AI** was tentatively characterized as a mixture of compound **H** and **I** as depicted in Figure 6.9.



Compound **H**



Compound **I**

Figure 6.9. The structure of compounds obtained from fraction **AI** isolated from ethyl acetate leaves extract of *Withania somnifera*.

Several studies have focused on the anti-inflammatory, anticonvulsant, anti-stress and anti-tumor activity of *Withania somnifera*. It was suggested that the pharmacological properties and

other biological activities of this plant are attributed to withaferin A and withanone presence in different parts of the plant (Kulkarni *et al*., 1993; Kulkarni and George, 1996; Sharada *et al*., 1996; Girish *et al*., 2006; Khare, 2007). There is limited data available on the antimicrobial activity of compounds isolated from *Withania somnifera* as compared to the evaluation of the extracts obtained from this plant (Dharajiya *et al*., 2014; Romha *et al*., 2018). However, a compound isolated from the roots of *Withania somnifera* and characterized as withaferin A has demonstrated antibacterial activity against human and animal pathogens (Kharela *et al*., 2011). Acetone extract from the fruit of *Withania somnifera* was reported to demonstrate a very good antifungal activity against *Fusarium culmorum* and *Rhizoctonia solani* (EL-Hefny *et al*., 2020). In another study, aqueous extract from the fruit was also reported to show potential antifungal activity against *F. oxysporum* strain (Nefzi *et al*., 2016). Our current study deals with antifungal activity of withanolide compound, specifically withaferin A glycoside isolated from the leaves of *Withania somnifera*. This compound demonstrated very strong antifungal activity (MIC = 0.16 mg/ml) against *Fusarium verticilloides*.

6.4.4. Phytotoxicity of isolated antifungal compounds against maize seed germination, shoots and root length

The isolated antifungal compounds were found to show no negative effect on maize seed germination (Figure 6.10). There is increase in shoots length between seeds treated with maslinic acid and boswellic acid as compared to water treatment (Figure 6.11). Moreover, isolated antifungal compounds showed no negative effect on roots length (Figure 6.12). These compounds can be studied further and be considered as potential maize seed treatment agents.

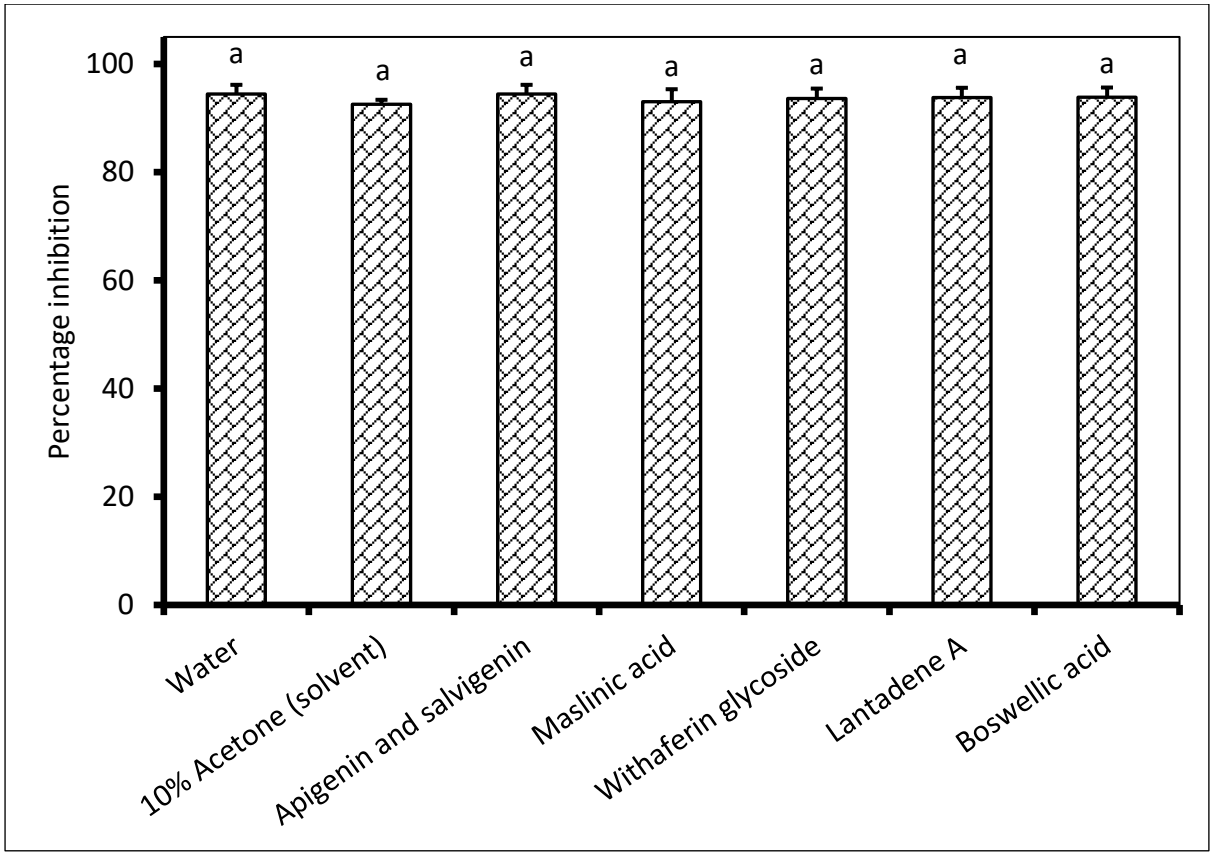


Figure 6.10. Percentage germination of maize seeds treated with isolated antifungal compounds. A mixture of apigenin and salvigenin, lantadene A, boswellic acid and maslinic acid were used at a concentration of 0.63 mg/mL in 10% acetone. Withaferein A glycoside was used at a concentration of 0.16 mg/ml in 10% acetone. Water and 10% acetone were used as negative controls. There were 5 replicates per treatment, each comprising 25 disinfected maize seeds, and the experiment was repeated twice. Bars bearing the same letters indicate no significant differences ($p = 0.05$), as determined by Duncan's Multiple Range Test.

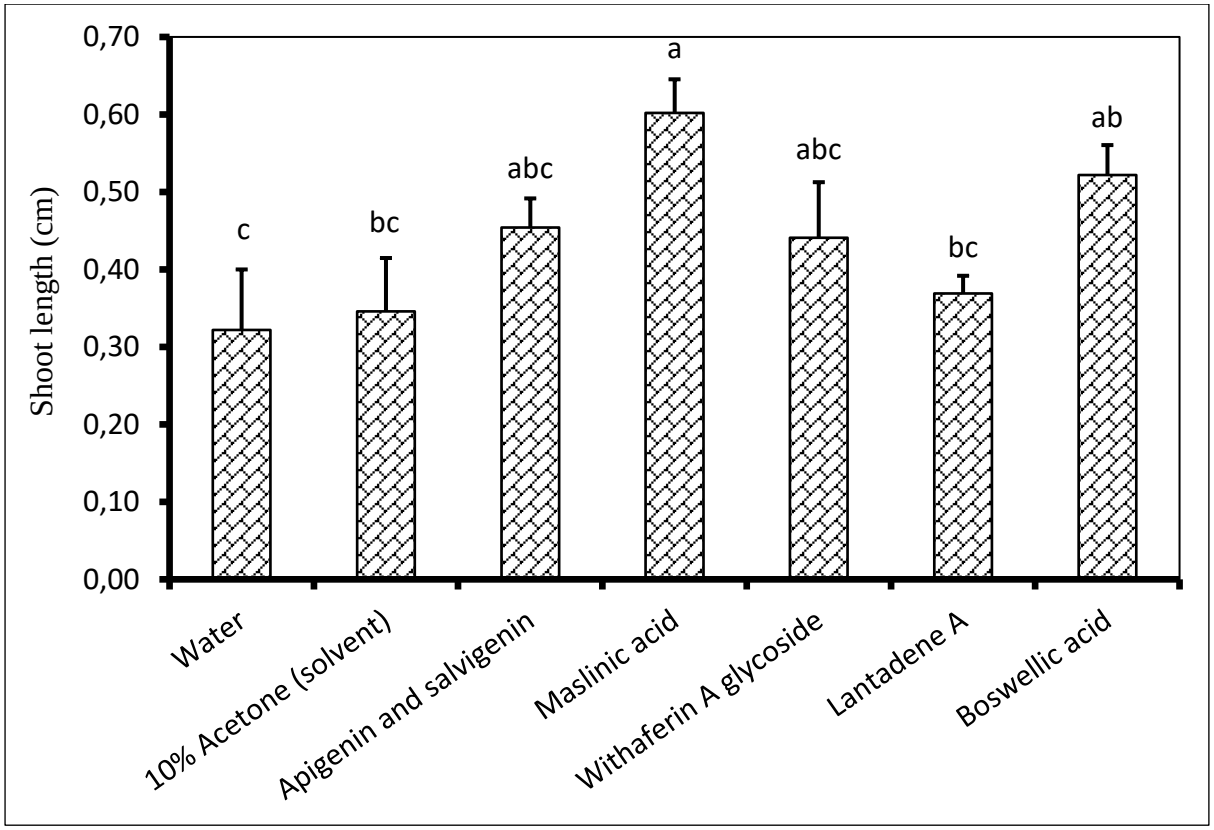


Figure 6.11. Shoots length of germinating maize seed treated with isolated antifungal compounds. A mixture of apigenin and salvigenin, lantadene A, boswellic acid and maslinic acid were used at a concentration of 0.63 mg/mL. Withaferein A glycoside was used at a concentration of 0.16 mg/mL. Water and 10% acetone were used as negative controls. There were 5 replicates per treatment, each comprising 25 disinfected maize seeds, and the experiment was repeated twice. Bars bearing the same letters indicate no significant differences ($p = 0.05$), as determined by Duncan's Multiple Range Test.

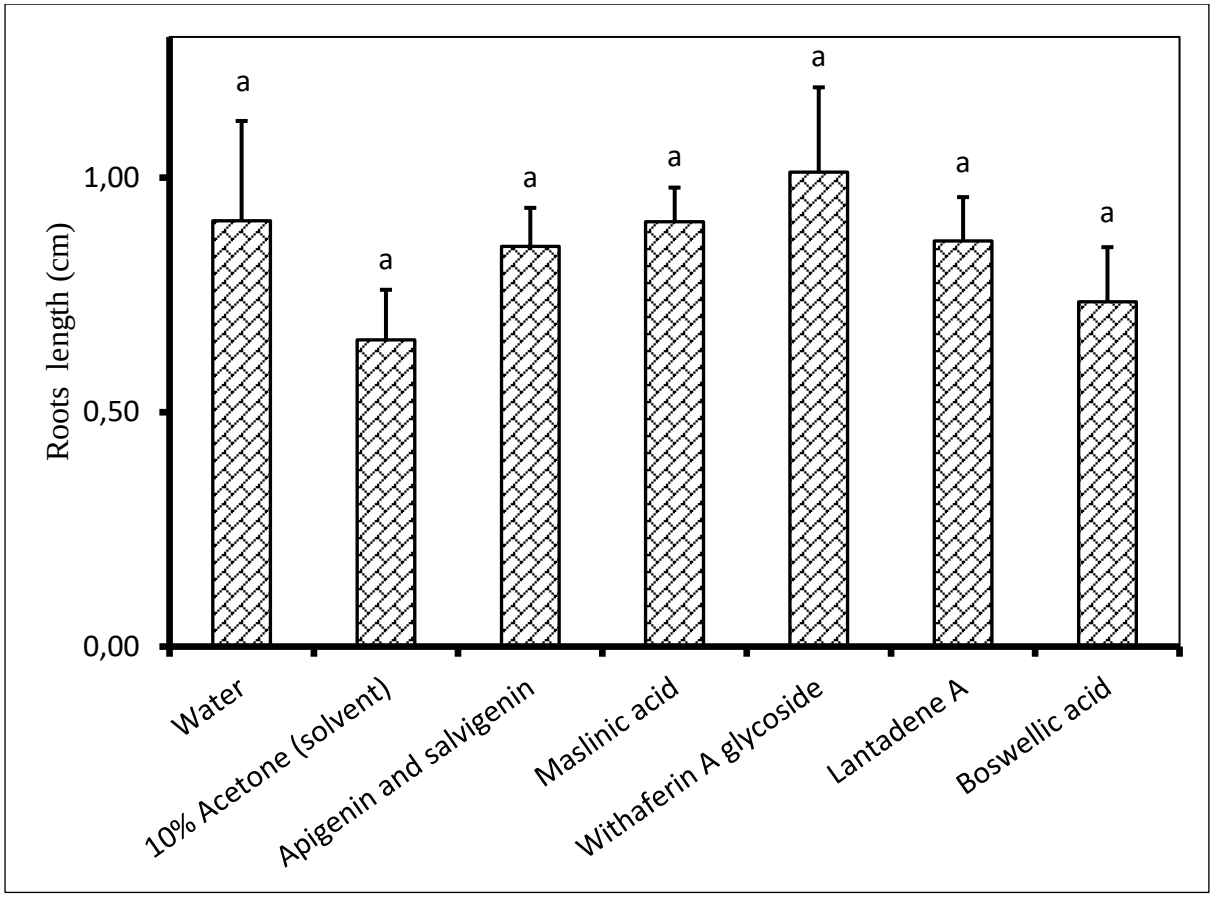


Figure 6.12. Roots length of germinating maize seed treated with isolated antifungal compounds. A mixture of apigenin and salvigenin, lantadene A, boswellic acid and maslinic acid were used at a concentration of 0.63 mg/mL. Withaferein A glycoside was used at a concentration of 0.16 mg/mL. Water and 10% acetone were used as negative controls. There were 5 replicates per treatment, each comprising 25 disinfected maize seeds, and the experiment was repeated twice. Bars bearing the same letters indicate no significant differences ($p = 0.05$), as determined by Duncan's Multiple Range Test.

6.5. CONCLUSIONS

In this study, different antifungal compounds were isolated from the leaves of *Combretum erythrophyllum*, *Lantana camara* and *Withania somnifera*. Antifungal activity of isolated compounds were lower compared to the activity of crude extract, however, in some cases

isolated antifungals such as compound **A** showed strong activity when compared to crude extract. The difference in the antifungal activity of crude extract and isolated compounds could be due to either antagonistic or synergistic interaction of the compounds present in the crude extract. Compounds isolated in this study include apigenin, salvigenin, a derivative of maslinic acid, boswellic acid, boswellic acid glycosides, lantadene A and withanolide glycoside. The structure of these antifungals could serve as scaffold compounds in crop protection chemistry during conventional synthesis of bio-pesticides and their derivatives. This approach will lead to conventional synthesis of plant-based fungicides in larger quantities for applications in agricultural sector.

REFERENCES

Adepoju, A.O., Ogunkunle, A.T.J., Femi-adepoju, A.G., 2014. Antifungal activities of seed oil of neem (*Azadirachta indica* A. Juss.). Global Journal of Biology, Agriculture and Health Sciences 1, 106–109.

Al-Harrasi, A., Rehman, N.U., Khan, A.L., Al- Broumi, M., Al-Amri, I., Hussain, J., Hussain, H., Csuk, R., 2018. Chemical, molecular and structural studies of *Boswellia* species: β-Boswellic Aldehyde and 3-*epi*-11β-Dihydroxy BA as precursors in biosynthesis of boswellic acids. PLoS ONE 13.

Al-Harrisi, Ahmed. Method of purifying 3-o-acetyl- 11-keto-beta-boswellic acid (AKBA). PCT/IB2015/053 177. 30 April 2015 (30.04.2015).

Begue, W.J., Kline, R.M., 1972. The use of tetrazolium salts in bioautographic procedure. Journal of chromatography 88, 182–184.

Chatterjee, G.K., Pal, S.D., 1984. Anti-inflammatory agents from Indian medicinal plants. Indian Drugs 21, 431.

Chaturvedula, V.S.P., Prakash, I., 2013. Flavonoids from *Astragalus propinquus.* Journal of Chemical and Pharmaceutical Research 5, 261–265.

Chaurasiya, N.D., Sangwan, N.S., Sabir, F., Misra, L., Sangwan, R.S., 2012. Withanolide biosynthesis recruits both mevalonate and DOXP pathways of isoprenogenesis in Ashwagandha *Withania somnifera* L. (Dunal). Plant Cell Reports, 31:1889–1897.

Chen, Y., Li, H., Chen, C.J., Zhou, M.G., 2008. Sensitivity of *Fusarium graminearum* to fungicide JS399-19: *in vitro* determination of baseline sensitivity and the risk of developing fungicide resistance. Phytoparasitica 36, 326–337.

Chen, Y., Zhou, M.G., 2009. Characterization of *Fusarium graminearum* isolates resistant to both carbendazim and a new fungicide JS399-19. Phytopathology 99, 441–446.

Dharajiya, D., Patel, P., Patel, M., Moitra, N., 2014. *In vitro* antimicrobial activity and qualitative phytochemical analysis of *Withania somnifera* (L.) dunal extracts. International Journal of Pharmaceutical Sciences Review and Research 27, 349–354.

Dong, Y., Zhang, M., Ling, B., 2005. Antifeeding effects of crude lantadene from *Lantana camara* on *Plutella xylostella* and *Spodeoptera litura* larvae. The Journal of Applied Ecology 16, 2361–2364.

EL-Hefny, M., Salem, M.Z.M., Behiry, S.I., Ali, H.M., 2020. The potential antibacterial and antifungal activities of wood treated with *Withania somnifera* fruit extract, and the phenolic, caffeine and flavonoid composition of the extract according to HPLC, Processes 8, 113.

Fandohan, P., Gbenou, J.D., Gnonlonfin, B., Hell, K., Marasas, W.F., Wingfield, M.J., 2004. Effect of essential oils on the growth of *Fusarium verticillioides* and fumonisin contamination in corn. Journal of Agricultural and Food Chemistry 52, 6824–6829.

Fatope, M.O., Salihu, L., Asante, S.K, Takeda, Y., 2002. Larvicidal activity of extracts and triterpenoids from *Lantana camara*. Pharmaceutical Biology 40, 564–567.

Fatore, M.O., Salihu, L., Asante, S.K., Takeda, T., 2002. Larvicidal activity of extracts and triterpenoids from *Lantana camara*. Pharmaceutical Biology 40, 564–567.

Girish, K. S., Machiah, K.D., Ushanandini, S., Kumar, K.H., Nagaraju, S., Govindappa, M., Vedavathi, M., Kemparaju, K., 2006. Antimicrobial properties of a non-toxic glycoprotein (WSG) from *Withania somnifera* (Ashwagandha). Journal of Basic Microbiology 46, 365–374.

Gupta, I., Parihar, A., Singh, G.B., Ludtke, R., Safayhi, H., Ammon, H.P., 1997. Effects of *Boswellia serrata* gum resin in patients with ulcerative colitis. European Journal of Medical Research 2, 37–43.

Harborne, J.B. Phytochemical Methods. A guide to modern techniques of plant analysis. 2nd Edition. London, New York: Chapman and Hall Ltd; 1973.

Hashmi, M. A., Khan, A., Hanif, M., Farooq, U., Perveen, S., 2015. Traditional uses, phytochemistry and pharmacology of *Olea europaea* (Olive). Evidence-Based Complementary and Alternative Medicine.

Hashmi, M.A.; Khan, A.; Hanif, M.; Farooq, U.; Perveen, S. "Traditional uses, phytochemistry, and pharmacology of *Olea europaea* (Olive)," Evidence-Based Complementary and Alternative Medicine. 2015.

HighChem LLC. mz Cloud. Advanced Mass Spectral Database. Bratislava, Slovakia. created or updated; 2013-2020. cited; 2019 May 29. https://www.mzcloud.org/.

Kartika, M.Z., Suryati, Mai, E., 2018. A triterpenoid from the leaves of *Tahi Ayam* (*Lantana camara Linn*). Indonesian Journal of Fundamental and Applied Chemistry 3, 18–22.

Karygianni, L., Cecere, M., Argyropoulou, A., Hellwig, E., Skaltsounis, A.L., Wittmer, A., Tchorz, J.P., Al-Ahmad, A., 2019. Compounds from *Olea europaea* and *Pistacia lentiscus* inhibit oral microbial growth. BMC Complementary and Alternative Medicine 19, 51.

Khare, C.P., Indian Medicinal Plants–An Illustrated Dictionary. 1st Edition. New Delhi, India: Springer-Verlag New York; 2007.

Kharela, P., Manandharb, M.D., Kalaunia, S.K., Awalec, S., Barala, J., 2011. Isolation, identification and antimicrobial activity of a Withanolide [WS-1] from the roots of *Withania somnifera.* Nepal Journal of Science and Technology 12, 179–186.

Kimmatkar, N., Thawani, V., Hingorani, L., Khiyani, R., 2003. Efficacy and tolerability of *Boswellia serrata* extract in treatment of osteoarthritis of knee–a randomized double blind placebo controlled trial. Phytomed 10, 3–7.

Krieglstein, C.F., Anthoni, C., Rijcken, E.J., Laukotter, M., Spiegel, H.U., Boden, S.E., Schweizer, S., Safayhi, H., Senninger, N., Schurmann, G., 2001. Acetyl-11-keto-betaboswellic acid, a constituent of a herbal medicine from *Boswellia serrata* resin, attenuates experimental ileitis. International Journal of Colorectal Disease 16, 88–95.

Kulkarni, S.K., George, B., 1996. Anticonvulsant action of *Withania somnifera* root extract against pentylene tetrazole (PTZ)-induced convulsions in mice. Phytotherapy Research 95, 447–449.

Kulkarni, S.K., Sharma, A., Verma, A., Ticku, M.K., 1993. GABA receptor mediated anticonvulsant action of *Withania somnifera* root extract. Indian Drugs, 305–312.

Litaudon, M., Jolly, C., Le Callonec, C., Cuong, D.D., Retailleau, P., Nosjean, O., Nguyen, V.H., Pfeiffer, B., Boutin, J.A., Guéritte, F., 2009. Cytotoxic pentacyclic triterpenoids from *Combretum sundaicum* and *Lantana camara* as inhibitors of Bcl-xL/BakBH3 domain peptide interaction. Journal of Natural Products 72, 1314–1320.

Lozano-Mena, G., S´anchez-Gonz´alez, M., Juan, M.E., Planas, J. M., 2014. Maslinic acid, a natural phytoalexin-type triterpene from olives—a promising nutraceutical. Molecules 19, 11538–11559.

Mangoyi, R., Midiwo, J., Mukanganyama, S., 2015. Isolation and characterization of an antifungal compound 5-hydroxy-7,4'-dimethoxyflavone from *Combretum zeyheri*. BMC Complementary and Alternative Medicine 15, 405.

Mansourabadi, A.H., Sadeghi, H.M., Razavi, N., Rezvani, E., 2015. Anti-inflammatory and analgesic properties of salvigenin, *Salvia officinalis* flavonoid extracted. Advanced Herbal Medicine 3, 31–41.

Martínez, J.A. Natural Fungicides Obtained From Plants. In: Dhanasekaran, D., Thajuddin, N., Panneerselvam, A., Editors. Fungicides for Plant and Animal Diseases. London, United Kingdom: IntechOpen; 2012.

Martini, N.D., Katerere, D.R.P., Eloff, J.N., 2004. Biological activity of five antibacterial flavonoids from *Combretum erythrophyllum* (Combretaceae). Journal of Ethnopharmacology 93, 207–212.

Masoko, P., Eloff, J.N., 2006. Bioautography indicates the multiplicity of antifungal compounds from twenty-four southern African *Combretum* species (*Combretaceae*). African Journal of Biotechnology 5, 1625–1647.

Masoko, P., Picard, J., Eloff, J.N., 2005. Antifungal activities of six South African *Terminalia* species (*Combretaceae*). Journal of Ethnopharmacology 99, 301–308.

Mena-Rejo´n, G.J.M., Pe´rez-Espadas, A.R., Moo-Puc, R.E., Cedillo-Rivera, R., Bazzocchi, I.L., Jime´nez-Diaz, I.A., Quijano, L. 2007. Antigiardial Activity of Triterpenoids from Root Bark of Hippocratea excels. Journal of Natural Products 70, 863–865.

Moghaddam, F.M., Farimani, M.M., Salahvarzi, S., Amin, G., 2007. Chemical constituents of dichloromethane extract of cultivated *Satureja khuzistanica*. Evidence Based Complementary and Alternative Medicine 4, 95–98.

Mohamed, A.A.A., Melati, K., Wong, K.C., 2015. Chemical constituents and antioxidant activity of *Teucrium barbeyanum* Aschers. Records of Natural Products 9, 159–163.

Nefzi, A., Ben Abdallah, R.A., Jabnoun-Khiareddine, H., Medimagh-Saïdana, S., Haouala, R., Daami-Remadi, M., 2016. Antifungal activity of aqueous and organic extracts from Withania somnifera L. against Fusarium oxysporum f. sp. radicis-lycopersici. Journal of Microbial and Biochemical Technology 8, 144–150.

Noori, S., Hassan, Z.M., Yaghmaei, B., Dolatkhah, M., 2013. Antitumor and immunomodulatory effects of salvigenin on tumor bearing mice. Cellular Immunology 286, 16–21.

Padvi, S.A., Hingole, D.G., Khaire, P.B., 2018. *In vitro* efficacy of fungicides against *Fusarium solani* incited by dry root rot of sweet orange. Journal of Pharmacognosy and Phytochemistry 7, 3270–3273.

Raja, AF., Ali, F., Khan, I.A., Shawl, A.S., Arora, D.S., Shah, B.A., Taneja, S.C., 2011. Antistaphylococcal and biofilm inhibitory activities of acetyl-11-keto-b-boswellic acid from *Boswellia serrata*. BMC Microbiology 11, 54.

Rashmi, Jyoti, B., Naveen, G., 2016. Phytochemical analysis and antibacterial activity of different leaf extracts of *Murraya koenigii.* International Journal of Biochemistry and Biomolecules 2, 1–5.

Reddy, G.K., Dhar, S.C., 1987. Effect of a new non-steroidal anti-inflammatory agent on lysosomal stability in adjuvant induced arthritis. Italian journal of biochemistry 36, 205–217.

Rishi, K., Singh, R., 2003. Chemical components and insecticidal properties of Bakain (*Melia azedarach* L.) - A Review. Agricultural Reviews 24, 101–115.

Romha, G., Admasu, B., Gebrekidan, T.H., Aleme, H., Gebru, G., 2018. Antibacterial activities of five medicinal plants in Ethiopia against some human and animal pathogens. Evidence-Based Complementary and Alternative Medicine.

Salehi, B., Venditti, A., Sharifi-Rad, M., Kre¸giel, D., Sharifi-Rad, J., Durazzo, A., Lucarini, M., Santini, A., Souto, B.E., Novellino, E., Antolak, H., Azzini, E., Setzer, W.N., Martins, N., 2019. The therapeutic potential of apigenin, International Journal of Molecular Science 20, 1305.

Seepe, H.A., Lodama, K.E., Sutherland, R., Nxumalo W., Amoo, S.O., 2020. In vivo antifungal activity of South African medicinal plant extracts against *Fusarium* pathogens and their phytotoxicity evaluation. Plants 9, 1668.

Sharada, A.C., Solomon, F.E., Devi, P.U., Udupa, N., Srinivasan, K.K., 1996. Antitumour and radiosensitizing effects of withaferin A on mouse Ehrlich ascites carcinoma *in vivo*. Acta. Oncology 35, 95–100.

Sharma, M., Sharma, P.D., Bansal, M.P., 2008. Lantadenes and their esters as potential antitumor agents. Journal of Natural Products 71, 1222–1227.

Shibata, C., Ohno, M., Otsuka, M., Kishikawa, T., Goto, K., Muroyama, R., Kato, N., Yoshikawa, K., Takata, A., Koike, K., 2014. The flavonoid apigenin inhibits hepatitis C virus replication by decreasing mature microRNA122 levels. Virology 42, 462–463.

Sofa, F., Megawati, Akhmad, D., 2016. Apigenin, an anticancer isolated from *Macaranga gigantifolia* leaves. The Journal of Tropical Life Science 6, 7–9.

Suresh, K., Deepa, P., Harisaranraj, R., Vaira, A.V., 2008. Antimicrobial and phytochemical investigation of the leaves of *Carica papaya* L., *Cynodon dactylon* L. Pers., *Euphorbia Hirta* L., *Melia azedarach* L. and *Psidium guajava* L. Ethnobotanical Leaflets 12, 1184–1191.

Taleb-Contini, S.H., Salvador, M.J., Watanabe, E., Ito, I.Y., Oliveira, D.C.R., 2003. Antimicrobial activity of flavonoids and steroids isolated from two *Chromolaena* species. Brazilian Journal of Pharmaceutical Sciences 39, 4.

Wicht, M.M. Oleanolic acid-its isolation and derivatisation to potential antimicrobial compounds. Cape Town: Cape Peninsula University of Technology; 2007.

Xu, S., Wang, J., Wang, H., Bao, Y., Li, Y., Govindaraju, M., Yao, Y., Chen, B., Zhang, M., 2019. Molecular characterization of carbendazim resistance of *Fusarium* species complex that causes sugarcane pokkah boeng disease. BMC Genomics 20, 115.

Zhao, J., Nakamura, N., Hattori, M., Kuboyama, T., Tohda, C., Komatsu, K. 2002. Withanolide derivatives from the roots of *Withania somnifera* and their neurite outgrowth activities. Chemical and Pharmaceutical Bulletin. 50, 760–765.

CHAPTER 7

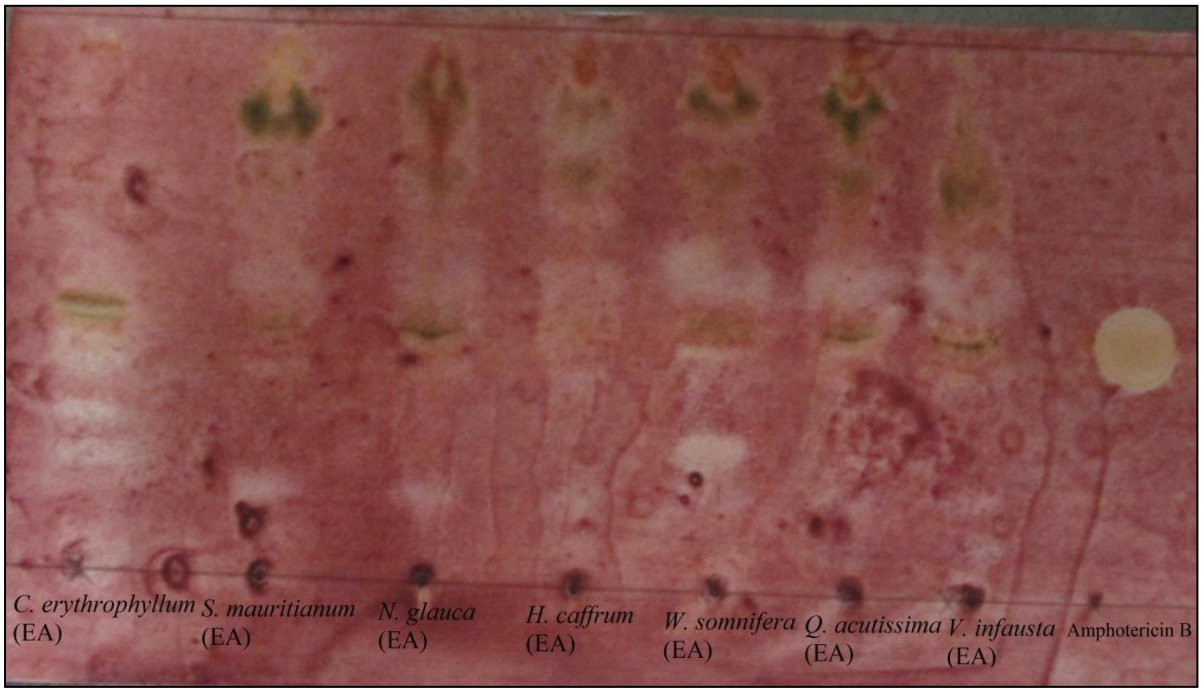
GENERAL SUMMARY AND CONCLUSIONS

Thirteen medicinal plant species were evaluated for antifungal activity against nine *Fusarium* species known to cause crop diseases in agriculture. Leafy were extracted using solvents of different polarities. The highest yield was obtained with water as an extractant, whereas petroleum ether had the lowest yield. The extracts were evaluated individually and others as combinations among different plant species. Of the individual extract solvents tested, ethyl acetate and acetone solvent extracts were found in most cases to exhibit stronger antifungal activity compared to water and petroleum ether extracts. These results have demonstrated that antifungal activity of medicinal plant species depends on the polarity of solvent used for extraction and to some extent, is pathogen-specific.

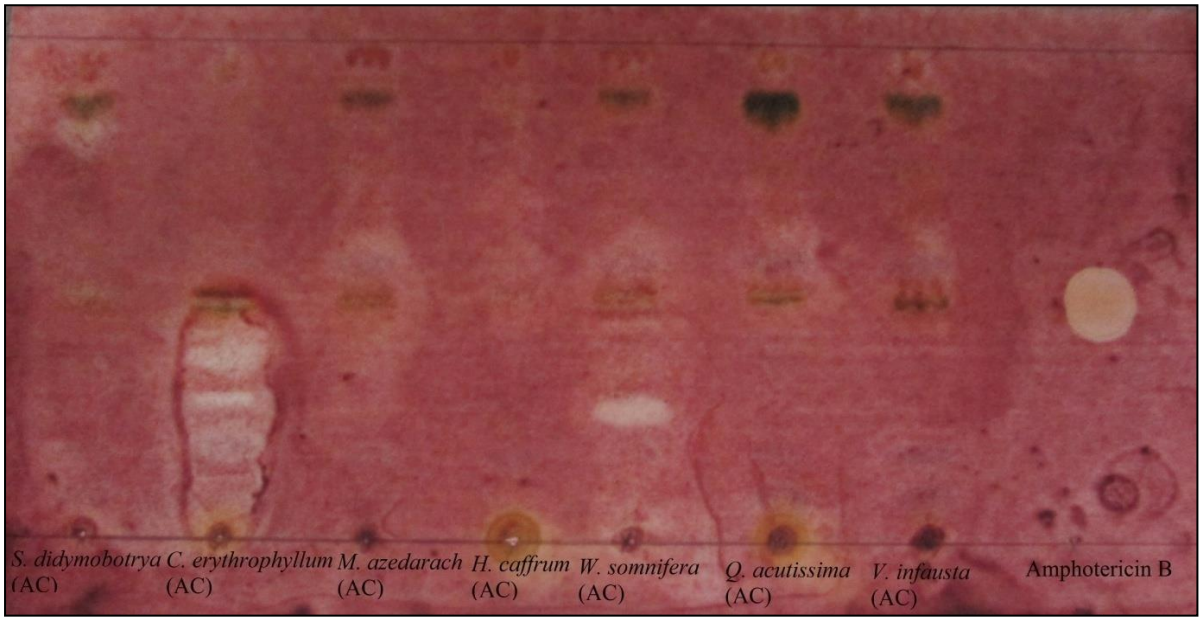
The combination of plant extracts demonstrated synergistic, additive, indifference and antagonistic antifungal activities. Of particular interest are combination extracts showing synergistic or additive antifungal activity. These extract combinations have potential to be developed into plant-based fungicides for agricultural application, especially in smallholder farming and organic crop production. The results showed that the extracts might be used individually or as combination from different plant species. *In vivo* activity of the plant extracts against *Fusarium* pathogens inoculated on maize seeds demonstrated their potential application in post-harvest storage to control spoilage of seeds. The use of leaves is sustainable, renewable. Moreover, the extracts have little or no negative effect on maize seed germination and seedling growth. Bio-autographic and phytochemical analysis of plant extracts revealed the presence of different chemical constituents or antifungals. Antifungal compounds characterised in this study include apigenin (*4',5,7-trihydroxyflavone*), salvigenin (*5-hydroxy-6,7,4'-trimethoxyflavone*) and derivative of maslinic acid [(*2α,3β)-2,3-dihydroxyolean-12-en-28-oic*

acid)] isolated from the leaves of *C. erythrophyllum*. Lantadene A (*22- angeloyloxy-9-hydroxy-3-oxo-olean-12-en-28-oic acid*), boswellic acid (*11-keto-β-boswellic acid)* and boswellic acid glycosides were isolated from the leaves of *Lantana camara*; while withaferin A (*4β,27-dihydroxy-1-oxo-5β,6β-epoxywitha-2-24-dienolide*) glycoside was isolated from the leaves of *Withania somnifera*. The structure of these antifungal compounds could serve as scaffold compounds in crop protection chemistry during synthesis of bio-pesticides and their derivatives. This approach allows conventional synthesis of plant-based fungicides in larger quantities for applications in both commercial and smallholder farming.

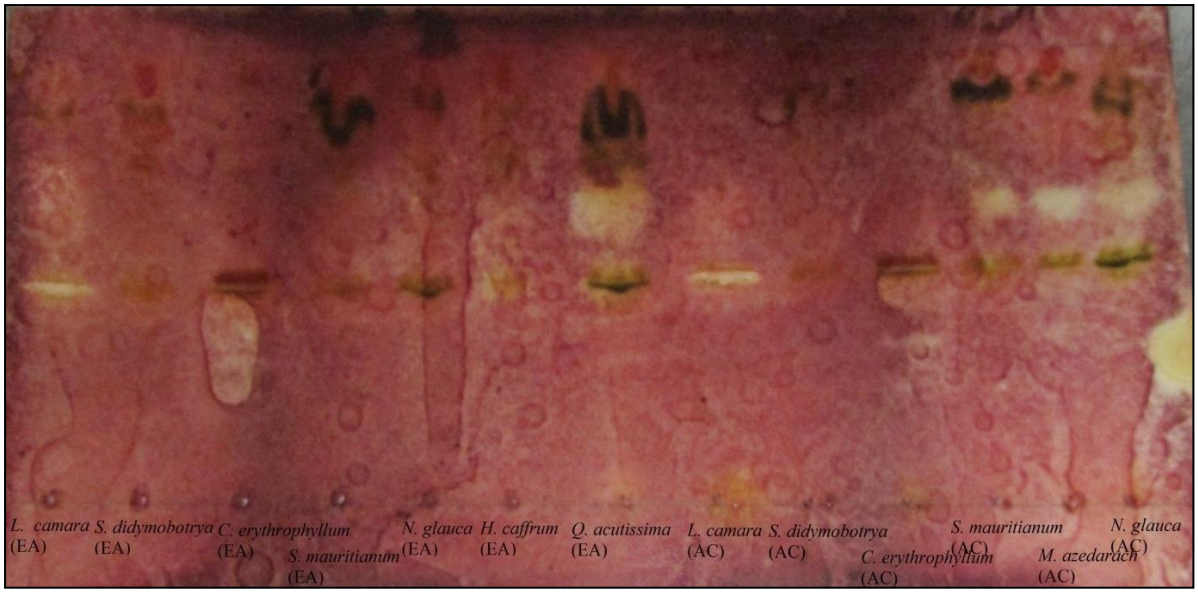
APPENDIXES



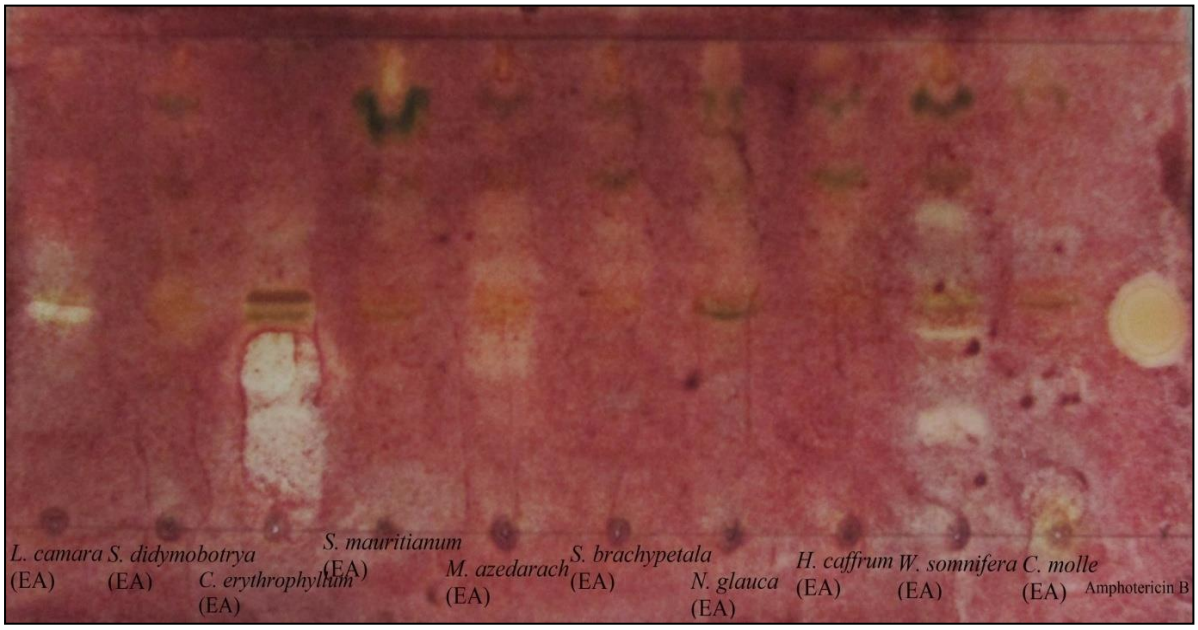
Appendix 1. TLC-bioautogram of different medicinal plant extracts against *F.* verticilloides



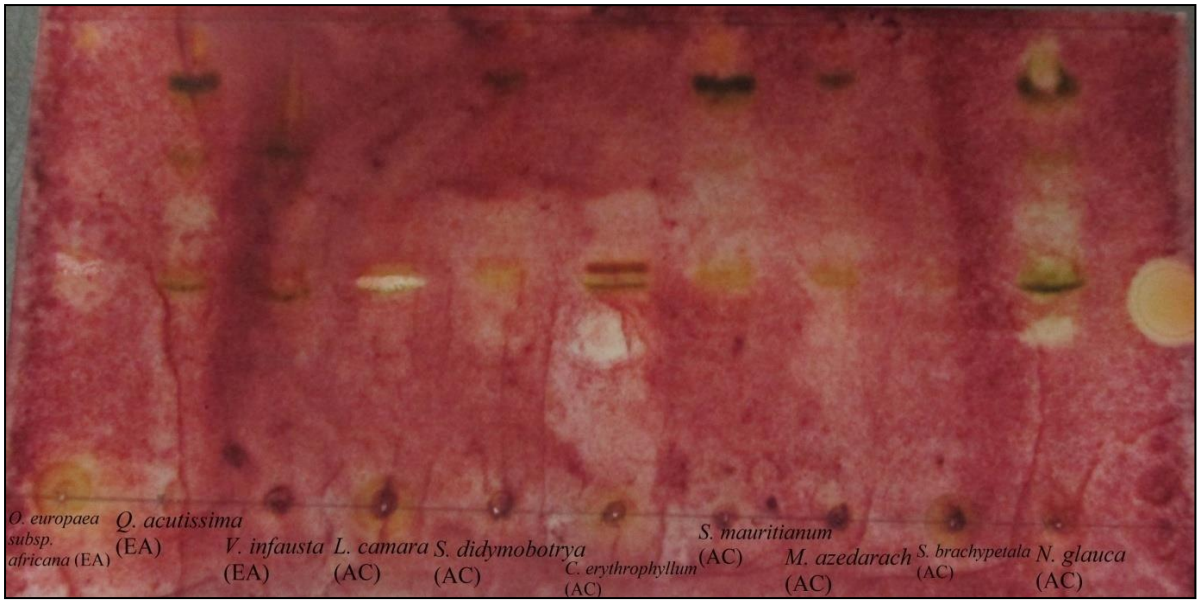
Appendix 1 continues. TLC-bioautogram of different medicinal plant extracts against *F.* verticilloides.



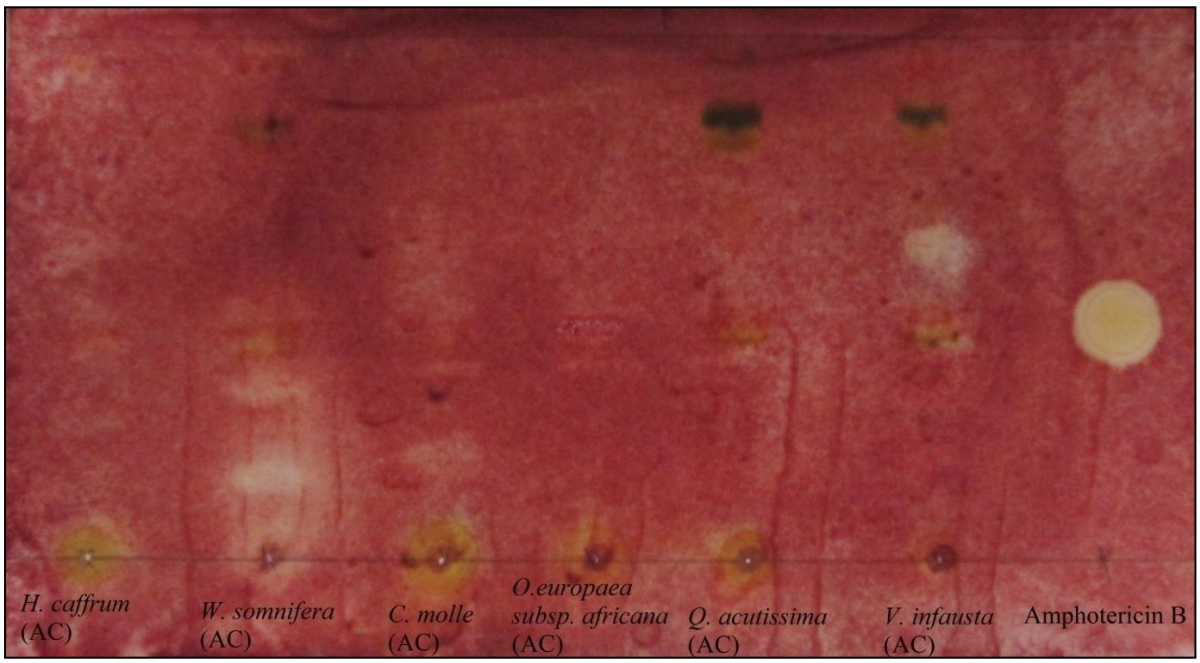
Appendix 2. TLC-bioautogram of different medicinal plant extracts against *F. subglutinans*



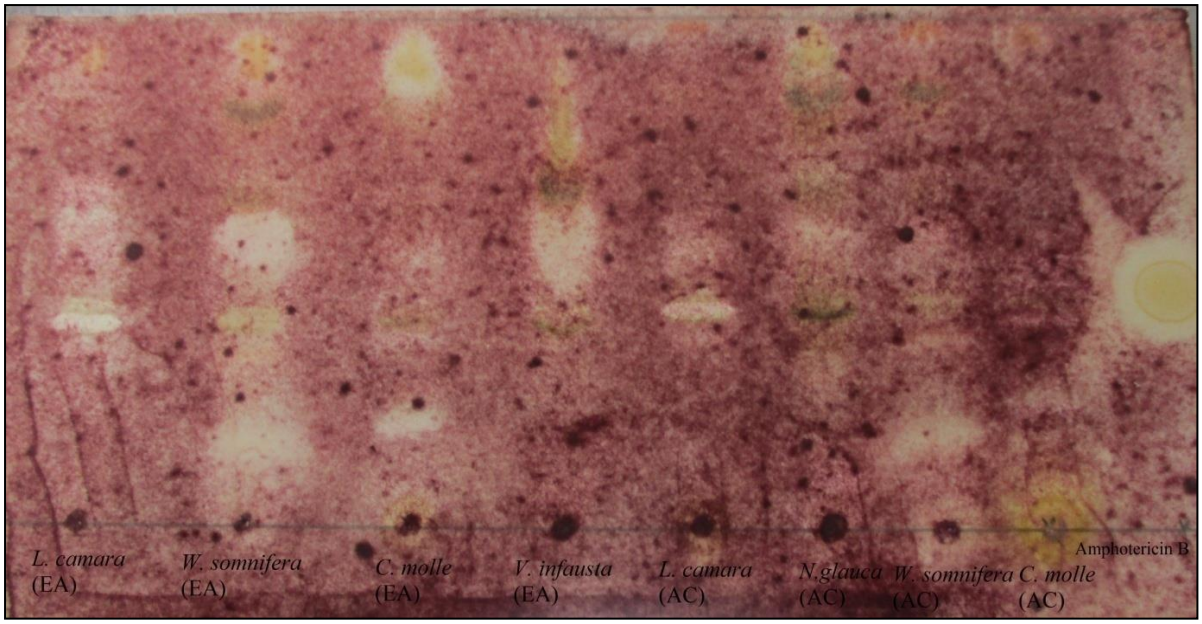
Appendix 3. TLC-bioautogram of different medicinal plant extracts against *F. proliferatum*



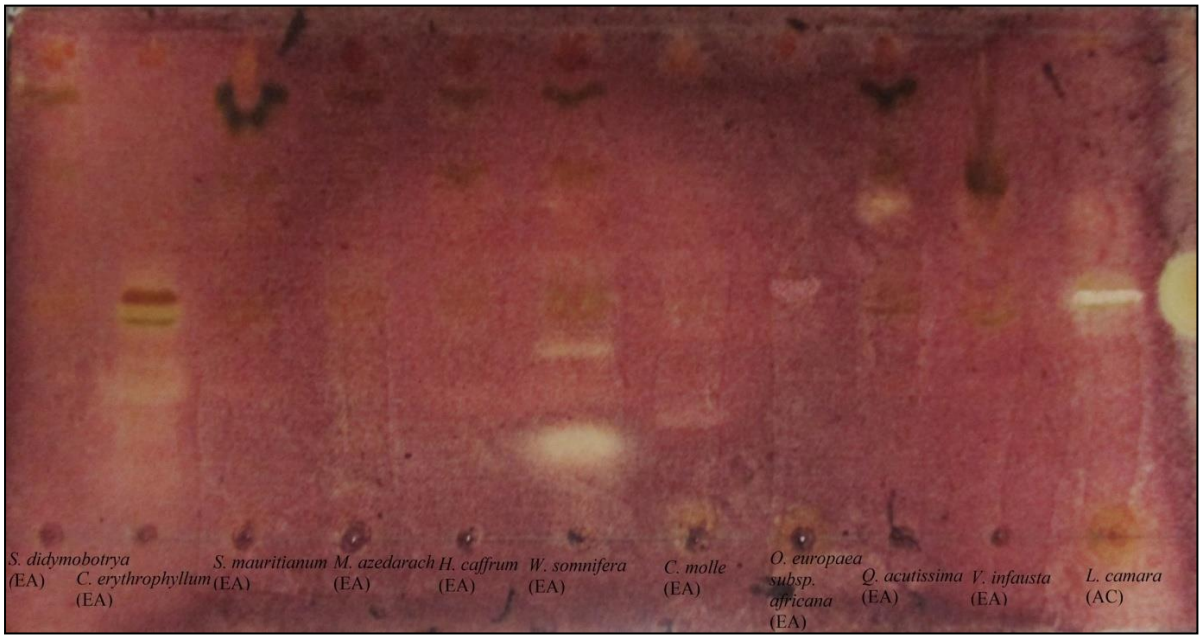
Appendix 3 continues. TLC-bioautogram of different medicinal plant extracts against *F. proliferatum*.



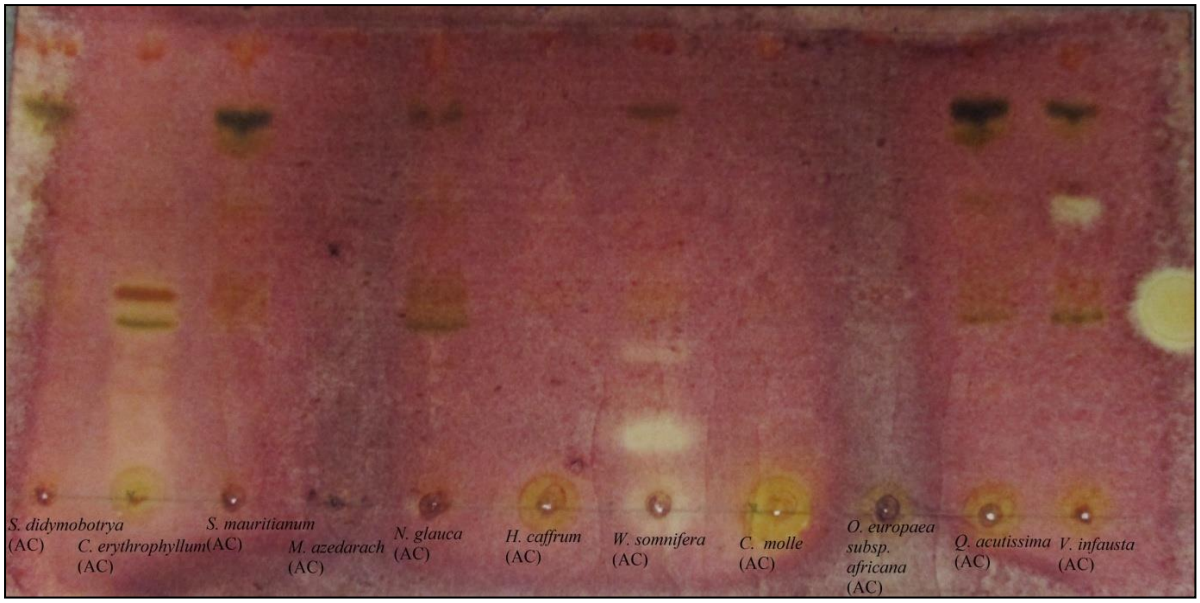
Appendix 3 continues. TLC-bioautogram of different medicinal plant extracts against *F. proliferatum*.



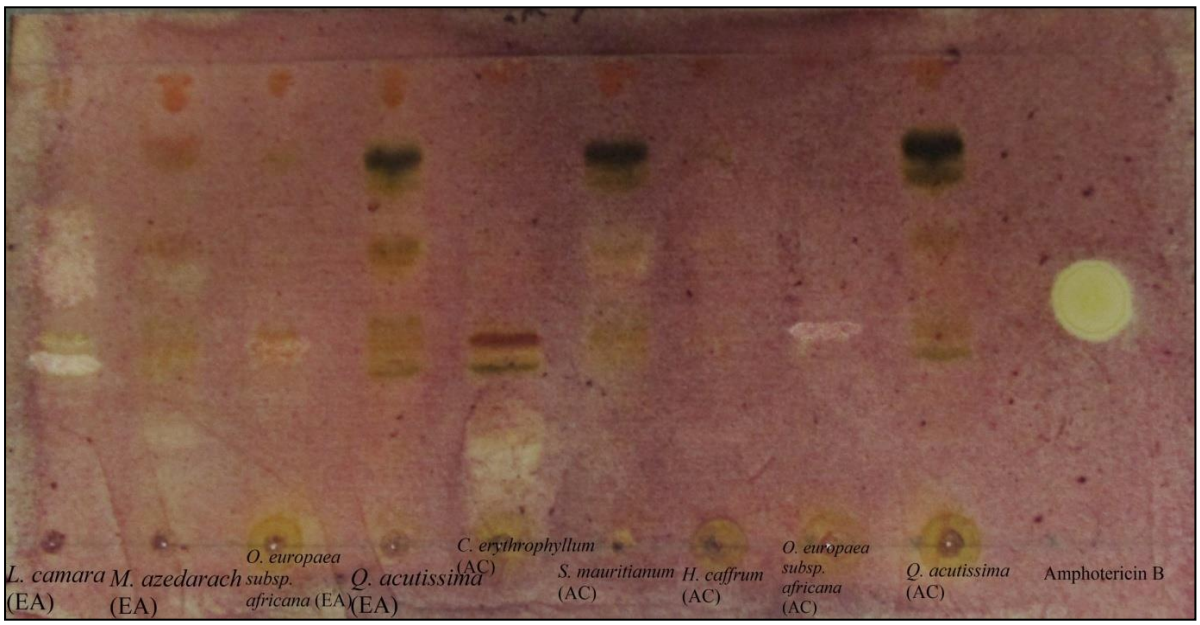
Appendix 4. TLC-bioautogram of different medicinal plant extracts against *F. semitectum*



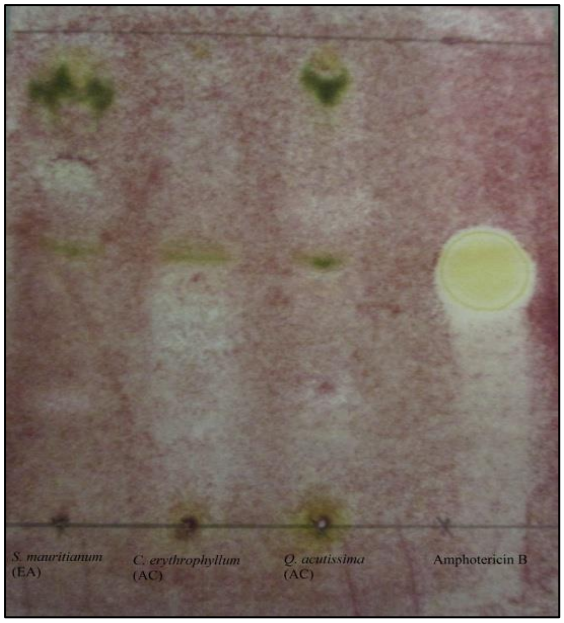
Appendix 5. TLC-bioautogram of different medicinal plant extracts against *F. solani*



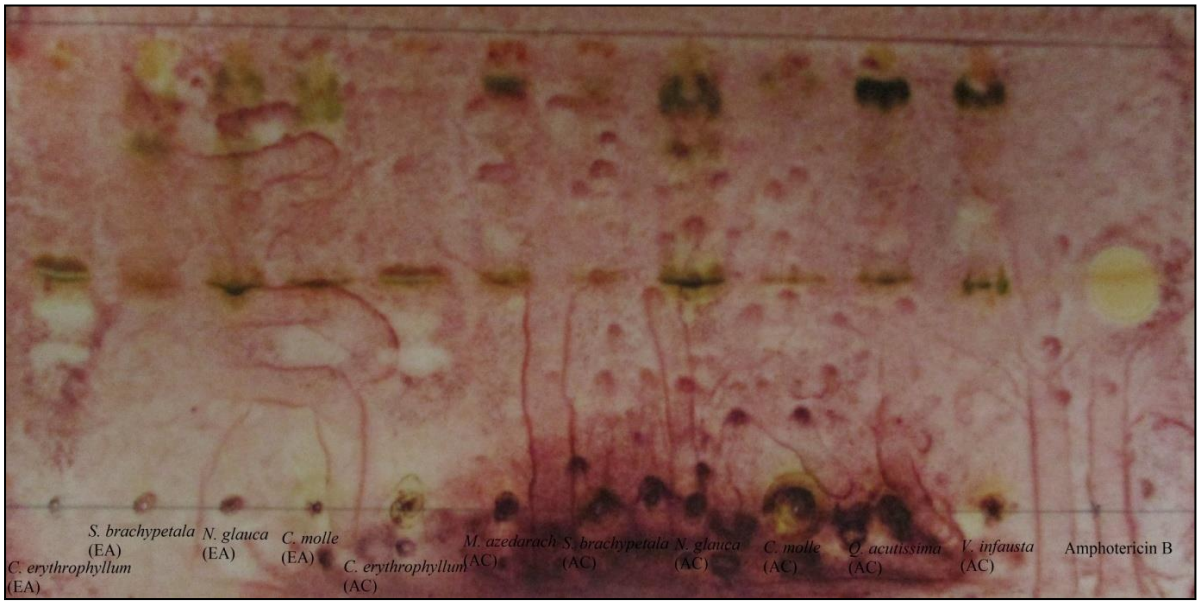
Appendix 5 continues. TLC-bioautogram of different medicinal plant extracts against *F. solani*.



Appendix 6. TLC-bioautogram of different medicinal plant extracts against *F. graminearum*



Appendix 7. TLC-bioautogram of different medicinal plant extracts against *F. equisite*

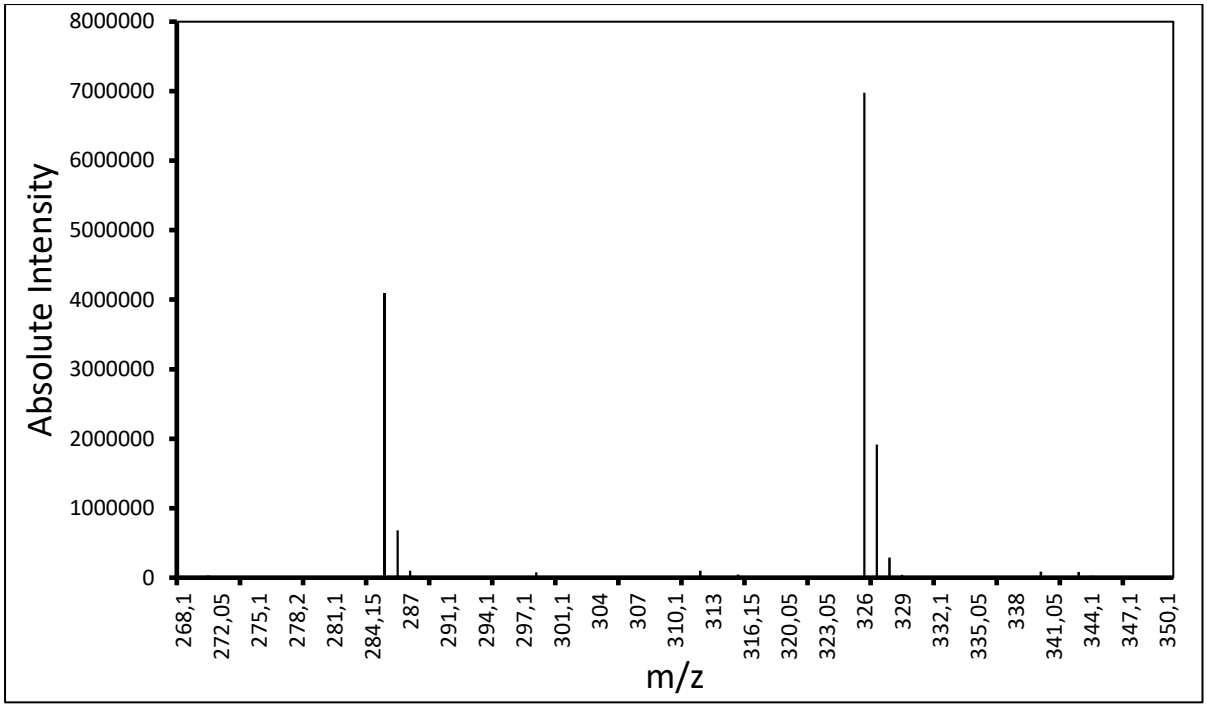


Appendix 8. TLC-bioautogram of different medicinal plant extracts against *F. chlamydosporum*

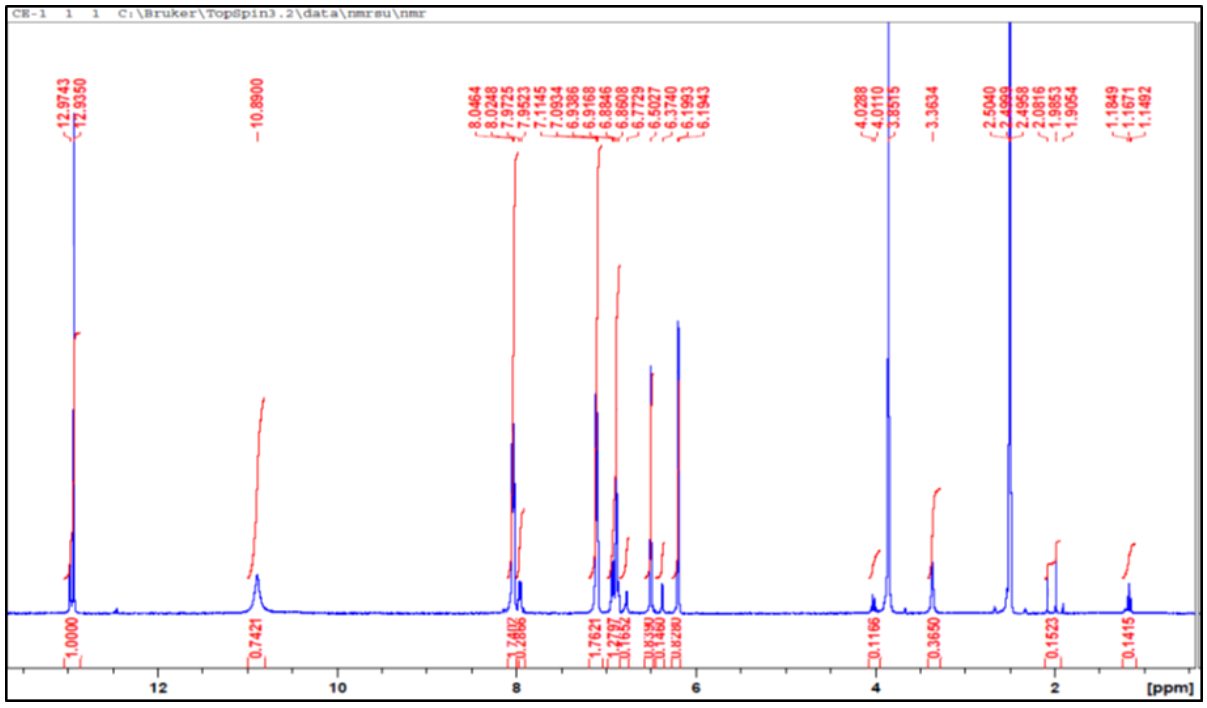
Appendix 9. The bioautographic qualitative inhibition of tested fungi growth by extracts of selected medicinal plant species separated by TLC with Toluene: Methanol: Acetonitrile: Acetic acid (80:10:5:5) as eluents. Rf values of active components.

Plant species	Solvents	*F. oxysporum*	*F. verticilloides*	*F. subglutinans*	*F. proliferatum*	*F. solani*	*F. graminearum*	*F. chlamydosporum*	*F. semitectum*	*F. equisite*
					Rf values					
C. erythrophyllum	EA	-	0.2	-	0.19	-	-	-		
		-	0.29	-	-	0.28	-	-		
		-	0.39	0.38	0.37	-	-	0.32		
		-	0.44	-	0.44	0.43	-	0.41		
		-	0.48	0.48	0.47	0.47	-	0.48		
		-	-	0.51	-	-	-	0.51		
	AC	-	0.30	-	-	-	-	0.32		
		-	0.38	0.36	0.36	0.37	0.38	-		
		0.46	0.47	0.51	0.47	0.44	0.43	0.41		
		0.51	0.49	0.52	0.50	-	-	0.48		
L. camara	EA			-	-	-	0.37		0.39	
				-	0.44	-	-		-	
				0.48	-	0.47	-			
				-	-	-	0.57		0.62	
	AC			-	-	-	-		0.42	
				-	0.44	-	-		-	
				0.49	-	-	-		-	
				-	-	-	-		0.53	
W. somnifera	EA	0.22	0.25		0.23	0.19	0.16			
		0.40	0.44		0.42	0.38	0.41			
		-	0.59		0.65	-	0.58			
	AC	0.27	0.26		0.17	0.16			0.18	
		-	-		0.33	0.33			-	
		0.44	-		-	-			0.38	
		-	-		-	-			0.46	
S. mauritianum	EA	-	0.14	-	-	-	-			-
		0.78	-	-	-	-	-			0.75
	AC	0.68	-	0.66	-	-	0.63			-

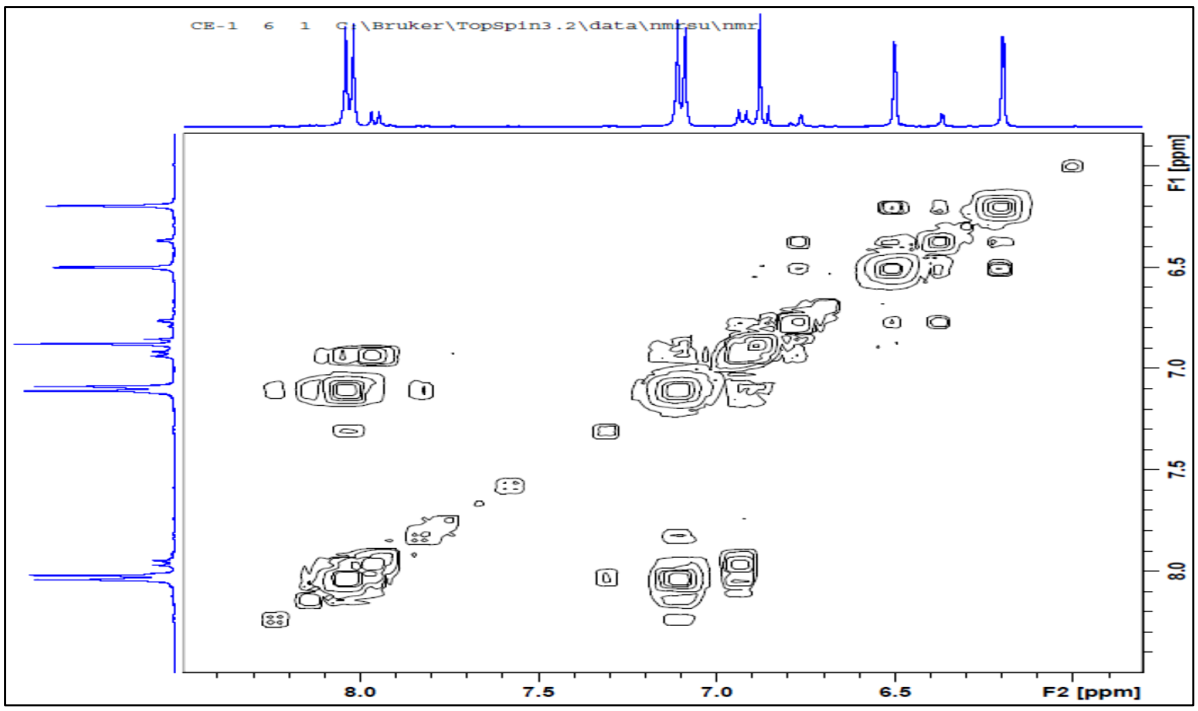
Plant species	Solvents	*F. oxysporum*	*F. verticilloides*	*F. subglutinans*	*F. proliferatum*	*F. solani*	*F. graminearum*	*F. chlamydosporum*	*F. semitectum*	*F. equisite*
C. molle	EA				-	-		0.32	0.16	
					-	-		-	0.92	
	AC				-	-		-	-	
O. europaea	EA				-	-	0.41			
					0.47	0.49	-			
	AC				-	-	0.45			
V. infausta	EA		0.59		-	-	-	-	0.56	
			-		-	-	-	-	0.77	
	AC		-		0.60	0.63	-	0.57	-	
Q. acutissima	EA	-	0.38	-	-	-	-	-		-
		-	0.59	-	0.58	-	-	-		-
		-	-	0.64	-	0.65	-	-		-
	AC	-	-	-	-	-	-	-		-
N. glauca	EA		0.14	-	-	-	-	-	-	
	AC		-	-	0.38	-	-	-	-	
			-	-	0.47	-	-	-	-	
			-	0.66	0.59	-	-	-	0.58	
M. azedarach	EA	-	-	-	-	-	0.22	-		
		-	-	-	0.36	-	0.38	-		
		-	-	-	0.53	-	0.57	-		
		-	-	-	0.65	-	-	-		
	AC	-	-	0.66	-	-	-	0.57		
S. didymobotrya	EA		-	-	-					
	AC		0.81	-	-					
S. brachypetala	EA				-			-		
	AC				-			-		
H. caffrum	EA		-	0.48	-	-				
	AC		-	-	-	-				



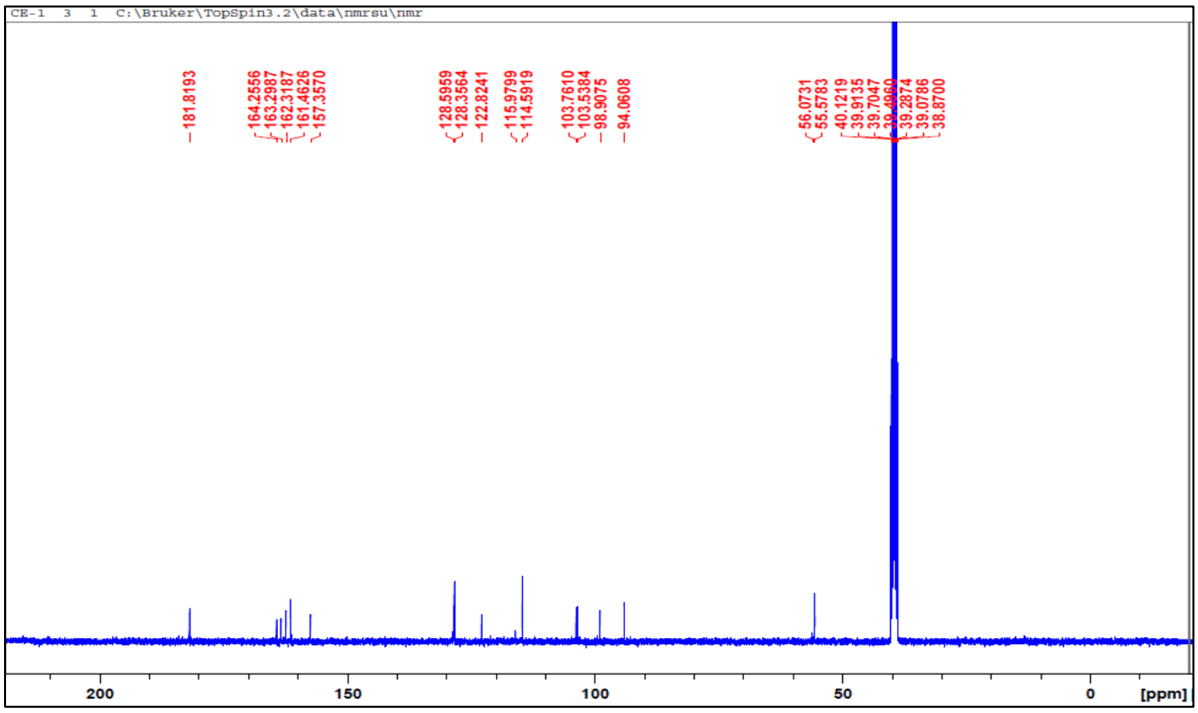
Appendix 10. MS fragmentation spectrum of fraction **A** isolated from acetone leaves extract of *Combretum erythrophyllum*.



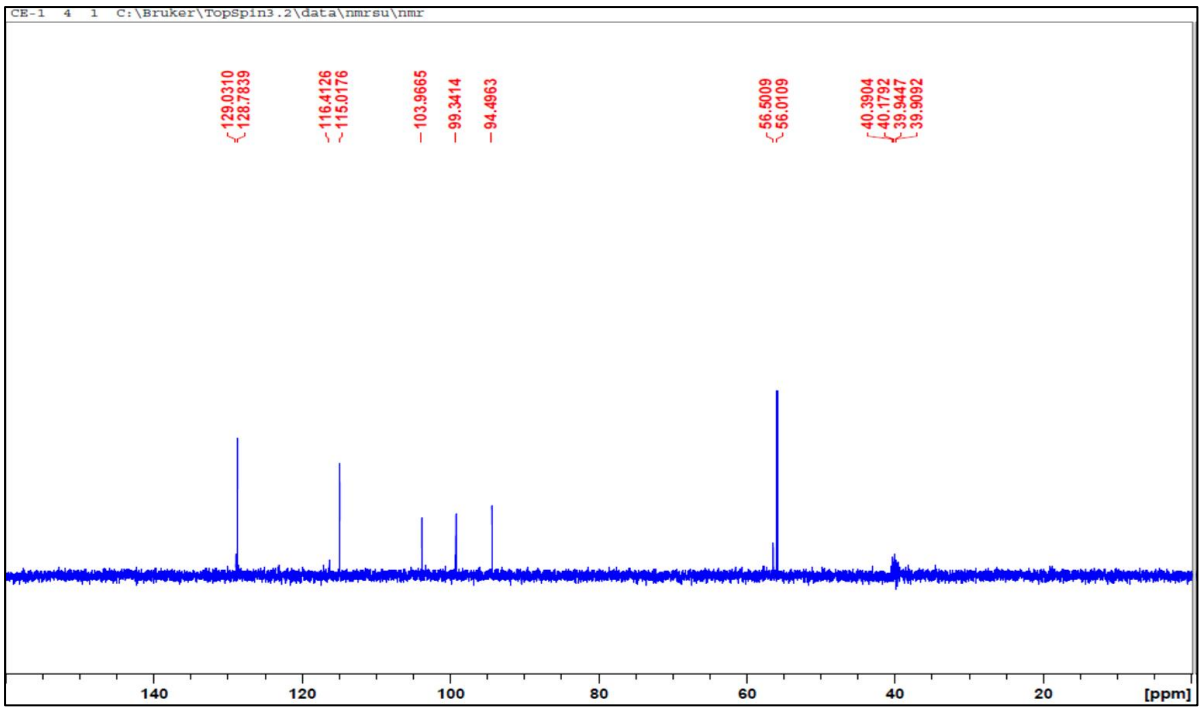
Appendix 11. ^{1}H-NMR spectrum of fraction **A** isolated from acetone leaves extract of *Combretum erythrophyllum*.



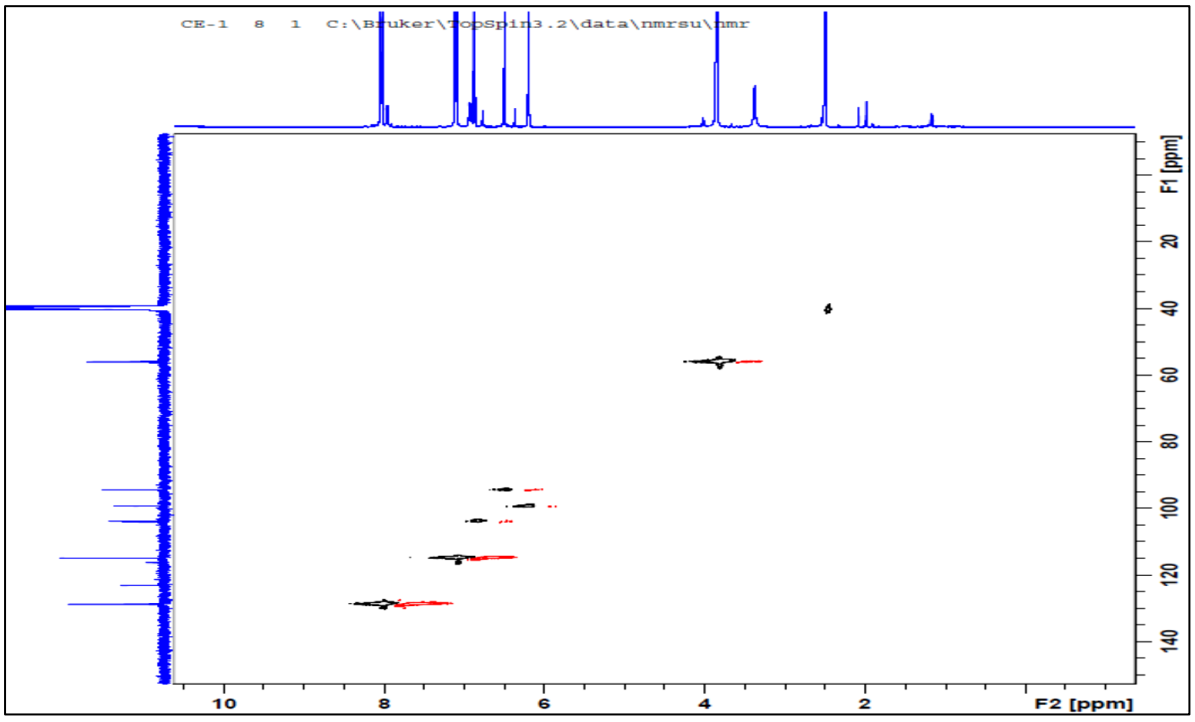
Appendix 12. ^{1}H-^{1}H COSY spectrum of fraction **A** isolated from acetone leaves extract of *Combretum erythrophyllum*.



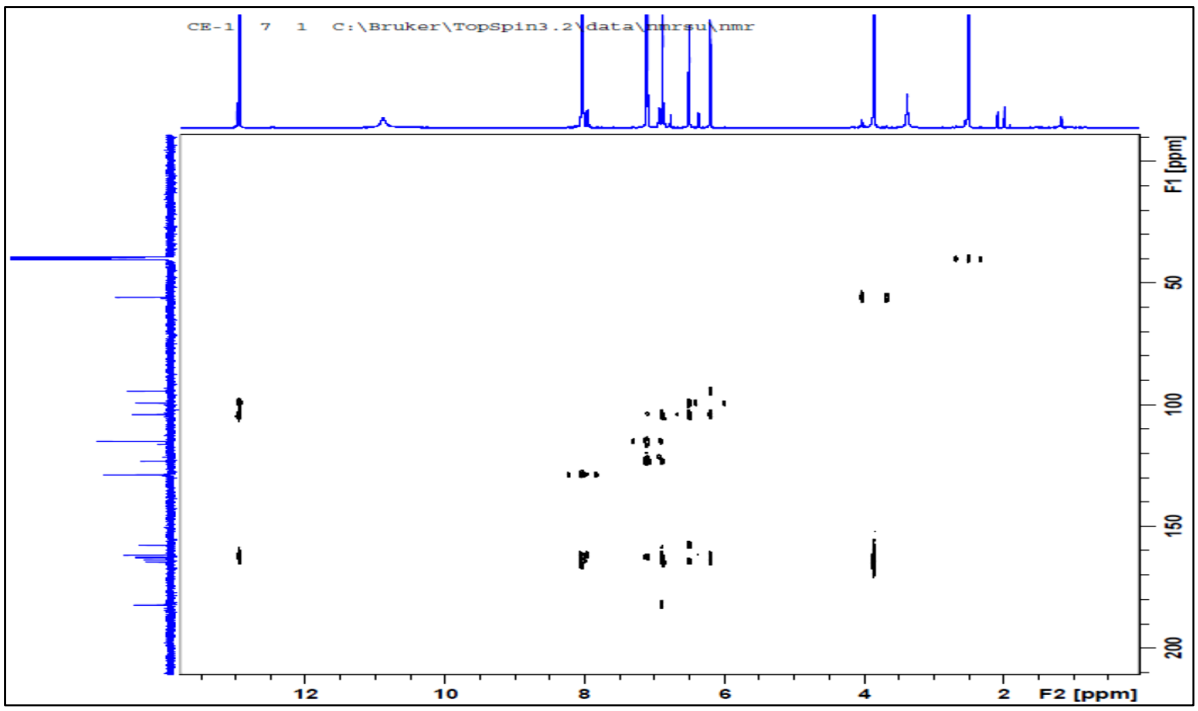
Appendix 13. ^{13}C-NMR spectrum of fraction **A** isolated from acetone leaves extract of *Combretum erythrophyllum*.



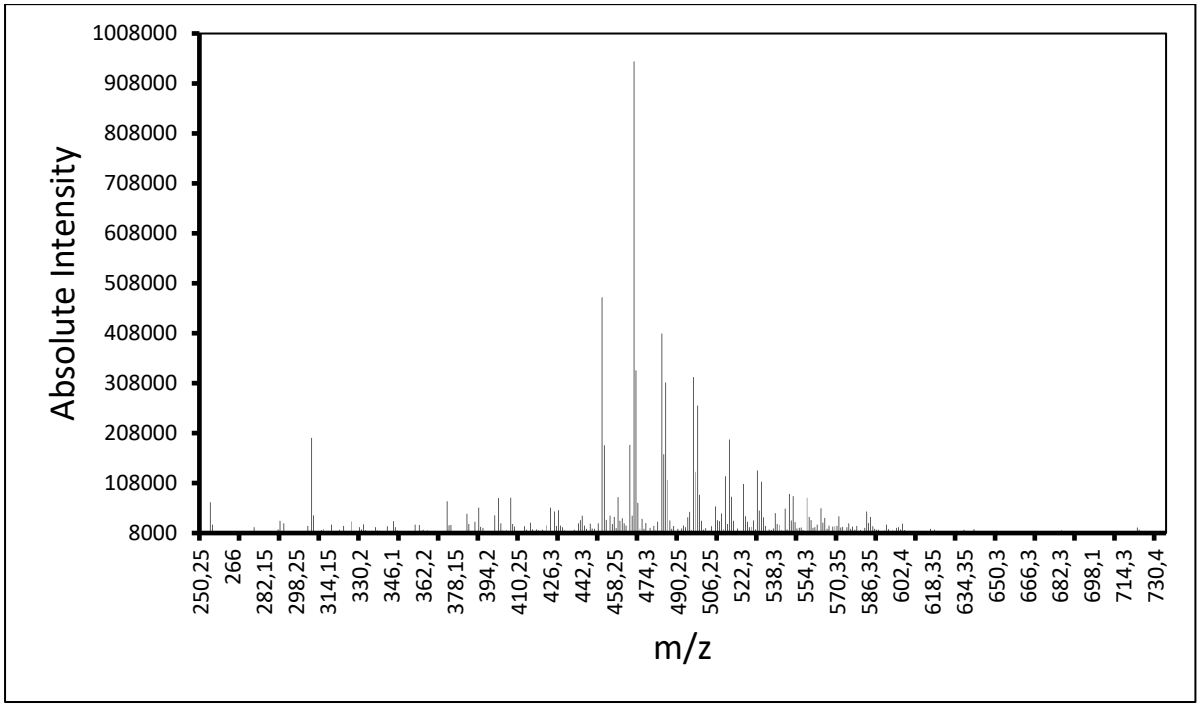
Appendix 14. DEPT spectrum of fraction **A** isolated from acetone leaves extract of *Combretum erythrophyllum*.



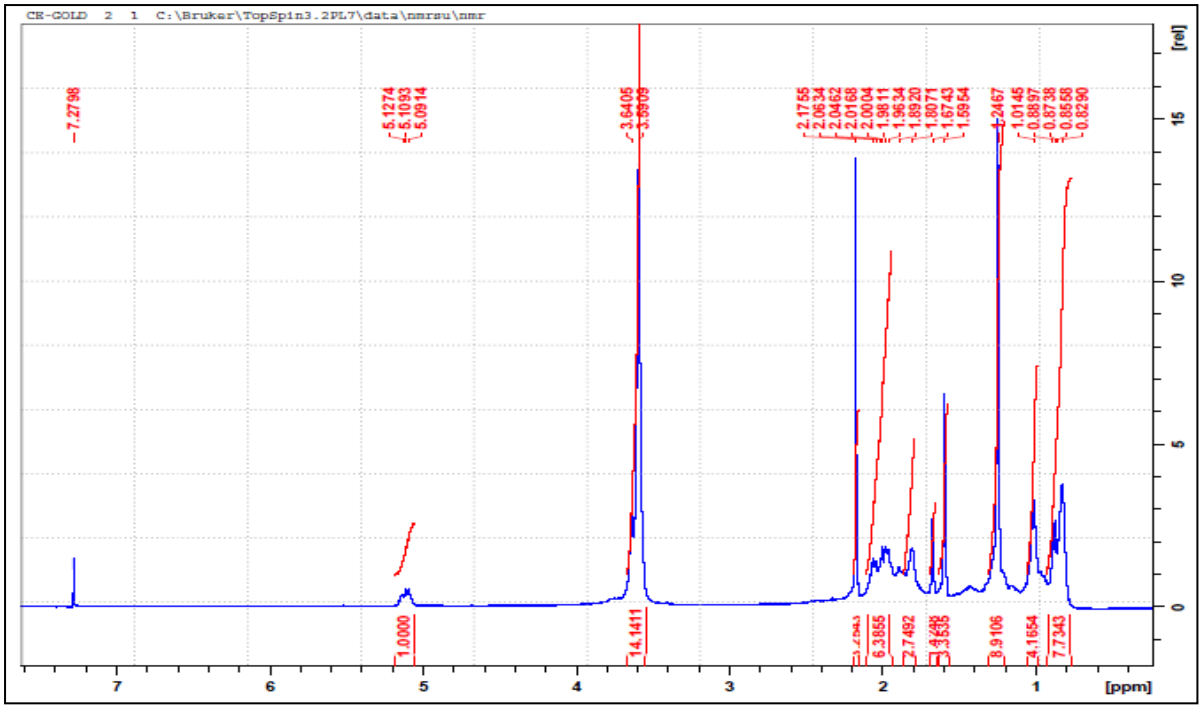
Appendix 15. HSQC spectrum of fraction **A** isolated from acetone leaves extract of *Combretum erythrophyllum*.



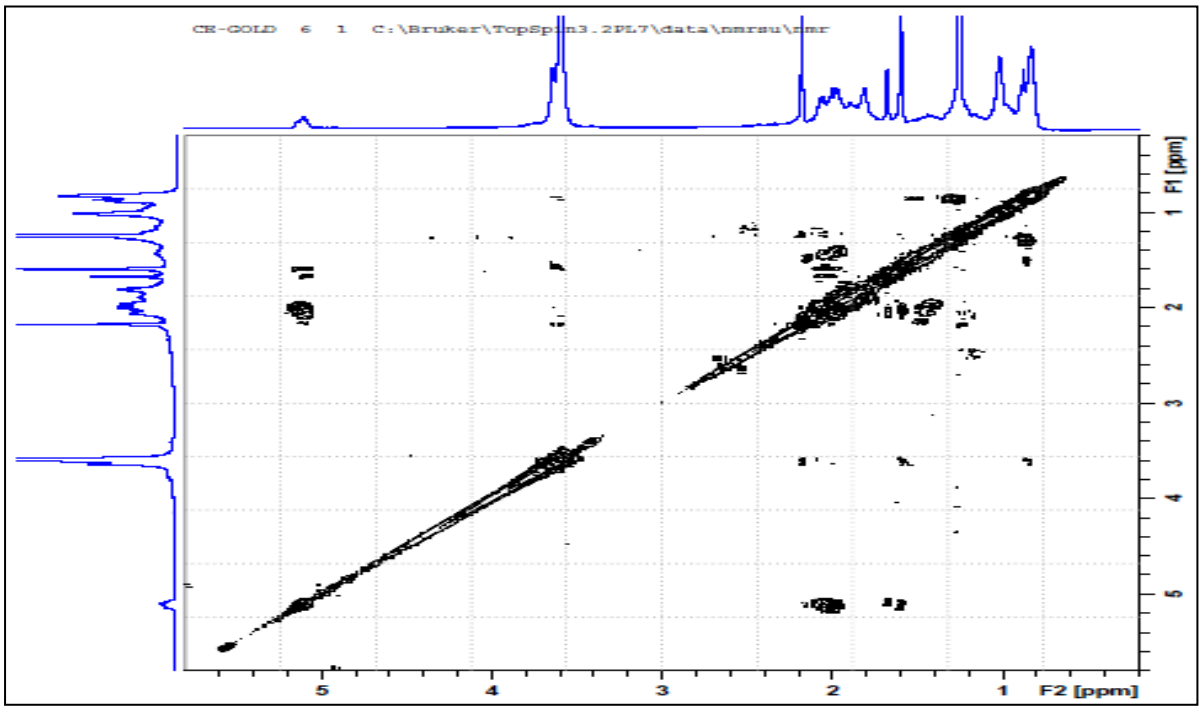
Appendix 16. HMBC spectrum of fraction **A** isolated from acetone leaves extract of *Combretum erythrophyllum*.



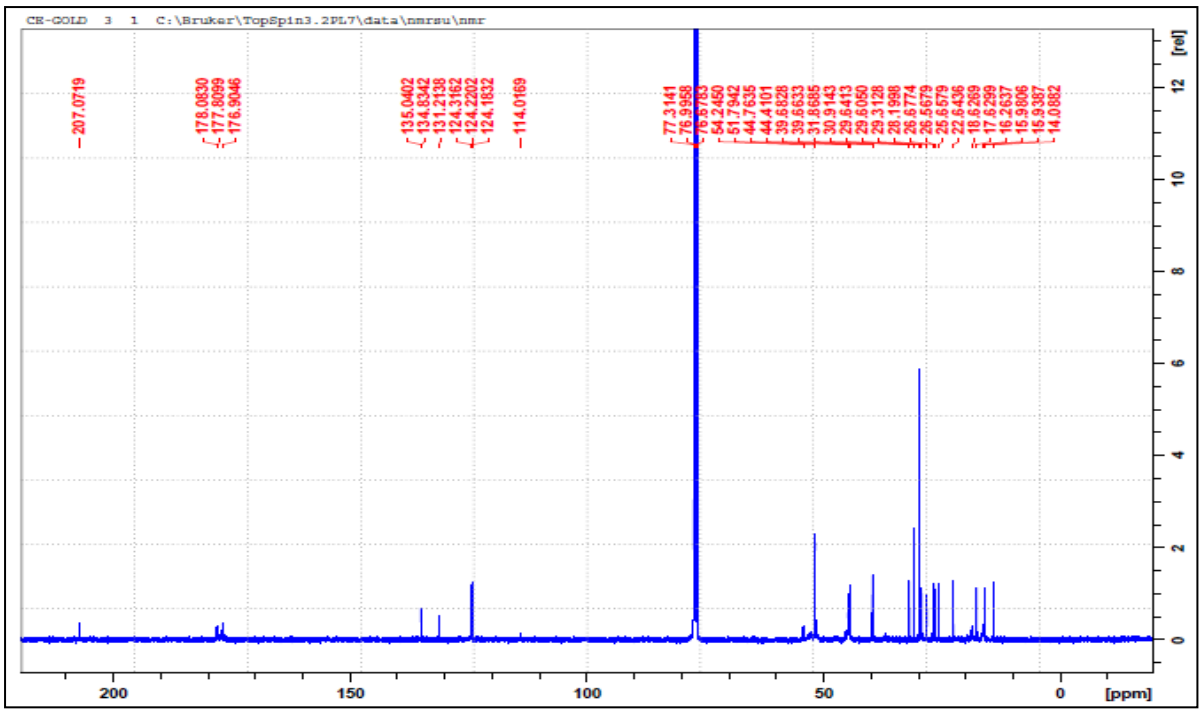
Appendix 17. MS fragmentation spectrum of fraction **B** isolated from acetone leaves extract of *Combretum erythrophyllum*.



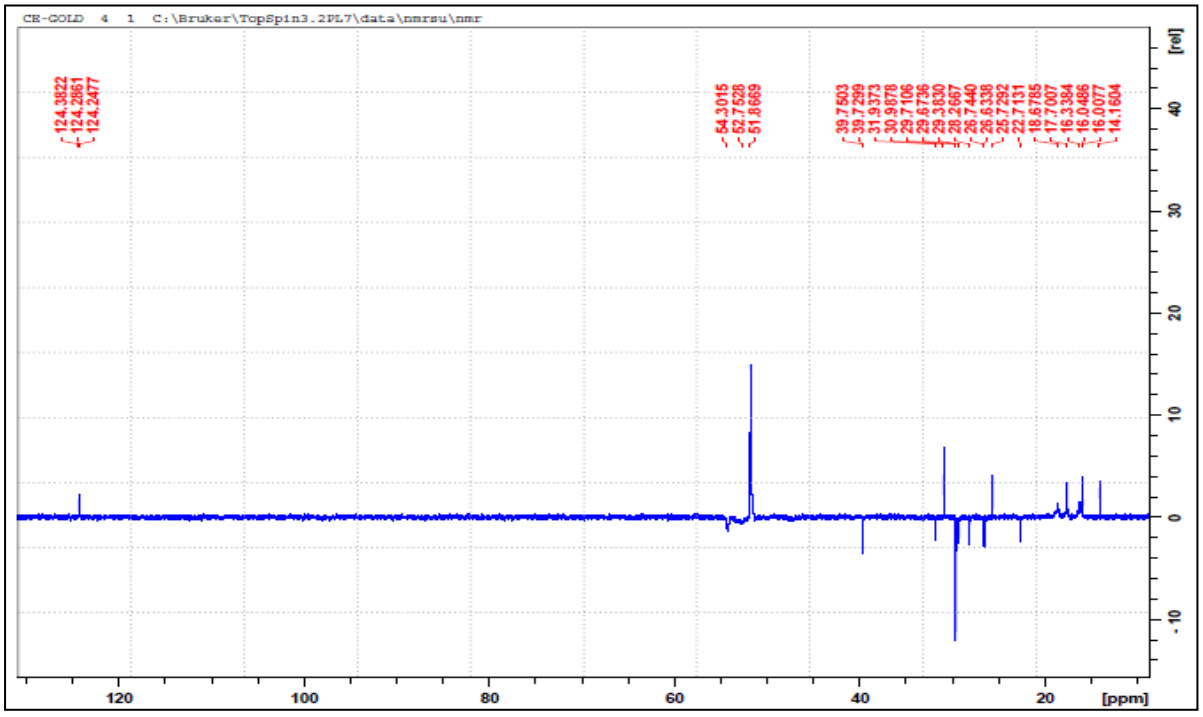
Appendix 18. ^{1}H-NMR spectrum of fraction **B** isolated from acetone leaves extract of *Combretum erythrophyllum*.



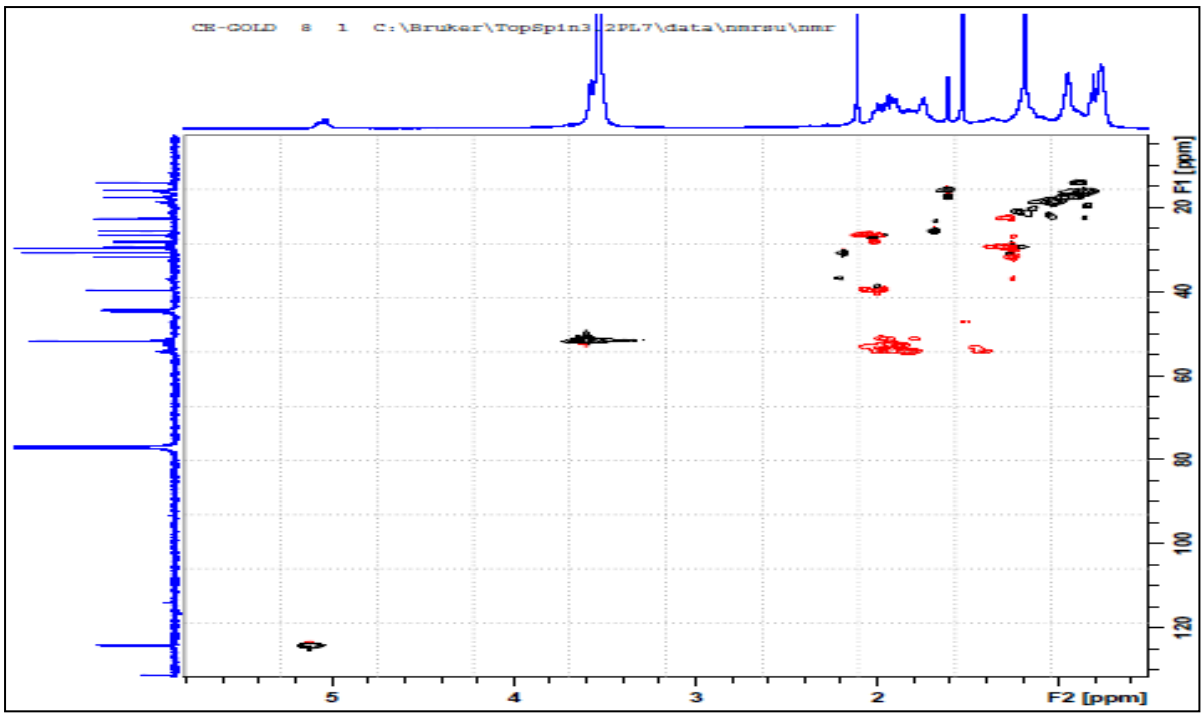
Appendix 19. ^{1}H-^{1}H COSY spectrum of fraction **B** isolated from acetone leaves extract of *Combretum erythrophyllum*.



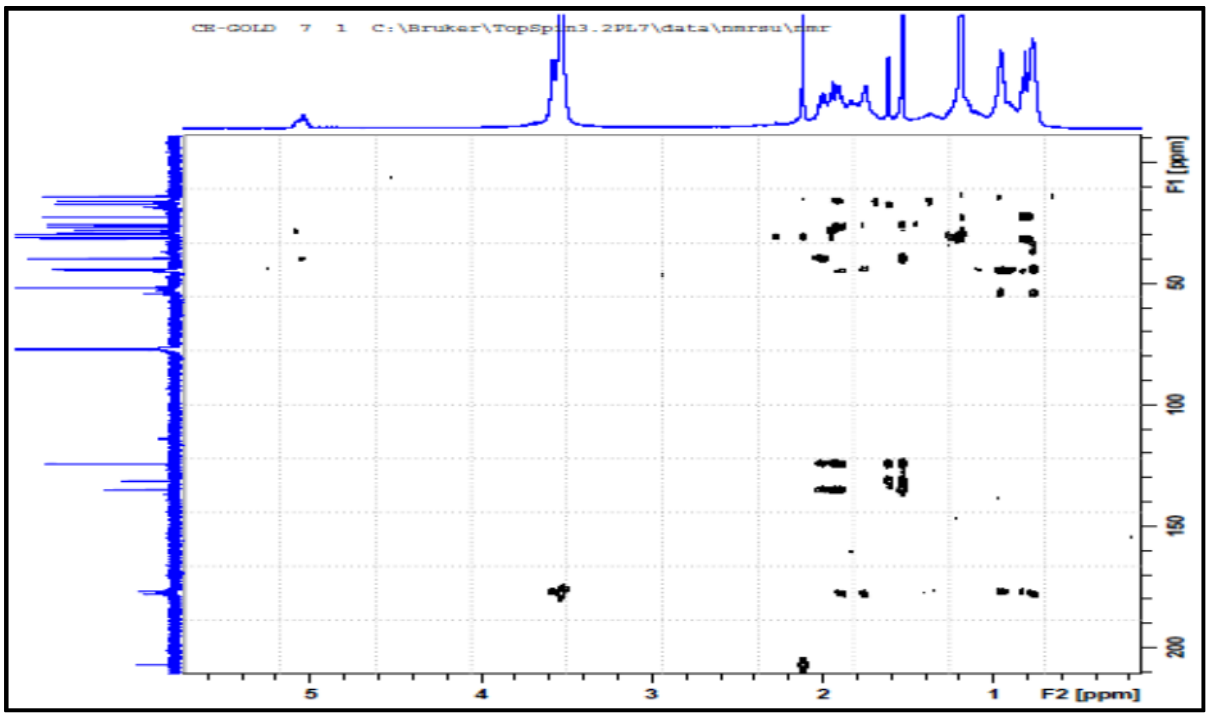
Appendix 20. ^{13}C-NMR spectrum of fraction **B** isolated from acetone leaves extract of *Combretum erythrophyllum*.



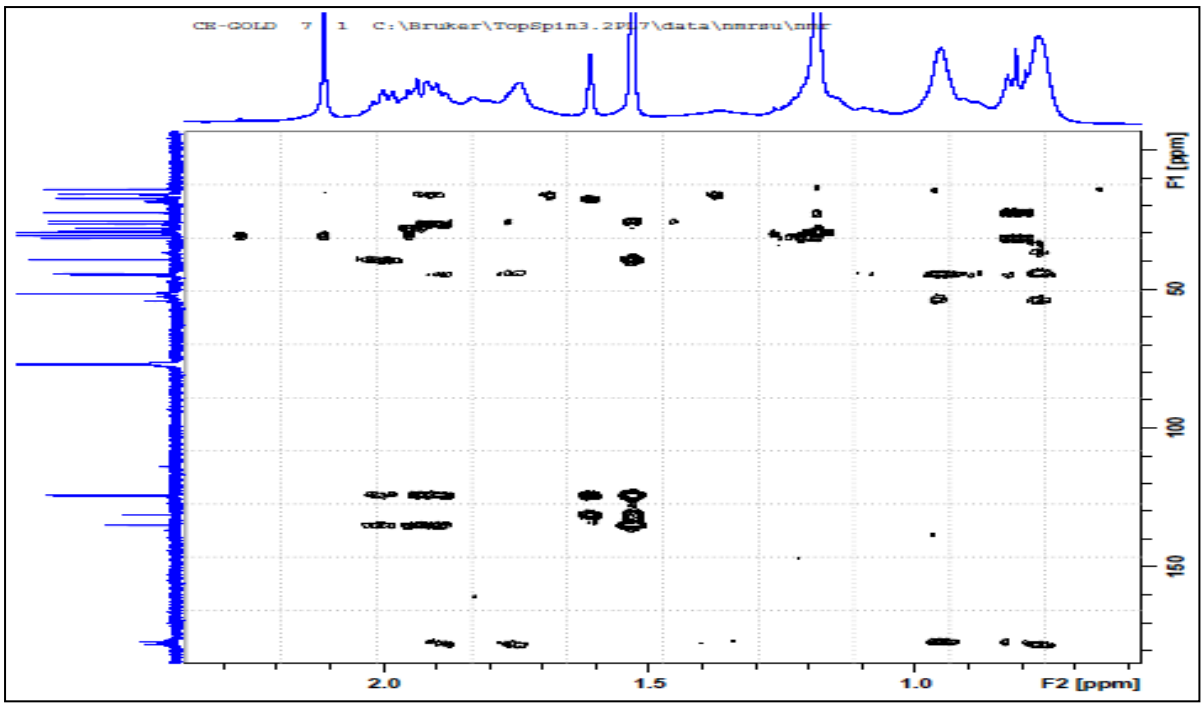
Appendix 21. DEPT spectrum of fraction **B** isolated from acetone leaves extract of *Combretum erythrophyllum*.



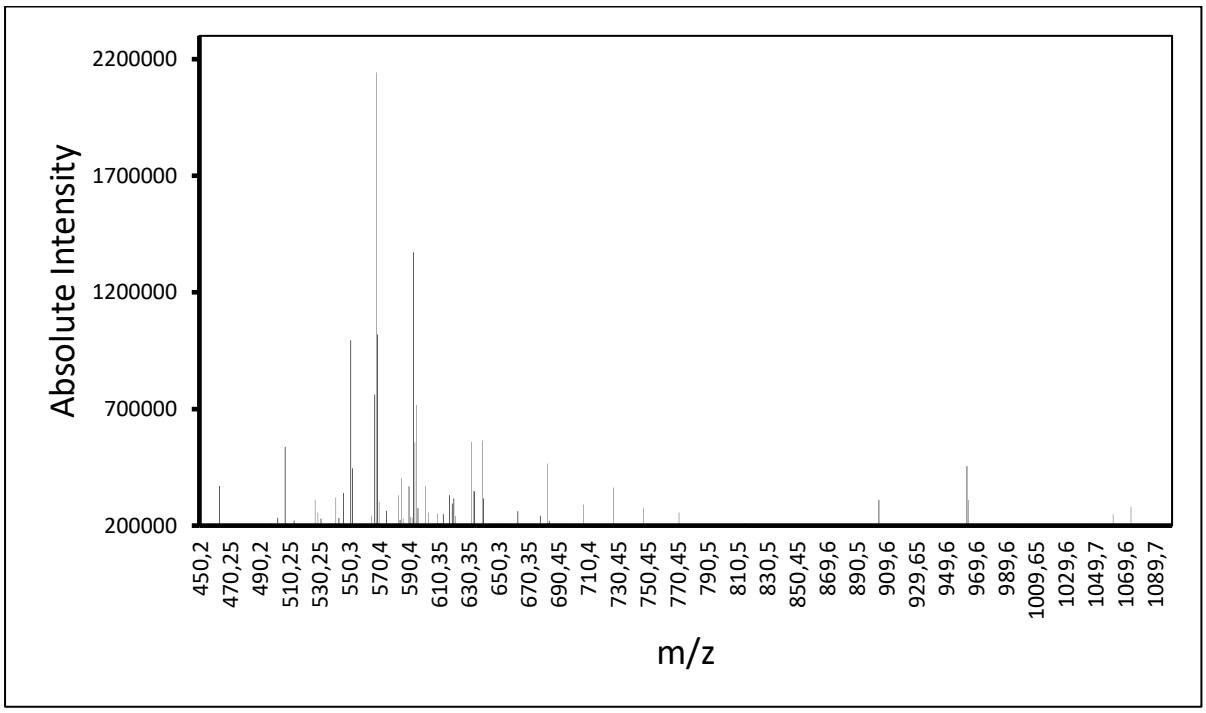
Appendix 22. HSQC spectrum of fraction **B** isolated from acetone leaves extract of *Combretum erythrophyllum*.



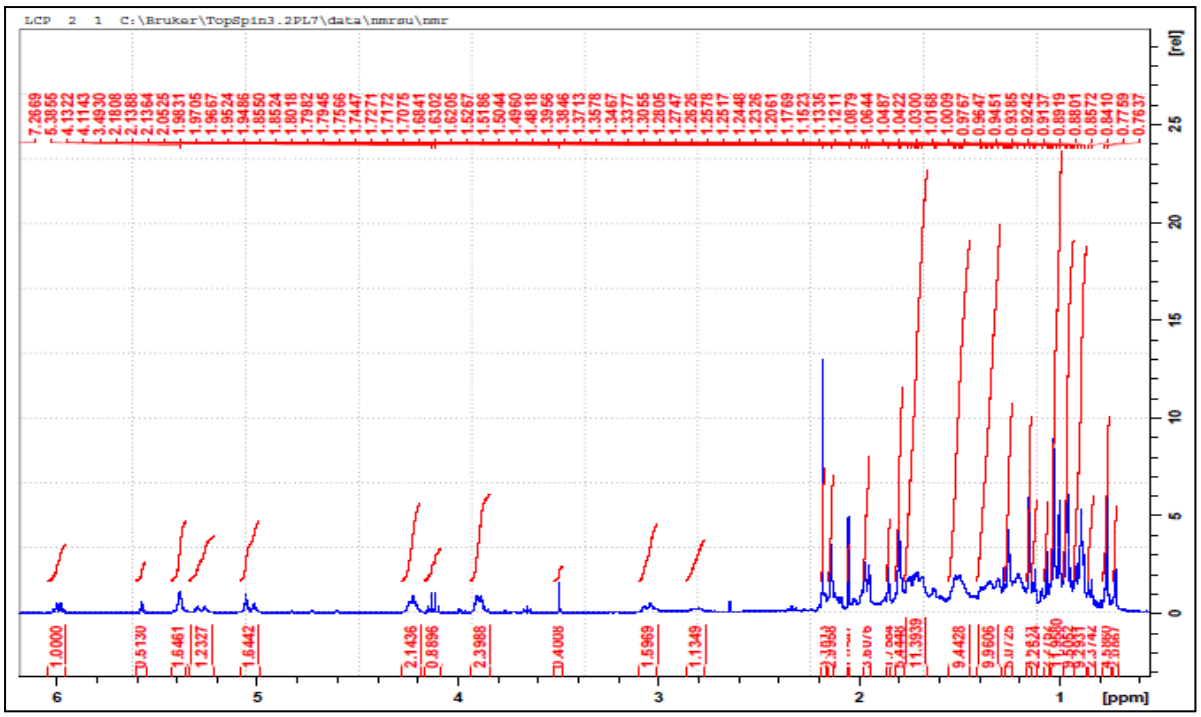
Appendix 23. HMBC spectrum of fraction **B** isolated from acetone leaves extract of *Combretum erythrophyllum*.



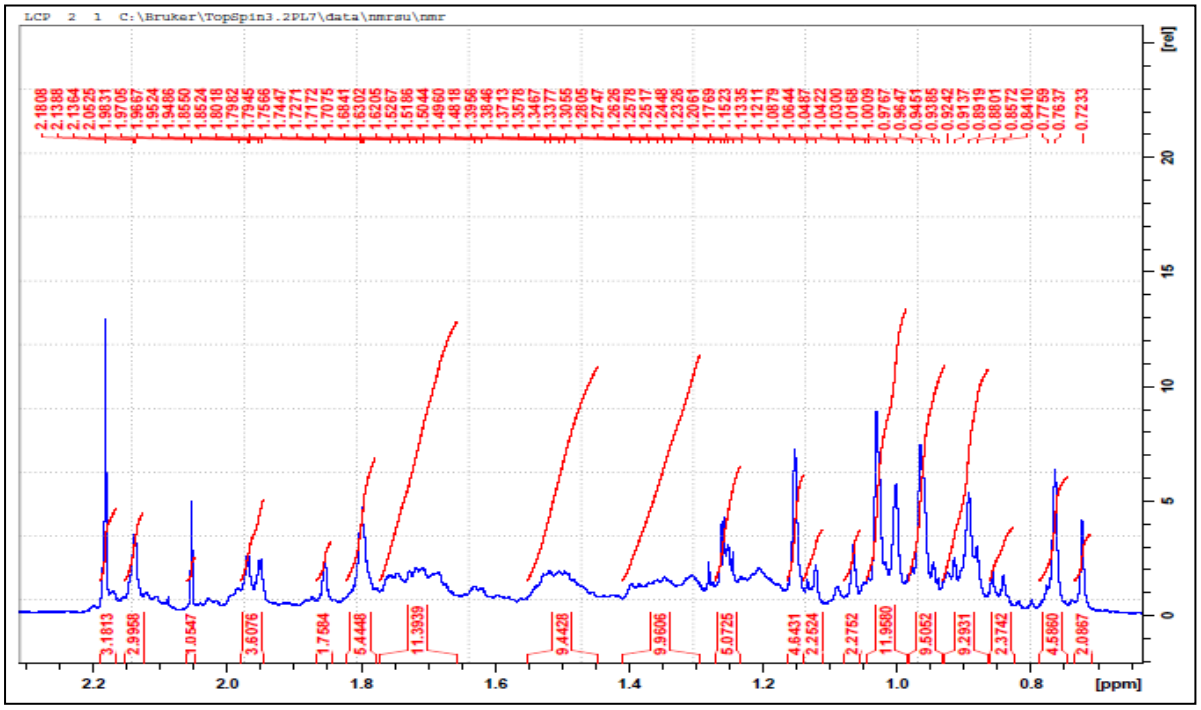
Appendix 24. Extended (0-2.5 ppm) HMBC spectrum of fraction **B** isolated from acetone leaves extract of *Combretum erythrophyllum*.



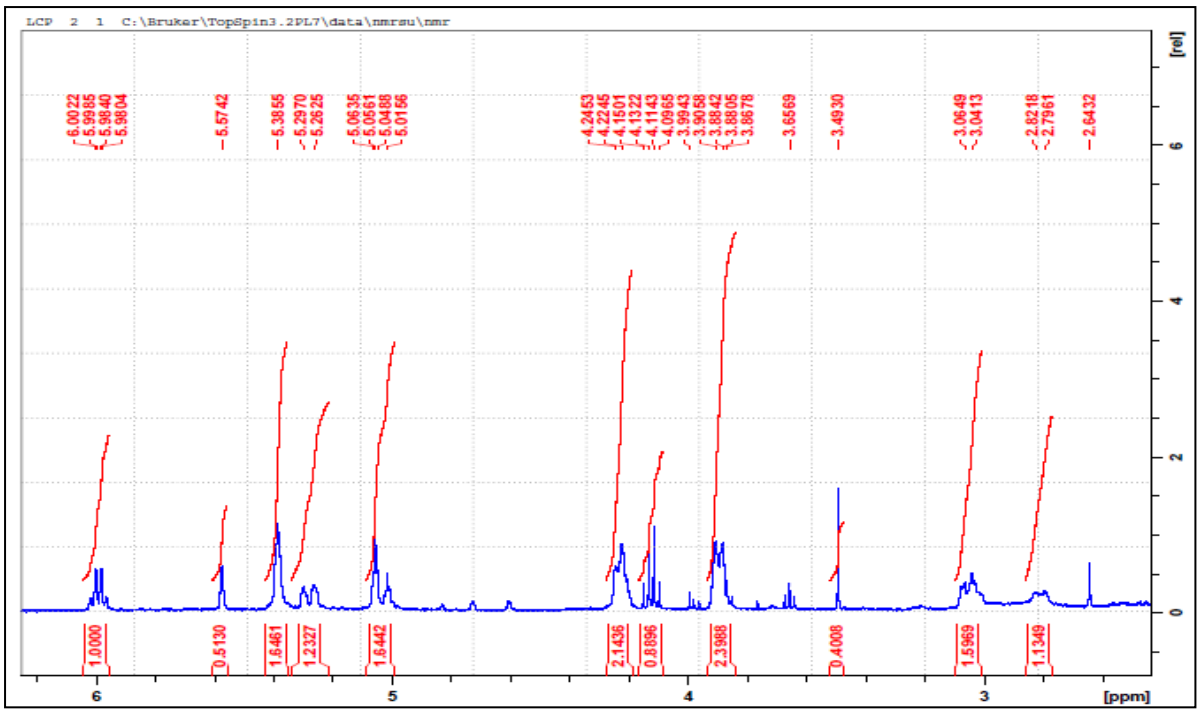
Appendix 25. MS fragmentation spectrum of fraction **L** isolated from ethyl acetate leaves extract of *Lantana camara*.



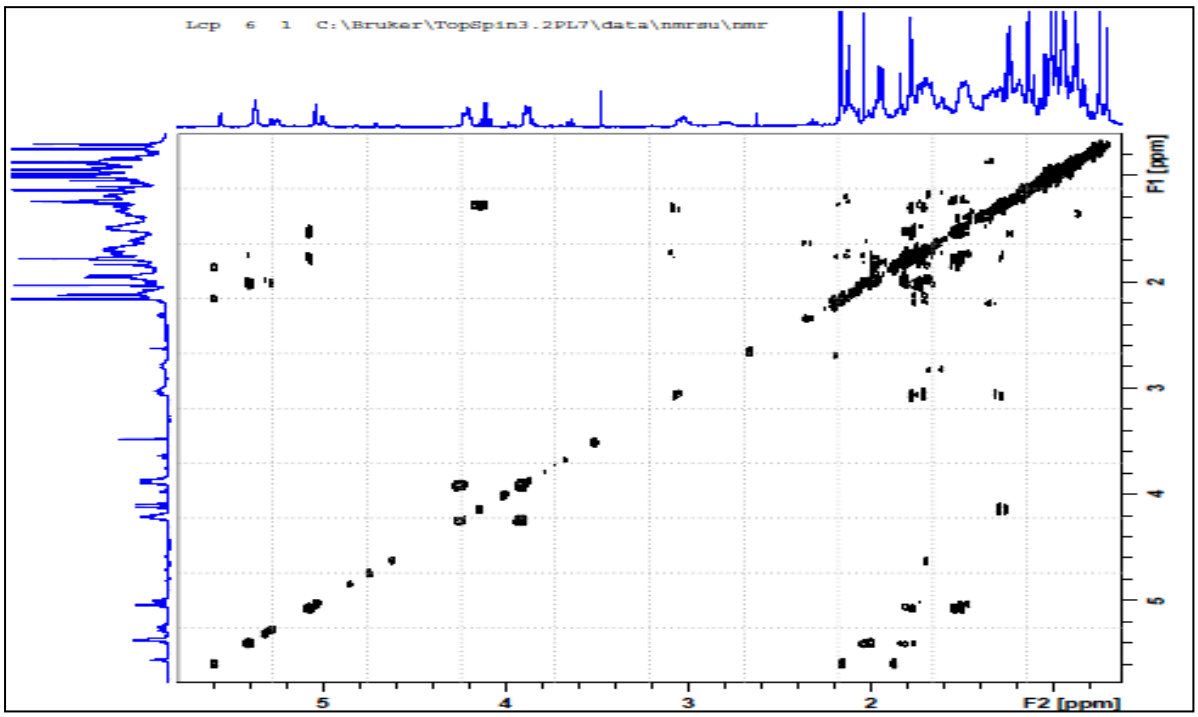
Appendix 26. ^{1}H-NMR spectrum of fraction **L** isolated from ethyl acetate leaves extract of *Lantana camara*.



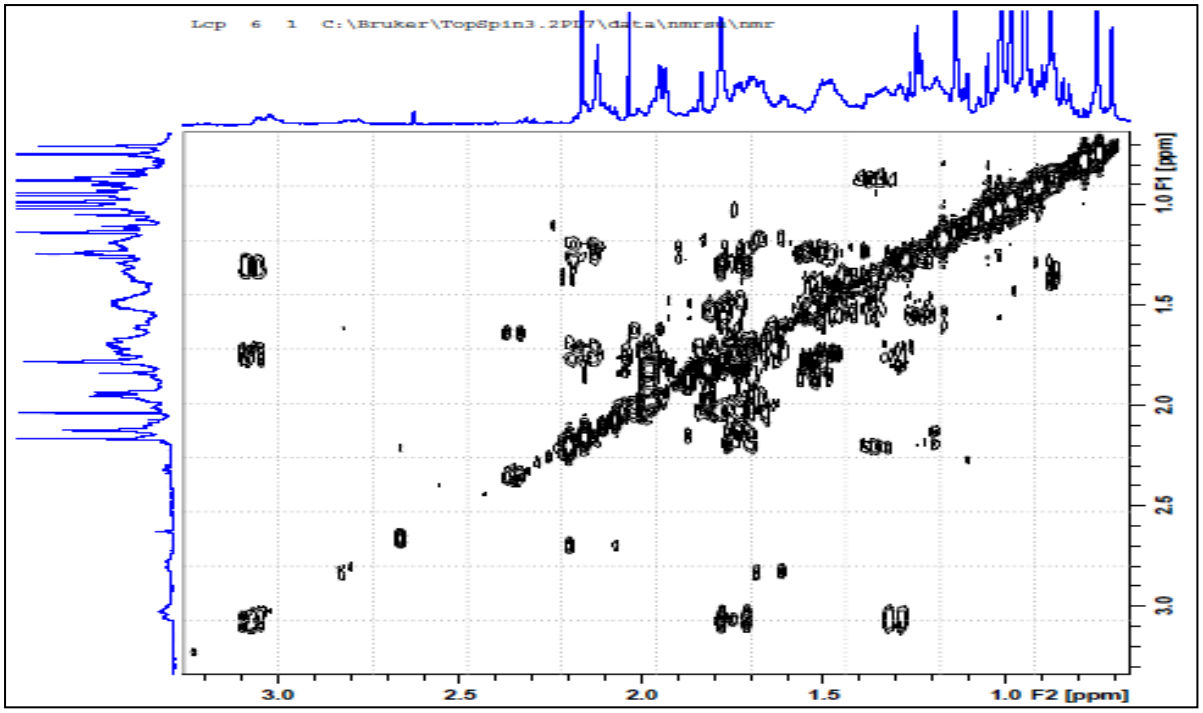
Appendix 27. Extended (0-2.2 ppm) ^{1}H-NMR spectrum of fraction **L** isolated from ethyl acetate leaves extract of *Lantana camara*.



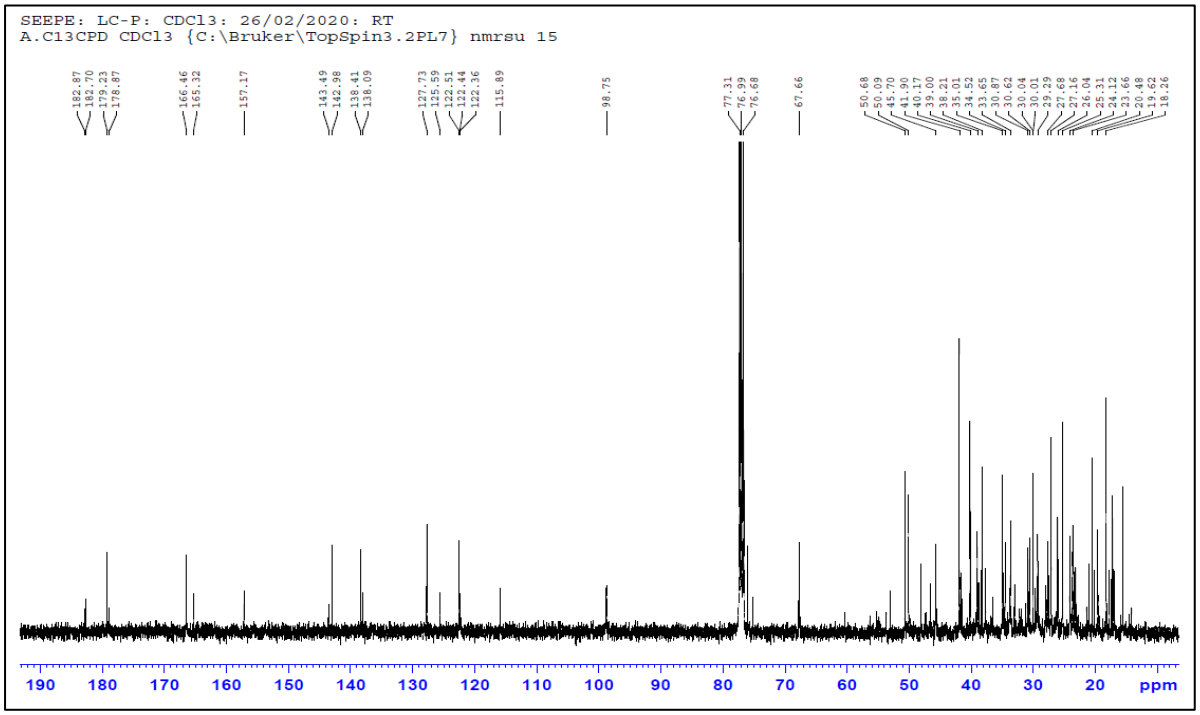
Appendix 28. Extended (2.5-6 ppm) ^{1}H-NMR spectrum of fraction **L** isolated from ethyl acetate leaves extract of *Lantana camara*.



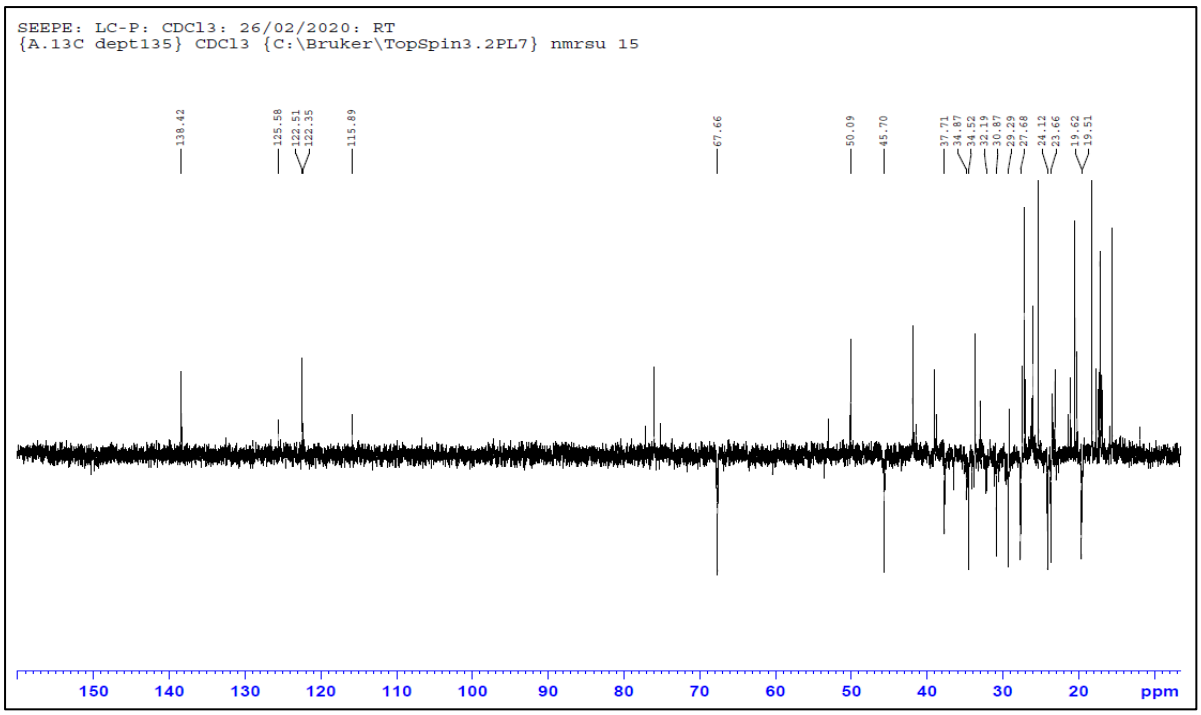
Appendix 29. ^{1}H-^{1}H COSY spectrum of fraction **L** isolated from ethyl acetate leaves extract of *Lantana camara*.



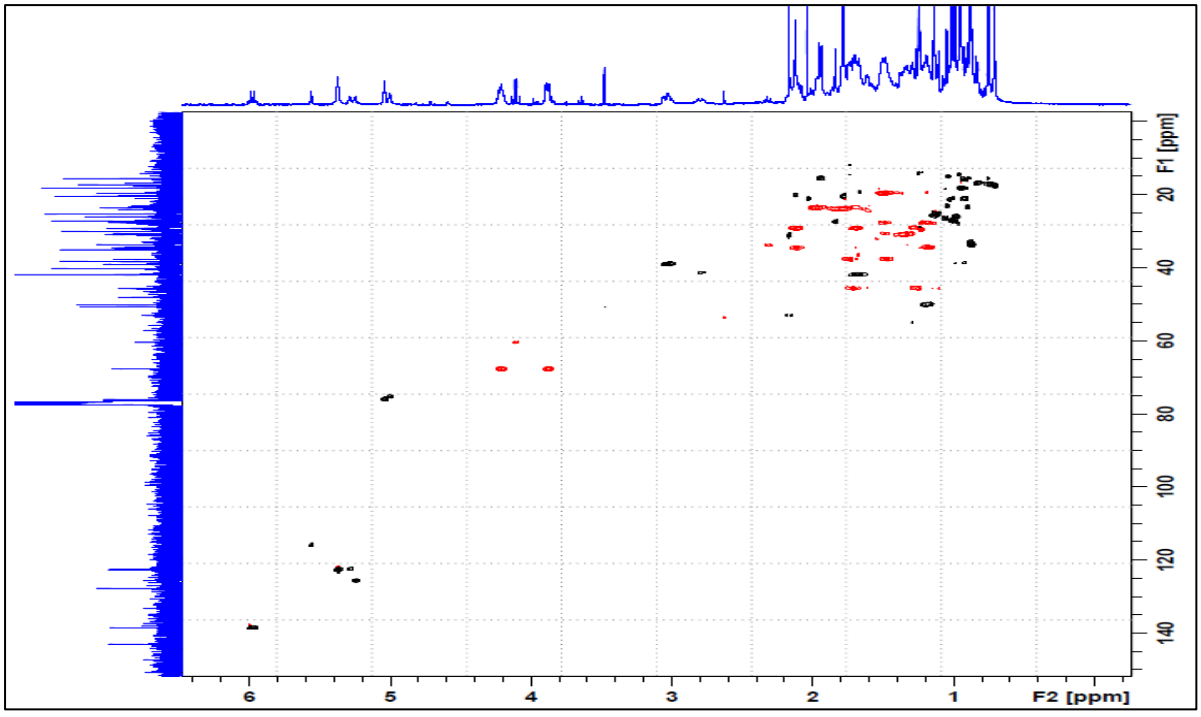
Appendix 30. Extended (0-3.5 ppm) ^{1}H-^{1}H COSY spectrum of fraction **L** isolated from ethyl acetate leaves extract of *Lantana camara*.



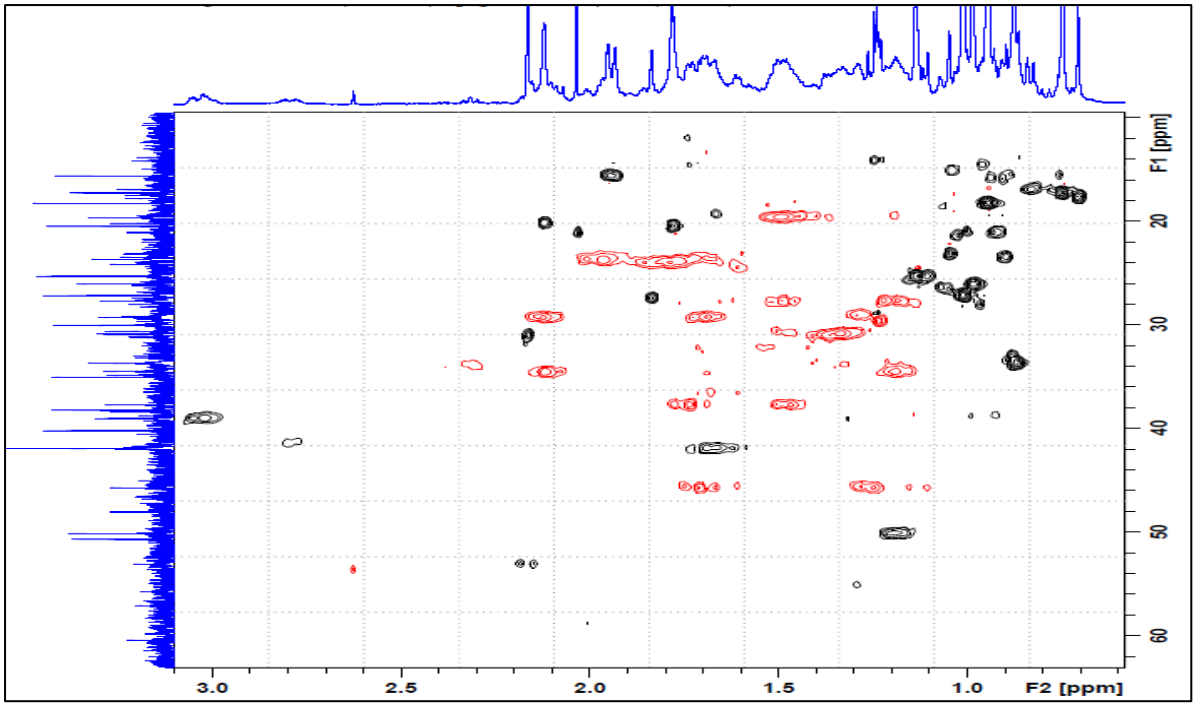
Appendix 31. ^{13}C-NMR spectrum of fraction **L** isolated from ethyl acetate leaves extract of *Lantana camara*.



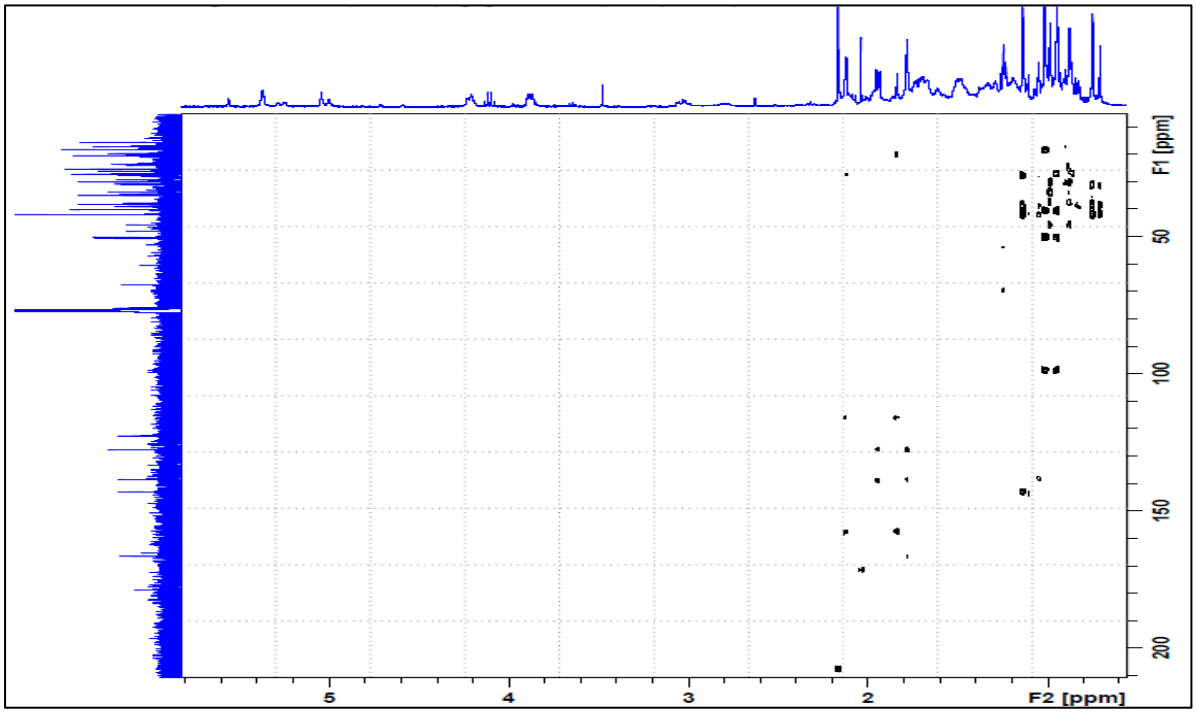
Appendix 32. DEPT spectrum of fraction **L** isolated from ethyl acetate leaves extract of *Lantana camara*.



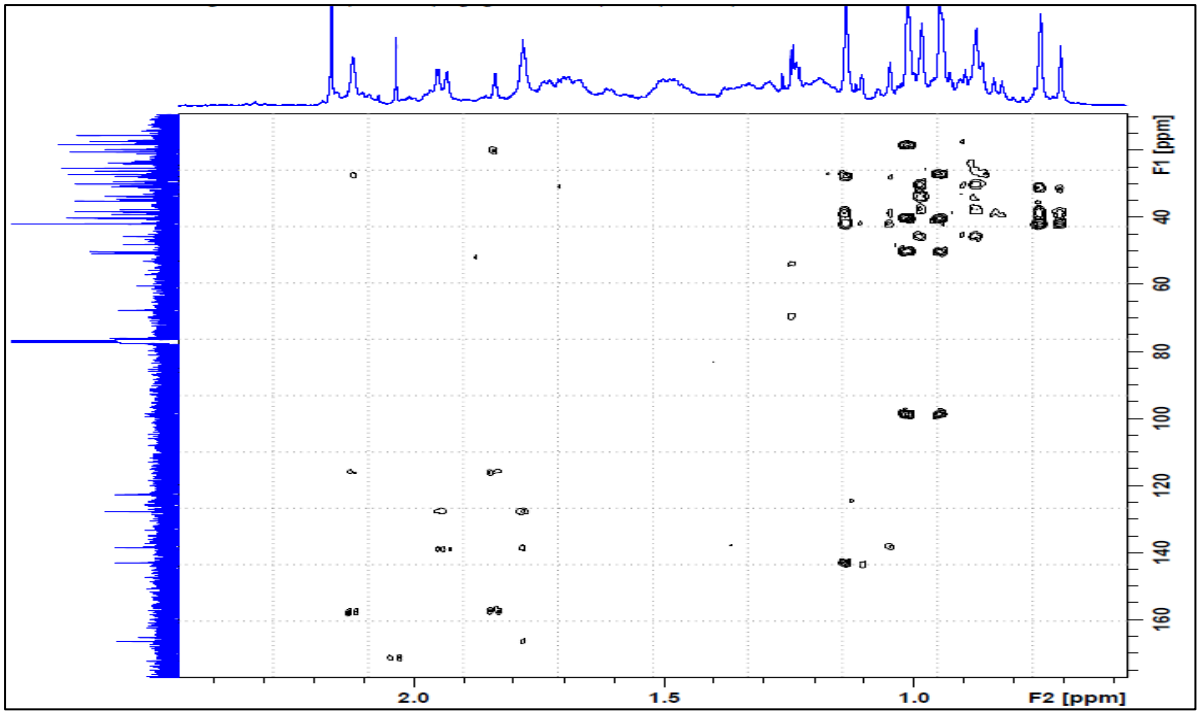
Appendix 33. HSQC spectrum of fraction **L** isolated from ethyl acetate leaves extract of *Lantana camara*.



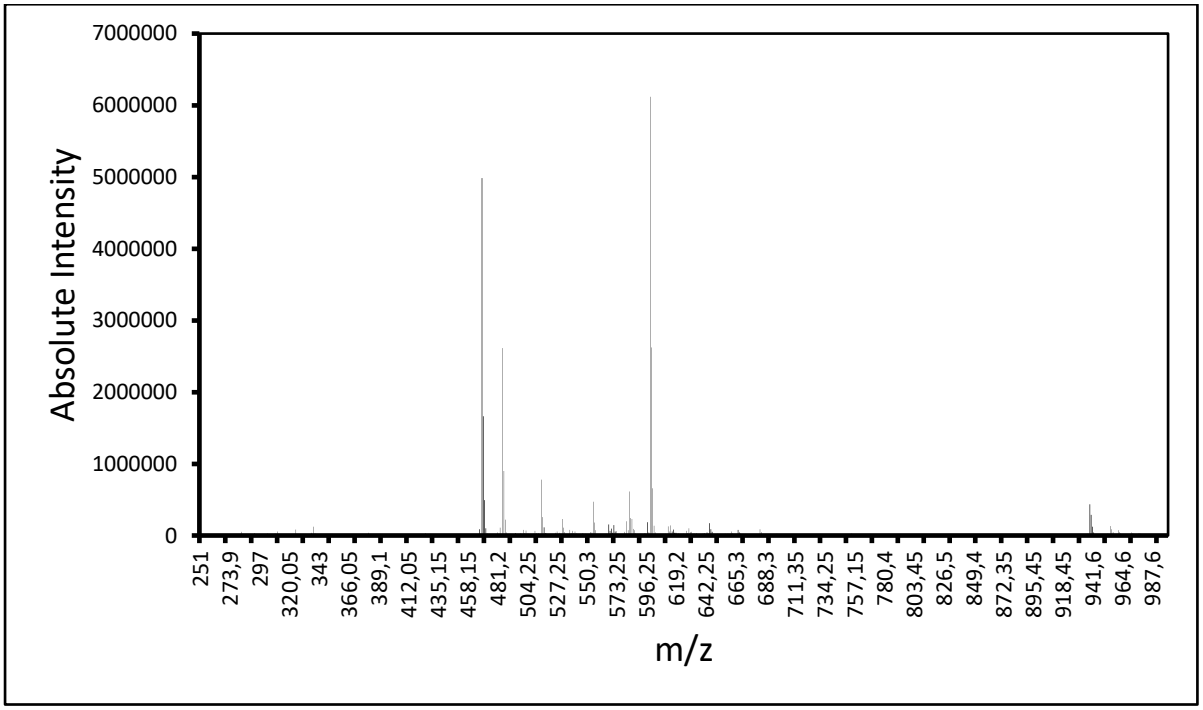
Appendix 34. Extended HSQC spectrum of fraction **L** isolated from ethyl acetate leaves extract of *Lantana camara*.



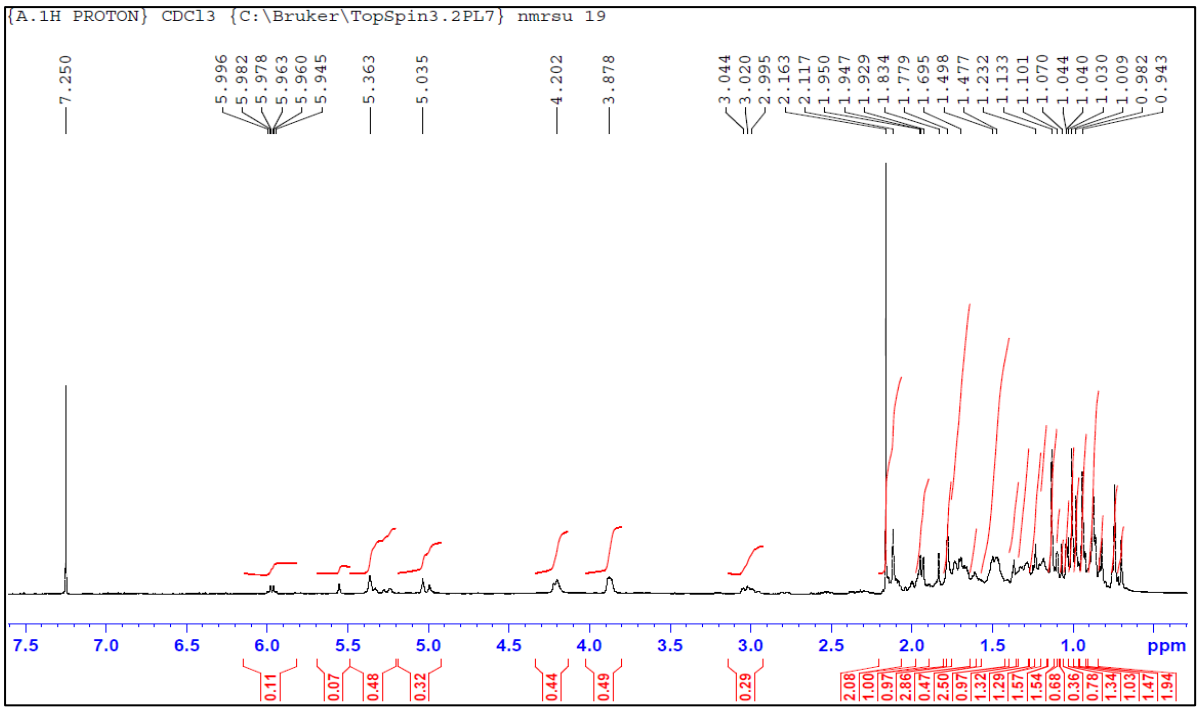
Appendix 35. HMBC spectrum of fraction **L** isolated from ethyl acetate leaves extract of *Lantana camara*.



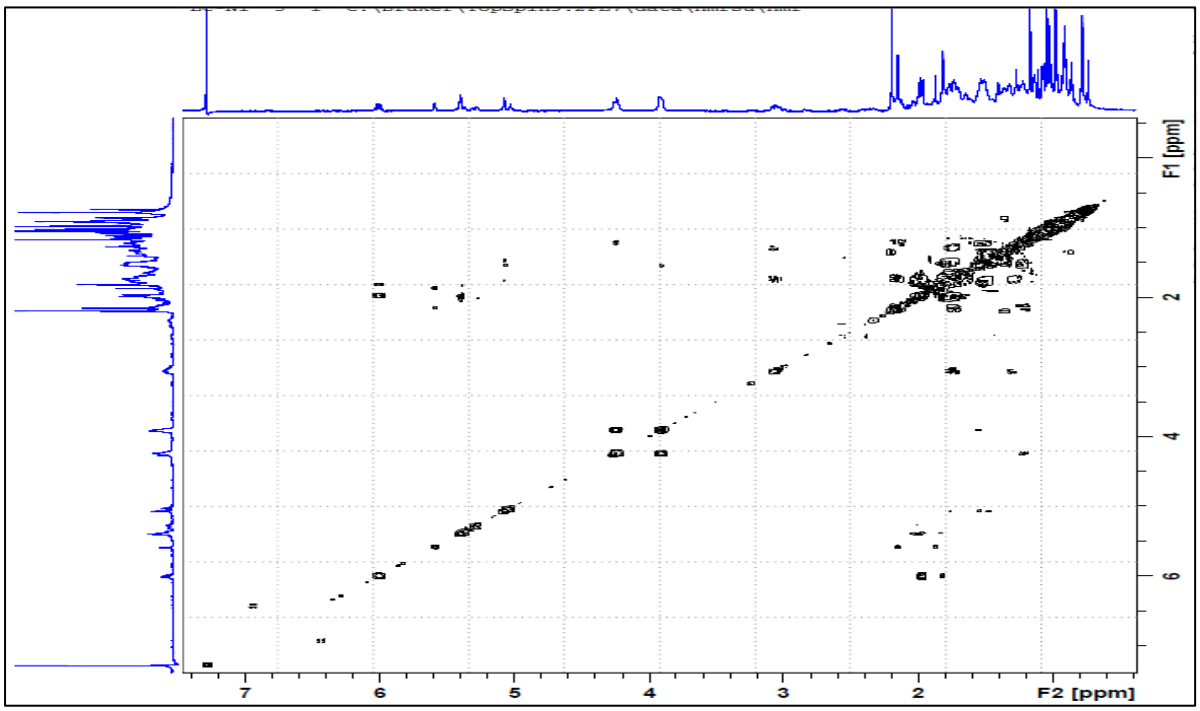
Appendix 36. Extended HMBC spectrum of fraction **L** isolated from ethyl acetate leaves extract of *Lantana camara*.



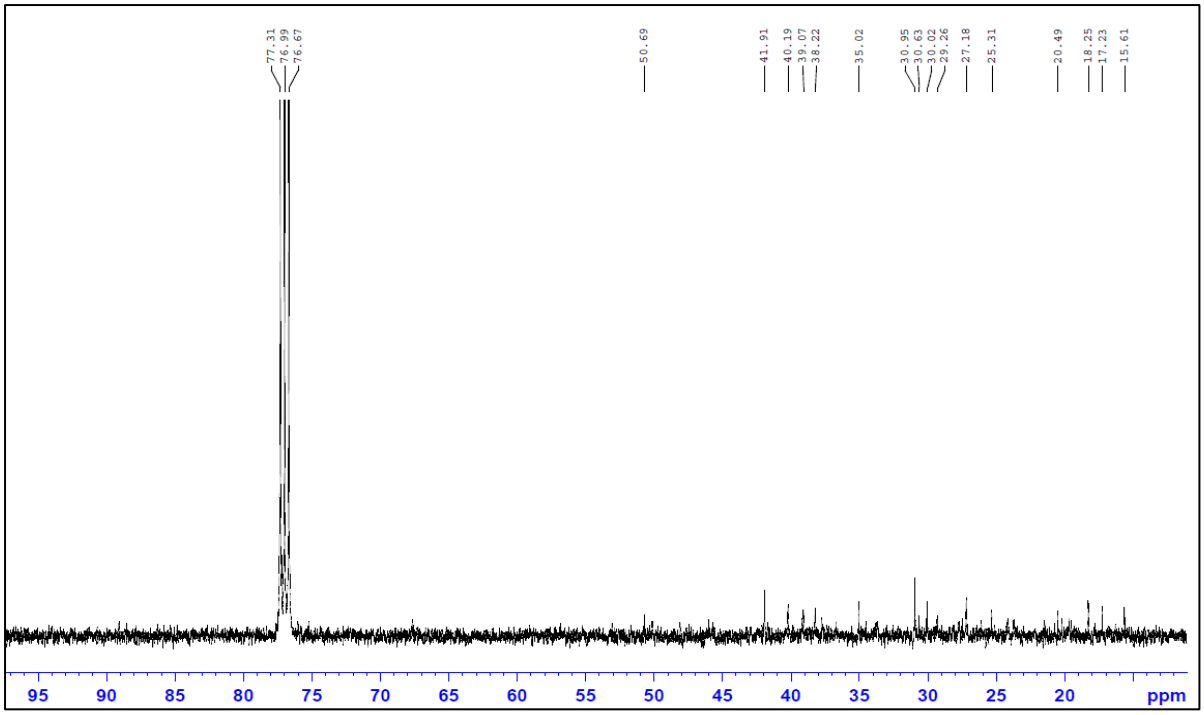
Appendix 37. MS fragmentation spectrum of fraction **N** isolated from ethyl acetate leaves extract of *Lantana camara*.



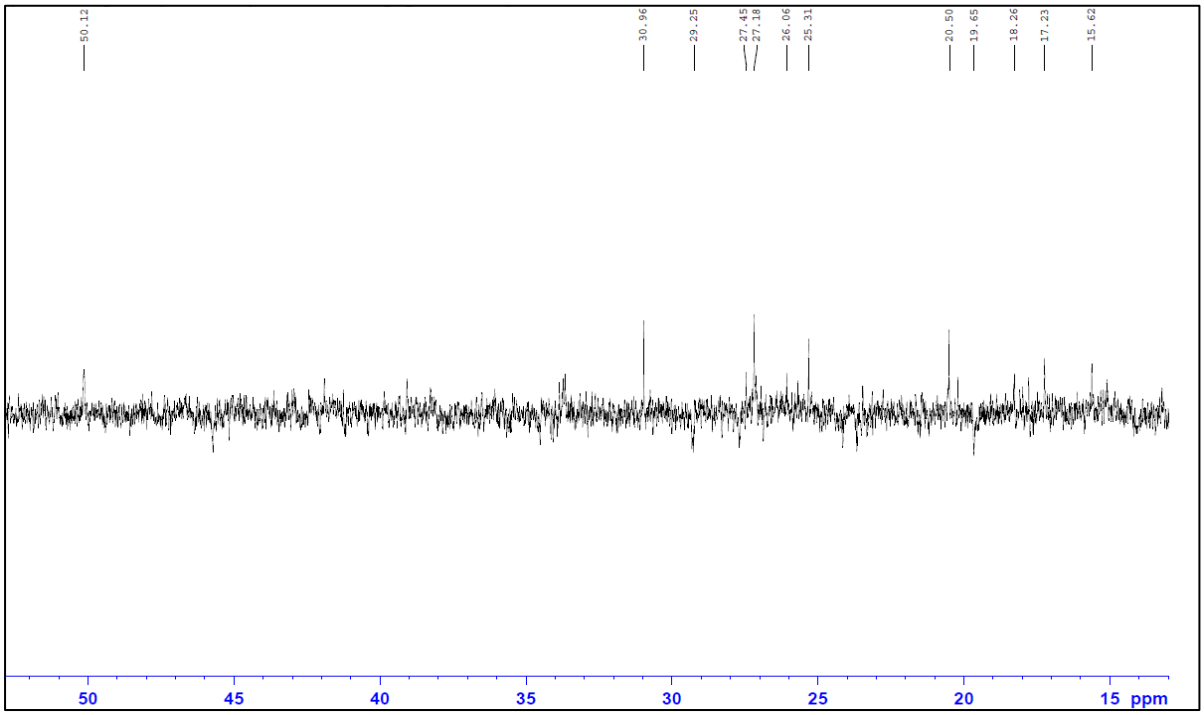
Appendix 38. ^{1}H-NMR spectrum of fraction **N** isolated from ethyl acetate leaves extract of *Lantana camara*.



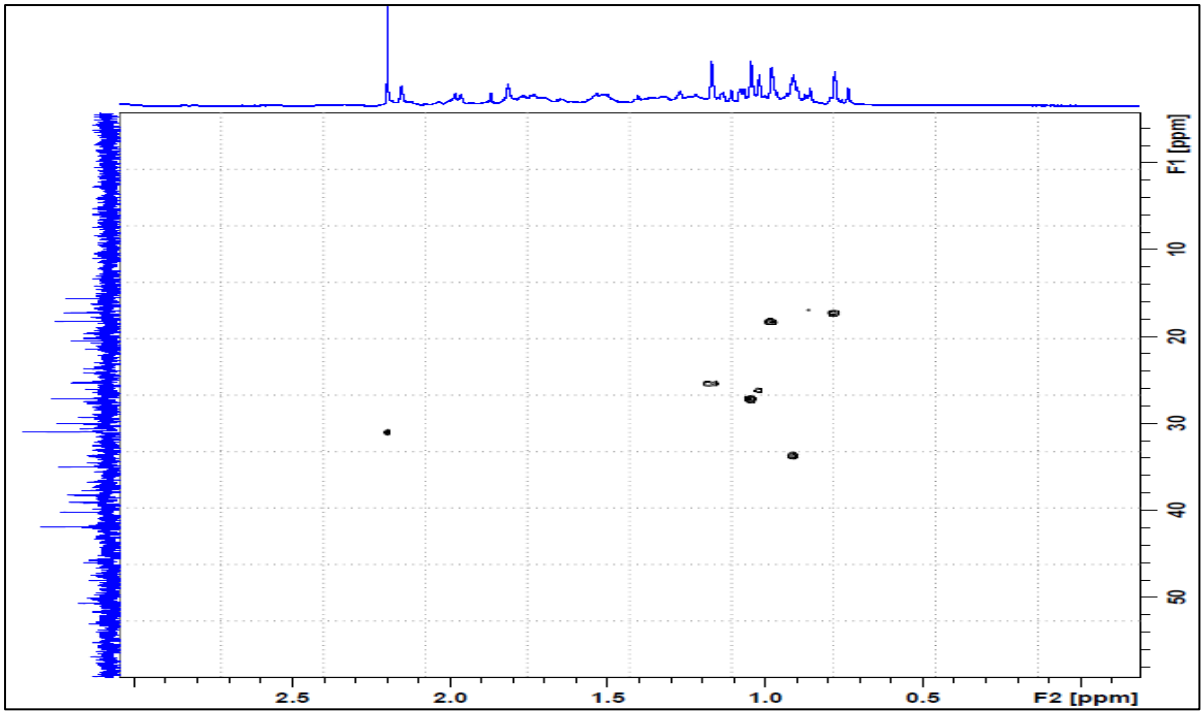
Appendix 39. ^{1}H-^{1}H COSY spectrum of fraction **N** isolated from ethyl acetate leaves extract of *Lantana camara*.



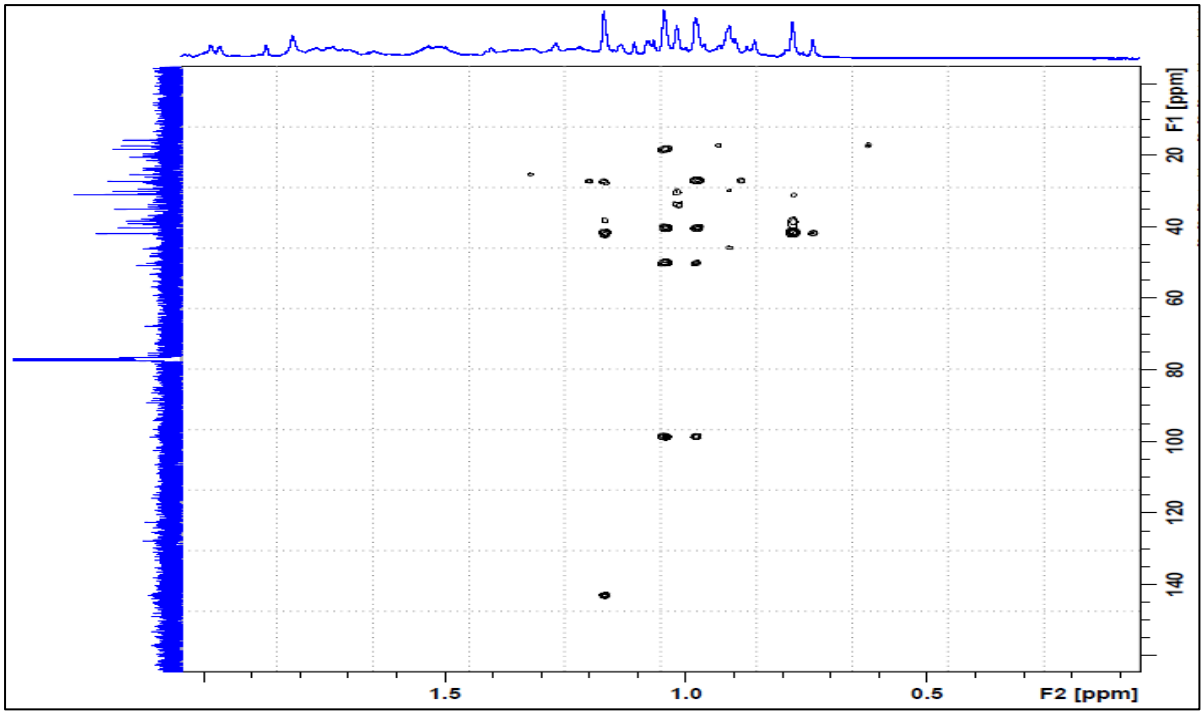
Appendix 40. ^{13}C-NMR spectrum of fraction **N** isolated from ethyl acetate leaves extract of *Lantana camara*.



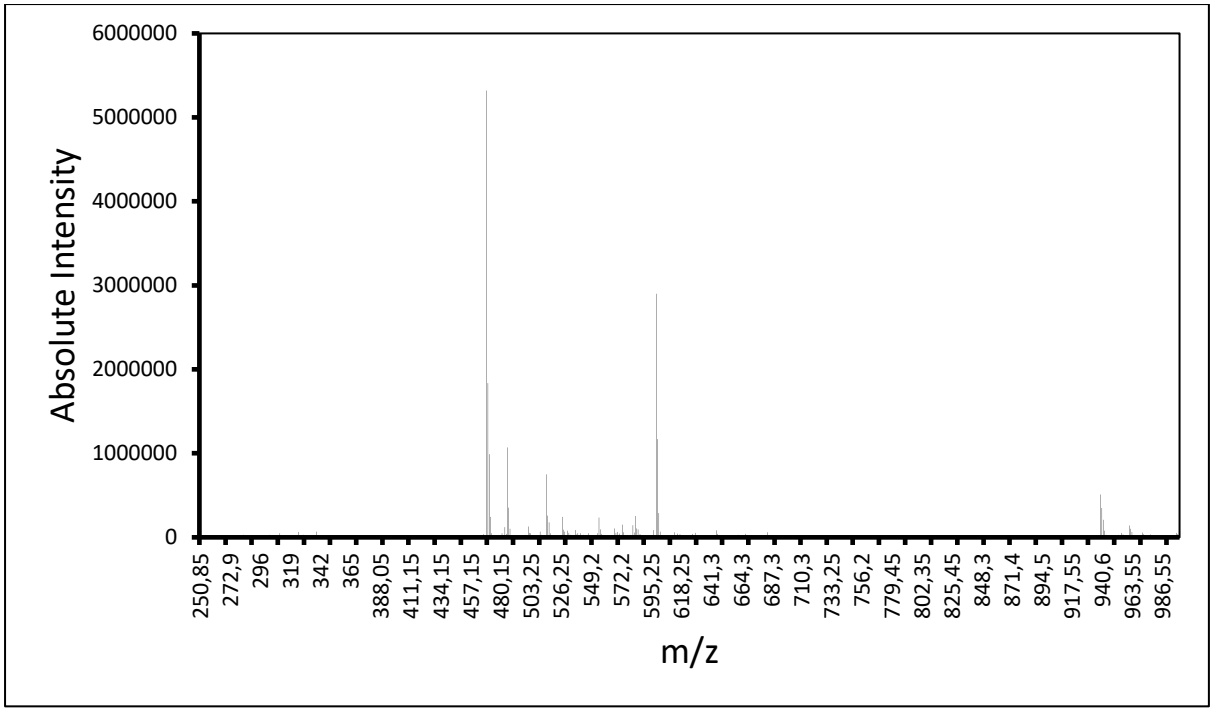
Appendix 41. DEPT spectrum of fraction **N** isolated from ethyl acetate leaves extract of *Lantana camara*.



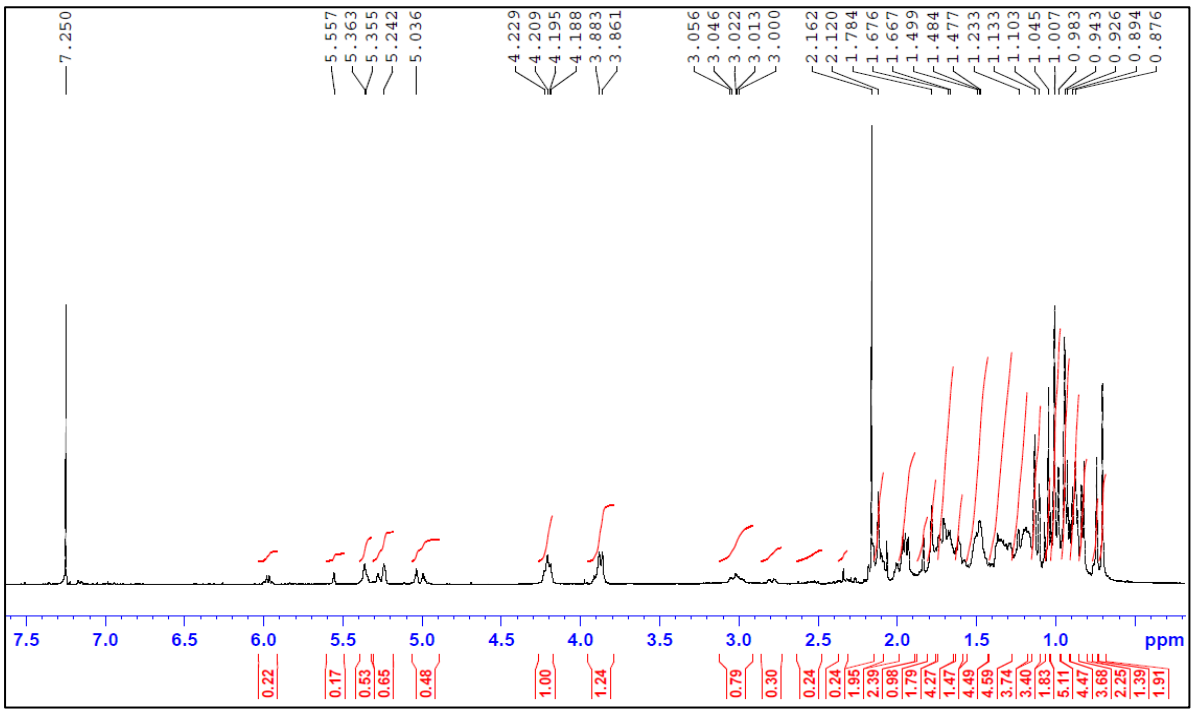
Appendix 42. HSQC spectrum of fraction **N** isolated from ethyl acetate leaves extract of *Lantana camara*.



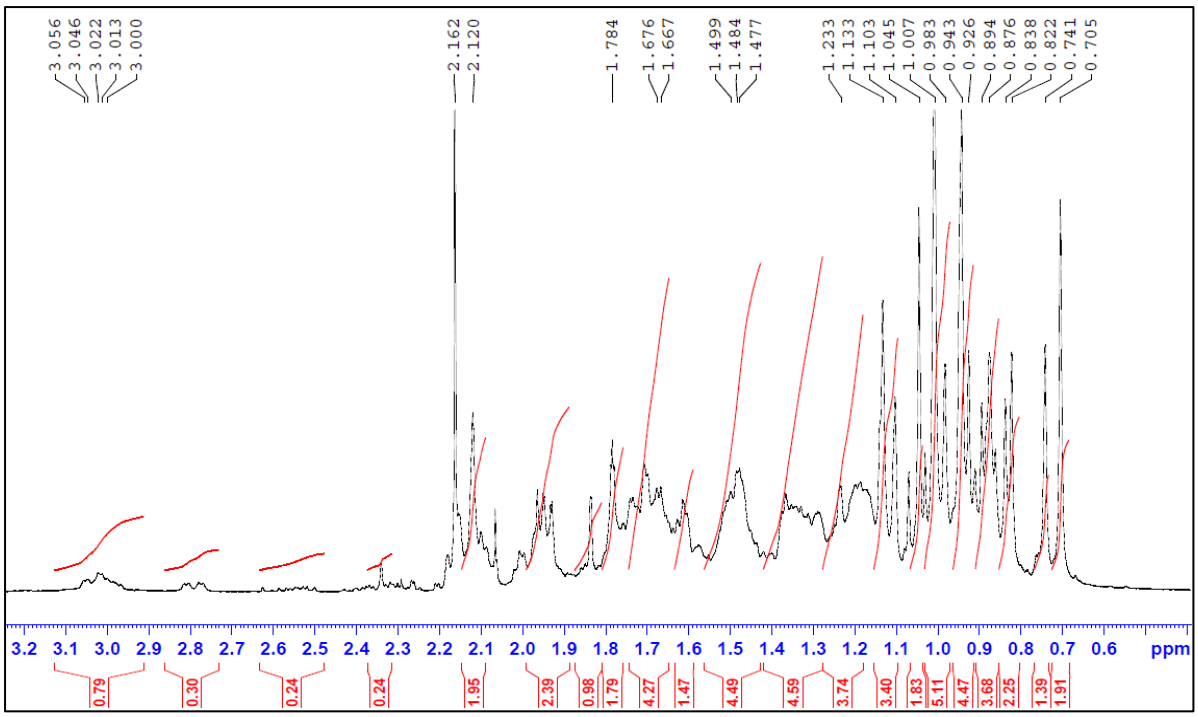
Appendix 43. HMBC spectrum of fraction **N** isolated from ethyl acetate leaves extract of *Lantana camara*.



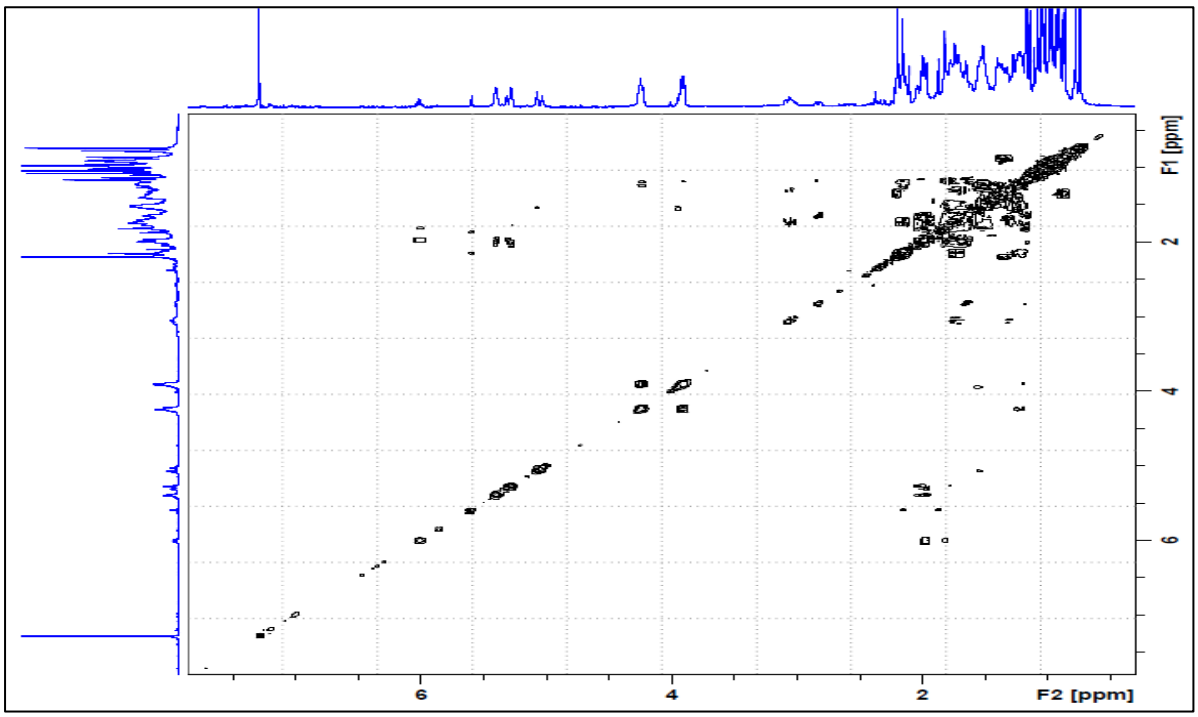
Appendix 44. MS fragmentation spectrum of fraction **R** isolated from ethyl acetate leaves extract of *Lantana camara*.



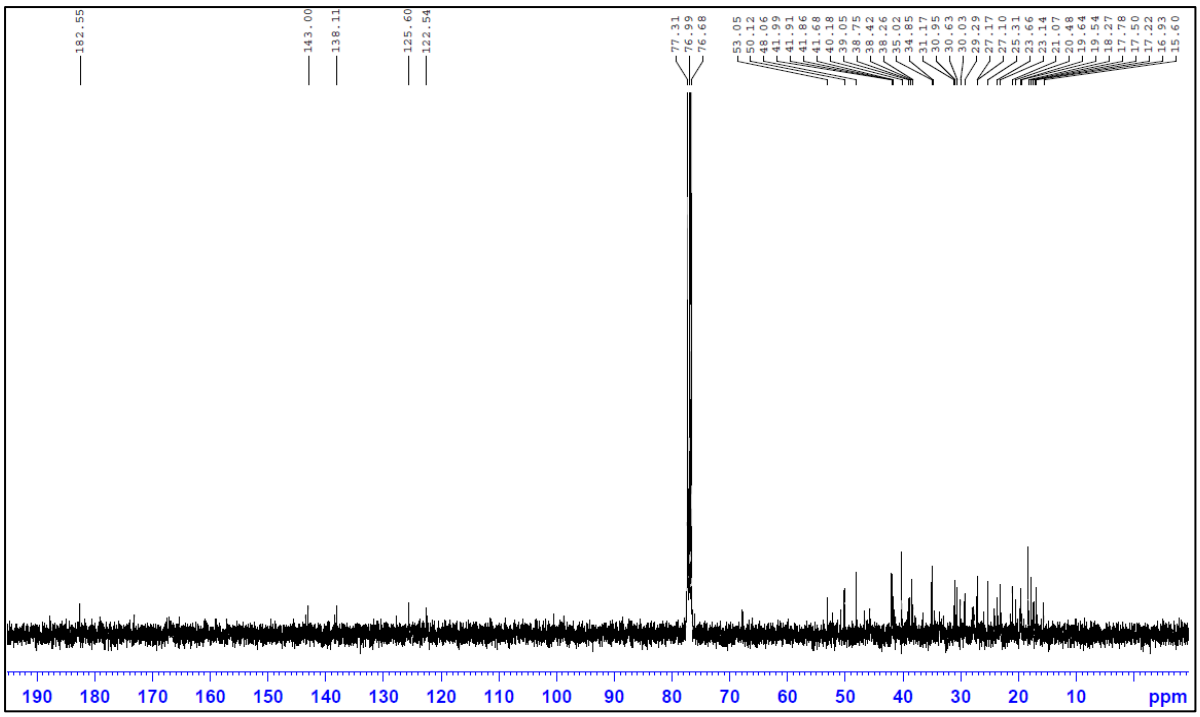
Appendix 45. ^{1}H-NMR spectrum of fraction **R** isolated from ethyl acetate leaves extract of *Lantana camara*.



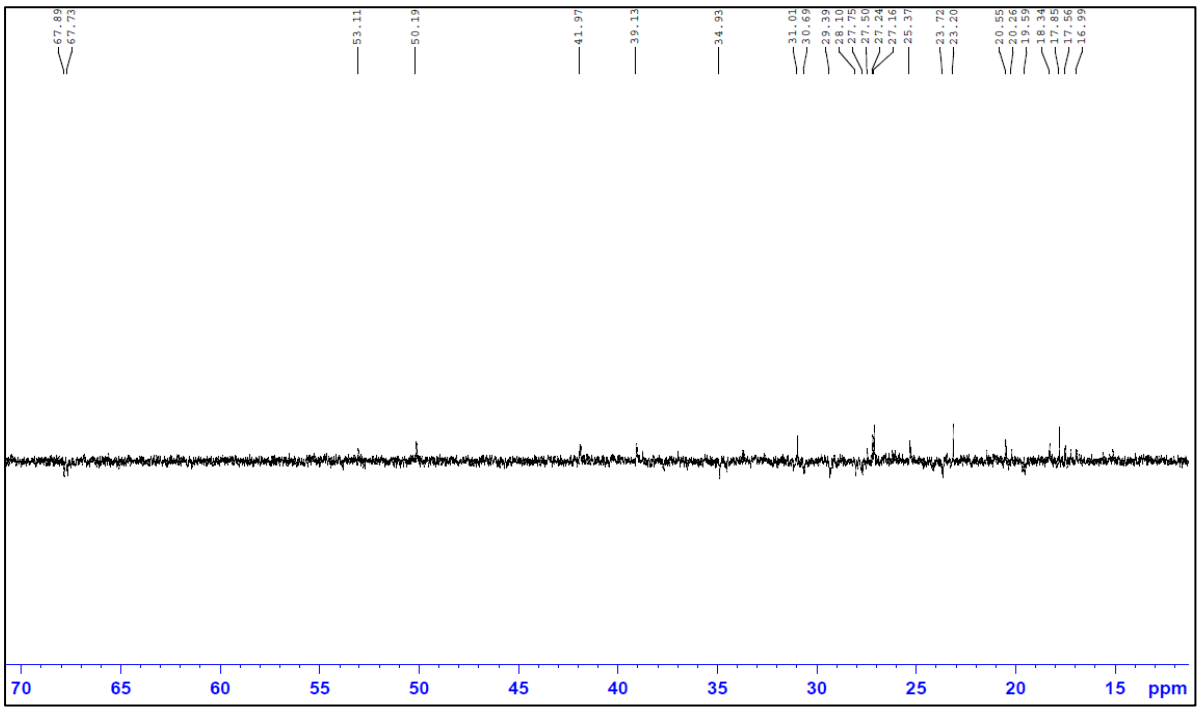
Appendix 46. Extended ^{1}H-NMR spectrum of fraction **R** isolated from ethyl acetate leaves extract of *Lantana camara*.



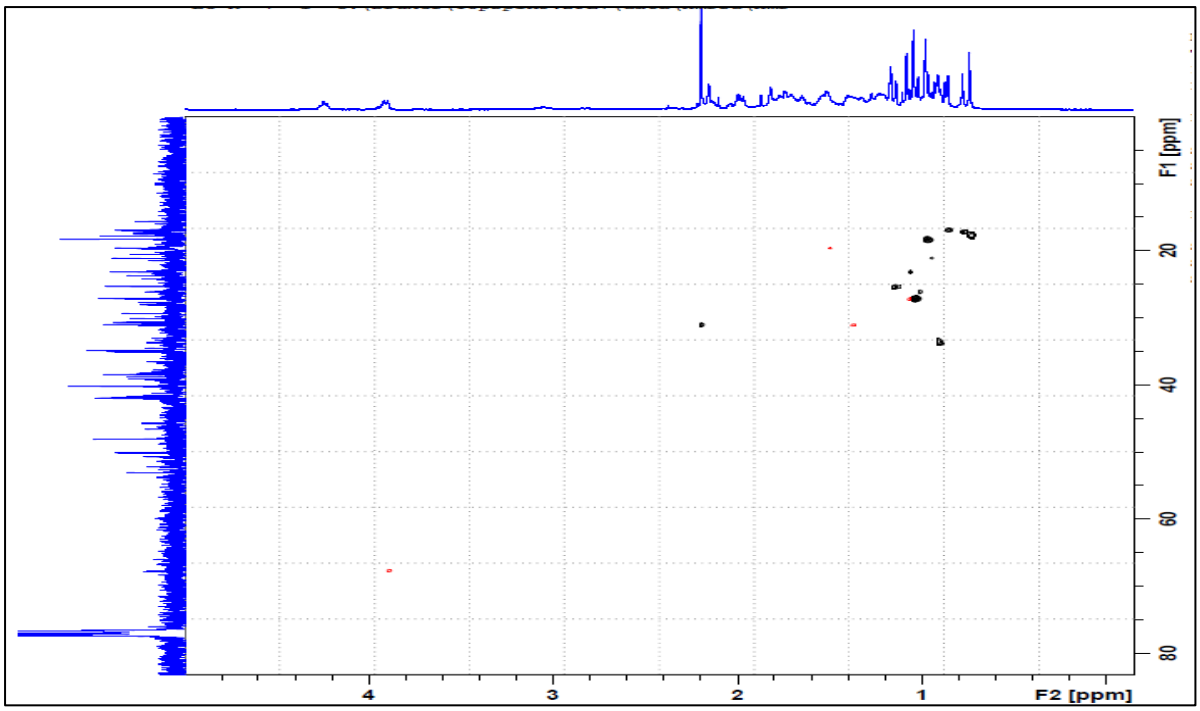
Appendix 47. ^{1}H-^{1}H COSY spectrum of fraction **R** isolated from ethyl acetate leaves extract of *Lantana camara*.



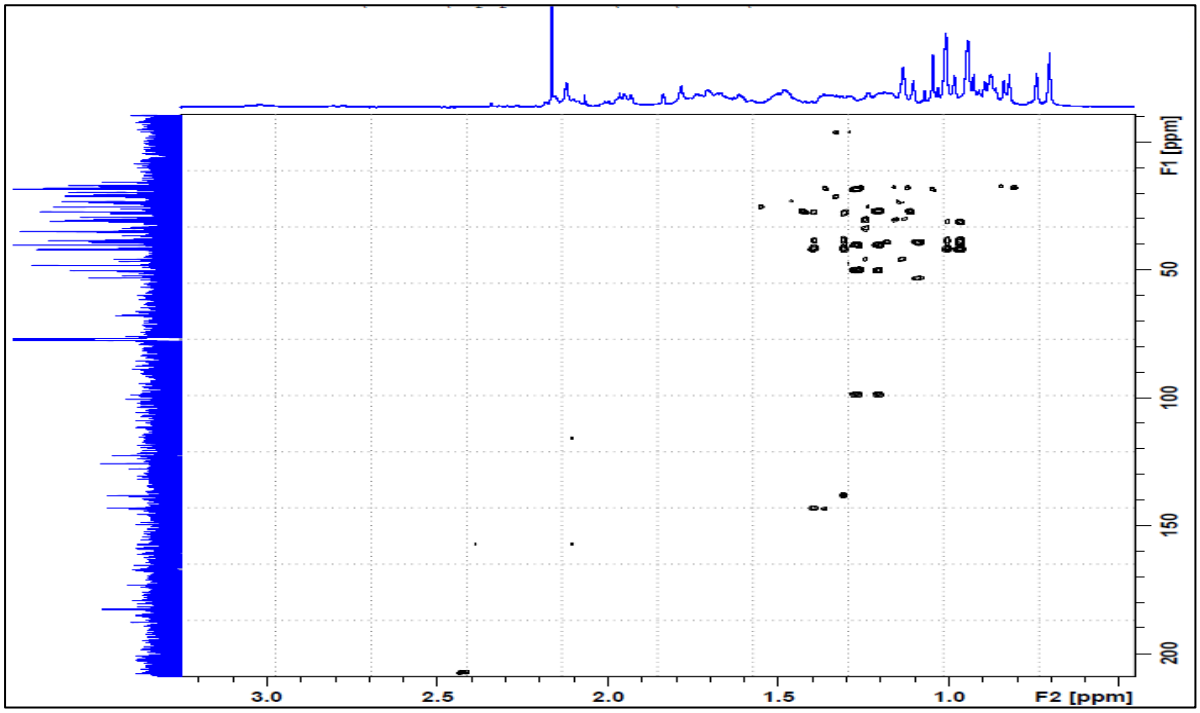
Appendix 48. ^{13}C-NMR spectrum of fraction **R** isolated from ethyl acetate leaves extract of *Lantana camara*.



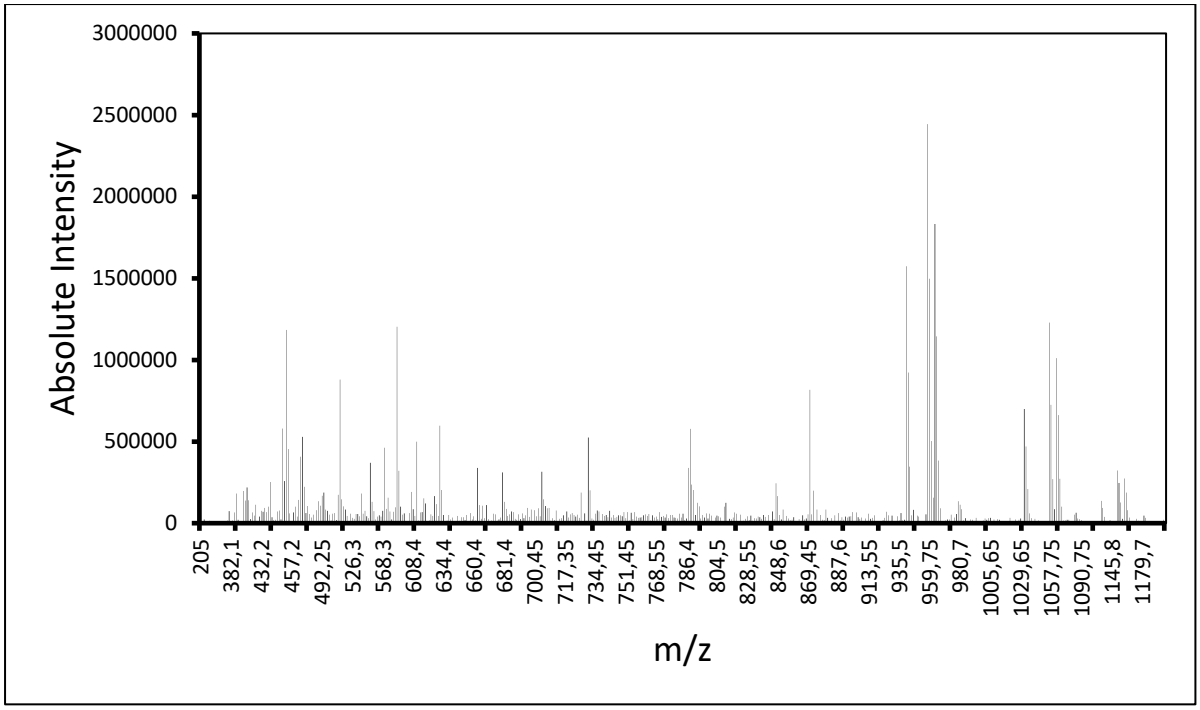
Appendix 49. DEPT spectrum of fraction **R** isolated from ethyl acetate leaves extract of *Lantana camara*.



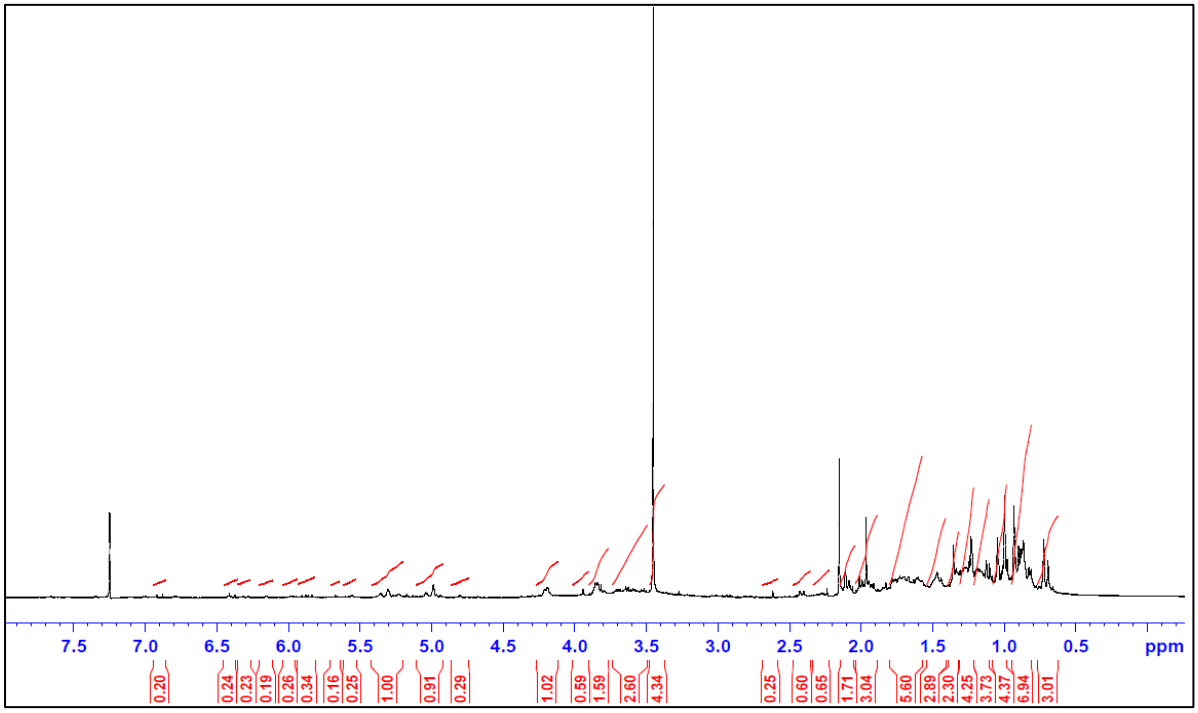
Appendix 50. HSQC spectrum of fraction **R** isolated from ethyl acetate leaves extract of *Lantana camara*.



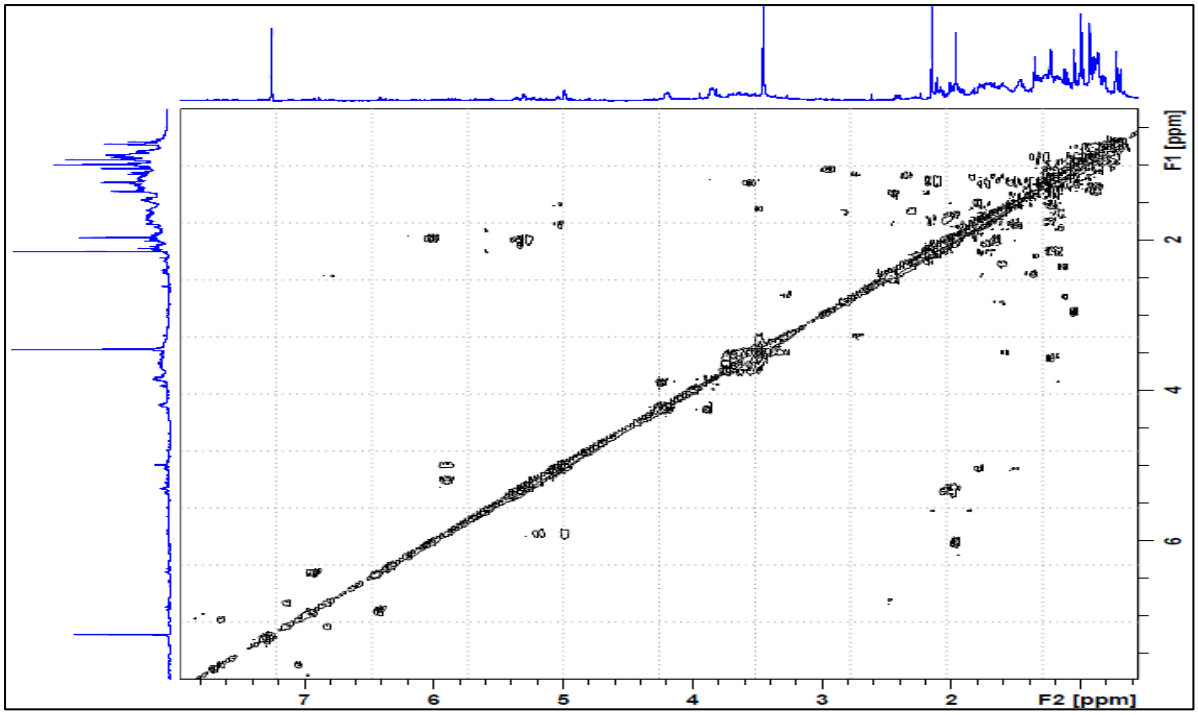
Appendix 51. HMBC spectrum of fraction **R** isolated from ethyl acetate leaves extract of *Lantana camara*.



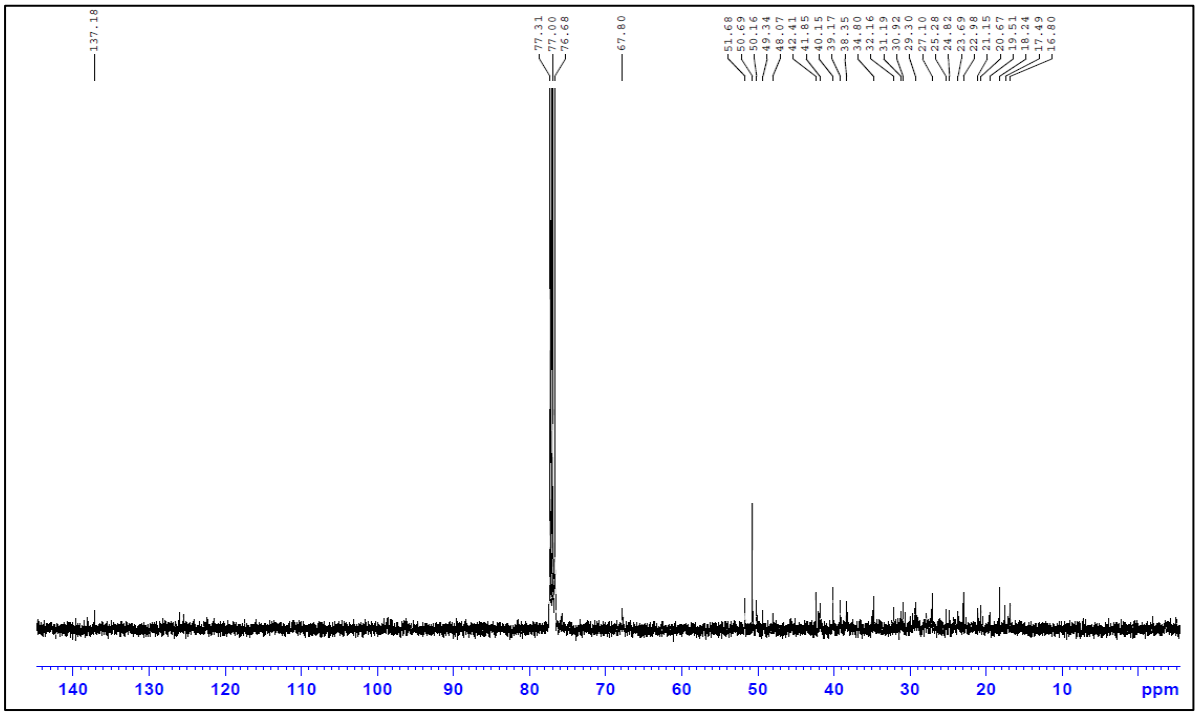
Appendix 52. MS fragmentation spectrum of fraction **U** isolated from ethyl acetate leaves extract of *Lantana camara*.



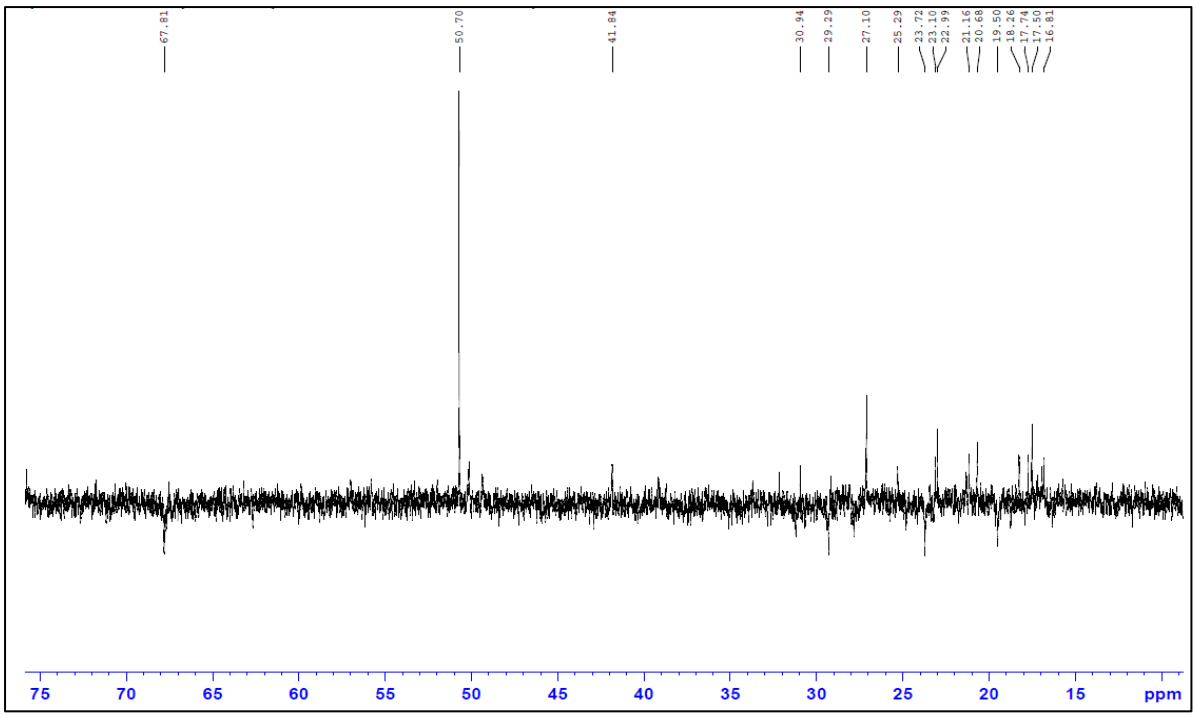
Appendix 53. ^{1}H-NMR spectrum of fraction **U** isolated from ethyl acetate leaves extract of *Lantana camara*.



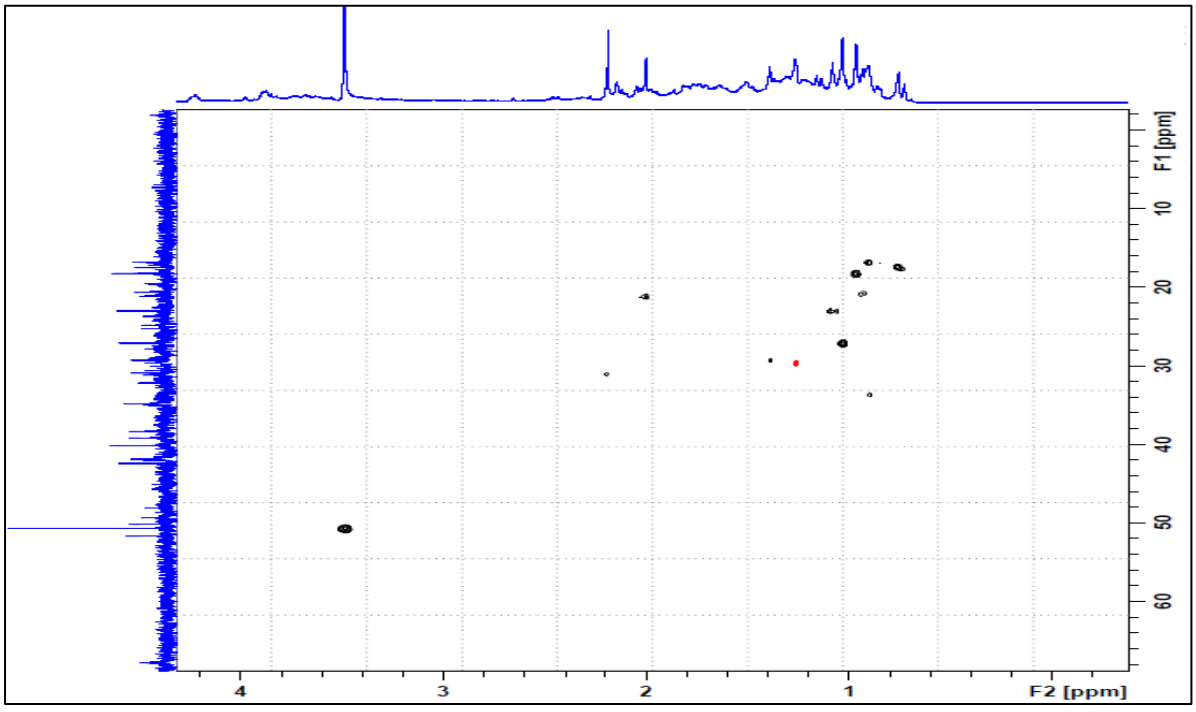
Appendix 54. ^{1}H-^{1}H COSY spectrum of fraction **U** isolated from ethyl acetate leaves extract of *Lantana camara*.



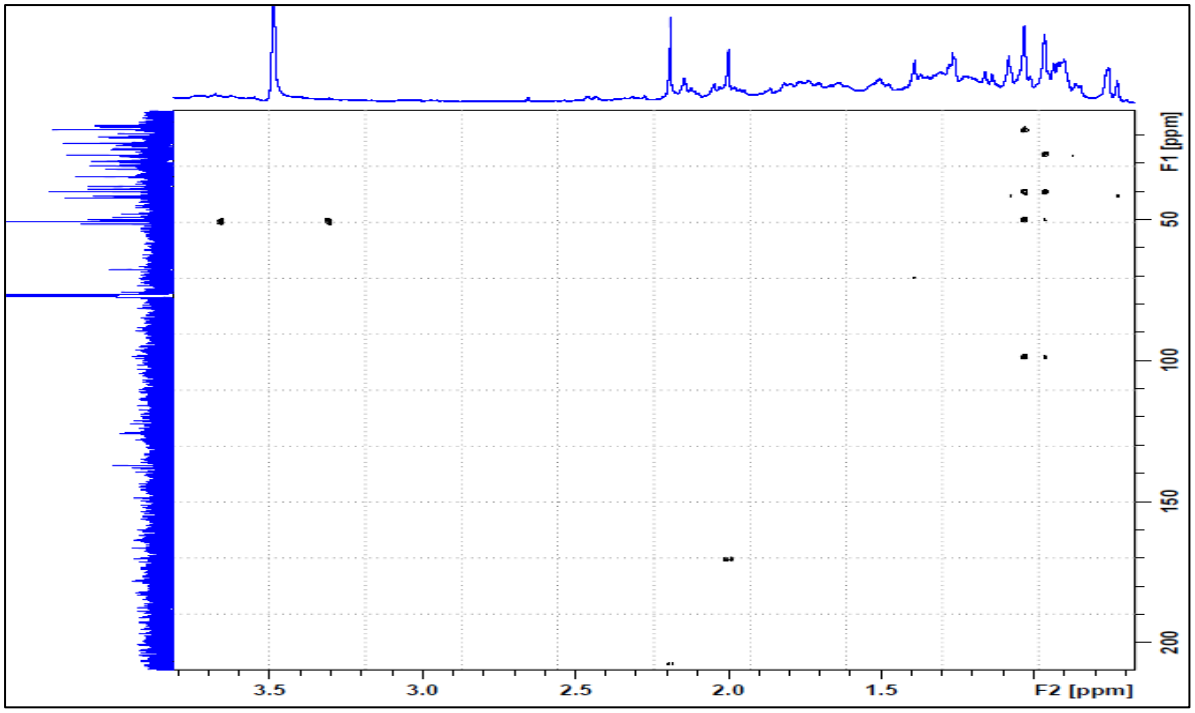
Appendix 55. ^{13}C-NMR spectrum of fraction **U** isolated from ethyl acetate leaves extract of *Lantana camara*.



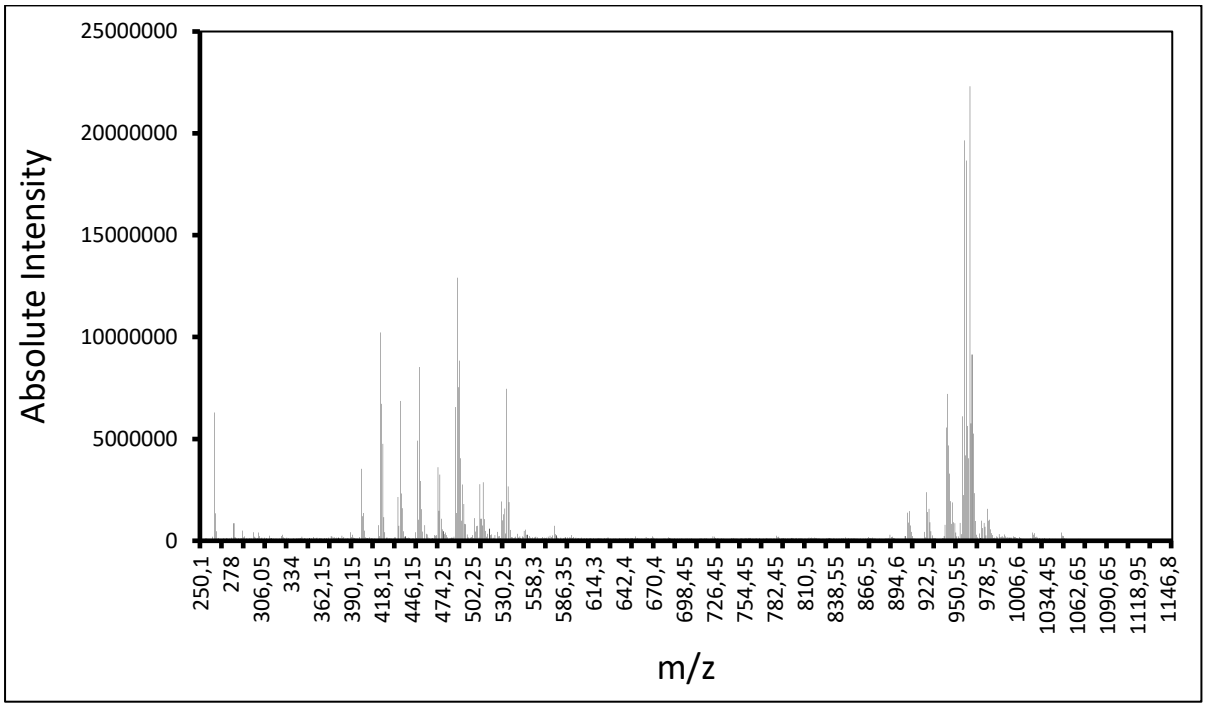
Appendix 56. DEPT spectrum of fraction **U** isolated from ethyl acetate leaves extract of *Lantana camara*.



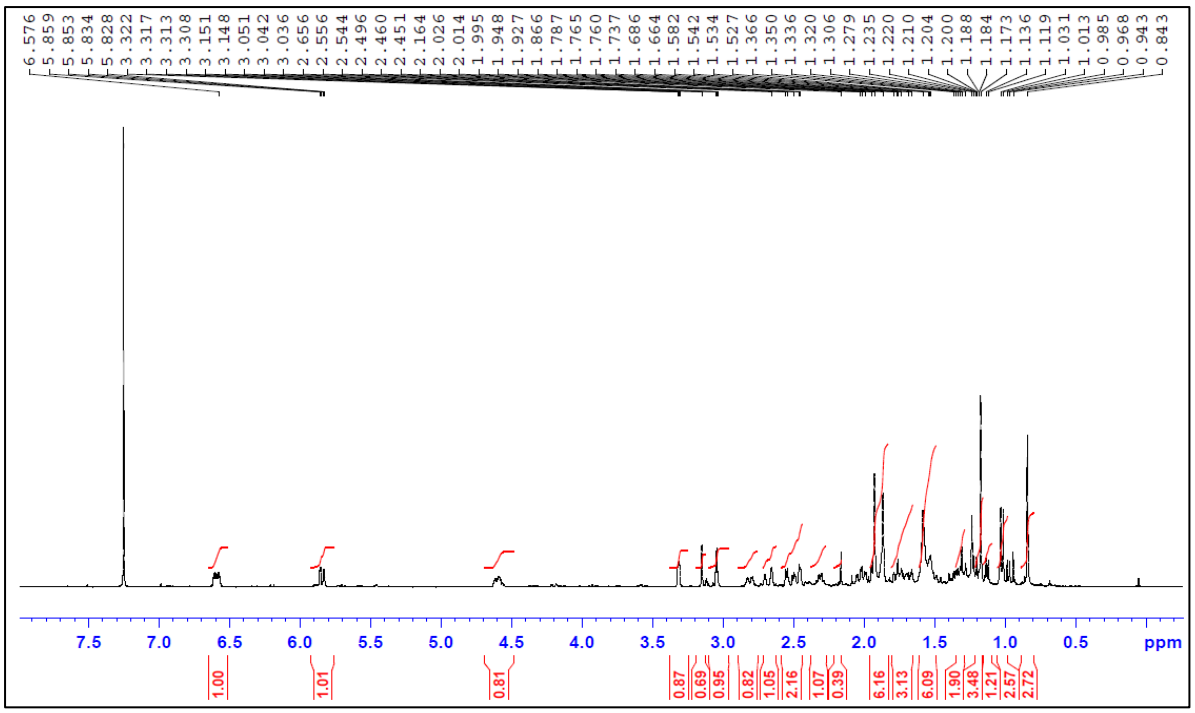
Appendix 57. HSQC spectrum of fraction **U** isolated from ethyl acetate leaves extract of *Lantana camara*.



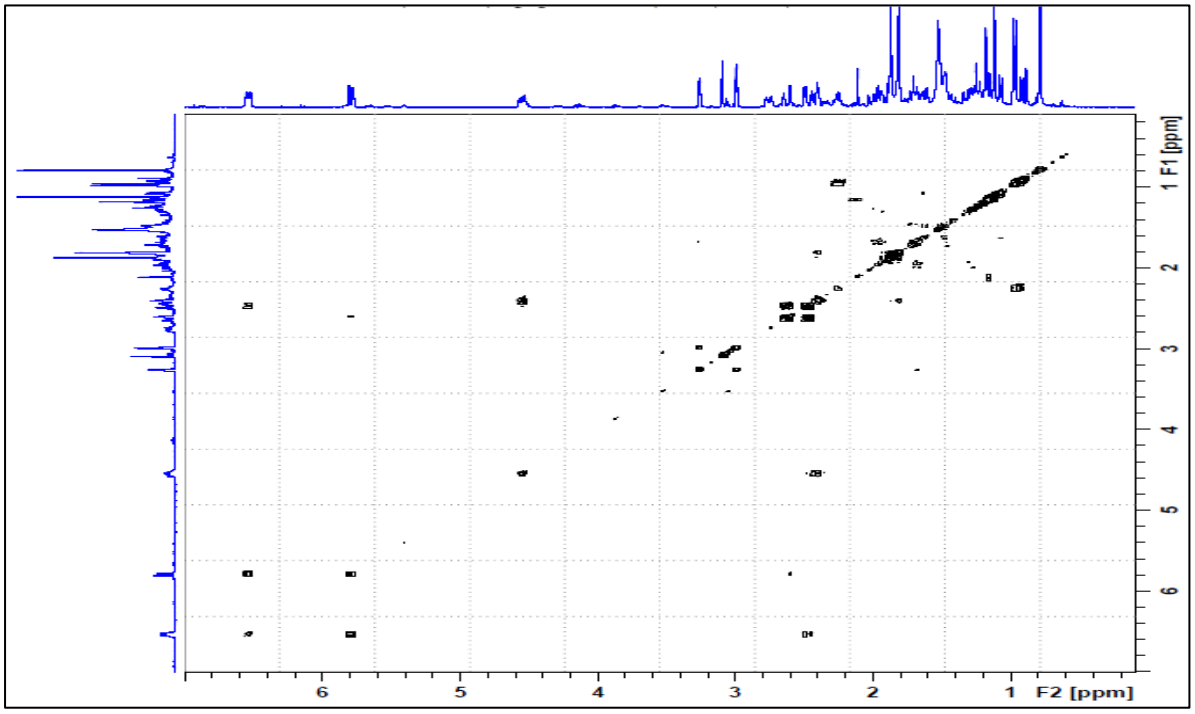
Appendix 58. HMBC spectrum of fraction **U** isolated from ethyl acetate leaves extract of *Lantana camara*.



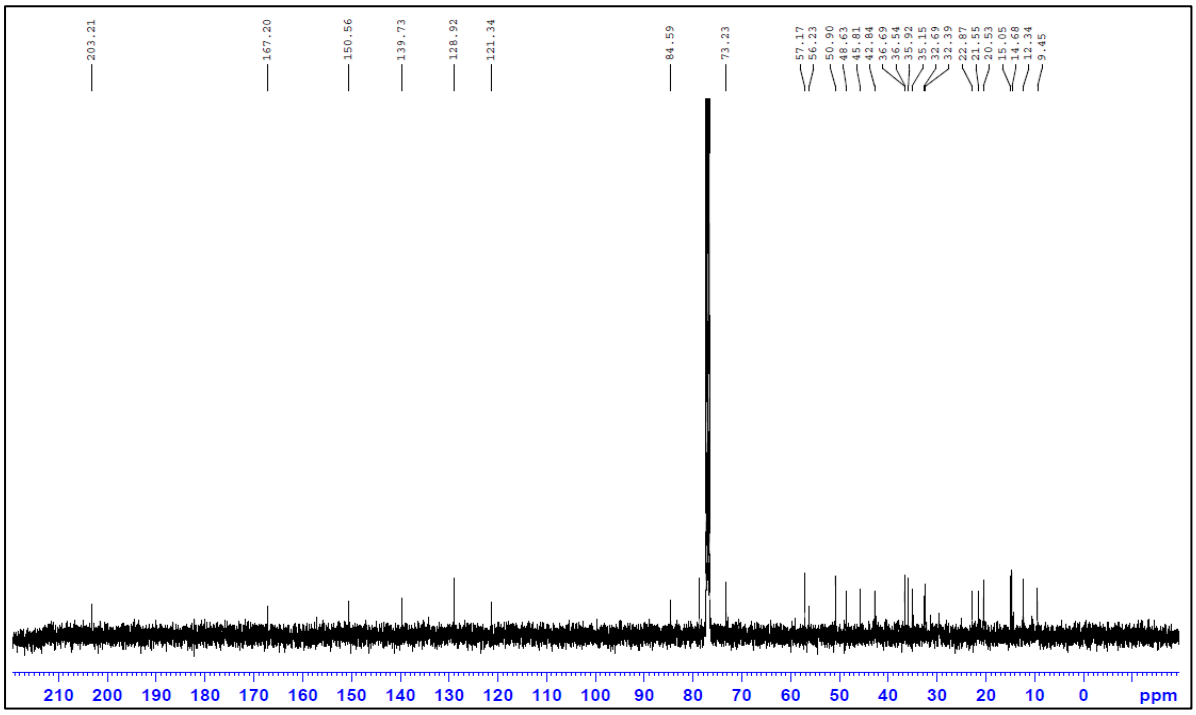
Appendix 59. MS fragmentation spectrum of fraction **AI** isolated from ethyl acetate leaves extract of *Withania somnifera*.



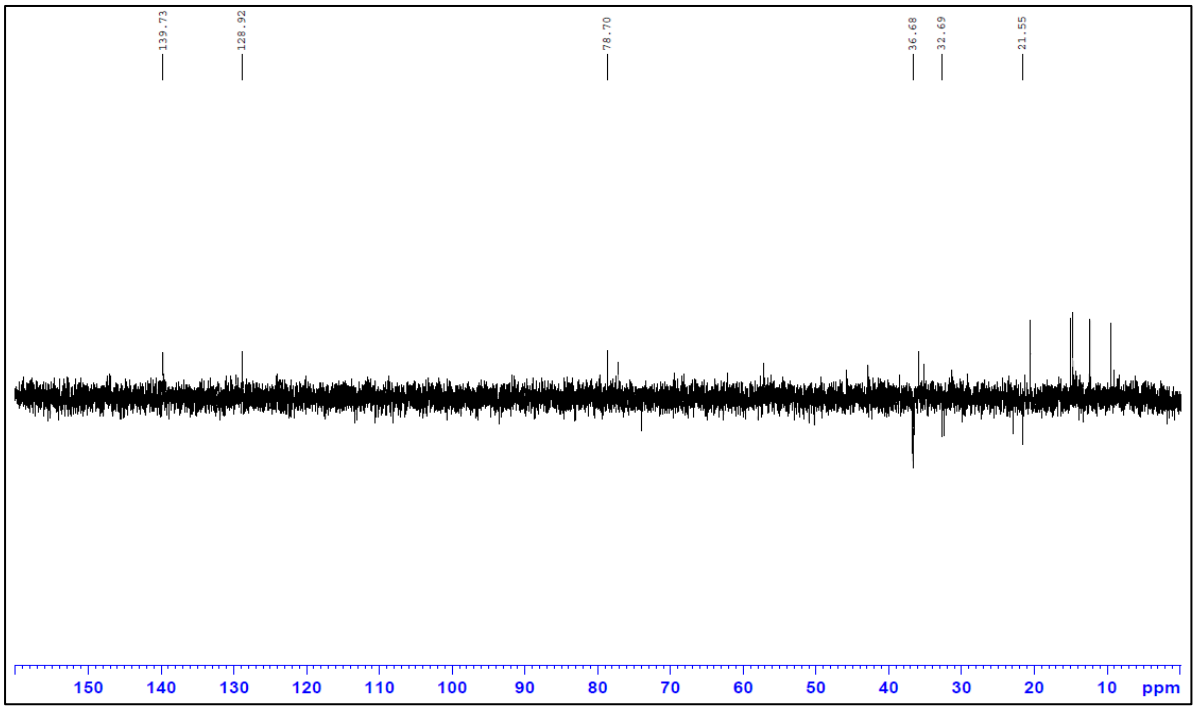
Appendix 60. ^{1}H-NMR spectrum of fraction **AI** isolated from ethyl acetate leaves extract of *Withania somnifera*.



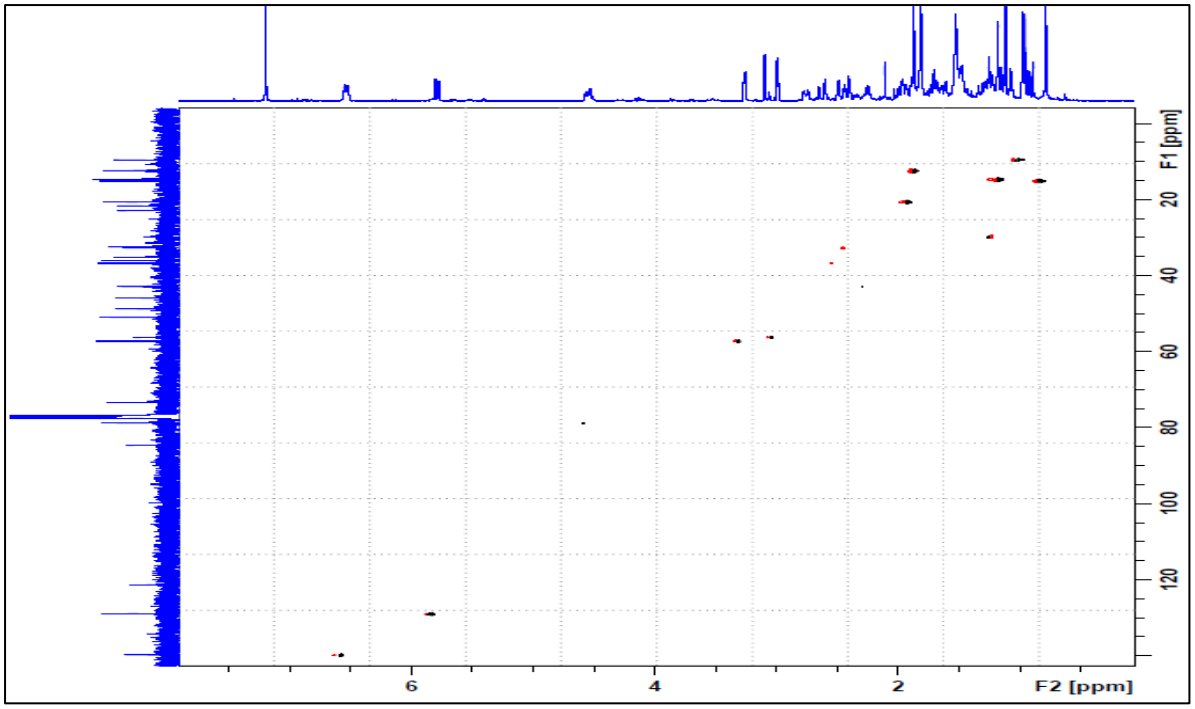
Appendix 61. ^{1}H-^{1}H COSY spectrum of fraction **AI** isolated from ethyl acetate leaves extract of *Withania somnifera*.



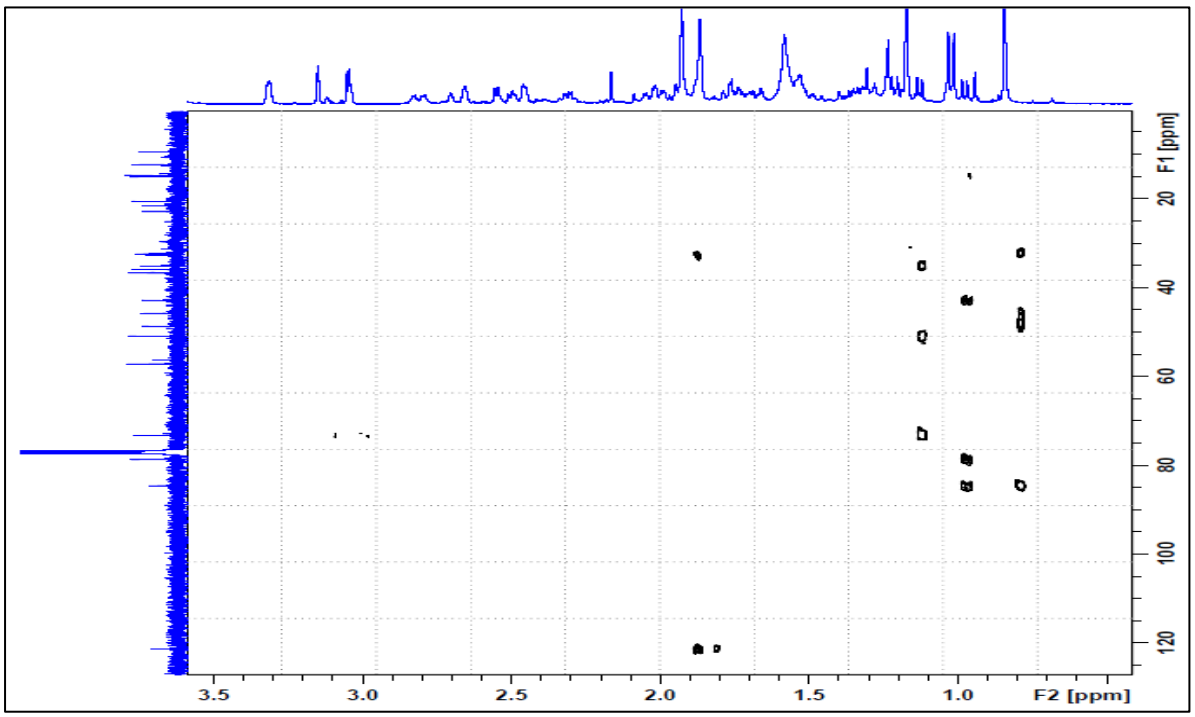
Appendix 62. ^{13}C-NMR spectrum of fraction **AI** isolated from ethyl acetate leaves extract of *Withania somnifera*.



Appendix 63. DEPT spectrum of fraction **AI** isolated from ethyl acetate leaves extract of *Withania somnifera*.



Appendix 64. HSQC spectrum of fraction **AI** isolated from ethyl acetate leaves extract of *Withania somnifera*.



Appendix 65. HMBC spectrum of fraction **AI** isolated from ethyl acetate leaves extract of *Withania somnifera*.