ETHNOBOTANICAL SURVEY AND CHARACTERIZATION OF ANTIMICROBIAL PRODUCING FUNGAL ENDOPHYTES FROM SELECTED MEDICINAL PLANTS FOUND IN CHERANGANI FOREST, ELGEYO-MARAKWET COUNTY, KENYA

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MAY, 2022

DECLARATION

This thesis is my original work and has not been presented for a degree or award in any other institution of higher learning.

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DEDICATION

I dedicate this work to my family for their unconditional love and constant support throughout this study. They have been source of inspiration to me in my academic journey.

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ABBREVIATIONS AND ACRONYMS

ANOVA	Analysis of Variance
BLAST	Basic Local Alignment Search Tool
CFU	Colony Forming Units
CTAB	Cetrimonium bromide
DDW	Double Distilled Water
DMSO	Dimethylsulfoxide
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetraacetic acid
FUV	Family Use Value
HCl	Hydrochloric acid
HSD	Tukey's Honest Significance Difference
ITS	Internal Transcribed Spacer
MH	Mueller Hilton media
MIC	Minimum Inhibitory Concentration
MSRA	Methicillin-Resistant Staphylococcus Aureus
NA	Nutrient Agar
NaOH	Sodium hydroxide
NCBI	National Council of Biotechnology Institute
PCR	Polymerase Chain Reaction
PDA	Potato Dextrose Agar
TE	Tris EDTA buffer

UV	Use value
WHO	World Health Organization

ABSTRACT

Infectious diseases remain a global health challenge, and contemporary medicine has been used for a long time to treat them. Apart from contemporary medicine, medicinal plants have been used repeatedly to treat some of these diseases. Due to these plants' overuse, the existence of these plants are threatened hence a need for conservation. Medicinal plants are host to fungal endophytes, which produce bioactive metabolites with antimicrobial activity. Therefore, fungal endophytes in these medicinal plants can be utilized since the intimate association with the plants may allow them to have similar antimicrobial properties. Owing to the importance of fungal endophytes, this study aimed to document medicinal plants in Cherangani forest, isolate and characterize antimicrobial fungal endophytes, and identify their bioactive metabolites. Structured questionnaires were used to gather information on uses of medicinal plants in Cherangani forest. It is from the questionnaires that two medicinal plants, namely; *Zehneria scabra* and *Croton macrostachyrus* were selected for fungal endophytes isolation. The leaves, roots and bark of the two plants were randomly collected and placed in polythene bags before transporting to Kenyatta University microbiology laboratories. Each plant's leaves, roots, barks, and stems were surface sterilized and plated on PDA at 25°C for five days. The fungal endophytes were selected based on morphology and fermented in PDA broth culture. The isolates' crude extracts were screened for antimicrobial activity against the test organisms; *Bacillus subtilis* ATCC 23857, *Staphylococcus aureus* ATCC 977, *E. coli* ATCC 35218 and *Candida albicans* ATCC 14053 using well and disk diffusion methods. Isolates with antimicrobial activity were identified based on morphology and sequencing the ITS region using ITS1F and ITS4R primers. To identify the bioactive compounds in the fungal extracts, ethyl acetate was used to extract the active compound from the isolate extracts with broad-spectrum antimicrobial activity. GC-MS analysis was used to profile the compounds present in active isolate extracts. A total of 26 medicinal plants were documented in Cherangani forest. A total of 172 isolates were isolated from the two plants and placed into 30 morphological groups. Out of the 30 groups, 18 had antimicrobial activity. These isolates were from genera; *Fusarium, Chaetomium, Clonostachys, Meyerozyma, Aporospora, Lasiodiplodia, Acremonium, Penicillium, Aspergillus, Leptosphaeria, Phyllosticta* and *Ampelomyces*. Antimicrobial bioassays showed 94.44% of the isolates showed activity against *Bacillus subtilis*, 63.1% against *Staphylococcus aureus* and 31.6% against *Escherichia coli* in disk diffusion method. However, none of the isolates had activity against *Candida albicans*. Antimicrobial bioassays from well diffusion method showed 83.33% of the isolates had activity against *Bacillus subtilis*, 61.11% activity against *Staphylococcus aureus*, and 38.89% had activity against *Escherichia coli*. The most active isolates in disk diffusion were; MR26, MR28 and MR29, with mean of inhibition ranging from 10mm to 18mm while in well diffusion isolates MR22, MR26, MR28, and MR29 were the most active isolates with mean zones of inhibition ranging from 9mm to 19mm. Analysis of variance showed a significant difference between the test organisms at $p = 0.0001$ in disk and well diffusion methods. There was a significant difference between the fungal extracts at $p = 0.0001$ in disk and well diffusion methods. In addition, there was a significant difference in interaction between the fungal extracts and the test organisms at $p = 0.0001$ in well and disk diffusion methods. Generally, the highest antibacterial activity was from isolate MR26 from *Croton macrostachyrus* bark, MR29 and MR22 from *Zehneria scabra* root, and MR28 from *Zehneria scabra* stem. The GC-MS analysis of the extracts revealed bioactive metabolites in chemical classes, monoterpenoids, carboxylic acid and long-chain fatty acids. This study has shown that medicinal plants are reservoir to fungal endophytes, which could be exploited as a source of natural products of pharmaceutical importance.

CHAPTER ONE: INTRODUCTION

1.1 Background Information

Medicinal plants have been source of significant curative and preventive medical therapy of humans in many parts of the world (Chen *et al*., 2016). The World Health Organization estimates that 80% of the world's population may be using medicinal plants for their primary healthcare purposes (Horrigan and Tourneau, 2003; WHO, 2013). With the development of contemporary drugs, some people combine contemporary medicine and herbal medicine (Mahwasane *et al*., 2013). According to Welz *et al*., (2018), 6.1% of German population use herbal remedies to promote health while 25% and 74% use herbal remedies as a result of positive past experience.

In third world countries, 80% of the population use herbal medicine because they are economical and are naturally available in most remote areas Popović *et al*., (2016), while in developing countries 40% - 90% of its population depend on traditional medicine for their primary healthcare purposes (Van and Carvalheiro, 2013). In contrast, contemporary medicine in developing countries are not easily available because hospitals and health facilities are beyond reach by locals (Kipkore *et al*., 2014). Additionally, costs are incurred on the drugs and services of the specialized personnel in the established health facilities (Ekor, 2014; Maroyi, 2015). In developed countries including North America, Australia, United Kingdom and European countries, complementary and alternative are becoming mainstream in their healthcare system (Anquez-Traxler, 2011). Due to the importance of the medicinal plants and their herbal remedies, populations tend to settle in areas where they can easily access them (Maroyi, 2017)

The indigenous plants usage is increasing with time (Jiang *et al*., 2015). For instance, in United Kingdom, some places are historically known for traditional herbal medicine practice (Nissen, 2010). Likewise, in European countries there are places within these countries which are known for specific traditional herbal medicine (Calapai, 2008). These are developed countries of the world but populations use herbal remedies because they believe it promotes healthy living (Ekor, 2014). Owing to the increased demand of herbal remedies, it is estimated that 15,000 medicinal plants species are threatened to extinction due to overharvesting and habitat destruction by human activities such as farming, timber harvesting, firewood and food (Van and Wink, 2018). These activities risk the loss of these indigenous plants that are commonly used as herbal medicine (Chen *et al*., 2016). The loss of indigenous plants used for herbal remedies call for governments to collaborate and document the plants as well as find a collaborative strategy of protecting the natural habitats of these plants (Maroyi, 2015).

The use of herbal preparations may be different between continents due to the plant species available in the different continents (Ozioma and Chinwe, 2019). The climatic conditions of continents might be different as well and this influences the medicinal plants that can be found in each region. Additionally, climate change affect the life cycle and distribution of medicinal plants with herbaceous medicinal plants being the most affected (Kaky and Gilbert, 2017). The climate change parameters especially the climatic factors such as rainfall and temperatures disrupt the pollinators which in turn affect the flowering and fruiting of the medicinal plants. According to (IPCC, 2012) climate change has significantly affected the distribution of medicinal plants by changing the photosynthesis, respiration and

transpiration. As a result, climate change is a threat to the availability of medically important plants (Barbosa *et al*., 2012).

Africa is rich in biodiversity of plants (Mahomoodally, 2013). The continent is rich in biodiversity estimated at 45,000 plant species out of which 5000 species are used as medicinal plants (Mahomoodally, 2013). Traditional medicine is known just like the contemporary medicine hence it is still in use in many parts of the world (Ozioma and Chinwe, 2019). In Africa, the use of medicinal plants has been linked to cultural and economic reasons therefore, it is commonly practiced (Amenu, 2007). Medicinal plants accumulate phytochemicals which when used to treat diseases, the active compounds act individually, or in synergy to kill microorganisms (Chen *et al*., 2021). The combined action of these phytochemicals from medicinal plants tend to increase the activity of the main constituents hence increase the effectiveness in healing the human diseases (Mahomoodally, 2013).

Despite the rich biodiversity, the documentation of these plants has not been fully done and there is risk loss of these plants owing to the deforestation in African forests (Gurib-Fakim and Mahomoodally, 2013). Each region within the continent has unique traditional medicine. Information on traditional medicine usage trickles down to tribal community where they still preserve and value it. Different communities have their own herbal remedies (Elujoba *et al*., (2005). There can be variations between communities in terms of the diseases they treat and the herbal preparation methods (Elujoba *et al*., 2005).

Kenya is one of the countries in East Africa with diversified flora estimated at 7,000 plant species (Kokwaro, 2009; Kimutai and Watanabe, 2016). Due to the rich flora diversity, many medicinal plants have been discovered in Kenya (Kigen *et al*., 2017). Ninety percent of Kenyan population have used herbal medicine at one point in their lives, while more than 70 % of the population use homemade herbal preparations for their primary healthcare (Kipkore *et al*., 2014).

The herbalists who practice herbal medicine pass the ethnobotanical information on the herbal remedies to close family members, and in most cases, it is shared orally (Ozioma and Chinwe, 2019). Oral sharing of knowledge on these medicinal plants is unreliable because there is no documentation on the plant parts used and the methods of preparation hence posing a risk to loss of the information (Ozioma and Chinwe, 2019).

According to Salako *et al*. (2018), community knowledge, cultures and their relationship with indigenous plant species remain instrumental in the protection of medicinal plants and their sources. Through it, utilization and conservation of the indigenous plant species can be achieved since the community knowledge has been used in other countries to conserve the indigenous plants (Calapai, 2008). Local approaches that include traditional knowledge are recommended when formulating policies, regulations and legislations (Potvin *et al*., 2017). Organizations such as the Convention on Biological Diversity have emphasized that the goal of protecting indigenous plants incorporates the use of traditional knowledge (Lovren, 2019)
In Kenya, forests and natural habitats are sources of medicinal plants (Hamilton, 2008). Apart from medicinal plants, the populations settling around the forests use the forest plants for food, firewood, and herding fields. Continuous use of the forest in harvesting of

medicinal plants, and farming risk loss of the indigenous plant species that are of medical importance and are found in such forests (Moore *et al*., 2020). The indigenous plant species may be vital for future use especially in finding active compounds that can be used against resistant pathogens; hence there is a need to document the knowledge on the plants and protect the forest due to its rich flora diversity (Moore *et al*., 2020).

According to Kimutai and Watanabe, (2016), Kenya has 6% forest cover which is lower than the 10% recommended by the United Nations. Apart from the low forest cover, the country still faces decline in the forest due to the intense human activities such as deforestation (Ongugo, 2008). To address the challenge, the government initiated the protection of the existing forests by enacting policies and institutions such as the Kenya forest service (KFS) that would pave way for involvement of stakeholders in the protection of all the forests in Kenya (Ongugo *et al*., 2008; Kimutai and Watanabe, 2016). One of the protected forests by the government of Kenya is the Cherangani forest. Despite the protection of the forest, the communities living around the forest encroach for timber harvesting and charcoal burning (Akotsi, 2006). Pastoralist communities like the Pokot and Marakwet use the forest as herding fields (Kigen *et al*., 2013). As a result, the forest risks losing its rich biodiversity (Gunlycke and Tuomaala, 2011). Forests such as Cherangani may be one of the ecosystems that will play critical role in the discovery of drugs in the future especially the search for natural products that can aid in the fight against resistant human pathogens.

The search for natural products used in the treatment of human diseases has been on-going over the years (Joana *et al*., 2013). It is estimated that around 50% of natural products and their derivatives obtained from herbal medicine find their way to clinical settings where they are used for diverse purposes (Gurib-Fakim, 2006). In the medical field, synthetic products have been embraced over the years. Despite the current focus on synthetic products, natural products such as bioactive metabolites greatly impact modern medicine (Newman and Cragg, 2016).

According to Newman and Cragg, (2007), 68% of the antibacterials are natural products or natural product derivatives that have diverse biological activities such as antibacterial and antifungal. Recently, it has been reported that 70% of the anticancer and antimicrobial agents are natural bioactive compounds or their derivatives obtained from endophytic fungi (Newman and Cragg, 2020). It is estimated that 51% of the natural products from fungal endophytes have been reported to have significant antimicrobial activity (Schulz *et al*., 2002). However, even with the existence of these natural products, cases of antibiotic resistance are still rampant hence calling for new drugs that can help manage these pathogens (Lu *et al*., 2007).

Fungal endophytes have demonstrated to be one of the potential sources of natural products because of their ability to produce bioactive metabolites with diverse biological activities (IDSA, 2010 , Selim *et al*., 2012, Newman and Cragg, 2016). Fungal endophytes colonize plants' internal tissues without causing any disease or any sign of disease (Strobel *et al*., 2004). The fungal endophytes have been isolated from diverse environments such as the

boreal forests, tropical and temperate forests, angiosperm, gymnosperms, ferns, oceans and xeric, extreme environments such as artic (Selim *et al*., 2017). In the extreme environments, the endophytic fungi produce the hydrolytic enzymes such as lipoidase, cellulace, pectinase, proteinase, and phenol oxidase (Krishnamurthy and Naik, 2017). In the endophytic relationship with plants hosts, the fungal endophytes produce bioactive metabolites, which in most cases, help the host plants by protecting them from both biotic and abiotic stresses (Rodriguez *et al*., 2012). Besides, the intimate relationship of the plants and the fungal endophytes make these endophytes produce bioactive compounds similar to those produced by the host plants. Additionally, the fungal endophytes may change the plant physiology while others have been reported to have plant promoting traits, siderophore production, indole acetic acid production, and phosphate solubilization (Strobel *et al*., 2004). On the other hand, the host plant helps the endophytic fungi survive by providing a protective environment for growth and multiplication of the endophytic fungi, protect them from harsh conditions during drought, and increasing the life cycle of the fungal endophyte via seed transmission to the next generation host (Khan *et al*., 2015).

Fungal endophytes accumulate the bioactive metabolites to survive in a hostile environment such as drought; hence it has been used in folk medicine (Bano *et al*., 2016). Moreover, the bioactive metabolites produced by these fungal endophytes have antimicrobial, anti-inflammatory, antitumor, antiparasitic, antifungal, and antioxidant properties (Gutierrez *et al*., 2012) . In addition, the fungal endophytes produce extracellular enzymes such as the hydrolases, lyases and oxidoreductases (Traving *et al*., 2015). As a result, researchers have focused on studies related to these fungal endophytes' biological activities with significant

interests in utilizing them to obtain natural products that can aid in the fight against drug resistance (Gutierrez *et al*., 2012). According to Gouda *et al*. (2016), fungal endophytes have been demonstrated to be a treasure house of bioactive metabolites with significant application in the medical field especially in controlling human pathogens. The bioactive metabolites from natural sources help in production of synthetic and semisynthetic drugs which have long term positive impacts (Subbulakshmi *et al*., 2012).

These fungal endophytes are isolated from plant sources (Selvanathan *et al*., 2011). According to Schulz *et al*., 2002 and Schulz and Boyle, 2006, fungal endophytes isolated from medicinal plants have intimate association with their host plants. The association enable them to produce bioactive compounds similar to those of the host medicinal plants. The species richness of the fungal endophytes in hosts are dependent on the climatic conditions such as temperature and humidity in which the plant grows (Selvanathan *et al*., 2011). In addition, the chemical profile of the host plant influences the bioactive metabolites produced the fungal endophytes (Kusari *et al*., 2012). According to Vinale *et al*., (2017), more than 4,000 bioactive metabolites of fungal origin have been identified and their biological activities described. The endophytic fungi are also known to have the highest number of bioactive metabolites more than any other endophytic microorganisms (Zhang *et al*., 2006; Zhang *et al*., 2011).

The fungal endophytes' diversity associated with each medicinal plant is not well understood (Nalini *et al*., 2014). Most of the fungal endophytes inhabit biotypes that are not well understood To date, only a few endophytes associated with medicinal plants have been

investigated on their ability to produce bioactive metabolites that are lead compounds in drug discovery (Omojate *et al*., 2014).

1.2 Statement of the Problem

Infectious diseases have been the leading cause of mortalities globally. Efforts to solve this challenge have been made and most infectious diseases are treatable (Pacios *et al*., 2020). However, one of the major setbacks of using antibiotics to treat infectious diseases are drug resistance to some of the commonly available antibiotics. Drug resistance is believed to be caused by the misuse of antibiotics (Thornber *et al*., 2020). Since drug resistance has become a global challenge, there is a need to come up with new measures and ideas to solve the problem by finding out new drugs and compounds that can be used to treat the infectious diseases. The search for new antimicrobials has necessitated the need to explore sources of secondary metabolites so that they can be utilized in the production of drugs against new and resistant pathogens.

The natural products are also obtained from the medicinal plants. Continuous use of the medicinal plants for isolation of the active compounds risk the loss of the plants since the plant parts used such as roots, leaves and barks are essential for survival of the medicinal plants (Chen *et al*., 2016). The intimate association between the medicinal plants and the fungal endophytes allow the fungal endophytes to produce bioactive metabolites similar to those of the host plant (Schulz and Boyle, 2006). Therefore, fungal endophytes can be utilized instead because a small part of the medicinal plant can be utilized in isolation of the fungal endophytes. Besides, the isolated fungal endophytes can be stored for future use in

fermentation and extraction of active compounds of medicinal importance. Considering that these medicinal plants are essential in isolation of fungal endophytes, there is need to document these plants. This is for purposes of both conservation of the threatened medicinal plants and future isolation of fungal endophytes which produce bioactive metabolites that are of medical importance (Fine *et al*., 2006).

Currently, the significant progress in the exploitation of endophytes is on the implementation of new technologies in the screening of natural products and bioprospecting of fungal secondary metabolites in drug discovery (Martinez *et al*., 2017). These technologies allow mass production of the natural products to meet the growing demand for the natural products (Martinez *et al*., 2017). However, the exploitation of the fungal endophytes with active bioactive metabolites is still limited with a few of the fungal endophytes successfully identified and their biological activities examined (Nalini *et al*., 2014) hence the need of this study.

1.3 Justification

According to Alves and Rosa, (2007), human population use the herbal preparations. However, with the advancement of drug discovery technologies, most population consider using complementary medicine over the herbal preparations. The medicinal plants are becoming rare because sources of these medicinal plants such as forests have been interfered with by human activities such as encroachment for settlement and farming purposes leading to exploitation of forest and natural habitats of medicinal plants (Maroyi, 2015). The exploitation of natural sources of medicinal plants used for herbal remedies in many parts of the world have made the populations go for contemporary medicine. However,

contemporary medicine have been reported to result in the emergence of drug resistance by infectious disease-causing pathogens among human populations (Buhner, 2012). Lately, there has been cases of multidrug-resistant pathogens such as MRSA (WHO, 2012; Yewale, 2014), and this has called for the development of new products that can help solve this challenge by exploring drugs from natural sources such as medicinal plants. Apart from medicinal properties, medicinal plants are host to many fungal endophytes that produce diverse secondary metabolites. These metabolites are lead compounds that will help in the discovery of new drugs that are reproducible and renewable (Wekesa, 2017); hence it can be used to treat infectious diseases and drug-resistant pathogens. The bioactive metabolites are diverse (Rai *et al*., 2021) therefore improved natural product sourcing that is aimed at one specific renewable, reproducible, and inexhaustible source of antimicrobials of pharmaceutical importance point it to these fungal endophytes (Godstime *et al*., 2014).

Globally, there is increasing demand for herbal drugs, natural health products and secondary metabolites derived from the medicinal plants (Chen *et al*., 2016). These grows the demand and use of the medicinal plants leading to significant loss of the wild medicinal plants species and threatening of approximately 50,000-80,000 of the medicinal plant species to extinction due to overharvesting and natural anthropogenic habitat destruction (Kala, 2000). Medicinal plants in the Cherangani forest are endangered due to human activities such as deforestation (Ongugo *et al*., 2009). This study provides information that will help in the conservation of the forest and medicinal plants for purposes of isolation of fungal endophytes. Isolation of fungal endophytes will help in protecting the medicinal plants because a small part is used in isolation of the fungal endophytes. Besides, the fungal

endophytes can be stored for future use in production of bioactive metabolites. The bioactive metabolites can be used in the development of compounds which can be utilized in solving the drug resistance menace.

1.4 Hypotheses of the study

i) Cherangani forest has no diverse medicinal plants

ii) *Croton macrostachyrus* and *Zehneria scabra* obtained from Cherangani forest do not harbor fungal endophytes.

iii) There is no significant difference in mean zones of inhibition of fungal endophytes isolated from *Croton macrostachyrus* and *Zehneria scabra* against bacterial and fungal test organisms.

iv) Fungal endophytes isolated from *Croton macrostachyrus* and *Zehneria scabra* hosts obtained from Cherangani forest do not produce bioactive metabolites.

1.5 Objectives of the study

1.5.1 General objective

To document the medicinal plants in Cherangani forest, and characterize antimicrobial fungal endophytes isolated from *Croton macrostachyrus* and *Zehneria scabra* obtained from Cherangani Forest.

1.5.2 Specific objectives

i) To document the medicinal plants used for antimicrobial purposes by the marakwet community in Cherangani Forest.

ii) To isolate and identify fungal endophytes from *Croton macrostachyrus* and *Zehneria scabra* plants obtained in Cherangani Forest.

iii) To compare the antimicrobial properties of fungal endophytes isolated from *Croton macrostachyrus* and *Zehneria scabra* using well diffusion and disk diffusion methods.

iv) To extract and characterize bioactive metabolites produced by fungal endophytes isolated from *Croton macrostachyrus* and *Zehneria scabra* plants in Cherangani forest.

1.6 Significance of this study

Medicinal plants have been used over time and recently, it has been reported that these plants risk extinction due to farming, and deforestation. Therefore, this study will give information that on documentation and conservation of the medicinal plants found in Cherangani forest for isolation of fungal endophytes and production of bioactive metabolites. In addition, the extracts obtained from the isolated fungal endophytes in this study showed broad antimicrobial activity against test organisms *Bacillus subtilis, Staphylococcus aureus,* and *E. Coli*. Therefore, this study forms a basis for isolation of the active compounds in the fungal endophyte extracts which can be used to determine the biological activity of the active compounds against other test organisms which were not used in this study. Besides, the study provided a database of ITS sequences from fungal endophytes obtained from *Zehneria scabra* and *Croton macrostachyrus* medicinal plants showing the species richness of the isolated fungal endophytes in these plants. Therefore, it will form a basis for more research on fungal endophytes in other medicinal plants found in the forest and have not been exploited for their potential to host endophytes of medicinal significance.

This study provides a data on bioactive metabolites from fungal endophytes isolated from *Zehneria scabra*, and *Croton macrostachyrus* in the Cherangani forest. The database can be utilized in bioprospecting in the process of finding new antimicrobials which can be useful in different fields such as medicine and agriculture.

CHAPTER TWO: LITERATURE REVIEW

2.1 Infectious diseases and antibiotic resistance

Antibiotics have played essential roles in the treatment of many bacterial infections. The use of antibiotics started since the introduction of penicillin in the 1940s by Alexander Fleming (Pacios *et al*., 2020). According to Mahady (2005), infectious diseases are the significant causes of morbidity and mortalities that accounts to 50% of all deaths reported in the tropical countries and 20% of deaths reported among the Americans.

Antibiotic resistance has been observed in many bacterial infections such as gastrointestinal tract, bloodstream, urinary tract infections, and respiratory tract infections (WHO, 2012). Cases of multi-drug resistance have been reported and one of them is Methicillin-Resistant *Staphylococcus aureus* (Yewale, 2014). The reported antibiotic resistance has negative impacts on the human population, due to high costs of treatment and a more extended stay in hospitals (Shrivastava *et al*., 2018). Apart from population health, antibiotic resistance has significant impacts to global development as indicated by Jasovský *et al*., (2016) and global economy (Jonas *et al*., 2017).

The antibiotic resistant pathogens have been categorized into three main groups which include critical, high and medium priority (WHO, 2017). These categories are comprised of twelve different bacteria that had been reported to be resistant to different antibiotics (Shrivastava *et al*., 2018). In this list, the bacteria under the high and medium priority categories are infectious disease causing pathogens responsible for common infections (Shrivastava *et al*., 2018). With such problems on the rise, there is a need for policy makers

to invest in research that can help in coming up with efficient and affordable solutions that will inform the production of pharmaceutical products that can help fight the antibiotic resistance challenge (Shrivastava *et al*., 2018).

Since antibiotic resistance is a global challenge, there is need for states to collaborate and find solutions to this challenge especially on releasing data and information that is needed to guide research and policy making (Hoffman *et al*., 2015). This is possible through the use of global antimicrobial surveillance systems and increasing education and awareness on the existence of antibiotic resistance, prevention and control methods (WHO, 2016). In addition to collaborations, health and research institutions given responsibilities of formulating policies and guidelines need to be strengthened by availing the needed resources, and providing a conducive environment for implementation of the set antimicrobial resistant policies (Hoffman *et al*., 2015).

2.2 Medicinal plants and treatment of infectious diseases

The use of plants to cure human diseases has long history where plant parts such as leaves barks, roots and stems were used to revert abnormal conditions in the body to normal (Mintah *et al*., 2019). Globally, 60% of human population depend on the medicinal plants for their primary healthcare while 80% of populations in the developing country depends on these medicinal plants for their medical uses (Shrestha and Dhillion, 2003). In the 21st century, natural products including those from medicinal plants accounts to more than 50% of the drugs that are in clinical use (Shakya, 2016). Currently, contemporary medicine has a history of herbal medicine (Shakya, 2016). About 80% of these synthetic medicine have a positive correlation between their current modern use and the traditional uses due to the

common active compounds in the synthetic drug and the medicinal plants used in herbal remedies (Sarkar *et al*., 2015). Examples are opium , aspirin and quinine (Ram *et al*., 2015; Mintah *et al*., 2019). These natural products are obtained from diverse sources including plants, animals, microorganisms such as fungi and bacteria (Mintah *et al*., 2019).

Some of medicinal plants have antimicrobial activity against the infectious disease-causing pathogens. For instance, methanolic extracts of *Terminalia chebula* have been reported to be bactericidal against *Vibrio Cholerae* strains and multi-drug resistant strains such as serotype O1, non-O1, O139 and non-O139 (Acharyya *et al*., 2009). Other plants with bactericidal activity against *Vibrio cholerae* include *Saraca indica* Acharyya *et al*., (2009), *Euphorbia serpens* Payne *et al*., (2015) and *Acacia farnesiana* (Sánchez *et al*., 2010). Additionally, Pneumonia is one of the commonly known infectious disease caused by some pathogenic bacteria, fungal and viral strains (Mintah *et al*., 2019). Some of the Pneumonia bacteria causative agents such as *Streptococcus pneumoniae* have been reported to be resistant to antibiotics (WHO, 2017). Consequently, researchers have focused on medicinal plants with activity against these pathogens (Mintah *et al*., 2019). Some of the medicinal plants with antibacterial activity against Pneumonia causing pathogens include *Echinops adenocaulos*, *Perietaria judaica* and *Urtica urens as* described by (Saleh *et al*., 2013).

According to Mahady, (2005), there are reduced number of antimicrobial drugs in the pharmaceutical industry hence there is a need for new compounds which can be used to treat the emerging pathogens and pathogens which have developed antibiotic resistance to the existing antibiotics. One of the potential sources of these compounds are medicinal plants

due to their ability to produce bioactive compounds commonly known as phytochemicals which have been reported to have therapeutic potential with minimal side effects. Also, they are economically viable (Shakya, 2016).

2.3 Medicinal plants in Cherangani forest

The Cherangani forest is one of the Kenyan forest which cuts across three counties namely: Elgeyo-Marakwet, Trans Nzoia and West Pokot. According to Mbuni *et al*., (2020), Cherangani forest is rich in medicinal plant biodiversity with more than 296 medicinal plants. The medicinal plants in this forest cut across 80 taxonomic families with *Asteraceae, Leguminosae, Lamiaceae, Rubiaceae* and *Euphorbiaceae* being the families with the highest numbers of medicinal plant species ranging from 32-12 species each. In addition, medicinal plants in Cherangani forest are distributed across different habits which include bushlands, grasslands, escarpments and highlands. Majority of the documented medicinal plants are found in escarpments while the highland part of the Cherangani forest has the least number of medicinal plants (Kipkore *et al*., 2014).

According to Kigen *et al*. (2013) and Kipkore *et al*. (2014), trees and herbs are the commonly used medicinal plants while shrubs and climbers are the least used medicinal plants by herbalists in Cherangani forest. Trees and herbs are the most preferred because they have a long lifespan as compared to climbers which are short lived and seasonal (Mbuni *et al*., 2020). Apart from being medicinal plants, these trees are used as firewood and the leaves used as animal feeds. Over use of these trees risk their availability in the future (Rotich, 2019).

Additionally, different medicinal plant parts such as leaves, roots, barks, stems, fruits, sap and wigs are used for medicinal purposes (Mbuni *et al*., 2020). According to Kipkore *et al*. (2014), 30% of herbalists around Cherangani forest use leaves to treat diverse ailments followed by barks (21%), roots(16%), fruits (8%) and seeds (5%). The medicinal plant parts can be used either singly or a decoction is made from different plant parts. Besides, the decoction can be from parts of the same medicinal plant or from different medicinal plants. Example of medicinal plants used by herbalists in Cherangani forest are; *Lippi javanica, Warbugia ugandensis, Acacia brevispica, Acadia hockii, Acacia nilotica, Cyperus esculentus, Zehneria scabra* and *Croton macrostachyrus* among others.

2.3.1 *Zehneria scabra*

Zehneria scabra is a perennial climber found in the kingdom plantae, phylum tracheophyta, class *Magnoliopsida*, order *Cucurbitales*, family *Cucurbitaceae* and genera *Zehneria* (Kokwaro, 2009). The plant is mainly found in hilly forests or forest margins. The plant grows at an altitude of between 1200m to 3580 m above sea level. The plant leaf shape is triangle with a costate and green in colour. The internode length is 6-7, while the petiole length 3-5. Besides, the leaves are lobed. The flowers are oval in shape and are yellow in colour. The primary fruit colour is light green while the secondary colour is dark green. When ripe, the fruit colour are always red. It has seeds whose coat are dark brown and rough in texture (Kokwaro, 2009; Agogbua *et al*., 2015).

According to Abebe, (2017), methanolic extracts from *Zehneria scabra* leaves contains phytochemicals in the chemical classes; alkaloids, tannins, phenols, flavonoids, terpenoids and glycosides while Bayu *et al*., (2018) reported that the tubers and leaves of this plants

have phytochemicals in the chemical classes quinones, diterpenes and steroids. These compounds have been previously reported to have antibacterial activity in both gram positive and gram negative bacteria (Thamacin *et al*., 2014). On the other hand, Tesfaye and Alamneh, (2014) reported that phytochemicals from the *Zehneria scabra* have antimalaria activity against *Plasmodium barghei*. Therefore, due to the phytochemicals produced by this plant, it has been used to treat various ailments (Van *et al*., 2004; Giday *et al*., 2016; Bayu *et al*., 2018).

According to traditional healers living around Cherangani forest, the plant's leaves and roots are used to treat stomach pains, common cold, and skin-related infections (Kipkore *et al*., 2014). Additionally, the leaves of *Zehneria Scabra* have been reported to be used in the treatment of leprosy, measles, wound dressing, and antihelminth (Geyid *et al*., 2005). The whole plant of *Zehneria scabra* has been used in treatment of fever and headaches in human (Ragunathan and Abay, 2009). Additionally, roots of this plant have been used to treat rabies in animals (Amenu, 2007). In other instances, the medicinal plant is grown within family compounds as a remedy against snakebites (Biologics *et al*., 2015).

Plate 2.1: *Zehneria scabra* plant. Source: (Woldeab *et al*., 2018)

2.3.2 *Croton macrostachyrus*

Croton macrostachyrus is a tree in the kingdom *Plantae*, division *Magnoliophyta*, class *Magnoliopsida,* order *Euphorbiales*, family *Euphorbiaceae* and genera *Croton Croton macrostachyrus* is a dioecious, medium-sized tree and grows to a height of approximately 30 m tall (Maroyi, 2017). The tree regenerates naturally, and it is referred to as a multipurpose tree by subsistence farmers in Kenya, Ethiopia, and Tanzania. *Croton macrostachyrus* is found in secondary forests, woodlands, wooded grasslands, and dry evergreen upland forests. The plant is one of those plants reported to have medically important phytochemicals (Kiristos *et al*., 2018)

According to Begeno and Abose, (2020), chloroform and methanol extracts from the stem bark of *Croton macrostachyrus* have compounds in the chemical classes alkaloids, saponins, tannins, terpenoids, glycosides, steroids and phenols. These compounds have wide range of pharmacological activities including antibacterial, antimycobacterial, antihelminth, antifungal, anti-inflammatory, and antiplasmodial among others (Begeno and Abose, 2020). In addition, this plant has been used in the herbal medicine in some countries such as Ethiopia (Kiristos *et al*., 2018)

Croton macrostachyrus is used as herbal medicine for at least 61 human and 20 animal diseases and ailments respectively (Maroyi, 2017). Moreover, the tree leaves, barks and roots are used in the treatment of skin related diseases, stomachache, and wounds among others (Mechesso *et al*., 2016; Maroyi, 2017). On the other hand, the seed oils are used as agents that aids in the stimulation or facilitate bowel movement (purgative) due the phorbol

esters in the seed oils (Salatino *et al*., 2007). In some communities, the plant parts are used in treatment of wounds, malaria, jaundice diarrhea, abdominal pain, pneumonia and gastrointestinal disorders (Begeno and Abose, 2020).

Plate 2.2 :*Croton macrostachyrus* plant

Source: Kenya Natural history; http://www.ngkenya.com/flora/croton_macrostachyus.html

2.4 Diversity of fungal endophytes

Endophytes are endosymbiotic organisms that are found in the intracellular and or extracellular space of plants (Bacon *et al*.,1997). Endophytes live in the host plants entirely without depicting any sign or evidence of a either disease or infection (Wilson, 1995). According to Nair and Padmavathy, (2014), endophytes have different relationships with their hosts including; antagonism and mutualism while a few of these endophytes have parasitic relationship. Endophytic fungi are in the family ascomycetes and are known to reproduce asexually while some reproduce sexually (Rodriguez *et al*., 2009). These fungi exist in three ecological groups which include; Mycorrhizal fungi, Pasture endophytic fungi, and Non-pasture endophytic fungi (Fine *et al*., 2006).

Depending on the species and the host interaction, endophytes can be isolated from barks, roots, stems and leaves (Grünig *et al*., 2008). Some of these fungal endophytes have been isolated from barks, stems, and leaves of medicinal plants. Examples include; *Fusarium oxysporioum*, *Culvurlaria culvata,* and *Culvularia lunata* Raviraja, (2005), *Alternaria* and *Penicillium* Jalgaonwala *et al*. (2011), *Pestaliotiopsis microspore* Strobel *et al.* (1996), and *Colletotrichum gloeosporioides* (Gangadevi and Muthumary, 2008).

The availability of endophytic fungi in different hosts and sources is dependent on the variations in environmental factors such as temperature, humidity, light, geographical location, nutrients, and vegetation cover (Song *et al*., 2004; Suryanarayanan *et al*., 2005). These factors affect the fungal endophyte life cycle and reproduction by interfering with the spore germination rate, and growth rate (Godstime *et al*., 2014). Apart from being host specific, fungal endophytes are known to be associated with hosts of specific genotypes (Dai *et al*., 2003 ; D'amico *et al*., 2008). Moreover, the genetic diversity, nutrient level, and ecological niche of the hosts influences colonization of fungal endophytes (Strobel, 2006; Kumar *et al*., 2014).

According to Strobel and Daisy, (2003), out of the 300,000 plants species that have been documented globally, each plant is a host to at least one endophyte. This shows that fungal endophytes are highly diverse organisms, and their genetic diversity remains unknown (Tan and Zou, 2001). Only 1% is known while 99% of these are not discovered yet (Davis *et al*., 2005). The undefined group of organisms includes the fungal endophytes found in extreme environments and unique biological niches (Strobel, 2006). According to Dreyfuss and

Chapela, (1994), there are approximately 1.3 million species of fungal endophytes. Their diversity is high in tropical forests because the angiosperm diversity is higher in such ecosystems (Banerjee, 2011). In temperate forests, the plant richness is high and as a result, the fungal diversity is also high since the plants and trees are hosts to the endophytes (Griffin and Carson, 2018).

2.5 Fungal endophytes as a source of antimicrobials

According to Strobel and Daisy, (2003), fungal endophytes produce bioactive metabolites which have significant applications in different fields such as medicine and agriculture. In the medical field, the bioactive compounds from endophytic fungi have been used in treatment of different diseases (Jalgaonwala *et al*., 2011; Omojate *et al*., 2014 and Shukla *et al*., 2014). Recently, comparison of bioactive metabolites from endophytic fungi and those of the host plants showed that fungal endophyte bioactive metabolites have high therapeutic potential more than the bioactive compounds from the host plants (Subbulakshmi *et al*., 2012).

Since the discovery of Taxol from endophyte, there has been a lot of efforts by researchers to discover other compounds that are of biological and medical importance from endophytes. As a result of these, compounds with antibacterial, antifungal, antiviral, antioxidant, immunosuppressant, and anti-neurogenerative properties have been discovered (Staniek *et al*., 2008). The search of compounds with biological and medical importance from fungal endophytes is still on-going. The rate at which the natural products are released to the world markets for medical purposes is high. An example is between 2001 and 2005, 20 natural products drug derivatives from endophytes have been released to world markets,

and around 140 have undergone stages of clinical development in significant therapeutics applications (Butler, 2005).

These secondary metabolites from fungal endophytes constitute a major portion of important natural products. For instance, according to Singh and Dubey, (2015) secondary metabolites produced by fungal endophytes contributes to approximately 80% of the natural products that are used as drugs and are available in current markets. The existence of these secondary metabolites from fungal endophytes presents a better alternative to overcoming the increasing level of drug resistance by some pathogenic microorganisms (Omojate *et al*., 2014)

The availability of secondary metabolites produced by fungal endophytes are affected by factors such as the sampling season, the geographical location and the climatic conditions of the study area (Shukla *et al*., 2014). The extraction of these metabolites has been challenging over the years. However, increased research on fungal endophytes has led to development of synthetic processes of bioactive compound extraction and the advancement of machines which have increased the efficiency and accuracy of fungal endophytes bioactive metabolite isolation and purification (Hussain *et al*., 2012).

According to Strobel and Daisy, (2003), the fungal endophytes and other endophytes such as the bacterial endophytes produce secondary metabolites as a way of surviving against biotic and abiotic stresses in their hosts. Furthermore, the metabolites produced by these endophytes go a long way to help the hosts from actions of insects, grazing animals and

some pathogenic microbes (Strobel *et al*., 2004; Kumar *et al*., 2014) while others induce growth and increases resistance of host plants to both herbivores and diseases (Rodriguez *et al*., 2009). In addition, the fungal endophytes help the host plants to accumulate secondary metabolites (Firakova *et al*., 2007).

Based on their properties and actions, the fungal endophyte bioactive metabolites have been grouped to different functional groups, including; antibiotics, flavonoids, phenols, tannins, steroids, terpenoids, xanthones, tetralones, chinones, quinones and among others (Schulz *et al*., 2002; Strobel and Daisy, 2003, and Omojate *et al*., 2014). Some of these bioactive compounds can be esters, lipids, alcohols, ketones, and acids (Bhardwaj *et al*., 2015). In the medical field, some of the bioactive metabolites produced by fungal endophytes have shown antibacterial activity against some pathogenic microorganisms Radić and Štrukelj, (2012) while some are new templates of antibacterials that have novel mechanisms of action. An example being Guanacastepene, which is a group of diterpenoids produced by fungus isolated from *Daphnopsis Americana*. The compound is currently used as a new class of antimicrobials against resistant pathogens such as Methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant *enterococcus faecium* (Singh *et al*., 2000). In addition to this, bioactive metabolites from *Chaetomium spp* isolated from *Astragalus Chinensis* showed antibacterial properties against *Staphylococcus aureus* and *Methicillin staphylococcus aureus* (Liu *et al*., 2019).

According to Phongpaichit *et al*. (2006) *Fusarium* species isolated from Garcinia plants produced bioactive compounds which were active against *Cryptococcus neoformans* and

Staphylococcus aureus. Similar findings were also reported in previous studies done by (Kumar *et al*., 2014). *Fusarium spp* have been reported to produce Sordaricin which is a compound with antibacterial properties while other compounds such as those in the functional group terpenoids have been reported to have active compounds with strong antibacterial properties (Manivasagan *et al*., 2014), while others such as ampicillin and oxacillin are known to be bactericidal (Akiyama *et al*., 2001).

Some endophytic fungi produce bioactive metabolites that have both antibacterial and antifungal properties. Examples are *Guignardia spp* isolated from *Spondias mombin* produced bioactive compounds that had both antibacterial and antifungal activities against *Escherichia coli, Staphylococcus aureus* and antifungal activities against *Saccharomyces cereviciae* and *Penicillium canadensis* (Rodrigues *et al*., 2000). In addition, *Xylaria spp* one of the endophytic fungi known to produce many bioactive metabolites that have been reported to have antifungal activity against *Candida albicans* (Stadler and Keller, 2008).

2.6 Properties of bioactive metabolites from fungal endophytes

Apart from producing bioactive metabolites that have antimicrobial properties, these bioactive metabolites have other properties one of them being anticancer. For instance, taxol one of the most successful anticancer drug was produced from endophytic fungi *Taxomyces adreanae* (Zhang *et al*., 2009). Similarly, *Metarhizium anisopliae* which is a fungal endophyte isolated from bark of Taxus tree produced taxol, one of the most synthesized anticancer agent today (Jalgaonwala *et al*., 2011). In addition, Penicillenols compound from *Penicillium spp* is an anticancer compound that is cytotoxic to many cell lines (Jalgaonwala *et al*., 2011).

Some of the bioactive metabolites have been used as antivirals. Examples are cytonic acid A and B produced by *Cytonaema spp.* which have been demonstrated to inhibit Cytomegalovirus protease (Guo *et al*., 2000). Hinnuliquinone compound was produced by fungal endophyte isolated from *Quercus coccifera* and its been reported to be a potent inhibitor of HIV type 1 protease while others such as podophyllotoxin produced by endophytic fungi *Podophyllum hexandrum Aspergillus fumigates*, and *Fusarium oxysposrum* have also been reported to antivirals and are used as precursors in antiviral production (Kumar *et al*., 2014).

Some of the bioactive metabolites have been reported to have antidiabetic properties example being compound L-783281 from endophytic fungus *Pseudomassaries spp* isolated from the Congo rainforest produce bioactive metabolite L-783281, which can cure diabetes by lowering blood glucose levels (Zhang *et al*., 1999) while other fungi produces immunosuppressive compounds and one of the examples is *Fusarium subglutinans* isolated from *T. wilfordii* which is reported to produce subgutinol A and B. These compounds are immunosuppressive (Lee *et al*.,1995).

2.7 Bioactive metabolites mode of action

The mode of action of the bioactive metabolites from fungal endophytes may vary. In most cases, the mode of action of microbial bioactive metabolites on pathogenic microorganisms is through inhibition of cell wall synthesis. In this case, the peptidoglycans forming bacterial cell wall is interrupted by glycopeptides, which are known to inhibit polymerization of a

disaccharide forming by N-acetylglucosamine and N-acetylmuramic acid. The beta-lactams and their derivatives inhibit peptidoglycan cross-linking (Běhal, 2006).

According to (Běhal, 2006), the bioactive metabolites from microbial sources use mode of actions similar to those of antibiotics. Some of these mechanisms are inhibition of cell wall and cytoplasmic membrane synthesis, protein synthesis, nucleic acid synthesis and bacterial two component signaling. Other bioactive metabolites such as Cyclosporine A, known to have immunosuppressants properties have mode of actions such as inhibiting the T cells from producing interleukin-2 and regulating T cell activation and metabolic pathways (Liddicoat and Lavelle, 2019).

The bioactive metabolites that have antiviral properties inhibit the reverse transcriptase of the disease casing virus by causing premature chain termination during the transcription stage of DNA from a single strand RNA template while others inhibit viral entry into the cells and inactivation of viral particles (Běhal, 2006).

2.8 Bioactive metabolites extraction methods

According to Soquetta *et al*. (2018), there are different methods of bioactive metabolites extraction from natural sources. The methods of extraction are dependent on several factors such as the raw materials and the extraction solvents that are used (Tiwari, 2015). Besides, the bioactive metabolite extraction techniques are grouped into two including conventional and non-conventional techniques. The conventional techniques require the use of organic solvents, temperature and agitation. Examples of the conventional techniques include

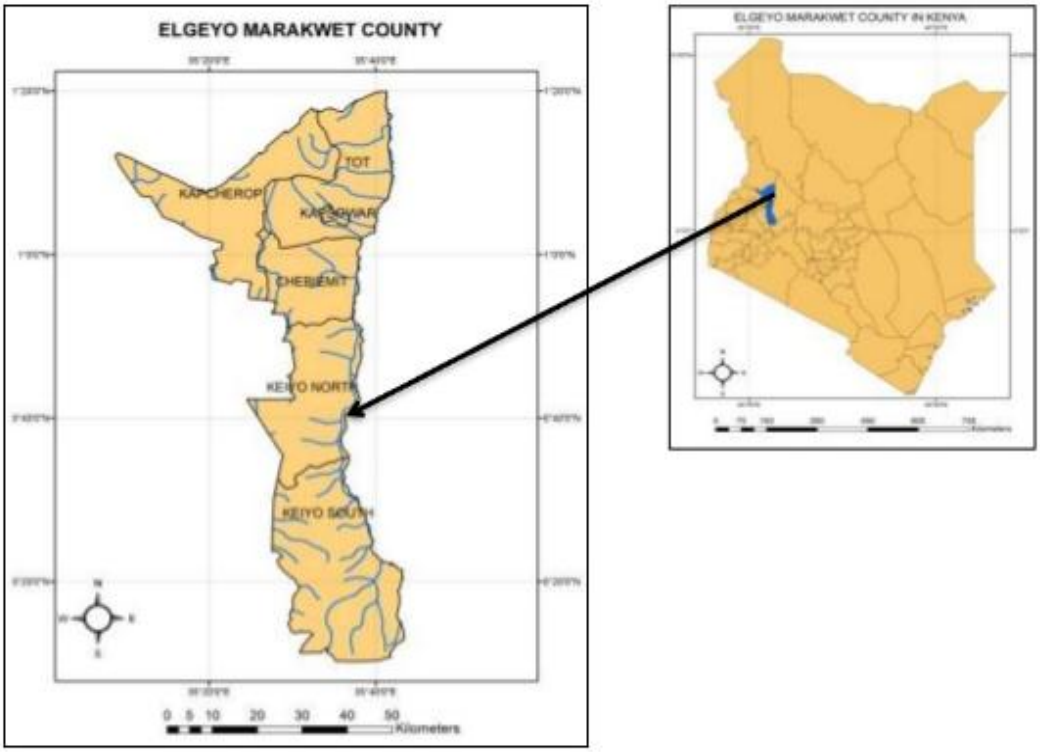
Soxholet maceration, and hydrodistillation. On the other hand, the non-conventional techniques also called modern techniques requires reduced use of energy and use of organic solvents (Rodríguez-Pérez *et al*., 2015).

The presence of bioactive metabolites from fungal endophytes is dependent on various factors which include season of sample collection, climatic conditions and the geographical location (Shukla *et al*., 2014). Globally, there are plants which are found in one geographical location and not other geographical areas. Therefore, the endophytic fungi may not be similar hence different bioactive compounds. In addition, bioactive metabolite extraction in fungal endophytes is dependent on the polarity of the target compounds and the solvents used in extraction (Azmir *et al*., 2013). According to Rahmawati *et al*. (2018), bioactive metabolites from fungal endophytes are mainly extracted using ethyl acetate because it is semi-polar and can extract polar and non-polar bioactive metabolites. On the other hand, methanol is used to extract bioactive metabolites from plant sources because methanol is polar and it yields high number of polar bioactive metabolites more than other solvents used in bioactive metabolite extraction such as ethanol, dichloromethane, and hexane (Oubihi *et al*., 2020). This is because, use of polar solvents favors the extraction of polar compounds which are the major bioactive metabolites in plants (Truong *et al*., 2019).

CHAPTER THREE: MATERIALS AND METHODS

3.1 Study area description

The study was carried out in Cherangani forest and surrounding. Cherangani forest borders three Counties, namely Trans-Nzoia, West Pokot, and Elgeyo-Marakwet. The areas is occupied by Luhya, Pokot, and Marakwet communities respectively.



Source: www.wri .org

Figure 3.1: Map of Elgeyo-Marakwet county

The area lies between Latitude: 1° 14’ 60.00” and N Longitude: 35° 26’ 59.99” E. The forest covers about 1,200 square kilometers. Out of the 1,200 square kilometers 956 square kilometers has been gazetted by the government as forest reserves. The forest is made of bushlands, grasslands, and croplands. The mountain parts of the forest are often rocky and stony with shallow soils (Forest, 2015). However, approximately 20% of the forest is rich in

vegetation cover consisting of Kapkanyar, Kapolet, Kiptaberr, Kipkunur, Kerer, and Lelan blocks. The soils are deep with high organic matter content. Some forest regions, such as the North-Western region and the Northern part of the forest, have shallow soils prone to soil erosions. Additionally, the soil acidity varies amongst various regions. The forest is majorly composed of metamorphic, quartz, marble, and precambrian rocks (Kenya Forest Service, 2015).

The area receives an average rainfall of 1200mm annually and 1400-1500mm in the rainy seasons. The area receives an uneven distribution of rains with April to May and August to November as the peak periods. The dry period is from December to February. The area experiences an average temperature of 24 ^{o} C annually (Ongugo *et al*., 2008). The sampling of the medicinal plants for isolation of the fungal endophytes was done in January 2020. This was within the dry season.

3.2 Selection of herbalists

Purposive and snowball sampling methods were used to select the herbalists. Purposive sampling was used where herbalists helped to pinpoint some of the practitioners of the herbal medicine in their areas of residence. Snowball sampling was used where some known herbalists were willing to lead us to where other practicing herbalists live.

3.3 Ethnobotanical data collection

The ethnobotanical data were collected in November and December 2019 through face-to-face interviews with the herbalists who were willing to participate in the study. Structured questionnaires were used to collect information on the medicinal plants. The questionnaires were divided into three sections which included; socio-demographic information, the

medicinal plants used by the herbalists and their uses and the third section had questions on method of preparation, administration, patient' response after use and the source of the medicinal plants (Appendix 1). The interviews were mostly at the herbalist's residence and market places namely; Kapsowar, Cheptongei, and Kapcherop. Information on the local name of the antimicrobial medicinal plants, the types of diseases treated, the recipes of preparing the medicinal plants, the mode of administration, the side effects of the medicinal plants, and the patient's response after use were documented. Information on parts of the plants frequently used was also noted. During the interviews, the emphasis was put on antimicrobial medicinal plants because of their crucial role in primary healthcare. Thirty-five herbalists were interviewed in the selected study site. The sample size was arrived at using the formula by Snedecor and Cochran, (1989)

$N= \frac{4pq}{(L)2}$

Where N is the sample size, p is the proportion of the target population, q=1-p, and L is the accepted error. The target population was the number of households obtained from the Kenya Bureau of statistics 2019 census. The target population was 119,968 persons, and the number of households was 2,700.

p=2,700/119968= 0.0225

q=1-0.0225= 0.9775

$l= (0.5)^2 =0.0025$

n=4(0.0225*0.9775)/0.0025= 35

3.4 Sample collection

Based on the questionnaires, the samples which were the most cited by the herbalist and had high use value (UV) were selected to be used in isolation of the fungal endophytes. A permit

to collect the medicinal plants was obtained from the Kenya Forest service through the County government of Elgeyo-Marakwet county (Appendix 6). The selected plants were sampled randomly and transported to Kenyatta university herbarium for taxonomic identification. During sampling, four samples from each plant part (leaves, stem, barks, and roots) of the two medicinal plants of *Zehneria scabra*, and *Croton macrostachyrus*) were sampled randomly in Cherangani Forest, Kenya as described by (Gamboa *et al*., 2003). The cut leaves, stems, roots, and barks were put in sterile polythene bags, sealed and put in a cool box then transported to Kenyatta university Microbiology laboratory for further identification of the plants and isolation of the fungal endophytes.

3.5 Isolation of fungal endophytes

The leaves, barks, and roots of the medicinal plants were thoroughly washed with running tap water to remove soil and any debris on the plant surface. After washing, the sampled plant parts from the two selected medicinal plants were surface sterilized in; 75% ethanol for a minute, Sodium hypochlorite for another five (5) minutes and rinsed thrice with double distilled water (DDW). The excess water in the surface sterilized samples was removed using sterilized paper towel (Froehlich and Petrini, 2000). The samples were placed on the surface of a sterile filter papers to absorb excess water. Once dried, the samples were cut into small pieces (0.5cm *0.5cm) using sterile blades. Each Petri dish with PDA supplemented with 200ug/ml of tetracycline were partitioned into four sections, and in each section, an explant was placed on its surface. The plates were sealed with parafilm and held at room temperatures. Regular observations were made and any mycelia of the fungi growing on the surface of the Petri dishes were isolated, sub-cultured, and pure cultures were maintained in PDA agar slants at 4℃ for further studies.

3.6 Fermentation of the fungal endophytes

An inoculum was prepared using 7mm of mycelial agar and 10-day fungal inoculum. The mixture was introduced into a 250 ml Erlenmeyer flask containing 200 ml of potato dextrose broth and incubated for 21 days at 25℃. After the incubation, the fungal biomass and fermentation broth was separated by filtration. The fermentation broth and the biomass were first filtered through Whatman filters, then through 0.45 pore membrane filters using a rotary pump to obtain the fungal endophyte crude extract. The secondary metabolites were obtained using ethyl acetate by solvent extraction procedure (Fernandes *et al*., 2009).

3.7 Screening for antimicrobial properties of the crude extracts obtained from the isolated fungal endophytes

Bioactivity of isolated fungal endophytes obtained from *Zehneria scabra* and *Croton macrostachyrus* was determined using antimicrobial assays; disk diffusion and well diffusion. The test organisms used in the study was acquired from Kenyatta university Microbiology Laboratory. These test organisms included; *Escherichia coli* (ATCC 35218), *Staphylococcus aureus* (ATCC 977), *Bacillus subtilis* (ATCC 23857), and *Candida albicans* (ATCC 14053). The antibacterial activity was determined. In well diffusion methods, Mueller Hilton media was prepared and sterilized and transferred aseptically into sterile Petri dishes and allowed to solidify. Approximately 100 µl (10^5 CFU/ml) of the bacterial cultures were spread on Mueller Hilton media surface. The cultures that were spread on the Mueller Hilton media were allowed to dry; and wells were made using a 6 mm cork borer. The wells were filled with 20 µl of the fungal endophyte culture filtrate, allowed to dry up and incubated for 24hours. In disk diffusion, Mueller Hilton media was prepared, sterilized and transferred aseptically into sterile Petri dishes, allowed to solidify, and 100 µl (10^5

CFU/ml) of bacterial cultures were spread on the surface of Mueller Hilton media. Paper disks were soaked on the fungal filtrate. The plates were petitioned into three, and one disk soaked with the fungal filtrate was placed on the partitioned petri dishes' surface. All the plates were incubated for 24 hours at 37℃. After incubation (24hours), zones of inhibition were measured using a ruler and compared with that of a plain disk (without soaking in fungal extracts), which were used as the negative control in disk diffusion. In well diffusion assay, sterile distilled water used as a negative control (Buatong *et al*., 2011). In each fungal extract, there was one Petri dish used as control with plain disks in the case of disk diffusion method and plain disk soaked in double distilled water in well diffusion method.

When evaluating the antifungal activity of isolated fungal endophytes, 100 µl (10^5 CFU/ml) of *Candida albicans* was evenly spread on Sabouraud dextrose agar's surface, and 20 µl of fungal endophyte filtrate was loaded into wells made using a 6 mm cork borer and allowed to diffuse to the media. The fungal crude extract that was positive for either the Gram-negative bacteria, Gram-positive bacteria, and antifungal activity were subjected to further analysis to identify the bioactive metabolites.

3.8 Extraction and characterization of the bioactive metabolites

The extraction and identification of the bioactive metabolites was done at the Government Chemist Laboratories. The secondary metabolites were obtained by solvent extraction procedure (Fernandes *et al*., 2009) using ethyl acetate as the solvent. In every 100 ml of the fungal endophyte extract, 40 ml of the ethyl acetate solvent (10:4) was added and mixed vigorously and allowed to settle until it formed two immiscible layers. The supernatant from the two immiscible liquids was separated using a separating funnel (Chatterjee *et al*., 2019). Again, equal volumes of ethyl acetate were added to the extract and filtered using the

separating funnel. The same procedure was repeated three times. The final extraction product was concentrated using a vacuum evaporator and subjected to GC-MS analysis.

Agient gas chromatography coupled with Agient series quadrupole mass spectrophotometry working in AI mode on a thermo column was used as the capillary column. One microliter of the sample was injected into the machine. The compounds were desorbed at a temperature of 260℃. The analysis was performed in programmed temperatures of 50℃ for five minutes, then 50-250℃ for forty minutes using ethyl acetate as the carrier gas. The flow of the machine was at a rate of 1.2ml min^{-1}. The GC-MS machine inferences were set at a temperature of 280℃. The compounds were identified based on the NIST library matches in the GC-MS machine.

3.9 Molecular identification of the fungal endophytes

3.9.1 Extraction of the genomic DNA

The genomic DNA was isolated based on the standard protocol described by Kariyawasam *et al*. (2012). The DNA was extracted from five-day old mycelia of the fungal endophytes growing in PDA. The mycelia cultures were harvested and put in 1.5 ml eppendorf tube, and to each sample, 400µl of lysis buffer (containing 1ml CTAB detergent, 2 ml double distilled water, 2.8 ml of 5M NaCl, Tris 1ml, 0.1ml EDTA, 0.005 mercaptoethanol) was added. They were then vortexed at maximum speed for 30 seconds and incubated for 30 minutes at 65 ℃ in a water bath with intermittent inversions. After incubation, the samples were centrifuged for 7 minutes at 13,000rpm, and the liquid phase transferred to a sterile eppendorf tube. Equal volumes of chloroform isoamyl in the ratio 24:1 was added and the samples incubated in a freezer for 30 minutes at -20 ℃. After incubation the samples were centrifuged at

13000rpm for five minutes and the supernatant transferred to another sterile eppendorf tube. Equal volume of absolute ethanol (96%) were added and incubated for 30 minutes at -20 ℃. The mixture was then centrifuged for 4 minutes at 13000rpm, and liquid phase decanted. Equal volume of 70% ethanol was added and the mixture centrifuged at 13000 rpm for 2 minutes to get rid of contaminants. The samples were dried by inversion on sterile paper towels followed by elution with 45µl of TE. To determine quality of DNA three microliters of each sample were stained with SYBR green and resolved in 1.0 % agarose gel at 80 volts in 0.5X TBE buffer. The gel was then visualized over UV trans-illuminator and documented by photographing using a 18 megapixel digital camera.

3.9.2 Polymerase chain reaction (PCR)

The isolated DNA was amplified using a protocol developed by Gul *et al*., (2017). The target segment was amplified using universal primers ITS1 F (TCCGTAGGTGAACCTGCGG) and ITS4 R (TCCTCCGCTTATTGATATGC) for 18S rDNA. PCR amplification was done in a total volume of 25µl, which contained 1µl of DNA template, 1.25µl of forward primer, 1.25µl of the reverse primer, 12.5µl of the master mix, and 9µl of PCR water.

Amplification was done in a thermal cycler (Techgene Thermal Cycler) (Techne) with an initial denaturation for 4 minutes at 95℃, followed by 35 cycles of initial denaturation for 30 seconds at 95 ℃, annealing for 1 minute at 55 ℃, synthesis for 1 minute 30 seconds at 72 ℃. Final extension was done for 10 minutes at 72℃ and final hold at 4 ℃. To confirm whether the PCR process was successful, 3 µl of the PCR product was loaded on 1% agarose gel

containing SYBR green (Thermal Fisher Scientific) and subjected to gel electrophoresis using 0.5X TBE buffer at 80V for 40 minutes. The bands were visualized using UV trans-illuminator and documented by photographing using 18-megapixel Sony digital camera.

3.9.3 DNA sequencing

The PCR product obtained was purified and subjected to Sanger sequencing using an automated genome sequence analyzer machine in Macrogen Europe B.V in the Netherlands. The ITS1 and ITS4 primers were utilized for sequencing.

3.10 Data analyses

Qualitative and quantitative data were generated using the Statistical Package for Social Sciences (SPSS) version 22.0. The normality of the collected data was tested using the SPSS. The data was analyzed and output generated in form of averages, percentages, figures, and tables.

The medicinal importance of each plant was determined based on the use-value index (UV) for each plant species using the formula by (Tardío and Pardo-de-Santayana, 2008). The UV values always range from 0-1. Zero (0) implies that none of the respondents in the study mentioned any use of the plant, while one (1) means that the plant was frequently mentioned to be used to treat various conditions.

$$\text{UVs} = \frac{U}{N}$$

Where: U= is the number of uses of each medicinal plant mentioned by the respondents

N=Number of informants interviewed during the survey citing the use of the species

The assumption made from this is that each informant had equal chances of mentioning any of the medicinal plants' species. In addition to the use-value index, the Family use-value

(FUV) was calculated to determine the medicinal plants' significant families based on the formula by (Hoffman and Gallaher, 2007). Where;

FUV= $\frac{UVs}{ns}$

UV=Use vale of the species

ns= total number of species within each family.

The data on isolation of the fungal endophytes from different plant parts was used to calculate their diversity using the Past software version 4.0. The morphological data was coded into numerical and Darwin software version 6.0 was used to calculate the dissimilarity index. The values obtained was used to draw the phenotypic tree.

The significant differences in mean zones of inhibition of the isolated fungal endophytes extracts against the test organisms was tested using Two-way ANOVA at $P<0.05$ (Park, 2009), while Tukey's Honest Significance Difference (HSD) test was used to separate the means (Lane and Salkin, 2010).

The sequences obtained from sequencing were used to create consensus sequences using the BioEdit software 7.2.5 version. Similar sequences of the consensus were searched using BLAST available on the NCBI website, and matching organisms obtained from the same database. Multiple sequence alignments of related species were done using CLUSTAL W. The phylogenetic analysis was done using Neighbor-joining method based on Tamura- Nei model using Mega X software. Additionally, 1000 bootstrap replicates were analyzed to assess reliable nodal support values.

CHAPTER FOUR: RESULTS

4.1 Socio-demographic information

The majority of the herbalists had formal education (48.6%), and out of the total sampled herbalists 8.6% had secondary education while 5.7% had tertiary education. However, 37.1% of the herbalists had no formal education. The duration of practice ranged from less than ten years (40%), while most (60%) have been practicing for more than ten years. The majority of the herbalists (85.71%) reported that the transfer of herbal knowledge is from close family members while 14.29% learned it through careful observation (Table 4.1).

Table 4.1: Social demographic information of the herbalists in areas surrounding Cherangani forest.

Gender	**Frequency**	**Percentage (%)**
Male	8	22.9
Female	27	77.1
Age bracket		
31-40	5	14.3
41-50	8	22.9
51-60	13	37.1
Above 60	9	25.7
Level of education		
N/A	13	37.1
Primary	17	48.6
Secondary	3	8.6
Tertiary	2	5.7
Duration of practice		
0-10	14	40
11-15	10	28.6
Above 15	11	31.4
Source of herbal knowledge		
Close relatives	30	85.71
Careful observations	5	14.29

4.2 Medicinal plants documented

The herbalists listed 26 medicinal plants used for antimicrobial purposes. Table 4.2 summarizes information on the local names, common names, botanical names, and family names of the documented medicinal plants. The plants are comprised of herbs, shrubs, trees, and climbers. However, their distribution differs. Trees recorded the highest percentage (38.5 %), herbs (30.8%), shrubs (26.9%), and climbers (3.8%). Each documented plant has its preparation method and uses, as outlined in (Table 4.2). The table also summarizes the plant parts used.

Table 4.2: Plants used for herbal remedies by Marakwet community in the areas surrounding Cherangani forest.

	Local name (Marakwet)	**Common name**	**Botanical name**	**Family name**	**Growth form**	**Uses**	**Parts used**	**Method of preparation**
1	Cheserya	Not found	*Zehneria scabra (L. f) Sond*	*Cucurbitaceae*	Climber	Stomachache and related complications Common cold and cough Oral thrush Sour throats and mouth ulcers Skin infections Cancer	Leaves Stem	Chew the leaves while fresh Dried and pound and taken
2	Toboswo	Broad leave croton	*Croton macrostachyrus Hohst*	*Euphorbiaceae*	Tree	Skin related infections Respiratory infections such as chest congestion Allergies Purgative	Leaves Barks Roots	Boiled and administered
3	Angurwo	Not found	*Plechrantus barbartus Andrews*	*Lamiaceae*	Herb	Skin rashes Soap Allergies	Leaves and roots	Boiled and administered
4	Remit	Wild olive	*Olea africana*	*Oleaceae*	Tree	Eye infections Itchy skin	Bark	The barks are boiled and

						rushes		administered
5	Chebokobil	Zanzibar plant	*Lippia javanica (Burn.f) spreng*	*Verbenaceae*	Herb	Amoebiasis Stomachache and stomach related complications	Leaves and twigs	The leaves and twigs are boiled and taken
6	Sokwon	East African Green Wood	*Warburgia ugandensis*	*Canellaceae*	Tree	Stomachache Oral thrush Common cold and cough	Bark and Leaves	The leaves and barks are boiled and soaked in hot water then administered
7	Korniswo	Pricky Thorn Bonsai	*Acacia brevispica Harms*	*Mimosaceae*	Shrub	Itchy skin rashes Wounds	Leaves	The leaves are crushed and applied on the affected area
8	Churur	Whistling thorn	*Acacia hockii De Wild*	*Mimosaceae*	Tree	Abdominal pains Stomachache	Bark	Boiled and administered
9	Ngopkwo	Thorn tree	*Acacia nilotica(L) Delile*	*Mimosaceae*	Tree	Abdominal pains and stomach related complications	Barks	The barks are chewed while raw. It can also be boiled and administered at an

								interval
10	Kurelwo	Orange leave croton	*Croton dichogamous pak*	*Euphorbiaceae*	Shrub	Oral thrush and mouth ulcers Stomach related pains	Leaves, barks, and roots	The whole plant is burnt, and the ashes licked
11	Murgut	Yellow nutsedge	*Cyperus esculentus*	*Cyperaceae*	Herb	Stomachache Colic pains in children Abdominal pains	Tubers	The tubers are chewed when raw or even when it's dry
12	Kalobotwo	Thorn apple	*Solanum incanum*	*Solanaceae*	Herb	Abdominal pains Colic pains in children Wounds	Roots	The roots are boiled and taken The roots can be chewed while raw
13	Chepkenderetwo	Burnt plant	*Aloe spp*	*Oleaceae*	Herb	Wounds and wound-related infections	Leaves	The leaves are crushed and applied to the affected area
14	Mochontopokot	Caralluma	*Caralluma acutangula*	*Asclepiadaceae*	Herb	Respiratory infections such as chest congestion, especially wheezing, cough, and chest pains	Leaves, Barks, and Roots	Parts of the whole plant are chewed when fresh and even when dried

15	Mororion	Stamperwood	*Ehretia cymose var*	*Boraginaceae*	Shrub	Stomach pains Colic pains in children	Barks	Soaked in water and boiled
16	Korkowo	Corpse flower	*Erythrina abyssinica*	*Fabaceae*	Tree	Mumps and oral thrush	Barks	Boiled and administered
17	Sumeyon	Orange bird berry	*Hoslundia opposite valn*	*Lamiaceae*	Shrub	Abdominal pains Amoebiasis Colic pain in children	Leaves	Boiled and administered
18	Sergellat	African indigo	*Indigofera arrecta Benth*	*Fabaceae*	Herb	Amoebiasis/ stomach related complications	Roots	The roots are boiled and mixed with other herbal and taken At times the roots are chewed when raw or even when dried
19	Chorwo	Brittle wood	*Nuxia congesta Var.*	*Loganiaceae*	Tree	Stomachache Colic pains	Roots	The toots are boiled and administered
20	Chebumbu	Bryophyta	*Schefflera volkensii* Harms	*Green moss*	Tree	Respiratory infections	Dried resins	The dried resins are sniffed to unblock

								blocked noses
21	Kibutkut		*Spilanthens Mauritania* (Pers.) D.C	*Asteraceae*	Herb	Oral thrushes Used during surgery	Leaves, Barks, and Roots	Leaves roots and barks and crushed and used as a mouthwash The leaves, roots, and leaves are crushed then applied to the region to be operated
22	Koloswo	Mbarao(Swahili) in Kenya	*Terminalia brownie Fresen*	*Combretaceae*	Tree	Oral thrush Abdominal pains	Barks	Boiled and administered
23	Turkukai	Red cherry	*Withania somnifera* (L) Dunal	*Solanaceae*	Shrub	Abdominal pains Respiratory infections Chronic skin ulcers Amoebiasis	Roots and leaves	The roots and leaves are boiled and administered They can be crushed and applied to the affected skin area
24	Tilomwo	Christ thorn	*Ziziphus mauritania var*	*Rhamnaceae*	Tree	Amoebiasis Abdominal pains	Bark	Boiled and administered

								Chewed while raw
25	Kochon	Knob wood	*Zanthoxylum chalybean* Engl	*Rotaceae*	Shrub	Amoebiasis Stomachache Abdominal pains Colic pains	Barks	Boiled and administered At times it is chewed while raw
26	Kunyotwo	Yellow plum	*Ximenia americana* Var	*Oleaceae*	Shrub	Wounds	Barks and Seeds	They are crushed and applied to the affected area

4.2.1 Diseases treated by the herbalists

The diseases described by the herbalists were put into four groups; gastrointestinal, respiratory, skin, and others. The gastrointestinal diseases included common ailments such as stomachache, diarrhea, colic pain in children, and vomiting. The skin infections include swellings, change of skin color and wounds. The common ailment treated, according to the herbalists, is gastrointestinal problems cited by 97.1% of the herbalists, followed by respiratory and skin infections, each recording 80%. Some of the herbalists (80%) treat other diseases (Figure 4.2).

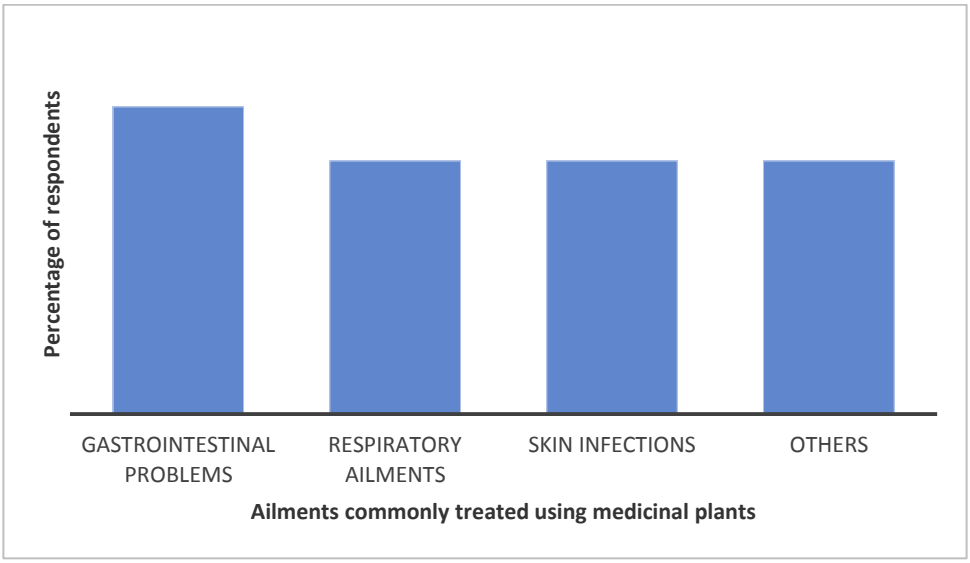


Figure 4.2: Common diseases treated by the herbalists using the medicinal plants. Y axis- percentage of herbalists treating gastrointestinal, respiratory, skin and other infections using medicinal plants obtained from Cherangani forest. X axis- Diseases treated by the herbalists using medicinal plants obtained from Cherangani forest.

4.2.2 Genera and species of the medicinal plants

The herbalists listed the use of different plants to treat various ailments. However, the use frequency of each plant species differed among the herbalists (Figure 4.3). The first three are *Croton macrostachyrus,* which recorded the highest frequency of herbalists (94 %) using it

in their daily practice, followed by *Zehneria scabra* with (91%) and *Cyperus esculentus* (89 %) while *Solanum incanum* and *Bryophta spp* recorded the least use frequency of the herbalists with 17.1% each

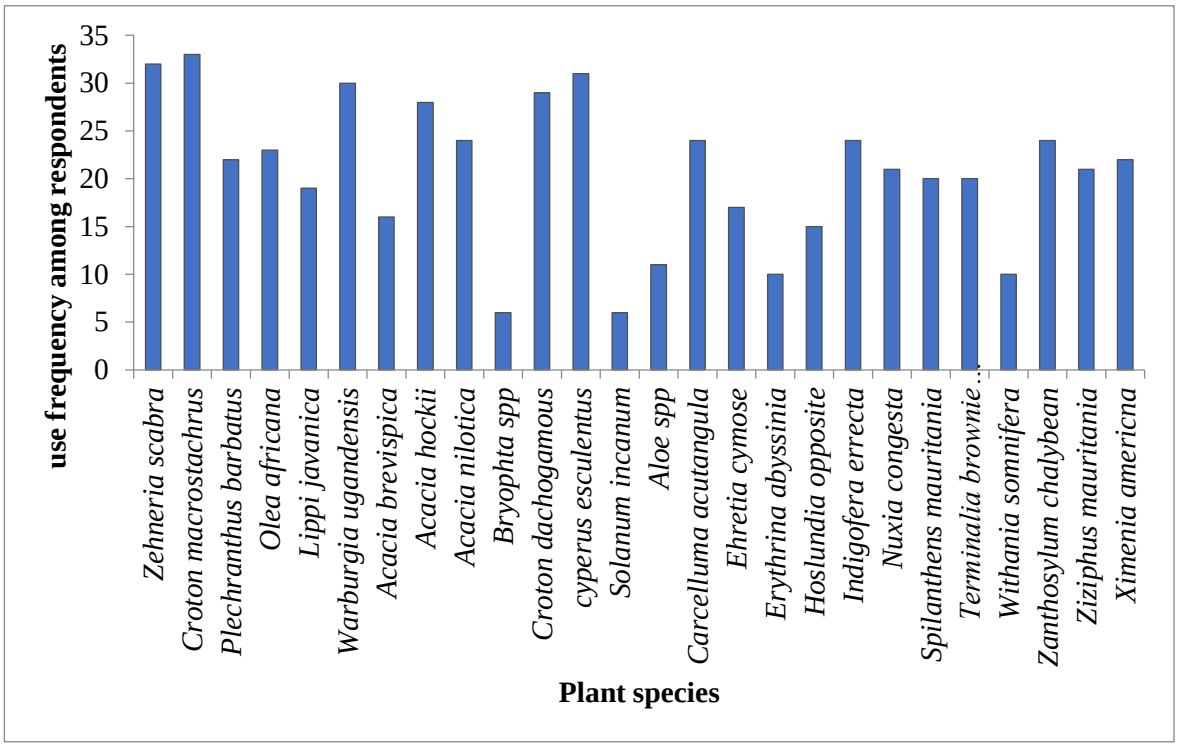


Figure 4.3: Species of medicinal plants used and the frequency use by the herbalists. Y axis- Frequency of herbalists cited to use each medicinal plant. X-axis- Plant species used as medicinal plants by the herbalists in Cherangani forest.

4.2.3 Families of the medicinal plants and their uses per family

The species of the medicinal plants documented were from 18 different families (Figure 4.3). Each family has its uses. The *Cucurbitaceae* family had the highest number of uses (8) followed by *Euphorbiaceae* and *Mimosaceae* with seven uses each. In contrast, *Loganiacea*e and *Cyperaceae* families recorded the least number of uses (one use each).

4.2.4 Use value and Family use-value of the medicinal plants

The calculated results of use-values (UV) showed that *Zehneria scabra* and *Acacia nilotica* scored the highest value (0.25) and (0.19), respectively, while the least was *Nuxia congesta* (0.05) (Table 4.3). The rest of the medicinal plants had use values (UV) ranging from 0.19 to 0.05. Based on the use-family values, *Cucurbitaceae, Baraginaceae,* and *Rotaceae* families of the medicinal plants recorded the highest values of 0.25, 0.18, and 0.15.

Table 4.3: Use value and Family use-value of the medicinal plants used by the herbalists in Cherangani Forest

	Plant	Family name	No of uses	Herbalists use frequency	UV	FUV
1	*Zehneria scabra*	*Cucurbitaceae*	8	32	0.25	0.25
2	*Croton macrostachyrus*	*Euphorbiaceae*	4	31	0.13	0.07
3	*Plechranthus barbatus*	*Lamiaceae*	3	22	0.14	0.07
4	*Olea africana*	*Oleaceae*	2	20	0.1	0.03
5	*Lippi javanica*	*Verbenaceae*	3	22	0.14	0.14
6	*Warburgia ugandensis*	*Canellaceae*	4	31	0.13	0.13
7	*Acacia brevispica*	*Mimosaceae*	2	16	0.13	0.04
8	*Acacia hockii*	*Mimosaceae*	2	28	0.07	0.02
9	*Acacia nilotica*	*Mimosaceae*	3	16	0.19	0.06
10	*Bryophta spp*	*Green moss*	3	28	0.12	0.11
11	*Croton dachogamous*	*Euphorbiaceae*	3	30	0.1	0.05
12	*cyperus esculentus*	*Cyperaceae*	1	12	0.08	0.08
13	*Solanum incanum*	*Solanaceae*	3	21	0.14	0.07
14	*Aloe spp*	*Oleaceae*	2	24	0.08	0.03
15	*Carcelluma acutangular*	*Asclepiadaceae*	2	17	0.12	0.12
16	*Ehretia cymose*	*Boraginaceae*	2	15	0.13	0.13
17	*Erythrina Abyssinia*	*Fabaceae*	3	20	0.15	0.15
18	*Hoslundia opposite*	*Lamiaceae*	2	24	0.08	0.04
19	*Indigofera errecta*	*Fabaceae*	2	21	0.10	0.1
20	*Nuxia congesta*	*Loganiaceae*	1	20	0.05	0.05
21	*Spilanthens Mauritania*	*Asteraceae*	2	19	0.11	0.11
22	*Terminalia brownie Fresen*	*Combretaceae*	2	19	0.11	0.2
23	*Withania somnifera*	*Solanaceae*	2	25	0.08	0.04
24	*Zanthosylum chalybean*	*Rotaceae*	4	22	0.18	0.18
25	*Ziziphus Mauritania*	*Rhamnaceae*	2	22	0.09	0.09
26	*Ximenia Americana*	*Oleaceae*	2	17	0.12	0.04

Key: Family name= Taxonomic family in which each medicinal plant belong, UV=use value based on each medicinal plant number of uses, FUV= Family use value based on cumulative number of uses of all plants in one family.

4.2.5 Plant parts used

The plant parts used were leaves, barks, roots, while other herbalists used the plant parts in combination (Figure 4.4). The use of leaves, barks, and roots recorded the highest percentage (77.1%) as compared to barks and roots (57.1%) and leaves and barks (11.4%). Use of leaves alone recorded the highest percentage (40%) as compared to barks alone (5.7%) and roots alone (2.9%).

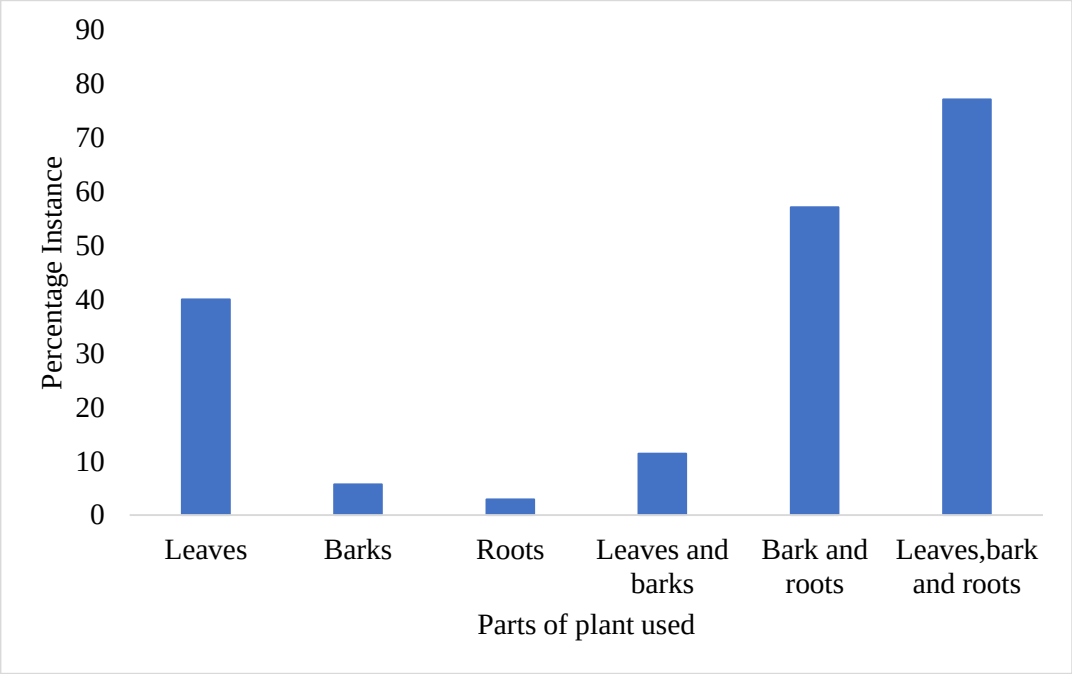


Figure 4.4: Parts of the medicinal plants used. Y axis-Percentage of herbalists using Leaves, Barks, and roots of medicinal plants obtained from Cherangani forest. X- Part of the medicinal plants used for medicinal purposes by herbalists in Cherangani forest.

4.2.6 Method of preparation

The respondent's reported that they use different ways of preparing the medicinal plants to treat the ailments which included boiling, chewing while raw and burning it into ashes. However, some of the herbalists reported preparing their medications by using more than one method. The most preferred way of preparation was both boiling (58%) and raw

consumption with 58% followed by burning the plant parts into ashes (28%), boiling 12%, and chewing the plants while raw (2%). The medicinal plants' prescription and dosage from one herbalist to another differed depending on the patient's age, the persistence of illness, physical fitness of the patient, and if the patient is pregnant.

4.2.7 Method of administration

The most preferred route of administration was oral 63%, followed by swabs 24% and inhalation 13%. The mode of administration was dependent on the type of ailment. In the gastrointestinal cases, oral administration was the preferred method, while both inhalations and oral were preferred for respiratory infections. Swabs and oral were used in the treatment of skin infections. The herbalists reported that some of the medicinal plants were bitter; therefore; they recommend their patients to mix it with honey or milk. In all diseases, oral administration was common. Additionally, all the herbalists reported giving verbal instructions to the patients. The majority of the herbalists (97. 1%) instructed their patients to use the prescribed dosages once a day, especially in the morning, while 11.4% instructed twice a day.

4.2.8 Source of medicinal plants

All the herbalists reported that the main source of the medicinal plants is the nearby forest within their residence (Cherangani forest). However, some of the herbalists (8.6%) have domesticated some medicinal plants within their family farms. Additionally, 82.9% of the herbalists reported that the scarce and rare species of the medicinal plants are outsourced from places outside Elgeyo-Marakwet County. Some herbalists cooperated with herbalists outside the county to help in the supply of these rare species of medicinal plants.

4.3 Medicinal plants selected for isolation of the fungal endophytes

The selection of the medicinal plants for the isolation of the fungal endophytes was based on the use values and family-use values. The UV value of each plant is calculated by dividing the number of uses per plant by the total number of herbalists who cited the specific plant and multiply it by the number of herbalists who participated in the study. On the other hand, FUV was calculated by dividing the UV of each plant by the total number of plants from the same family. The plants that had high UV and FUV values, were cited by majority of the herbalists and available were selected (Table 4.3). The selected medicinal plants therefore; were *Zehneria scabra* (Plate 4.3) with (UV- 0.25 and FUV-0.25) and *Croton macrostachyrus* (Plate 4.4) with (UV-0.13 and FUV-0.07).

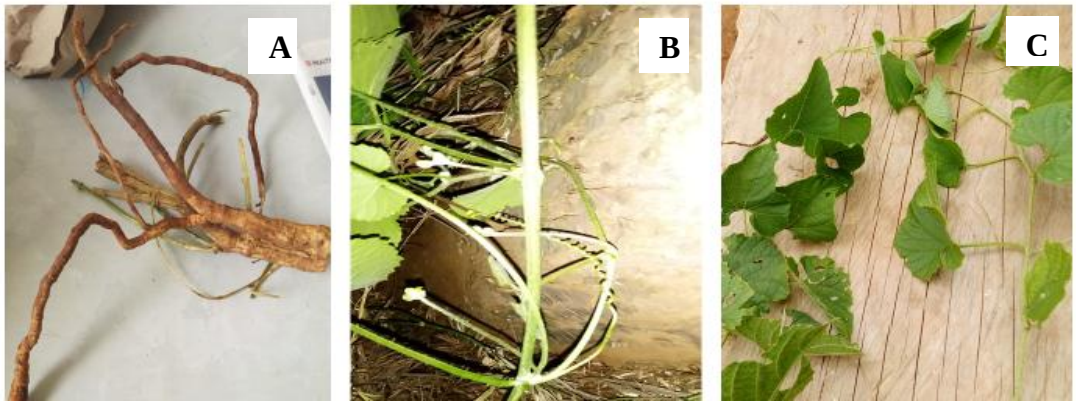


Plate 4.3: Parts of *Zehneria scabra* used to isolate the fungal endophytes.
Key: A-Roots, B-Stem and C-Leaves.

Plate 4.4: Parts of *Croton macrostachyrus* host used to isolate the fungal endophytes.
Key: A-Young *Croton macrostachyrus* plant, B-*Croton macrostachyrus* bark and C-*Croton macrostachyrus* leaves.

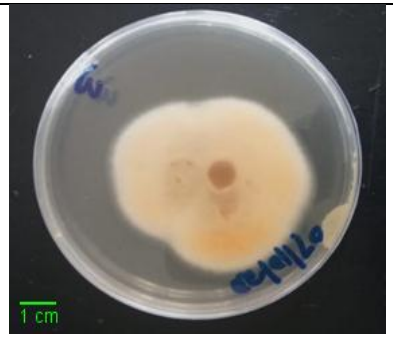
4.4 Isolation of the fungal endophytes

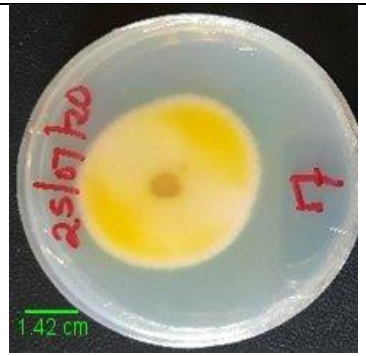
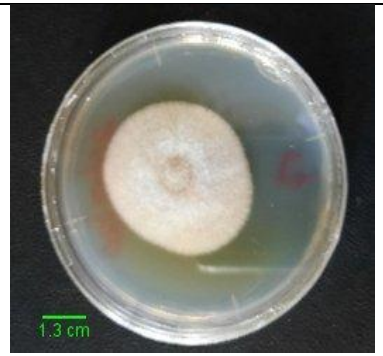
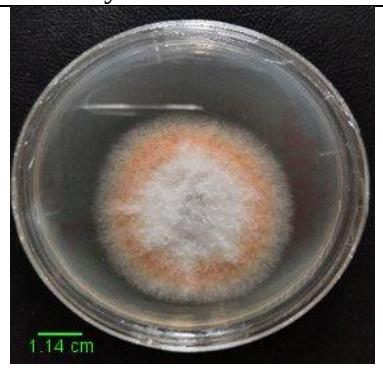
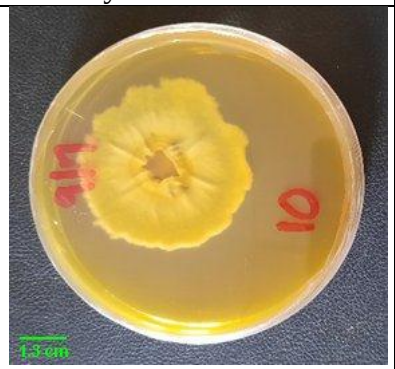
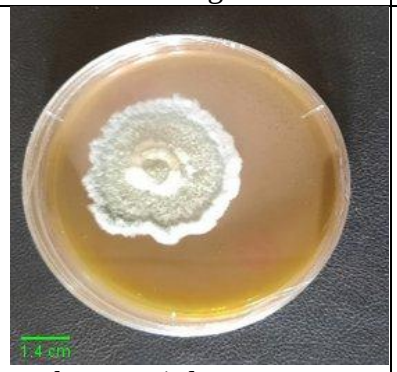
A total of 172 fungal endophytes were isolated from *Croton macrostachyrus* and *Zehneria scabra*. Out of these, 56.40% were from *Croton macrostachyrus,* while 43.60% were isolated from *Zehneria scabra*. Additionally, 61.63% of the fungal endophytes isolated were from leaves, 19.77% from roots, 10.47% from barks, and 8.14% from stems.

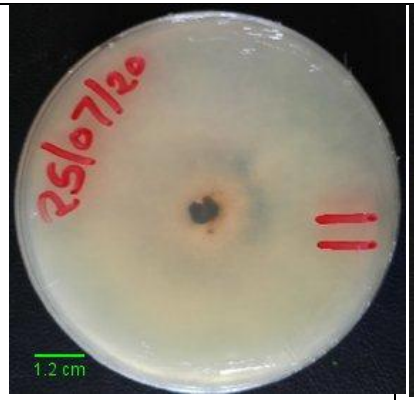
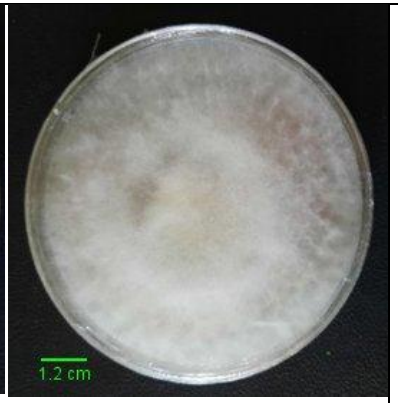
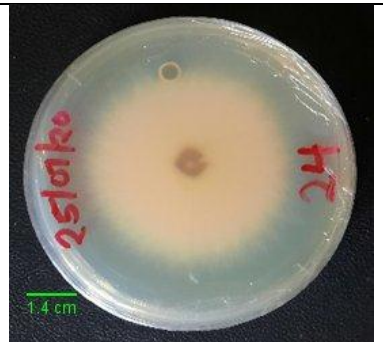
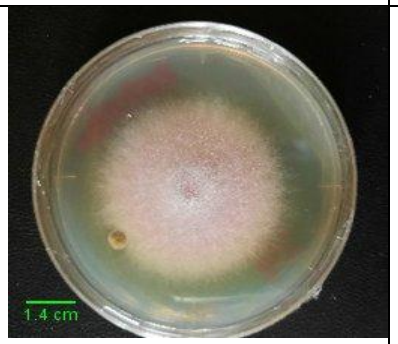
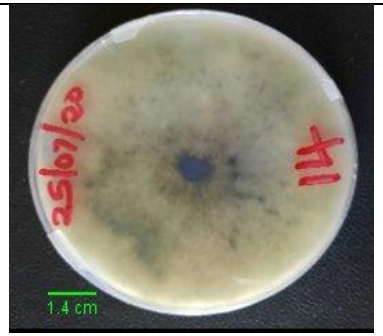
4.5 Morphological identification

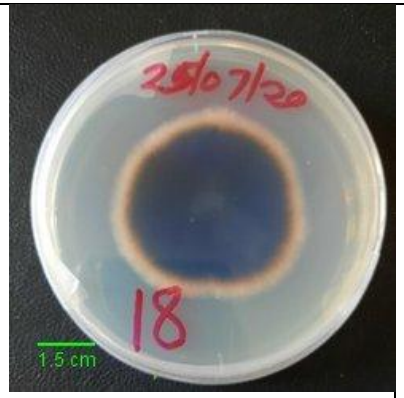
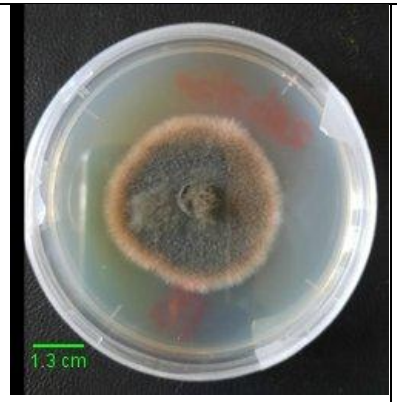
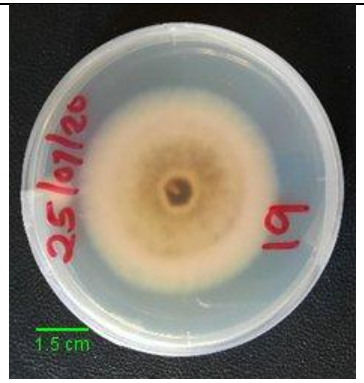
Morphological identification of the isolates was based on colour (front and reverse), margin (regular, irregular), elevation (raised, flat, umbonate) (Table 4.4). shape of the conidia (globulose, oval, circular, asymmetrical) (Plate 4.5) mycelia (dense or sparse) and the nature of the hyphae (septate or aseptate) (plate 4.7)

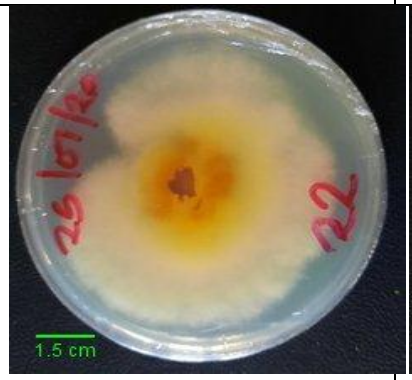
Table 4.4: Cultural characteristics of the fungal endophytes isolated from *Zehneria scabra* and *Croton macrostachyrus* obtained from Cherangani Forest.

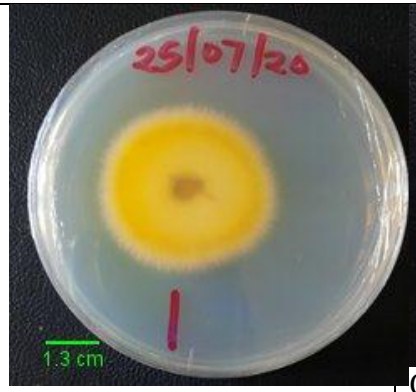
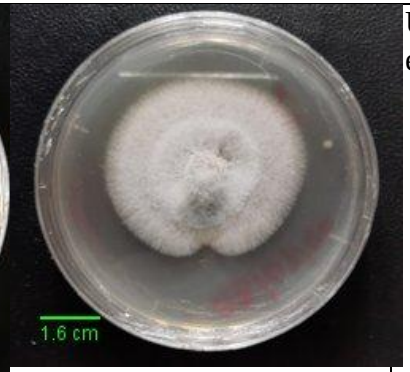
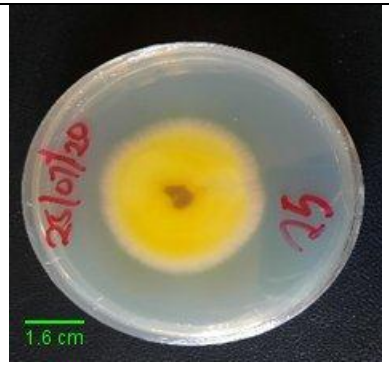
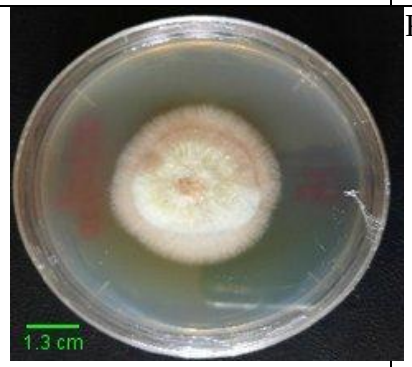
Isolates	Reverse	Front	Elevation	Margin	Form	Features
MR3	 Cream yellow	White	Flat	Regular	Filamentous	Develops concentric ring with age The rigs are brown and cream white
MR6	White initially but changes to yellow with age	Initially White but changes to black with age	Flat	Irregular	Filamentous	Has grooves on the reverse There is media alteration to yellow

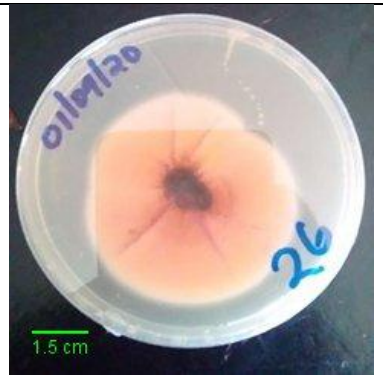
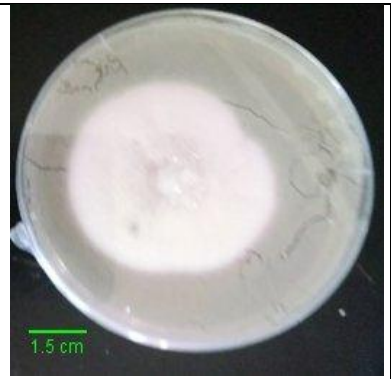
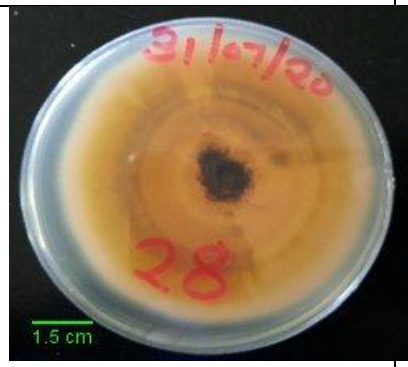
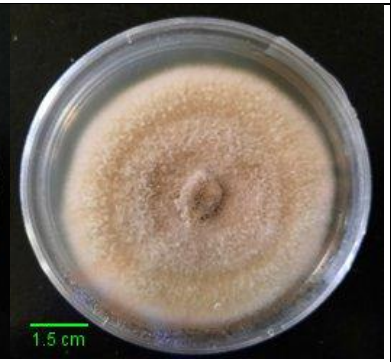
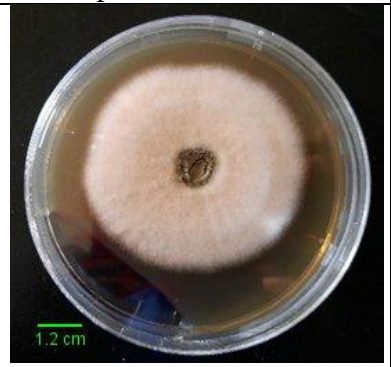
MR7	 White	 Cream yellow	Convex	Smooth/Entire/regular	Filamentous	Has concentric rings both on front and reverse Dense and raised mycelia
MR8	Cream yellow	 White and orange	Flat	Regular and white	Filamentous	Sparsely mycelia Has concentric ring of cream yellow and brown
MR10	 Cream yellow	 Light green/Blue-green	Raised	Irregular white margin	Powdery to velvety appearance	Slow growing Has grooves on the reverse Dense mycelia reverse

MR11	 White	 White	Raised	White and filamentous	Filamentous	Dense raised mycelia Fast growing and fills the plat in fve days
MR13	 White	 White	Umbonate	Undulate/Wavy	Regular and white	Slow growing Flat and Dense mycelia
MR14	 Grey	Grey	convex	Lobate	Filamentous	Fast growing. Initialy with but chngesto grey and finally black with age

MR18 Alternaria	 Black	 Grey	Flat	Entire	Filamentous	Dense flat mycelia
MR19	 Grey and cream white	Initially white and changes to grey with age	Flat	Entire/ smooth	Cream white	Has a concentric ring Grey center White margins The concetric ring is in the reverse

MR20	Brown	White	Flat	Smooth and regular	Filamentous and velvety	Sparse mycelia Media alteration to brown
MR21	White	White	Convex	Entire/smooth	Filamentous and velvety	Slow growing and has sparse mycelia
MR22	 Cream yellow	Cottony cream white	Convex	Undulate and cream colour	Filamentous	Media alteration to yellow

MR23	 Cream white	 Cream white	Umbonate	Regular	Filamentous	Had concentric ring in both the front and reverse view The mycelia was raised and dense
MR25	 Cream yellow	 Cream yellow and has the concentric ring	Raised	Entire/ smooth Cream white	Filamentous	The concentric ring is cream and yellow on both front and the reverse The mycelia is raised separately

MR26	 Brown	 White	Flat	Regular/Smooth White	Velvety appearance	Has grooves on the reverse Slow growing
MR28	 Cream yellow	 White pink	Flat	Entire/ Smooth	Filamentous	Has concentric ring on both front view and the reverse Slow growing
MR29	Yellow	 White pink	Flat	Regular/Entire/ smooth	Velvety	Has grooves

The morphological features used in characterization of the fungal endophytes included the conidia and septation of the conidia (Plate 4.10). The shape of the conidia was also different. Some were circular (Plate 4.6) while some were curved (4.6). Isolates such as MR6 produce a clump of spores which were circular and small in size (Plate 4.7). In addition, isolates such as MR14 had three spores per conidiophore (Plate 4.8)

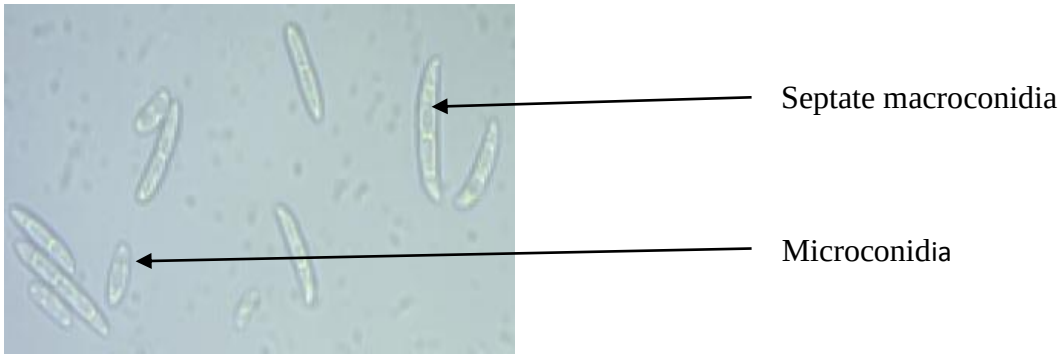


Plate 4.5: Microscopic features of MR13 under light microscope X400. Macroconidia are septate, 3-5 celled, and gradually pointed and curved towards the ends. Microconidia are smaller than macroconidia, and 1-2 celled.

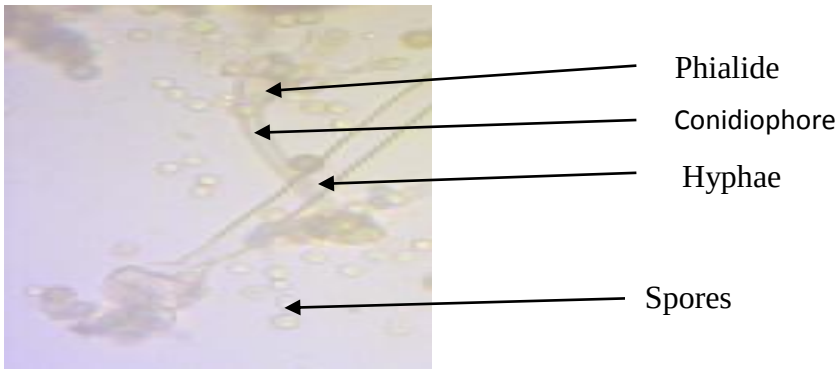


Plate 4.6: Microscopic features of isolate MR10 under light microscope X400. Has brush like spore bearing structures (phialides). The spores are produced in chains at the Phialides.

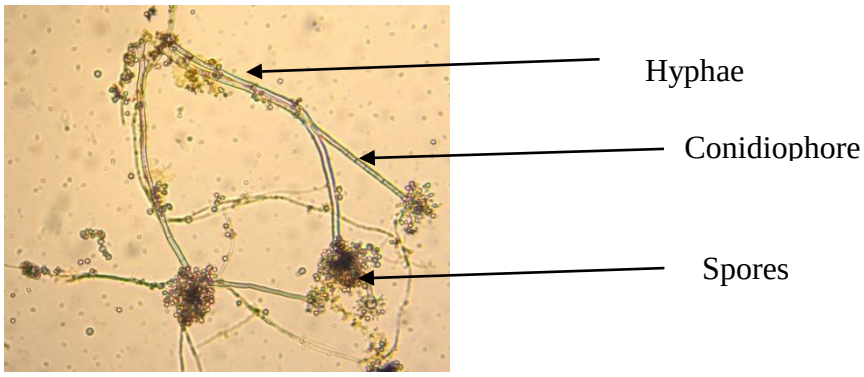


Plate 4.7: Microscopic features of isolate MR6 under light microscope X400. It has septate hyphae, and globose conidia. The conidiophores are septate

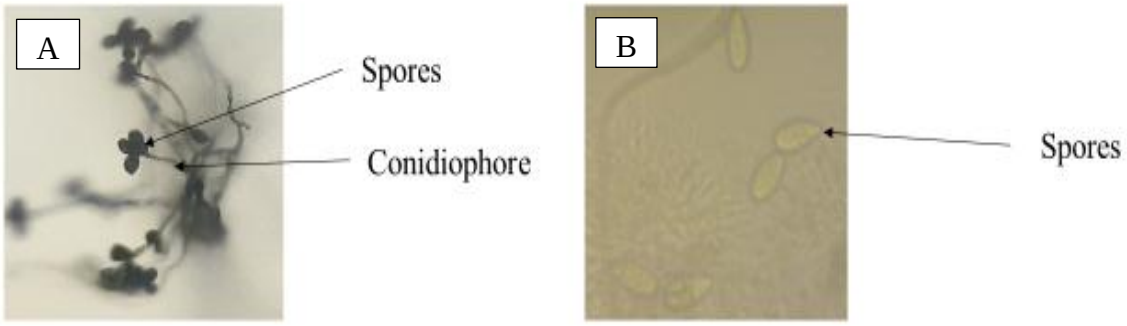


Plate 4.8: Microscopic features of isolate MR14 under light microscope X400. Key: A- spores and conidiophore of isolate MR14, B- Isolate MR14 spores

Morphologically, the isolated fungal endophytes clustered into three major phenotypic clusters. Cluster A was comprised of isolates MR22, MR18, MR20, MR28, MR25, MR23, MR21 and MR8. Cluster B was comprised of isolates MR3, MR7, MR11, MR13, MR14 and MR29 while Cluster C was comprised of isolates MR6, MR10, MR26, and MR19. In this study, majority of the isolates clustered in Cluster A (Figure 4.8). Additionally, Clusters A and cluster B had two sub clusters each while cluster C had one sub cluster.

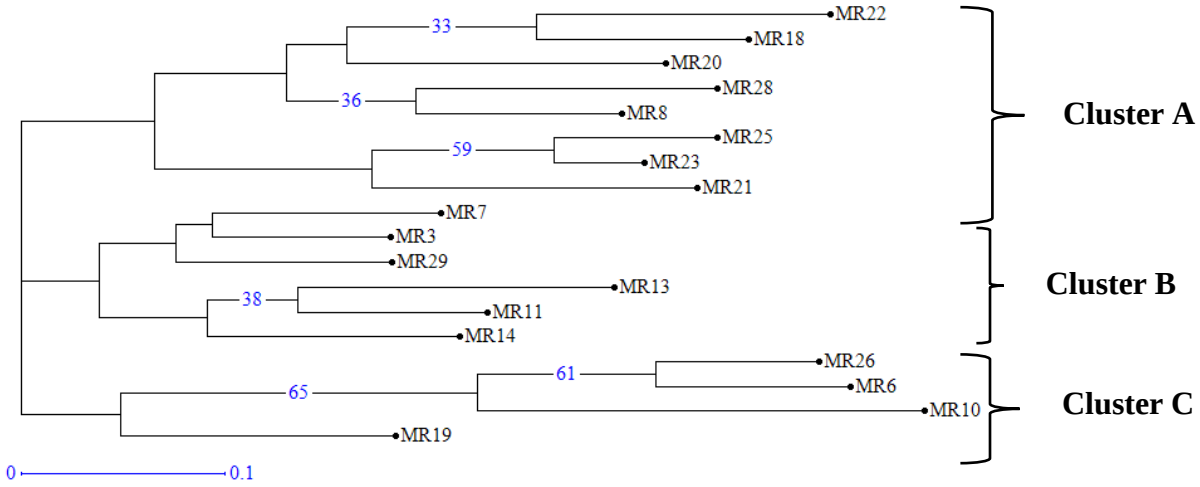


Figure 4.5: Cluster analysis on morphology of endophytes isolated from *Zehneria scabra* and *Croton macrostachyrus* using the neighbor joining method and Euclidean similarity index on Past Software Version 3. Numbers shown at the nodes indicate the percentage bootstraps for 1000 iterations.

Based on the morphological characterization, the 172 isolated fungal endophytes were placed on 30 morphological groups. The morphological features used in morphological characterization of the isolated fungal endophytes included the shape of the spores, size, type of hyphae and the type of conidiophores (Plate 4.5, Plate 4.6, Plate 4.8). The number of isolates in each plant and plant parts were recorded and used to calculate the morphological diversity of the isolated fungal endophytes.

4.6 Diversity of isolated fungal endophytes in *Croton macrostachyrus* plant

In *Croton macrostachyrus*, 61.86% of the isolated fungal endophytes were from leaves, 18.56% from barks and 19.58% from roots. Additionally, 16 species of fungal endophytes were isolated from leaves, 12 from barks and 12 from roots. The Shannon (H) diversity index showed that leaves had the highest diversity (2.712) followed by barks (2.37) and roots (2.306) (Table 4.5). Based on Simpson (1-D) diversity index, the highest diversity was

recorded from leaves (0.93) followed by barks (0.8951) and roots (0.892). Moreover, the highest diversity evenness was recorded in leaves (0.9416), followed by roots (0.9119) and barks (0.8916). The highest species dominance was recorded roots (0.108) followed by barks (0.1049) and the lowest was roots (0.07) (Table 4.5).

Table 4.5: Diversity indices of fungal endophytes isolated from *Croton macrostachyrus* host

	Leaves	**Barks**	**Roots**
Taxa_S	16	12	11
Individuals	60	18	19
Dominance_D	0.07	0.1049	0.108
Simpson_1-D	0.93	0.8951	0.892
Shannon_H	2.712	2.37	2.306
Evenness_e^H/S	0.9416	0.8916	0.9119

Key: S-Number of species, D-Dominance, 1-D-Simpson diversity index, H- Simpson diversity index, and e^H/S- Eveness based on Past software version 3.

4.6.1 Individual rarefaction in *Croton macrostachyrus* host

The diversity in leaves was high as compared to other samples. In leaves, there was increase in species taxa as the samples increase. In contrast, in barks and roots, the diversity of species taxa increased even with decrease in samples (Figure 4.6)

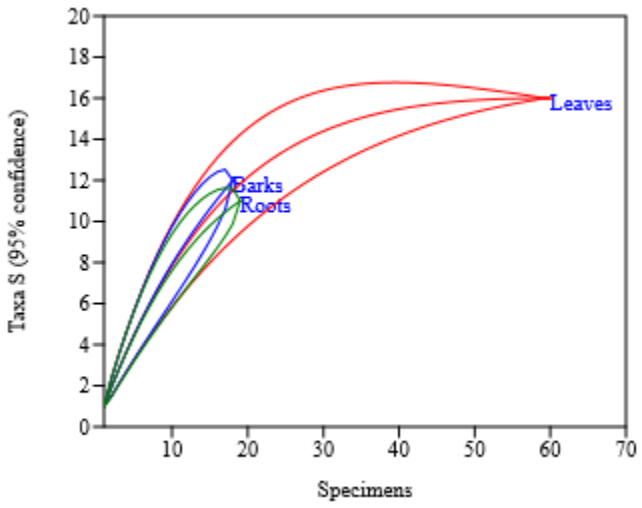


Figure 4.6: Diversity of isolated fungal endophytes in leaves, barks and roots of *Croton macrostachyrus* host based on individual rarefaction.

4.7: Diversity of isolated fungal endophytes in *Zehneria scabra*

In *Zehneria scabra*, 61.33% of the isolated fungal endophytes were from leaves, 18.67% from stem and 20% from roots (Table 4.6). A total of 17 fungal species were isolated from leaves, 11 species from stem and 13 species from roots. The highest species dominance was recorded in stem with 0.102 followed by roots with 0.08444 and leaves with 0.06994. The Simpson (1-D) index showed that leaves had the highest diversity with 0.9301 followed by roots with 0.9156 and stem with 0.898. On the other hand, Shannon (H) diversity index showed that the highest diversity was in leaves with 2.74, followed by roots with 2.523. On evenness, roots recorded the highest species evenness with 0.9591, followed by stem with 0.9456 and leaves with 0.9111 (Table 4.6)

Table 4.6: Diversity indices of isolated fungal endophytes in *Zehneria scabra* host

	Leaves	**Stem**	**Roots**
Taxa_S	17	11	13
Individuals	46	14	15
Dominance_D	0.06994	0.102	0.08444
Simpson_1-D	0.9301	0.898	0.9156
Shannon_H	2.74	2.342	2.523
Evenness_e^H/S	0.9111	0.9456	0.9591

Key:

4.7.1 Individual rarefaction in Zehneria scabra host

In *Zehneria scabra*, the diversity in leaves was high with increase in species taxa as the sample size increased. In contrast, in roots and stems the species taxa increased with decrease in the samples (Figure 4.10).

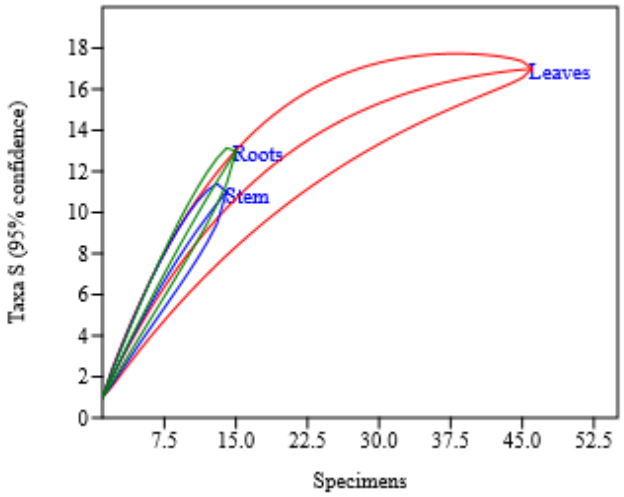


Figure 4.7: Diversity of the isolated fungal endophytes in leaves, roots and stems of *Zehneria scabra* based on Individual rarefaction

4.8 Antimicrobial activity of the isolated fungal endophytes extracts

Out of the 30 morphological groups subjected to fermentation, extracts from 18 isolates (63.3%) showed activity against at least two test organisms used in the study. Out of the 18

active isolates, extracts from 17 active isolates (94.44%) inhibited *Bacillus subtilis* while 12 isolates (63.1%) inhibited *Staphylococcus aureus* and six isolates (31.6%) inhibited *E. coli*. However, none of the active fungal isolates inhibited *Candida albicans*.

4.8.1 Antimicrobial activity of the isolated fungal endophyte extracts in disk diffusion method

In disk diffusion, there was significant difference between the isolate extracts at Df=19, f = 46.19, p = 0.0001. In *Bacillus subtilis*, extracts from isolates MR8, MR25, MR26, MR28 and MR29 (Plate 4.9) showed high antimicrobial activity with mean zones of inhibition ranging from 13.00 mm to 15.67 mm while the lowest inhibition was from extracts MR3, MR6 and MR7 with mean zones of inhibitions close to that of control. In *Staphylococcus aureus* extracts from isolates MR22, MR26, MR28, and MR29 (Plate 4.9) showed high antibacterial activity with mean zones of inhibition ranging from 12 mm to 18 mm. In *E. coli*, extracts from isolates MR8, MR19, MR20 and MR28 showed high antibacterial activity with mean zones of inhibition ranging from 8.00mm to 11 mm. However, none of the fungal extracts inhibited *Candida albicans* (Table 4.7). Additionally, there was significant difference between the test organisms at Df=3, f = 484.93, p = 0.0001 with *Bacillus subtilis* being the most inhibited test organism with mean zones of inhibition of 11. 00 followed by *Staphylococcus aureus* with 9.63 mm and *E. coli* with 6.67mm. There was significant interaction at Df=55, f = 21.02, p = 0.0001 between the isolate extracts and the test organisms in determining the antimicrobial activity (Table 4.7).

Table 4.7: Antimicrobial activity of isolated fungal endophyte extracts in disk diffusion method

		Zones of inhibition			
Isolate Extracts	**Isolate source**	***Bacillus subtilis* ATCC 23857**	***Staphylococcus aureus* ATCC 977**	***E. coli* ATCC 35218**	***Candida albicans* ATCC 14053**
MR3	ZsL	6.67 ± 0.33^{ef}	6.00 ± 0.00^{g}	6.00 ± 0.00^{c}	6.00±0.00
MR6	ZsR	6.66 ± 0.33^{ef}	7.33 ± 0.88^{fg}	6.00 ± 0.00^{c}	6.00±0.00
MR7	CmL	6.00 ± 0.00^{f}	6.00 ± 0.00^{g}	6.00 ± 0.00^{c}	6.00±0.00
MR8	Cml	15.67 ± 0.67^{a}	6.00 ± 0.00^{g}	8.33 ± 1.33^{abc}	6.00±0.00
MR10	ZsS	9.67 ± 1.86^{cdef}	6.00 ± 0.00^{g}	6.00 ± 0.00^{c}	6.00±0.00
MR11	CmL	12.67 ± 0.88^{abc}	9.00 ± 0.00^{ef}	6.00 ± 0.00^{c}	6.00±0.00
MR13	CmB	11.33 ± 0.88^{bcd}	6.00 ± 0.00^{g}	6.00 ± 0.00^{c}	6.00±0.00
MR14	ZsS	8.00 ± 1.00^{def}	6.00 ± 0.00^{g}	6.00 ± 0.00^{c}	6.00±0.00
MR18	CmB	11.33 ± 0.33^{bcd}	10.33 ± 0.33^{de}	7.67 ± 0.88^{bc}	6.00±0.00
MR19	CmB	10.33 ± 0.33^{bcde}	11.00 ± 0.58^{cde}	8.00 ± 0.00^{bc}	6.00±0.00
MR20	ZsR	10.67 ± 0.33^{bcd}	12.33 ± 0.33^{cd}	8.67 ± 0.67^{ab}	6.00±0.00
MR21	CmR	11.33 ± 1.20^{bcd}	6.00 ± 0.00^{g}	6.00 ± 0.00^{c}	6.00±0.00
MR22	ZsR	12.67 ± 0.33^{abc}	12.67 ± 0.33^{c}	6.00 ± 0.00^{c}	6.00±0.00
MR23	CmL	12.00 ± 0.58^{abc}	11.00 ± 0.58^{cde}	6.00 ± 0.00^{c}	6.00±0.00
MR25	ZsL	13.00 ± 0.00^{abc}	7.00 ± 0.00^{fg}	6.00 ± 0.00^{c}	6.00±0.00
MR26	CmB	14.00 ± 0.58^{ab}	18.00 ± 0.00^{a}	6.00 ± 0.00^{c}	6.00±0.00
MR28	ZsS	14.00 ± 0.58^{ab}	16.00 ± 1.00^{ab}	10.67 ± 0.88^{a}	6.00±0.00
MR29	ZsR	13.33 ± 0.33^{abc}	15.33 ± 0.33^{b}	6.00 ± 0.00^{c}	6.00±0.00
Control		6.00 ± 0.00^{f}	6.00 ± 0.00^{g}	6.00 ± 0.00^{c}	6.00±0.00
P values					
Endophyte extracts		<.0001	<.0001	<.0001	>0.05
Test organisms		<.0001	<.0001	<.0001	>0.05
Endophytes extracts* Test organisms		<.0001	<.0001	<.0001	>0.05

Key: Values with the same superscript$^{(a\text{-}j)}$ within the columns are not significantly different from each other according to Tukey's Honest Significant Difference (HSD) at $P<0.05$. ZsL-*Zehneria scabra* leave, ZsR- *Zehneria scabra* root, ZsS- *Zehneria scabra* stem, CmL-*Croton macrostachyrus* leave, CmR-*Croton macrostachyrus* root, and CmB-*Croton macrostachyrus* bark

4.8.2 Antimicrobial activity of the isolated fungal endophyte extracts in well diffusion method

In well diffusion method, there was significant difference between the isolate extracts at Df=19, f = 58.52, p = 0.0001. Extracts from isolates MR19, MR22, MR26, MR28 and MR29 showed high antibacterial activity against *Bacillus subtilis* test organism with mean

zones of inhibition ranging from 14.00 mm to 17 mm while the lowest inhibition was from extracts MR3, MR10 and MR13 with mean zones of inhibitions close to that of control. In *Staphylococcus aureus* extracts from isolates MR22, MR26, MR28, and MR29 (Plate 4.9) showed high antibacterial activity with mean zones of inhibition ranging from 18 mm to 19 mm. In *E. coli*, extracts from isolates MR18, MR20, MR25 and MR26 showed high antibacterial activity with mean zones of inhibition ranging from 8.00 mm to 11 mm. However, none of the fungal extracts inhibited *Candida albicans* (Table 4.8). Additionally, there was significant difference between the test organisms at Df=3, f = 451.49, p = 0.0001 with *Bacillus subtilis* being the most inhibited test organism with mean zones of inhibition of 12.12 mm followed by *Staphylococcus aureus* with 11.05 mm and *E. coli* with 7.40 mm respectively. There was significant interaction at Df=55, f = 18.01, p = 0.0001 between the isolate extracts and the test organisms in determining the antimicrobial activity (Table 4.8).

Table 4.8: Antimicrobial activity of the isolated fungal endophyte extracts in well diffusion method

		Zones of inhibition			
Isolate Extracts	**Isolate source**	***Bacillus subtilis* ATCC 23857**	***Staphylococcus aureus* ATCC 977**	***E. coli* ATCC 35218**	***Candida albicans* ATCC 14053**
MR3	ZsL	9.00 ± 1.00^{de}	6.00 ± 0.00^{g}	6.00 ± 0.00^{b}	6.00 ± 0.00
MR6	ZsR	13.00 ± 0.58^{abcd}	11.00 ± 1.00^{de}	6.00 ± 0.00^{b}	6.00 ± 0.00
MR7	CmL	10.00 ± 0.58^{cde}	6.00 ± 0.00^{g}	6.00 ± 0.00^{b}	6.00 ± 0.00
MR8	CmL	11.33 ± 0.88^{bcd}	6.00 ± 0.00^{g}	6.00 ± 0.00^{b}	6.00 ± 0.00
MR10	ZsS	6.00 ± 0.00^{e}	6.00 ± 0.00^{g}	6.00 ± 0.00^{b}	6.00 ± 0.00
MR11	CmL	15.00 ± 0.58^{ab}	9.33 ± 0.33^{ef}	6.00 ± 0.00^{b}	6.00 ± 0.00
MR13	CmB	6.00 ± 0.00^{e}	6.00 ± 0.00^{g}	6.00 ± 0.00^{b}	6.00 ± 0.00
MR14	ZsS	6.00 ± 0.00^{e}	6.00 ± 0.00^{g}	6.00 ± 0.00^{b}	6.00 ± 0.00
MR18	CmB	12.33 ± 0.33^{abcd}	12.67 ± 0.33^{cd}	11.00 ± 0.58^{a}	6.00 ± 0.00
MR19	CmB	16.33 ± 1.86^{a}	13.00 ± 0.58^{cd}	9.00 ± 0.58^{ab}	6.00 ± 0.00
MR20	ZsR	14.67 ± 0.88^{ab}	14.67 ± 0.88^{c}	10.00 ± 1.15^{a}	6.00 ± 0.00
MR21	CmR	9.00 ± 1.00^{de}	6.00 ± 0.00^{g}	6.00 ± 0.00^{b}	6.00 ± 0.00
MR22	ZsR	16.00 ± 1.15^{a}	18.00 ± 1.15^{ab}	9.33 ± 0.88^{a}	6.00 ± 0.00
MR23	CmL	14.00 ± 0.00^{abc}	11.00 ± 1.00^{de}	6.00 ± 0.00^{b}	6.00 ± 0.00
MR25	ZsL	13.33 ± 1.20^{abcd}	7.67 ± 0.88^{fg}	10.00 ± 0.58^{a}	6.00 ± 0.00
MR26	CmB	14.68 ± 0.33^{ab}	19.00 ± 0.58^{a}	9.33 ± 0.33^{a}	6.00 ± 0.00
MR28	ZsS	15.33 ± 0.88^{ab}	18.33 ± 0.67^{a}	8.00 ± 1.53^{ab}	6.00 ± 0.00
MR29	ZsR	15.33 ± 0.88^{ab}	18.33 ± 0.88^{a}	6.00 ± 0.00^{b}	6.00 ± 0.00
Control		6.00 ± 0.00^{e}	6.00 ± 0.00^{g}	6.00 ± 0.00^{b}	6.00 ± 0.00
P values of the main effects and their interaction					
Endophyte extracts		<.0001	<.0001	<.0001	>0.05
Test organisms		<.0001	<.0001	<.0001	>0.05
Endophyte extract*Test organisms		<.0001	<.0001	<.0001	>0.05

Key: Values with the same superscript$^{(a\text{-}j)}$ within the columns are not significantly different from each other according to Tukey's Honest Significant Difference (HSD) at $P<0.05$. ZsL-*Zehneria scabra* leave, ZsR- *Zehneria scabra* root, ZsS- *Zehneria scabra* stem, CmL-*Croton macrostachyrus* leave, CmR-*Croton macrostachyrus* root, and CmB-*Croton macrostachyrus* bark

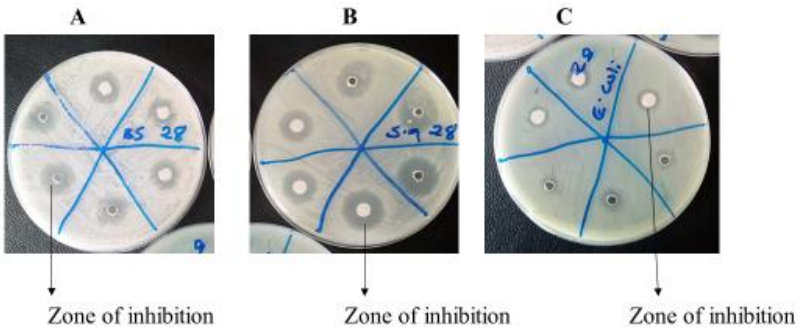


Plate 4.9: Antibacterial activity of extracts obtained from isolate MR28 extracts in disk and well diffusion methods. A-*Bacillus subtilis*, B- S*taphylococcus aureus* and C- *E. coli*.

4.9 Molecular identification of the isolates

4.9.1 Gel electrophoresis

Gel electrophoresis was done to confirm if the genomic DNA extraction was successful. Also, it was used to confirm the quality of the genomic DNA. Band size of approximately 3000 base pairs on the ladder showed that genomic DNA was in the right quality for PCR (Plate 4.10)

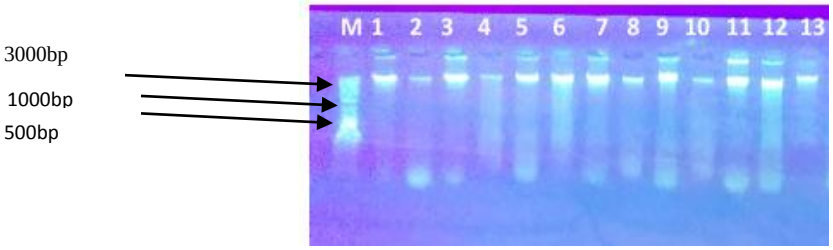


Plate 4.10: Gel electrophoresis of genomic DNA of the endophytic fungal isolates on 1% agarose gel at 80 Volts for 30 minutes. M-Quick-load Purple 2-log DNA ladder (Biolabs).

4.9.2 Amplified DNA product

Full length of the genomic DNA extracted from the 18 active endophytic isolates were amplified by PCR using IT1 and IT4 primers. The amplified PCR products was between 500bp and 1000bp in size (Plate 4.11)

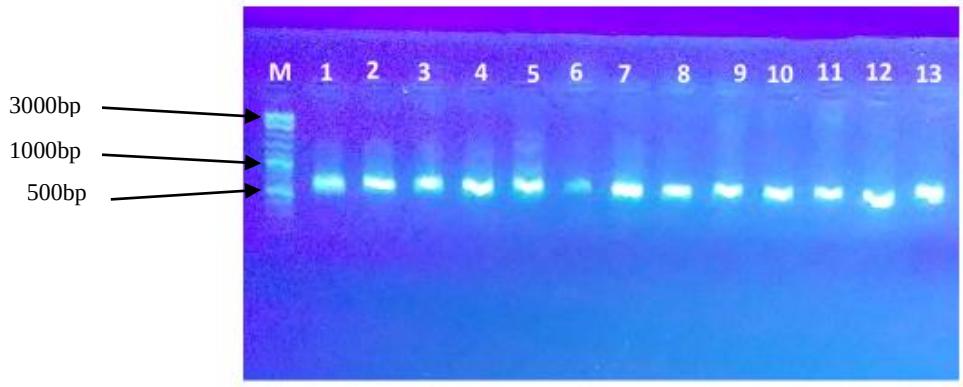


Plate 4.11: Gel electrophoresis of PCR amplified ITS of the endophytic isolates on 1% agarose gel at 80 volts for 40 minutes. Lanes 1-13- amplified ITS of 13 isolates. M-Quick-load Purple 2-log DNA ladder (Biolabs)

4.9.3 Phylogenetic analysis

The isolated fungal endophytes were identified based on ITS sequences and their closest neighbors retrieved from NCBI database. The BLAST analysis showed that majority of the isolated fungal isolates displayed diverse taxonomic affinities. The phylogenetic analysis based on Neighbor-joining showed that that the fungal isolates could be placed in phylum Ascomycota and three taxonomic subclasses; hyphomycetes, coelomycetes, and ascomycetes. In addition, the isolated fungal endophytes were in ten taxonomic genera including; *Fusarium, Chaetomium, Clonostachys, Meyerozyma, Aporospora, Lasiodiplodia, Acremoninum, Penicillium, Aspergillus, Laptophaeria, Phyllosticta* and *Ampelomycetes* (Figure 4.8*)*. The phylogenetic tree based on ITS1 and ITS 4 showed that fungal isolates were in two major Clades (Clade I and Clade II). Clade I was comprised of isolates closely related to *Fusarium,* and *Acremonium, Chaetomium,* and *Clonostachys* species. Clade I had 11 isolates and eight of them were in genera *Fusarium* while 3 were from genera *Clonostachys, Chaetomium,* and *Meyerozyma*. Isolate MR3 was isolated from *Zehneria*

scabra leave and was closely related to *Fusarium solani* with similarity index of 99.63%. The second isolate within clade I is MR7 which was closely related to *Fusarium solani* with similarity index of 99.44% and was isolated from *Croton macrostachyrus* leaves. Also, isolate MR8 was isolated from *Croton macrostachyrus* leaf and was closely related to *Fusarium solani* with similarity index of 96.41%. In the same host plant, isolate MR11 was isolated from the leaf and was closely related to *Acremonium sp.* with similarity index of 99.46% (Appendix 4).

Isolate MR13 was isolated from *Croton macrostachyrus* bark and was closely related to *Fusarium oxysporium* with similarity index of 94.57%. Isolate MR18 which was closely related to *Fusarium solani* with 100% similarity index and was isolated from *Croton macrostachyrus* bark. In addition, isolate MR20 was isolated from the *Zehneria scabra* root and was closely related to *Fusarium solani* with similarity index of 99.46%. Also, isolate MR29 which was closely related to *Fusarium solani* with similarity index of 99.64% was isolated from roots of *Zehneria scabra* (Appendix 4).

Apart from isolates in the genera *Fusarium*, three isolates from genera *Clonostachys, Chaetomium* and *Meyerozyma* clustered in clade I. Isolate MR21 was isolated from *Croton macrostachyrus* root and was closely related to *Clonostachys rosea* with similarity index of 100%. In addition, isolate MR22 was isolated from *Zehneria scabra* root and was closely related to *Chaetomium globosum* with similarity index of 98.96%. Lastly in clade I is isolate MR23 which was isolated from *Croton macrostachyrus* leave and was closely related to *Meyerozyma guilliermondii* with similarity index of 93.35% (Appendix 4).

Clade II was comprised of 7 isolates from genera *Leptospaheria, Ampelomyces, Aporospora, Lasiodiplodia, Phyllostica*, *Penicillium,* and *Aspergillus*. All the isolates in this clade were from different genera (Figure 4.8). Isolate MR14 was isolated from *Zehneria scabra* stem and was closely related to *Lasiodiplodia theobromae* with similarity index of 96.67%. Isolate MR25 was isolated from *Zehneria scabra* leaf and was closely related to *Phyllosticta citricarpa* with similarity index of 100%. Isolate MR6 was isolated from *Zehneria scabra root* and was closely related to *Aspergillus niger* with similarity index of 97.79%. Also, isolate MR10 was isolated from *Zehneria scabra* stem and was closely related to *Penicillium chrysogenum* with similarity index of 90.73%. Furthermore, isolate MR19 was isolated from *Croton macrostachyrus* bark and was closely related to *Aporospora terricola* with similarity index of 98.17%. Moreover, isolate MR26 was isolated from *Croton macrostachyrus* bark and was closely related to *Ampelomyces Sp*. with similarity index of 99.41%. In clade II, isolate MR28 which was isolated from *Zehneria scabra* stem and was closely related to *Leptosphaeria spegazzanii* with similarity index of 98.24%

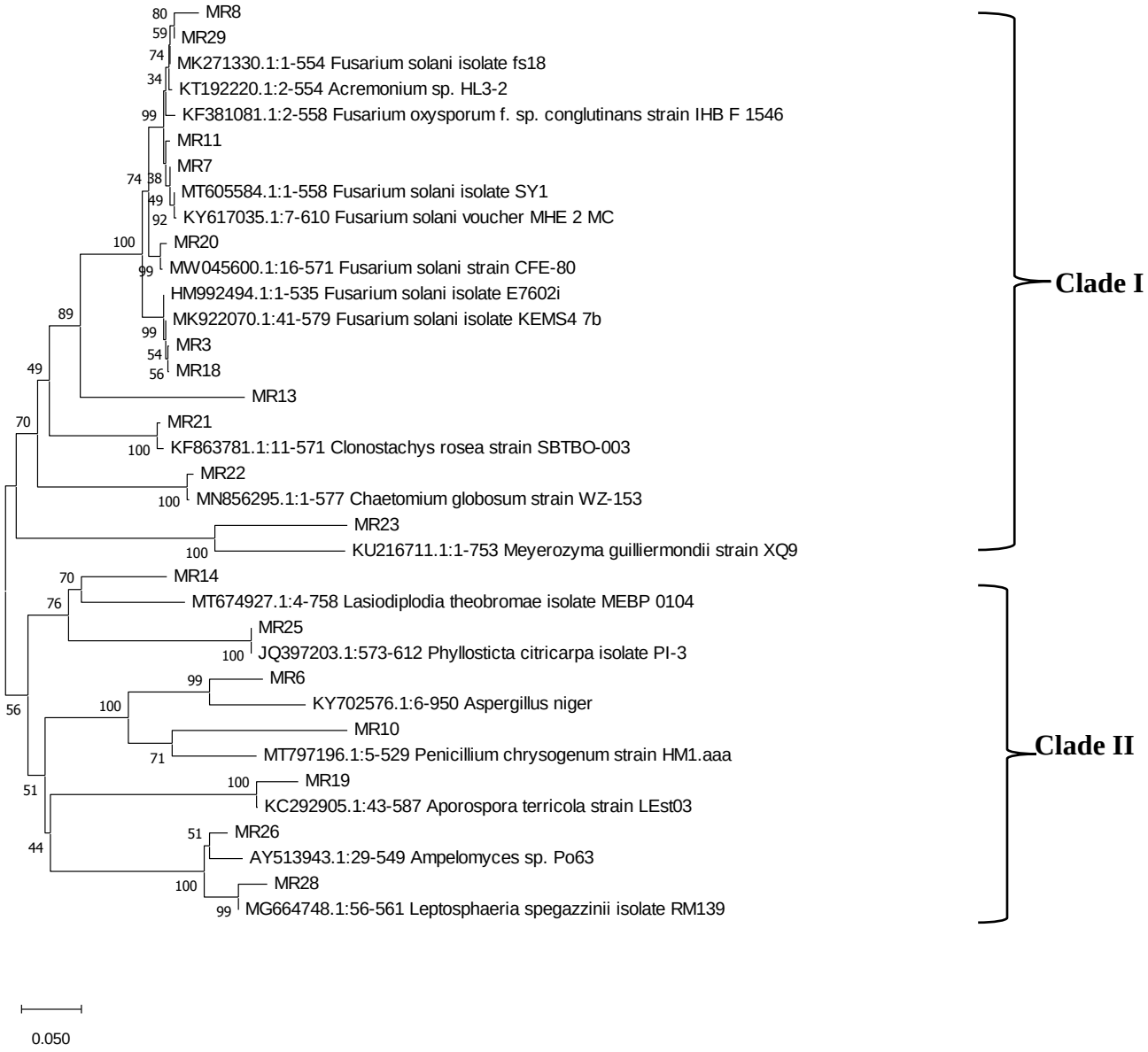


Figure 4.8: Neighbor-joining Phylogenetic tree showing the identity of the 18 active isolates from *Croton macrostachyrus* and *Zehneria scabra* hosts based on ITS gene. The values at each node represent the bootstrap values from 1000 replicates. The scale bar represents 0.05 substitutions per nucleotide.

4.10 Identification of compounds obtained from ethyl acetate extracts of the isolated fungal endophytes using Gas Chromatography-Mass spectrophotometry

In this study, the identified fungal endophytes were fermented and the bioactive metabolites from the isolated fungal endophyte extracts were obtained using ethyl acetate solvent. The

ethyl acetate extracts from these isolates showed diverse compounds from different chemical classes. This included compounds in the chemical classes; alkane, primary alcohols, phenols, monoterpenoids and long-chain fatty acid.

Based on the GC-MS- analysis, the bioactive metabolite profile of ethyl acetate extracts from isolate MR8 isolated from *Croton macrostachyrus* and closely related to *Fusarium solani* (96.41%), showed that the ethyl acetate extracts of this isolate had compounds in the chemical class phenols, benzyl alcohol, primary alcohols, long chain fatty acids, alkanes, and alkylphenylketone. When compared to other *Fusarium* isolates in this study, the only unique compound from the ethyl acetate extracts of this isolate is Ethanone,1-(2-hydroxyphenyl)- (Table 4.9)

Table 4.9 Compounds identified from the ethyl acetate extracts of obtained from isolate MR8 isolated from *Croton macrostachyrus* leave and closely related to *Fusarium solani* with similarity index of 96.41%

S/N	Compound	Retention time(min)	ID	Molecular weight(g/mol)	Molecular formula	Chemical class
1	Benzyl Alcohol	12.5536	100-51-6	108.14	C_7H_8O	Benzyl alcohols
2	1-propanol,2-methyl	5.0538	78-83-1	89.14	$C_4H_{10}O$	Primary alcohols
3	1-Butanol,3-methyl-	6.5855	123-51-3	88.15	$C_5H_{12}O$	Primary alcohols
4	Phenol	11.2209	108-95-2	94.11	C_6H_6O	Phenols
5	Phenol,4-methyl-	12.9889	106-44-5	296.4	$C_{21}H_{28}O$	Phenols
6	Dodecane	15.1019	112-40-3	170.33	$C_{12}H_{26}$	Alkanes
7	1-Tetradecanol	17.9580	112-72-1	214.39	$C_{14}H_{30}O$	Long-chain Fatty acid alcohols
8	Tetradecane	18.0575	629-59-4	198.39	$C_{14}H_{30}$	Alkanes
9	1-Hexadecanol	20.5923	36653-82-4	242.44	$C_{16}H_{34}O$	Long-chain Fatty alcohols
10	1-Octadecanol	22.9468	112-92-5	270.49	$C_{20}H_{43}NO_3$	Long-chain fatty alcohols
11	Octadecane	23.0111	593-45-3	245.5	$C_{18}H_{38}$	Alkanes
12	Ethanone,1-(2-hydroxyphenyl)-	18.7208	118-93-4	136.14	$C_8H_8O_2$	Alkyl phenylketones
13	Benzeneacetamide	18.2140	103-81-1	135.16	C_8H_9NO	Benzene

Key: ID- GC-MS NIST library identification number, min-Minutes, g/mol- grams per mole.

The GC-MS analysis of compounds from the ethyl acetate extracts of isolate MR18 showed compounds in the chemical classes; primary alcohols, Carboxylic acids, benzaldehydes, unsaturated aliphatic hydrocarbons, long chain fatty acids, ketones, monoterpenoids and

pyridazines (Table 4.10). The unique compounds from this isolate that were not in the ethyl acetate extracts of other isolates are propanoic acid, Maleic hydrazide, and 1-Tridecene.

Table 4.10: Compounds identified from the ethyl acetate extracts obtained from isolate MR18 isolated from *Croton macrostachyrus* bark and closely related to *Fusarium solani* with similarity index of 100%

S/N	Compound	Retention time(min)	ID	Molecular weight (g/mol)	Molecular formula	Chemical class
1	1-propanol,2-methyl	5.0538	78-83-1	89.14	$C_4H_{10}O$	Primary alcohols
2	Propanoic acid, anhydride	6.1139	123-62-6	130.14	$C_6H_{10}O_3$	Carboxylic acid
3	1-Butanol,3-methyl-	6.5855	123-51-3	88.15	$C_5H_{12}O$	Primary alcohols
4	Phenol	11.2209	108-95-2	94.11	C_6H_6O	Phenols
5	Benzaldehyde	11.2758	100-52-7	106.12	C_7H_6O	Benzaldehyde
6	Benzoic acid	14.3091	65-85-0	122.12	$C_6H_7O_2$	Benzoic acids
7	1-Tridecene	24.1459	2437-56-1	182.35	$C_{13}H_{26}$	Unsaturated aliphatic hydrocarbons
8	n-Hexadecanoic acid	24.7044	57-10-3	256.42	$C_{16}H_{32}O_2$	Long-chain fatty acids
9	Benzaldehyde,4-hydroxy-	17.4936	123-08-0	122.12	C_7H_6O	Hydroxybenzaldehyde
10	Acetic acid,2-methylpropyl ester	7.2808	110-19-0	116.16	$C_6H_{12}O_2$	Carboxylic acid esters
11	Acetic acid,1-methylpropyl ester	7.2860	105-46-4	116.16	$C_6H_{12}O_2$	Carboxylic acid esters
12	Camphene	11-1317	79-92-5	136.23	$C_{10}H_{16}$	Bicyclic monoterpenoids
13	Naphthalene	15.4427	91-202-3	128.17	$C_{10}H_8$	Naphalenes
14	Benzeneacetic acid	15.6728	103-82-2	136.15	$C_9H_{10}O_2$	Benzene
15	Acetophenone,2-chloro	6.3119	532-27-4	154.59	C_8H_7ClO	Ketone
16	Hexanoic acid	10.8831	142-62-1	116.16	$C_6H_{12}O_2$	Saturated fatty acid
17	Maleic	14.7321	123-33-	112.09	$C_4H_4N_2O_2$	Pyridazines

hydrazide	1	

Key: ID-GC-MS NIST library identification number, min-Minutes, g/mol- grams per mole.

Additionally, the ethyl acetate extracts from isolate MR19 closely related to *Aporospora terricolla* had the highest number of bioactive metabolites when compared to other isolates ethyl acetate extracts. The compounds included were chemical classes; Benzyl alcohols, primary alcohols, styrenes, phenols, Benzoic acids, carboxylic acids, alkanes, Phthalates, chlorobenzenes, bicyclic monoterpenoids, long chain fatty acids naphalenes and ketones (Table 4.11). In addition, the ethyl acetate extracts from this isolate had different compounds when compared to ethyl acetate extracts from other isolates in this study. These compounds include; Ethanone,1-(4-hydroxy-3-methoxyphenyl)-, Acetophenone,2-chloro, Cyclohexanone, Bis(2-ethylhexyl) phthalate, Benzothiazole and Ethanone,1-(2,5-dimethoxyphenyl)- (Table 4.11).

Table 4.11: Compounds identified from ethyl acetate extracts obtained from isolate MR19 isolated from *Croton macrostachyrus* bark to closely related *Aporospora terricola* with similarity index of 98.17%

S/N	Compound	Retention time(min)	ID	Molecular weight(g/mol)	Molecular formula	Chemical class
1	Benzyl Alcohol	12.5536	100-51-6	108.14	C_7H_8O	Benzyl alcohols
2	1-propanol,2-methyl	5.0538	78-83-1	89.14	$C_4H_{10}O$	Primary alcohols
3	1-Butanol,3-methyl-	6.5855	123-51-3	88.15	$C_5H_{12}O$	Primary alcohols
4	Styrene	9.8809	100-42-5	104.15	C_8H_8	Styrenes
5	Phenol	11.2209	108-95-2	94.11	C_6H_6O	Phenols
6	Benzaldehyde	11.2758	100-52-7	106.12	C_7H_6O	Benzaldehyde
7	Phenol,4-methyl-	12.9889	106-44-5	296.4	$C_{21}H_{28}O$	Phenols
8	Benzoic acid	14.3091	65-85-0	122.12	$C_6H_7O_2$	Benzoic acids
9	Pentadecane	16.2761	629-62-9	72.15	C_5H_{12}	Alkane
10	1-Tetradecanol	17.9580	112-72-1	214.39	$C_{14}H_{30}O$	Long-chain Fatty acid alcohols
11	Tetradecane	18.0575	629-59-4	198.39	$C_{14}H_{30}$	Alkanes
12	1-Hexadecanol	20.5923	36653-82-4	242.44	$C_{16}H_{34}O$	Long-chain Fatty alcohols
13	Hexadecane	20.6721	544-76-3	226.44	$C_{16}H_{34}$	Alkanes
14	1-Octadecanol	22.9468	112-92-5	270.49	$C_{20}H_{43}NO_3$	Long-chain fatty alcohols
15	Octadecane	23.0111	593-45-3	245.5	$C_{18}H_{38}$	Alkanes
16	1-Tridecene	24.1459	2437-56-1	182.35	$C_{13}H_{26}$	Unsaturated aliphatic hydrocarbons
17	n-Hexadecanoic acid	24.7044	57-10-3	256.42	$C_{16}H_{32}O_2$	Long-chain fatty acids
18	Benzaldehyde, 4-hydroxy-	17.4936	123-08-0	122.12	C_7H_6O	Hydroxybenzaldehyde

Table 4.12: Compounds identified from ethyl acetate extracts obtained from isolate MR19 isolated from *Croton macrostachyrus* bark to closely related *Aporospora terricola* with similarity index of 98.17% **(Continuation)**

19	Acetic acid,2-methylpropyl ester	7.2808	110-19-0	116.16	$C_6H_{12}O_2$	Carboxylic acid esters
	Acetic acid,1-methylpropyl ester	7.2860	105-46-4	116.16	$C_6H_{12}O_2$	Carboxylic acid esters
20	Camphene	11-1317	79-92-5	136.23	$C_{10}H_{16}$	Bicyclic monoterpenoids
21	Naphthalene	15.4427	91-202-3	128.17	$C_{10}H_8$	Naphalenes
22	Benzeneacetic acid	15.6728	103-82-2	136.15	$C_9H_{10}O_2$	Benzene
23	Ethanone,1-(4-hydroxy-3-methoxyphenyl)-	20.1727	498-02-2	182.17	$C_9H_{10}O_4$	Alkyl phenyl ketones
24	Acetophenone,2-chloro	6.3119	532-27-4	154.59	C_8H_7ClO	Ketone
25	Hexanoic acid	10.8831	142-62-1	116.16	$C_6H_{12}O_2$	Saturated fatty acid
26	n-Hexadecanoic acid	24.7034	57-10-3	253.4	$C_{16}H_{29}O_2^-$ 3	Long-chain saturated fatty acid
27	Benzeneacetamide	18.2140	103-81-1	135.16	C_8H_9NO	Benzene
28	Bis(2-ethylhexyl) phthalate	24.0038	117-81-7	390.6	$C_{24}H_{38}O_4$	Phthalates
29	Benzene,chloro-	9.0210	108-90-7	112.55	C_6H_5Cl	Chlorobenzenes
30	Ethanone,1-(2,5-dimethoxyphenyl)-	20.1778	1201-38-3	212.20	$C_{10}H_{12}O_5$	Alkyl phenylketones
31	Cyclohexanone	9.9556	108-94-1	98.14	$C_6H_{10}O$	Ketone
32	Benzothiazole	16.0616	95-16-9	135.19	C_7H_5NS	Benzothiazoles

Key: ID-GC-MS NIST library identification number, min-Minutes, g/mol- grams per mole.

The ethyl acetate extracts from this isolated showed compound in the chemical classes Benzyl alcohols, primary alcohols, alkanes, monocyclic monoterpenoids, and long chain fatty acids, (Table 4.12). When compared to other *Fusarium* isolates, the unique compounds

from this isolate were; 2,5-cyclohexadiene-1,4-dione,2,6-bis(1,1-dimethylethyl)-, Maleic hydrazide, Pentanoic acid and Benzoic acid, 4-methyl-,methyl ester.

Table 4.13: Compounds from the ethyl acetate extracts obtained from isolate MR20 isolated from *Zehneria scabra* root closely related to *Fusarium solani* with similarity index of 99.46%

S/N	Compound	Retention time(min)	ID	Molecular weight(g/mol)	Molecular formula	Chemical class
1	Benzyl Alcohol	12.5536	100-51-6	108.14	C_7H_8O	Benzyl alcohols
2	1-propanol,2-methyl	5.0538	78-83-1	89.14	$C_4H_{10}O$	Primary alcohols
3	1-Butanol,3-methyl-	6.5855	123-51-3	88.15	$C_5H_{12}O$	Primary alcohols
4	Styrene	9.8809	100-42-5	104.15	C_8H_8	Styrenes
5	Phenol	11.2209	108-95-2	94.11	C_6H_6O	Phenols
6	Benzaldehyde	11.2758	100-52-7	106.12	C_7H_6O	Benzaldehyde
7	Fumaric acid	14.2003	110-17-8	116.07	$C_4H_4O_4$	Dicarboxylic acids
8	Dodecane	15.1019	112-40-3	170.33	$C_{12}H_{26}$	Alkanes
9	Pentadecane	16.2761	629-62-9	72.15	C_5H_{12}	Alkane
10	1-Tetradecanol	17.9580	112-72-1	214.39	$C_{14}H_{30}O$	Long-chain Fatty acid alcohols
11	1-Hexadecanol	20.5923	36653-82-4	242.44	$C_{16}H_{34}O$	Long-chain Fatty alcohols
12	Hexadecane	20.6721	544-76-3	226.44	$C_{16}H_{34}$	Alkanes
13	1-Octadecanol	22.9468	112-92-5	270.49	$C_{20}H_{43}NO_3$	Long-chain fatty alcohols
14	Octadecane	23.0111	593-45-3	245.5	$C_{18}H_{38}$	Alkanes
15	1-Tridecene	24.1459	2437-56-1	182.35	$C_{13}H_{26}$	Unsaturated aliphatic hydrocarbons
16	n-Hexadecanoic acid	24.7044	57-10-3	256.42	$C_{16}H_{32}O_2$	Long-chain fatty acids
17	Benzaldehyde,4-hydroxy-	17.4936	123-08-0	122.12	C_7H_6O	Hydroxybenzaldehyde
18	Acetic acid,2-methylpropyl ester	7.2808	110-19-0	116.16	$C_6H_{12}O_2$	Carboxylic acid esters
19	Acetophenone,2-chloro	6.3119	532-27-4	154.59	C_8H_7ClO	Ketone
20	n-Hexadecanoic acid	24.7034	57-10-3	253.4	$C_{16}H_{29}O_2^{-3}$	Long-chain saturated fatty acid
21	Benzeneacetic acid	15.8926	103-82-2	244. 08	$C_9H_{10}BrNO_2$	Monocarboxylic acid amide

Key: ID-GC-MS NIST library identification number, min-Minutes, g/mol- grams per mole.

Table 4.14: Compounds identified from the ethyl acetate extracts obtained from isolate MR20 isolated from *Zehneria scabra* root closely related to *Fusarium solani* with similarity index of 99.46%. (**Continuation**)

22	Octadecanoic acid	27.4465	57-11-4	284.5	$C_{18}H_{36}O_2$	Fatty acids
23	Pentanoic acid	10.8831	109-52-4	130.13	$C_5H_{10}O_2$	Straight chain saturated fatty acids
24	2,5-cyclohexadiene-1,4-dione,2,6-bis(1,1-dimethylethyl)-	19.2368	719-22-2	220.31	$C_{14}H_{20}O_2$	Monocyclic monoterpenoids
25	Benzene,chloro-	9.0210	108-90-7	112.55	C_6H_5Cl	Chlorobenzenes
26	Maleic hydrazide	14.7321	123-33-1	112.09	$C_4H_4N_2O_2$	Pyridazines
27	Benzoic acid, 4-methyl-,methyl ester	15.4120	99-75-2	150.17	$C_9H_{10}O_2$	Benzoic acids

Key: ID-GC-MS NIST library identification number, min-Minutes, g/mol- grams per mole.

In this study, the ethyl acetate extracts obtained from isolate MR22 showed diverse bioactive compounds. When compared to other isolates, this isolate had unique compounds which include Acetic acid,2-methylpropyl ester, Benzeneacetic acid, Acetophenone,2-chloro, 2,5-cyclohexadiene-1,4-dione,2,6-bis(1,1-dimethylethyl)-, and Benzeneacetamide (Table 4.13).

Table 4.15: Compounds identified from the ethyl acetate extracts obtained from isolate MR22 isolated from *Zehneria scabra* root closely related to *Chaetomium globosum* with similarity index of 98.96%.

S/N	Compound	Retention time(min)	ID	Molecular weight(G/mol)	Molecular formula	Chemical class
1	Benzyl Alcohol	12.5536	100-51-6	108.14	C_7H_8O	Benzyl alcohols
2	Benzoic acid	14.3091	65-85-0	122.12	$C_7H_6O_2$	Benzoic acids
3	1-propanol,2-methyl	5.0538	78-83-1	89.14	$C_4H_{10}O$	Primary alcohols
4	Propanoic acid, anhydride	6.1139	123-62-6	130.14	$C_6H_{10}O_3$	Carboxylic acid
5	1-Butanol,3-methyl-	6.5855	123-51-3	88.15	$C_5H_{12}O$	Primary alcohols
6	Styrene	9.8809	100-42-5	104.15	C_8H_8	Styrenes
7	Phenol	11.2209	108-95-2	94.11	C_6H_6O	Phenols
8	Benzaldehyde	11.2758	100-52-7	106.12	C_7H_6O	Benzaldehyde
9	Dodecane	15.1019	112-40-3	170.33	$C_{12}H_{26}$	Alkanes
10	Pentadecane	16.2761	629-62-9	72.15	C_5H_{12}	Alkane
11	1-Tetradecanol	17.9580	112-72-1	214.39	$C_{14}H_{30}O$	Long-chain Fatty acid alcohols
12	Tetradecane	18.0575	629-59-4	198.39	$C_{14}H_{30}$	Alkanes
13	1-Hexadecanol	20.5923	36653-82-4	242.44	$C_{16}H_{34}O$	Long-chain Fatty alcohols
14	Hexadecane	20.6721	544-76-3	226.44	$C_{16}H_{34}$	Alkanes
15	1-Octadecanol	22.9468	112-92-5	270.49	$C_{20}H_{43}NO_3$	Long-chain fatty alcohols
16	1-Tridecene	24.1459	2437-56-1	182.35	$C_{13}H_{26}$	Unsaturated aliphatic hydrocarbons
17	n-Hexadecanoic acid	24.7044	57-10-3	256.42	$C_{16}H_{32}O_2$	Long-chain fatty acids

Key: ID-GC-MS NIST library identification number, min-Minutes, g/mol- grams per mole.

Table 4.16: Compounds identified from the ethyl acetate extracts obtained from isolate MR22 isolated from *Zehneria scabra* root closely related to *Chaetomium globosum* with similarity index of 98.96% (**Continuation)**

18	Benzaldehyde,4-hydroxy-	17.4936	123-08-0	122.12	C_7H_6O	Hydroxybenzaldehyde
19	Acetic acid,2-methylpropyl ester	7.2808	110-19-0	116.16	$C_6H_{12}O_2$	Carboxylic acid esters
20	Camphene	11-1317	79-92-5	136.23	$C_{10}H_{16}$	Bicyclic monoterpenoids
21	Naphthalene	15.4427	91-202-3	128.17	$C_{10}H_8$	Naphalenes
22	Acetophenone,2-chloro	6.3119	532-27-4	154.59	C_8H_7ClO	Ketone
23	Benzeneacetamide	18.2140	103-81-1	135.16	C_8H_9NO	Benzene
24	Benzeneacetic acid	15.8926	103-82-2	244. 08	$C_9H_{10}BrNO_2$	Monocarboxylic acid amide
25	2,5-cyclohexadiene-1,4-dione,2,6-bis(1,1-dimethylethyl)-	19.2368	719-22-2	220.31	$C_{14}H_{20}O_2$	Monocyclic monoterpenoids

Key: ID-GC-MS NIST library identification number, min-Minutes, g/mol- grams per mole.

The ethyl acetate extracts obtained from isolate MR23 closely related to *Meyerozyma guillermondii* showed different bioactive metabolites. These included Benzoic acid, Benzaldehyde,4-hydroxy-, 1-Butanol, 3-methyl-,acetate, Phenol, 4,4'-(1-methylethylidene)bis-, Acetophenone,2-chloro, 1,2-Benzenedicarboxylic acid,bis(2-methylpropyl) ester, Aziridine, 2-methyl-, Dibutyl phthalate, Ethanone,1-(2-hydroxyphenyl)-,Ethanone,1-(4-hydroxy-3-methoxyphenyl)-, Methane,isocyanato-, Methyl

vinyl ketone, Acetic acid,1-methylpropyl ester, and Acetic acid,2-methylpropyl ester (Table 4.14)

Table 4.17: Compounds identified from the ethyl acetate extracts obtained from isolate MR23 isolated from *Croton macrostachyrus* leave and closely related to *Meyerozyma guillermondii* with similarity index 93.35%

S/N	Compound	Retention time(min)	ID	Molecular weight(G/mol)	Molecular formula	Chemical class
2	Benzoic acid	14.3091	65-85-0	122.12	$C_7H_6O_2$	Benzoic acids
3	1-propanol,2-methyl	5.0538	78-83-1	89.14	$C_4H_{10}O$	Primary alcohols
4	Propanoic acid, anhydride	6.1139	123-62-6	130.14	$C_6H_{10}O_3$	Carboxylic acid
5	1-Butanol,3-methyl-	6.5855	123-51-3	88.15	$C_5H_{12}O$	Primary alcohols
6	Acetic acid, butyl ester	7.2829	123-86-4	116.16	$C_6H_{12}O_2$	Carboxylic acid esters
7	Styrene	9.8809	100-42-5	104.15	C_8H_8	Styrenes
8	Benzyl-chloride	11.1380	100-44-7	126.58	C_7H_7Cl	Benzene Chlorides
9	Phenol	11.2209	108-95-2	94.11	C_6H_6O	Phenols
10	Benzaldehyde	11.2758	100-52-7	106.12	C_7H_6O	Benzaldehyde
11	Phenol,4-methyl-	12.9889	106-44-5	296.4	$C_{21}H_{28}O$	Phenols
12	Fumaric acid	14.2003	110-17-8	116.07	$C_4H_4O_4$	Dicarboxylic acids

13	Benzoic acid	14.3091	65-85-0	122.12	$C_6H_7O_2$	Benzoic acids
14	Pentadecane	16.2761	629-62-9	72.15	C_5H_{12}	Alkane
15	1-Tetradecanol	17.9580	112-72-1	214.39	$C_{14}H_{30}O$	Long-chain Fatty acid alcohols
16	Tetradecane	18.0575	629-59-4	198.39	$C_{14}H_{30}$	Alkanes
17	1-Hexadecanol	20.5923	36653-82-4	242.44	$C_{16}H_{34}O$	Long-chain Fatty alcohols
18	Hexadecane	20.6721	544-76-3	226.44	$C_{16}H_{34}$	Alkanes

Table 4.18: Compounds identified from the ethyl acetate extracts obtained from isolate MR23 isolated from *Croton macrostachyrus* leave and closely related to *Meyerozyma guillermondii* with similarity index 93.35% (**continuation**)

S/N	**Compound**	**Retention time(min)**	**ID**	**Molecular weight(G/mol)**	**Molecular formula**	**Chemical class**
19	1-Octadecanol	22.9468	112-92-5	270.49	$C_{20}H_{43}NO_3$	Long-chain fatty alcohols
20	Octadecane	23.0111	593-45-3	245.5	$C_{18}H_{38}$	Alkanes
21	1-Tridecene	24.1459	2437-56-1	182.35	$C_{13}H_{26}$	Unsaturated aliphatic hydrocarbons
22	n-Hexadecanoic acid	24.7044	57-10-3	256.42	$C_{16}H_{32}O_2$	Long-chain fatty acids
23	Benzaldehyde,4-hydroxy-	17.4936	123-08-0	122.12	C_7H_6O	Hydroxybenzaldehyde

24	Acetic acid,2-methylpropyl ester	7.2808	110-19-0	116.16	$C_6H_{12}O_2$	Carboxylic acid esters
25	Acetic acid,1-methylpropyl ester	7.2860	105-46-4	116.16	$C_6H_{12}O_2$	Carboxylic acid esters
26	Camphene	11-1317	79-92-5	136.23	$C_{10}H_{16}$	Bicyclic monoterpenoids
27	Naphthalene	15.4427	91-202-3	128.17	$C_{10}H_8$	Naphalenes
28	Benzeneacetic acid	15.6728	103-82-2	136.15	$C_9H_{10}O_2$	Benzene
29	Methyl vinyl ketone	6.5959	78-74-4	70.09	C_4H_6O	Enones
30	Methane, isocyanato-	6.6768	624-83-9	58.06	C_2H_4NO -	Isocyanate
31	Ethanone,1-(4-hydroxy-3-methoxyphenyl)-	20.1727	498-02-2	182.17	$C_9H_{10}O_4$	Alkyl phenyl ketones
32	Ethanone,1-(2-hydroxyphenyl)-	18.7208	118-93-4	136.14	$C_8H_8O_2$	Alkyl phenylketones

Table 4.19: Compounds identified from the ethyl acetate extracts obtained from isolate MR23 isolated from *Croton macrostachyrus* leave and closely related to *Meyerozyma guillermondii* with similarity index 93.35% (**continuation**)

S/N	**Compound**	**Retention time(min)**	**ID**	**Molecular weight(G/mol)**	**Molecular formula**	**Chemical class**
33	Phthalic anhydride	17.2472	85-44-9	148.11	$C_8H_4O_3$	Cyclic dicarboxylic anhydride
34	Dibutyl phthalate	24.8931	84-74-2	278.34	$C_{16}H_{22}O_4$	Benzoic acid esters
35	Aziridine, 2-methyl-	6.6786	75-55-	57.09	C_3H_7N	Aziridine

			8			
36	Squalene	26.6257	7683-64	410.7	$C_{30}H_{50}$	Polyunsaturated hydrocarbon
37	1,2-Benzenedicarboxylic acid,bis(2-methylpropyl) ester	24.8940	84-69-5	278.34	$C_{16}H_{22}H_4$	Phthalate esters
38	Methane, diazo-	4.1625	334-88-3	42.04	CH_2N_2	Diazo
39	Acetophenone,2-chloro	6.3119	532-27-4	154.59	C_8H_7ClO	Ketone
40	Phenprobamate	15.8926	673-31-4	179.22	$C_{10}H_{13}NO_2$	Benzenes
41	Hexanoic acid	10.8831	142-62-1	116.16	$C_6H_{12}O_2$	Saturated fatty acid
42	n-Hexadecanoic acid	24.7034	57-10-3	253.4	$C_{16}H_{29}O_2^{-3}$	Long-chain saturated fatty acid
43	Phenol, 4,4’-(1-methylethylidene)bis-	28.3979	80-05-7	228.29	$C_{15}H_{16}O_2$	Bisphenols
44	Benzeneacetamide	18.2140	103-81-1	135.16	C_8H_9NO	Benzene
45	Benzeneacetic acid	15.8926	103-82-2	244. 08	$C_9H_{10}BrNO_2$	Monocarboxylic acid amide
46	Benzenepropanol	15.8926	122-97-4	136. 19	$C_9H_{12}O$	Benzene
47	1-Butanol, 3-methyl-,acetate	9.3099	123-92-2	130.18	$C_7H_{14}O_2$	Carboxylic acid esters

Table 4.20: Compounds identified from the ethyl acetate extracts obtained from isolate MR23 isolated from *Croton macrostachyrus* leave and closely related to *Meyerozyma guillermondii* with similarity index 93.35% (**continuation**)

S/N	Compound	Retention time(min)	ID	Molecular weight(G/mol)	Molecular formula	Chemical class
48	Benzenepropanoic acid	17.0626	501-52-0	150.17	$C_9H_{10}O_2$	Benzenes
49	Bis(2-ethylhexyl) phthalate	24.0038	117-81-7	390.6	$C_{24}H_{38}O_4$	Phthalates
50	Octadecanoic acid	27.4465	57-11-4	284.5	$C_{18}H_{36}O_2$	Fatty acids
51	Pentanoic acid	10.8831	109-52-4	130.13	$C_5H_{10}O_2$	Straight chain saturated fatty acids
52	Benzaldehyde,2-hydroxy	17.4917	90-02-8	122.12	$C_7H_6O_2$	Hydroxybezaldehydes
53	2,5-cyclohexadiene-1,4-dione,2,6-bis(1,1-dimethylethyl)-	19.2368	719-22-2	220.31	$C_{14}H_{20}O_2$	Monocyclic monoterpenoids
54	Benzene,chloro-	9.0210	108-90-7	112.55	C_6H_5Cl	Chlorobenzenes
55	Maleic hydrazide	14.7321	123-33-1	112.09	$C_4H_4N_2O_2$	Pyridazines
56	Benzoic acid, 4-methyl-,methyl ester	15.4120	99-75-2	150.17	$C_9H_{10}O_2$	Benzoic acids
57	Ethanone,1-(2,5-dimethoxyphenyl)-	20.1778	1201-38-3	212.20	$C_{10}H_{12}O_5$	Alkyl phenylketones
58	Cyclohexanone	9.9556	108-94-1	98.14	$C_6H_{10}O$	ketone
59	Benzothiazole	16.0616	95-16-9	135.19	C_7H_5NS	Benzothiazoles

60	Acetic acid	4.9346	64-19-7	60.05	$C_2H_4O_2$	Monocarboxylic acid
62	Propanoic acid	5.4403	79-09-4	74.08	$C_3H_6O_2$	Short chain saturated fatty acids

Key: ID-GC-MS NIST library identification number, min-Minutes, g/mol- grams per mole.

The GC-MS analysis of ethyl acetate extracts from isolate MR25 which was closely related to *Phyllosticta citricapa* showed unique compound which includes; 1-propanol,2-methyl, Benzaldehyde,4-hydroxy-, Acetic acid,2-methylpropyl ester, Dibutyl phthalate, Acetophenone,2-chloro, Acetic acid and propanoic acid (Table 4.15.

Table 4.21: Compounds identified from ethyl acetate extracts from isolate MR25 from *Zehneria scabra* leave and closely related to *Phyllosticta citricapa* with 100 % similarity

S/N	Compound	Retention time(min)	ID	Molecular weight(g/mol)	Molecular formula	Chemical class
1	1-propanol,2-methyl	5.0538	78-83-1	89.14	$C_4H_{10}O$	Primary alcohols
2	1-Butanol,3-methyl-	6.5855	123-51-3	88.15	$C_5H_{12}O$	Primary alcohols
3	Styrene	9.8809	100-42-5	104.15	C_8H_8	Styrenes
4	Phenol	11.2209	108-95-2	94.11	C_6H_6O	Phenols
5	Benzaldehyde	11.2758	100-52-7	106.12	C_7H_6O	Benzaldehyde
6	Phenol,4-methyl-	12.9889	106-44-5	296.4	$C_{21}H_{28}O$	Phenols
7	Fumaric acid	14.2003	110-17-8	116.07	$C_4H_4O_4$	Dicarboxylic acids
8	Benzoic acid	14.3091	65-85-0	122.12	$C_6H_7O_2$	Benzoic acids
9	1-Tridecene	24.1459	2437-56-1	182.35	$C_{13}H_{26}$	Unsaturated aliphatic hydrocarbons
10	n-Hexadecanoic acid	24.7044	57-10-3	256.42	$C_{16}H_{32}O_2$	Long-chain fatty acids
11	Benzaldehyde,4-hydroxy-	17.4936	123-08-0	122.12	C_7H_6O	Hydroxybenzaldehyde
12	Acetic acid,2-methylpropyl ester	7.2808	110-19-0	116.16	$C_6H_{12}O_2$	Carboxylic acid esters
13	Camphene	11-1317	79-92-5	136.23	$C_{10}H_{16}$	Bicyclic monoterpenoids
14	Benzeneacetic acid	15.6728	103-82-2	136.15	$C_9H_{10}O_2$	Benzene
15	Dibutyl phthalate	24.8931	84-74-2	278.34	$C_{16}H_{22}O_4$	Benzoic acid esters
16	Acetophenone,2-chloro	6.3119	532-27-4	154.59	C_8H_7ClO	Ketone
17	Acetic acid	4.9346	64-19-7	60.05	$C_2H_4O_2$	Monocarboxylic acid
18	Propanoic acid	5.4403	79-09-4	74.08	$C_3H_6O_2$	Short chain saturated fatty acids

Key: ID-GC-MS NIST library identification number, min-Minutes, g/mol- grams per mole.

Isolate MR26 ethyl acetate extracts showed compounds in the chemical classes; Benzyl alcohols, Benzoic acids, benzene chlorides, benzaldehydes, primary alcohols, carboxylic acids, styrenes, phenols, alkanes, monoterpenoids and long chain fatty acids (Table 4.16). The unique compounds produced by this isolate included 1-propanol,2-methyl, Acetic acid,butyl ester, Fumaric acid, Benzyl-chloride, 1-Tridecene, and Propanoic acid, anhydride.

Table 4.22: Compounds identified from the ethyl acetate extracts obtained from isolate MR26 isolated from *Croton macrostachyrus* bark and closely related to *Ampelomyces sp*. with similarity index of 99.41%

S/N	Compound	Retention time(min)	ID	Molecular weight(g/mol)	Molecular formula	Chemical class
1	Benzyl Alcohol	12.5536	100-51-6	108.14	C_7H_8O	Benzyl alcohols
2	1-propanol,2-methyl	5.0538	78-83-1	89.14	$C_4H_{10}O$	Primary alcohols
3	Propanoic acid, anhydride	6.1139	123-62-6	130.14	$C_6H_{10}O_3$	Carboxylic acid
4	1-Butanol,3-methyl-	6.5855	123-51-3	88.15	$C_5H_{12}O$	Primary alcohols
5	Acetic acid, butyl ester	7.2829	123-86-4	116.16	$C_6H_{12}O_2$	Carboxylic acid esters
6	Styrene	9.8809	100-42-5	104.15	C_8H_8	Styrenes
7	Benzyl-chloride	11.1380	100-44-7	126.58	C_7H_7Cl	Benzene Chlorides
8	Phenol	11.2209	108-95-2	94.11	C_6H_6O	Phenols
9	Benzaldehyde	11.2758	100-52-7	106.12	C_7H_6O	Benzaldehyde
10	Phenol,4-methyl-	12.9889	106-44-5	296.4	$C_{21}H_{28}O$	Phenols

Key: ID-GC-MS NIST library identification number, min-Minutes, g/mol- grams per mole.

Table 4.23: Compounds identified from the ethyl acetate extracts obtained from isolate MR26 isolated from *Croton macrostachyrus* bark and closely related to *Ampelomyces sp*. with similarity index of 99.41% (**Continuation**)

11	Fumaric acid	14.2003	110-17-8	116.07	$C_4H_4O_4$	Dicarboxylic acids
12	Benzoic acid	14.3091	65-85-0	122.12	$C_6H_7O_2$	Benzoic acids
13	Dodecane	15.1019	112-40-3	170.33	$C_{12}H_{26}$	Alkanes
14	Pentadecane	16.2761	629-62-9	72.15	C_5H_{12}	Alkane
15	1-Tetradecanol	17.9580	112-72-1	214.39	$C_{14}H_{30}O$	Long-chain Fatty acid alcohols
16	Tetradecane	18.0575	629-59-4	198.39	$C_{14}H_{30}$	Alkanes
17	1-Hexadecanol	20.5923	36653-82-4	242.44	$C_{16}H_{34}O$	Long-chain Fatty alcohols
18	Hexadecane	20.6721	544-76-3	226.44	$C_{16}H_{34}$	Alkanes
19	1-Octadecanol	22.9468	112-92-5	270.49	$C_{20}H_{43}NO_3$	Long-chain fatty alcohols
20	Octadecane	23.0111	593-45-3	245.5	$C_{18}H_{38}$	Alkanes
21	1-Tridecene	24.1459	2437-56-1	182.35	$C_{13}H_{26}$	Unsaturated aliphatic hydrocarbons
22	n-Hexadecanoic acid	24.7044	57-10-3	256.42	$C_{16}H_{32}O_2$	Long-chain fatty acids

Key: ID-GC-MS NIST library identification number, min-Minutes, g/mol- grams per mole.

The ethyl acetate extracts obtained from Isolate MR28 showed in chemical classes. The unique compounds include; fumaric acid, 1-Butanol,3-methyl, Phenyl,4-methyl-, Benzeneacetic acid, and Benzaldehyde,4-hydroxy- (Table 4.17).

Table 4.24: Compounds identified from ethyl acetate extracts obtained from isolate MR28 isolated from *Zehneria scabra* stem and closely related to *Leptosphaeria spegazzanii* with similarity index of 98.24%

S/N	Compound	Retention time(min)	ID	Molecular weight(g/mol)	Molecular formula	Chemical class
1	Benzyl Alcohol	12.5536	100-51-6	108.14	C_7H_8O	Benzyl alcohols
2	Benzoic acid	14.3091	65-85-0	122.12	$C_7H_6O_2$	Benzoic acids
3	1-propanol,2-methyl	5.0538	78-83-1	89.14	$C_4H_{10}O$	Primary alcohols
4	Propanoic acid, anhydride	6.1139	123-62-6	130.14	$C_6H_{10}O_3$	Carboxylic acid
5	1-Butanol,3-methyl-	6.5855	123-51-3	88.15	$C_5H_{12}O$	Primary alcohols
6	Styrene	9.8809	100-42-5	104.15	C_8H_8	Styrenes

Key: ID-GC-MS NIST library identification number, min-Minutes, g/mol- grams per mole.

Table 4.25: Compounds identified from ethyl acetate extracts obtained from isolate MR28 isolated from *Zehneria scabra* stem and closely related to *Leptosphaeria spegazzanii* with similarity index of 98.24% (**Continuation)**

7	Phenol	11.2209	108-95-2	94.11	C_6H_6O	Phenols
8	Benzaldehyde	11.2758	100-52-7	106.12	C_7H_6O	Benzaldehyde
9	Phenol,4-methyl-	12.9889	106-44-5	296.4	$C_{21}H_{28}O$	Phenols
10	Fumaric acid	14.2003	110-17-8	116.07	$C_4H_4O_4$	Dicarboxylic acids
11	Benzoic acid	14.3091	65-85-0	122.12	$C_6H_7O_2$	Benzoic acids
12	Dodecane	15.1019	112-40-3	170.33	$C_{12}H_{26}$	Alkanes
13	Pentadecane	16.2761	629-62-9	72.15	C_5H_{12}	Alkane
14	1-Tetradecanol	17.9580	112-72-1	214.39	$C_{14}H_{30}O$	Long-chain Fatty acid alcohols
15	Tetradecane	18.0575	629-59-4	198.39	$C_{14}H_{30}$	Alkanes
16	1-Hexadecanol	20.5923	36653-82-4	242.44	$C_{16}H_{34}O$	Long-chain Fatty alcohols
17	Hexadecane	20.6721	544-76-3	226.44	$C_{16}H_{34}$	Alkanes
18	1-Octadecanol	22.9468	112-92-5	270.49	$C_{20}H_{43}NO_3$	Long-chain fatty alcohols
19	Octadecane	23.0111	593-45-3	245.5	$C_{18}H_{38}$	Alkanes
20	n-Hexadecanoic acid	24.7044	57-10-3	256.42	$C_{16}H_{32}O_2$	Long-chain fatty acids
21	Benzaldehyde,4-hydroxy-	17.4936	123-08-0	122.12	C_7H_6O	Hydroxybenzaldehyde
22	Camphene	11-1317	79-92-5	136.23	$C_{10}H_{16}$	Bicyclic monoterpenoids
23	Naphthalene	15.4427	91-202-3	128.17	$C_{10}H_8$	Naphalenes
24	Benzeneacetic acid	15.6728	103-82-2	136.15	$C_9H_{10}O_2$	Benzene

Key: ID-GC-MS NIST library identification number, min-Minutes, g/mol- grams per mole.

The identified compounds from isolate MR29 closely related to *Fusarium solani* were in the chemical classes Benzyl alcohols, Benzoic acids, benzaldehydes, chlorobenzenes, primary

alcohols, carboxylic acids, styrenes, phenols, alkanes, monoterpenoids, naphthalenes, and long chain fatty acids (Table 4.18). Moreover, the isolate extracts showed unique bioactive compounds that were not identified in ethyl acetate extracts of other isolates in this study. The compounds include; Acetic acid,2-methylpropyl ester, Acetic acid,1-methylpropyl ester, 2,5-cyclohexadiene-1,4-dione,2,6-bis(1,1-dimethylethyl)- and Benzene,chloro-.

Table 4.26: Compounds identified from the ethyl acetate extracts obtained from isolate MR29 closely related to *Fusarium solani* and isolated from *Zehneria scabra* root

S/N	Compound	Retention time(min)	ID	Molecular weight(G/mol)	Molecular formula	Chemical class
1	Benzyl Alcohol	12.5536	100-51-6	108.14	C_7H_8O	Benzyl alcohols
2	Propanoic acid, anhydride	6.1139	123-62-6	130.14	$C_6H_{10}O_3$	Carboxylic acid
3	1-Butanol,3-methyl-	6.5855	123-51-3	88.15	$C_5H_{12}O$	Primary alcohols
4	Phenol	11.2209	108-95-2	94.11	C_6H_6O	Phenols
5	Benzaldehyde	11.2758	100-52-7	106.12	C_7H_6O	Benzaldehyde
6	Phenol,4-methyl-	12.9889	106-44-5	296.4	$C_{21}H_{28}O$	Phenols
7	Fumaric acid	14.2003	110-17-8	116.07	$C_4H_4O_4$	Dicarboxylic acids
8	Benzoic acid	14.3091	65-85-0	122.12	$C_6H_7O_2$	Benzoic acids
9	Dodecane	15.1019	112-40-3	170.33	$C_{12}H_{26}$	Alkanes
10	Pentadecane	16.2761	629-62-9	72.15	C_5H_{12}	Alkane
11	1-Tetradecanol	17.9580	112-72-1	214.39	$C_{14}H_{30}O$	Long-chain Fatty acid alcohols

Key: ID-GC-MS NIST library identification number, min-Minutes, g/mol- grams per mole.

Table 4.27: Compounds identified from the ethyl acetate extracts obtained from isolate MR29 closely related to *Fusarium solani* and isolated from *Zehneria scabra* root **(Continuation**)

12	Tetradecane	18.0575	629-59-4	198.39	$C_{14}H_{30}$	Alkanes
13	1-Hexadecanol	20.5923	36653-82-4	242.44	$C_{16}H_{34}O$	Long-chain Fatty alcohols
14	Hexadecane	20.6721	544-76-3	226.44	$C_{16}H_{34}$	Alkanes
15	1-Octadecanol	22.9468	112-92-5	270.49	$C_{20}H_{43}NO_3$	Long-chain fatty alcohols
16	n-Hexadecanoic acid	24.7044	57-10-3	256.42	$C_{16}H_{32}O_2$	Long-chain fatty acids
17	Acetic acid,2-methylpropyl ester	7.2808	110-19-0	116.16	$C_6H_{12}O_2$	Carboxylic acid esters
18	Acetic acid,1-methylpropyl ester	7.2860	105-46-4	116.16	$C_6H_{12}O_2$	Carboxylic acid esters
19	Camphene	11-1317	79-92-5	136.23	$C_{10}H_{16}$	Bicyclic monoterpenoids
20	Naphthalene	15.4427	91-202-3	128.17	$C_{10}H_8$	Naphalenes
21	n-Hexadecanoic acid	24.7034	57-10-3	253.4	$C_{16}H_{29}O_2^{-3}$	Long-chain saturated fatty acid
22	2,5-cyclohexadiene-1,4-dione,2,6-bis(1,1-dimethylethyl)-	19.2368	719-22-2	220.31	$C_{14}H_{20}O_2$	Monocyclic monoterpenoids
23	Benzene,chloro-	9.0210	108-90-7	112.55	C_6H_5Cl	Chlorobenzenes

Key: ID-GC-MS NIST library identification number, min-Minutes, g/mol- grams per mole.

CHAPTER FIVE: DISCUSSION, CONCLUSIONS, AND RECOMMENDATIONS

5.1 DISCUSSION

5.1.1 Socio-demographic information

In the study area, the 35 participating herbalists treated human diseases. The ages of the herbalists ranged from 30 years to 76 years, with an average of 55 years. The knowledge on the medicinal plants requires time of learning and this can be found among the older and experienced herbalists. In addition, knowledge on the medicinal plants is not easily passed to the younger generation since they might not be trusted to keep the secrets on these plants. The younger could be disregarded by the older ones as well. Similar findings were reported by Minja *et al*. (2001) where knowledge of medicinal plants is passed from the experienced and older generation to close relatives. However, there are discrepancies in the number of men and women who are herbalists (Zhang *et al*., 2015).

In this study, the majority of the herbalists were women. The herbalists regularly sell their herbal products in the market places within the county (Elgeyo-Marakwet). The high percentage of women practicing medicinal plants to treat various ailments in the study area is attributed to the role of women in the family, which is in agreement with findings by Miara *et al*. (2018) where women were the majority of the practicing herbalists, and they got to learn through their family roles. The women are often responsible for providing meals to the family, which in most cases, are plant-based recipes so they get to learn more about the medicinal plants that are medically important. Besides that, women are also responsible for family health (Eddouks *et al*., 2017).

On duration of practice, some of the herbalists had practised for longer periods than others. The respondent's duration of practice was directly proportioned to the knowledge of medicinal plants. The older herbalists cited many plants more than the younger herbalists. Moreover, age was directly proportional to the level of education where the younger herbalists had formal education ranging from primary to tertiary levels. In contrast, a majority of those who had no formal education. Similar findings were reported by Bouasla and Bouasla, (2017) where the experienced and older herbalists had no formal education but they are well versed with the medicinal plants information more than the younger herbalists. Also, the older herbalists cited many uses of each medicinal plants more than the younger herbalists. The older might have learned this through their long experience in using these plants as compared to those who had practiced for a short duration.

In the study area, the sources of herbal knowledge were close relatives who have been practicing over time. The knowledge is passed from one generation to another. Those who get the privilege of this information are advised to keep the secrets since they are the trusted members of the family. Similarly, such findings were reported by Beyene *et al*., 2016 and Gabriel *et al*., 2017 where knowledge on medicinal plants is shared with close relatives only. However, some of the herbalists indicated that their source of knowledge on medicinal plants was through careful observation and continuous practice. Elsewhere, the source of herbal knowledge through observation was reported by Kebebew and Mohamed, (2017).

5.1.2 Medicinal plants used by the Marakwet community

A total of 26 medicinal plants belonging to 18 families and are used for diverse purposes were recorded in the study area. The growth forms of these plants range from herbs, trees, shrubs, and climbers. The use of trees and herbs more than climbers and shrub could be attributed to the availability of the trees and herbs in the study area. The study area is also in the tropical and subtropical region where the climatic conditions favor the growth of trees and this could explain why trees were many (Mahomoodally, 2013). The socio-cultural beliefs and practice of the herbalists could have made them believe that the herbs and trees are efficient in the treatment of human ailments. This is similar with previous studies by Kebebew and Mohamed, (2017) where trees and herbs used as medicinal plants were more than shrubs or climbers is because of the availability of the trees plant parts such as barks more than those of the shrubs and climbers. In the same study area Kigen *et al*. (2017) , had indicated the percentage of trees used as medicinal plants was higher as compared to herbs, climbers, scrubs , seeds, fruits and tubers.

The diseases treated by the herbalists were grouped into four categories gastrointestinal, respiratory, skin infections. Gastrointestinal ailments had the highest percentage cited by the herbalists in the study area, while respiratory and skin infection was cited at equal proportions. Similarly, a study by Sørensen *et al*. (2013) reported the highest percentage of herbalists treating gastrointestinal diseases. This can be linked to many ailments along the gastrointestinal tract. In addition, some of the herbalists had specialized in treating specific diseases. Previously, specialization of disease treatment had been reported by (Kipkore *et al*., 2014). In addition, the experienced herbalists were knowledgeable on the disease

presenting symptoms, diagnosis, the medicinal plants to use and the best way of administering the herbal preparations. Some of them knew the duration at which the herbal preparation would take to heal although they indicated that in some cases there can be discrepancies from one person to another.

In this study, some plant families had more than one species while some of the plant species had more than one use. High use values and family use demonstrate that the plants in these families could be having extensive uses and can be used for the treatment of diverse ailments. The findings are in agreement with those of Umair *et al*. (2017) where plants with high UV and FUV have many uses. Additionally, these plants could be having active compounds that give positive results when used for the treatment of human diseases hence preferred more than others. The lowest UV value in some plant species may be due to less ethnobotanical uses reported by the community and a little knowledge of these medicinal plants by the users.

In addition, the plant families with many uses may be the best families to carry out future investigations on their active compounds. They could be having many species that are important in the medical field, especially in the development of drugs. This is in line with findings by Aadhan and Anand, (2017), where the *Cucurbitaceae* family was reported to have many plant species used in the treatment of diabetes. Despite others having a high family use-values, some of the families reported low family use-values. The findings therefore demonstrate that the utilization of medicinal plants by the Marakwet community

cuts across diverse families and there is need to evaluate each one of them to discover their uses.

Even though all plant parts are essential for medicinal purposes, the herbalists had diverse views. The herbalists were very categorical in that some plant parts are used singly while others are used in combination. The use of leaves alone reported the highest percentage compared to roots and barks. Leaves can be picked more often than the barks and roots. The herbalists also cited the need to protect the medicinal plants, and the use of roots and barks risk them losing the plants. Leaves can be chewed while raw more easily compared to barks and roots. Similar findings on the use of leaves more than barks and roots because it is a method of protecting the plants for future use have been reported by previous studies done by Umair *et al*. (2017). The findings were also in agreement with Moore *et al*. (2020) who indicated that the largest proportion of plant parts used by the Marakwet community is leaves although in most cases, the leaves were used in combination with other plant parts such as roots and barks.

On the other hand, a combination of different plant parts was also used. A combination of leaves, barks, and roots recorded the highest percentage compared to barks and roots and leaves and barks. The mixture of plant parts could be a way of increasing the concentration of active ingredients essential for medication purposes. It could also be attributed to increasing the efficiency of treatment hence; reducing the period in which the patient is supposed to use it. However, the methods of preparing the mixture differ. These findings are

in agreement with those reported by Kigen *et al*. (2017) in the same study area where plant parts are used in combination in herbal preparations.

The herbalists in the study area use different methods of preparing the medicinal plants before administering to their patient. One of the common methods is soaking the medicinal plant then boiling it with water to make a decoction. According to the herbalists in the study area, boiling is done until the amount of water is reduced to half or quarter of the initial water in the boiling pots The preparation method is a way of making the decoction concentrated so that it can increase its efficiency. Simultaneously, the herbalists instructed their patients to chew some of the plant parts, especially the leaves.

Additionally, others were burnt, and the ashes licked. It is important to note that the herbalists in the study area combined plant parts from different plant species. This is consistent with other studies done in the same region by Kigen *et al*., 2017 and Mbuni *et al*., 2020 where plant parts were used in combination to treat human and animal diseases. The reason for this is to increase efficiency, and, in some instances, the decoction can be bitter, and other plants can reduce the bitterness. Alternatively, milk or honey can be added to the decoction, especially in children. The reason most herbalists preferred decoction is that it is easier to administer, and easier to prepare. This is in agreement with previous studies by Madani *et al*. (2017) where decoction was more preferred because it is easier to prepare, and administer. According to Mahomoodally, (2013), the medicinal plants accumulate phytochemicals which when used, act individually, or in synergy to improve health. The combined action of these phytochemicals from medicinal plants tend to increase the activity

of the main constituents found in the medicinal plants by speeding up or slowing down the assimilations in the body of the user (Mahomoodally, 2013).

In all preparations of the medicinal plants, the solvent used by the herbalists in the study area was water. This is in line with other studies by Aadhan and Anand, 2017 where water was used as a medium in all herbal preparations because it is available and anyone can use it. Also, Nafiu *et al*. 2017 indicated that in most herbal preparations, the common and universal solvent is water.

While the majority of the herbalists stated different methods of administration, oral was common among all the herbalists. Oral administration was also common in all the type of diseases that the herbalists treated. This can be attributed to the infections and diseases that occur along the gastrointestinal tract (Maroyi, 2015). Again, it could be because the herbalists have carefully observed that oral administration of their herbal preparations has positive impacts on the treatment of all diseases. The methods of administration are similar to those reported by Kigen *et al*. (2017). Swabs were common in the treatment of skin and skin-related infections. The herbalists reported that the decoction is swabbed in the affected areas in some cases. Some of the plant parts were ground into a powder and applied to the affected areas as well, while in other cases, leaves of some of the plants were pounded when fresh and applied to the skin affected areas on the skin.

In the study area, the herbalists indicated variations in dosages and prescriptions. The dosage of a patient was determined by age, physical fitness of the patient based on the herbalist

experience, the persistence of the disease, and if the patient is pregnant. The herbalists also indicated that they were careful not to overdose their patients because they are aware that excessive use of the herbal preparations can negatively impact the patient's health. As a result, they recommend the amount and the number of times to use in a day. In most cases, the amount of decoction is measured using a glass, and they estimate it to quarter, half, or full glass. The majority of the herbalists indicated that the best time to use is early in the morning before any meal. This could be attributed to the need to monitor the reaction of the body after using the herbal preparations. However, some herbalists recommended the patients to use the herbal preparations twice a day or once a day for a specific number of days. Lack of precision and standardization of herbal preparations have been discussed widely and are a significant drawback of traditional medicine systems, as reported by Nafiu *et al*. (2017).

Most of the herbalists reported that the response of the patients was good, while a few were fair. However, there are side effects of the herbal preparations. The common side effects are headache, diarrhea, fatigue, and vomiting. In some instances, the patient can experience more than two side effects. In other situations, the side effects such as diarrhea show the effectiveness of the herbal preparations, and the herbalists advise the patients to drink enough water. This to rehydrate in case the patient gets dehydrated. The cause of the side effects can be attributed to the excessive or unquantified amount of the herbal preparations; hence they become toxic to the body at some level. Cases of side effects in herbal or medicinal plants have been reported in previous studies by Yatoo *et al*. (2017). However, patients reporting side effects are few according to the herbalists. Additionally, the findings

are in agreement with previous study by Ekor, (2014) who indicated that some herbal remedies are capable of producing adverse reactions to the users which can lead to adverse injuries, life threatening conditions and in some instances it can lead to death. This is because there is no toxicological test or mandatory safety evaluation of these products before it is released to the market or usage.

The primary source of medicinal plants in the study area is the Cherangani forest. The forest is located near the herblists's residence. Although the government has put in tough measures to protect the forest from encroachment, the herbalists still find ways to collect the medicinal plants. This means that the herbalists have not cultivated most of these medicinal plants, and they rely on the wild medicinal plants. The overdependence of the medicinal plants in wild habitats may risk the availability of these plants in the future. This is in line with the previous study by Kigen *et al*. (2017). Therefore, documentation of these plants is very essential (Hamilton, 2008; Chen *et al*., 2016). Despite relying on the forest as the main source of these medicinal plants, an exceptional number of herbalists cited that they have domesticated some medicinal plants in their family farms. Domestication of medicinal plants has been reported by Ganie *et al*. (2020). Herbalists who have domesticated the medicinal plant supplement it with those from the forest. Apart from being medicinal plants, the domesticated medicinal plants have other uses such as source of firewood or fencing posts.

The medicinal plants thrive in diverse habitats such as the highland, lowlands, and the escarpements. The medicinal plants that thrive in lowlands are rare, and the herbalists

outsource them from other places outside Elgeyo-Marakwet County. This can be attributed to differences in climatic conditions and soils (Kipkore *et al*., 2014). In such instances, the herbalists reported to outsource the medicinal plants from neighboring counties such as from West Pokot, Transnzoia, and Baringo counties, respectively. The herbalists indicated that they know other practicing herbalists in those counties who help in collecting the medicinal plants and supply to them at a negotiable cost.

5.1.3 Identification and morphological diversity of the isolated fungal endophytes

In the present study, the isolated fungal endophytes belonged to phylum Ascomycota. The isolated fungal endophytes were distributed across four classes including, *Eurotiomycetes, Sordariomycetes, Saccharomycetes* and *Dothideomycetes*. The finding agrees with those of Mohinudeen *et al*. (2019) who indicated that most fungal endophytes isolated from medicinal plants are found in division Ascomycota. In other studies, endophytic fungi from medicinal plants are found in the phyla Ascomycota and Basidiomycota (Zhou *et al*., 2017).

Isolates in cluster A had a cream to cream yellow mycelia on both the front and reverse. Additionally, they had flat surface and some had concentric rings. These morphological features were similar to those described by Kidd *et al*. (2016). The septation of these isolates were in agreement with those described by Crous *et al*. (2016). Moreover, the morphological features were similar to those of *Lasiodiplodia* species. Similar features had been previously described by Somasekhara, (2016). However, the septation and shape of conidia were different. Some were globulose, while others were ovoid. Similar characteristics had been described by Crous *et al*. (2016) and are features of fungi in genera *Fusarium* and *Clonostachys*. The isolated fungal endophytes in cluster C had morphological

features similar to those of *Penicillium* species, *Aspergillus*, *Ampelomyces* and *Aporospora*. The endophytes clustering together means that they are closely related phenotypically (Tsang *et al*., 2018).

5.1.4 Diversity of the isolated fungal endophytes in hosts

In this study, *Croton macrostachyrus* recorded the highest number of endophytic fungi as compared to *Zehneria scabra*. *Croton macrostachyrus* is a tree and considering the slow-growing nature of fungal endophytes, many endophytes could finish their life cycle in this host compared to *Zehneria scabra*, a climber and might have a short life span thus reducing the number of endophytes colonizing this host plant. Therefore, this could have contributed to the discrepancies in the endophytes isolated from each. The finding are in agreement with those reported by Yu *et al*. (2018), who reported that the difference in endophytes from one plant to another is due to the lifespan of the host. Additionally, some endophytes are host-specific; hence might prefer one host to another (Kouipou *et al*., 2019). Some fungal endophytes prefer hosts of a certain genotypes, Dudeja and Giri, (2014) and this could have also brought in the discrepancies reported between the two hosts. Apart from differences in the number and diversity of fungal endophytes isolated from each plant, there was also discrepancies in the number of fungal endophytes isolated from the different plant parts such as leaves, roots, bark and stem.

The fungal endophytes isolated from leaves were higher as compared to stems, barks, and roots. The leaf structure and chemical composition might be the reason that high number of fungal endophytes isolated from the leaves. Similarly, Luo *et al*. (2015) reported that many fungal endophytes were isolated from leaves of *Tephrosia purpurea* more than its roots and

barks due to chemical composition of the leaves. Additionally, the higher number of fungal endophytes in the leaves could probably be due to the dispersal mechanisms. The fungal spores can be dispersed by wind, insect vectors or rain splash. The leave location in a plant allows it to be the recipient of the endophytic spores. In the leaves, the spores germinate, and successful colonization results to high number of endophytes in the leaves. Other plant parts such as barks and roots may have chemical and structural barriers that may affect the colonization of the fungal endophytes. Some of the structural barriers are lignin layers, cuticular layers and outer barks while the chemical barriers can be lignin, cyanide and allelochemicals (Gazis and Chaverri, 2010).

Furthermore, fungal endophytes invade their hosts through penetration through wounds and natural openings such as stomata and lenticels (Gazis and Chaverri, 2010). In leaves, these features may be abundant than roots thus influencing their colonization. Similarly, some organism such as arbuscular endomycorrhiza are involved in mutualistic relationship with hosts and as a result, they can prevent the invasion of the fungal endophytes by producing antagonistic substances. This is according to previous studies done by Li *et al*. (2016) who reported that the mutualistic relationship of fungal hosts with endomycorrhiza inhibits the invasion of fungal endophytes by producing antagonistic compounds.

In the present study, the above ground samples (leaves, barks and stem) had higher number of isolates as compared to below-ground samples (roots). The soil conditions such as moisture, pH and soil substrates contributes to the number of endophytes isolated from roots either positively or negatively (Li *et al*., 2020). These factors contribute to the diversity of

the endophytic isolates as well. However, this finding disagrees with that of Jin *et al*. (2017) who reported that the root samples have more endophytic fungi than the leaves. Also, Jin *et al.* (2017) established that the number of fungal endophytes isolated from the roots are many due to the horizontal dispersal, invasion and the rhizosphere soils which provide good habitat for the endophytic fungi.

There was uneven distribution of the isolated fungal endophytes in the two hosts. This comprised of a few frequent and some infrequent. *Fusarium* species is known as a dominant cosmopolitan fungus and is found in habitats of other living plants (Cui *et al*., 2012). In this study, it was one of the dominant genera of fungal endophytes (Jin *et al.*, 2017). Its dominance in all plant parts demonstrate that endophytic fungi in this genera are not tissue specific (Chen *et al*., 2011). Additionally, endophytic *Fusarium* species have been documented to be endophytic in woody, herbs and in tropical hosts (Crozier *et al*., 2006). In contrast, Yuan *et al*. (2009) indicated that some *Fusarium* species such as *Fusarium solani* were localized to roots alone while Chen *et al*., (2011) indicated that some of the isolated fungal endophytes such as *Colletotricum* sp. were from leaves and stem and not roots. This therefore mean that the number of endophytes isolated from a plant depend on the host plant and the plant tissues.

Apart from *Fusarium* endophytes, *Acremonium sp*. has been reported to be closely related to *Fusarium* and one of the dominant fungal endophyte (Jin *et al*., 2017). Some of the isolated fungal endophytes such as *Meyerozyma gulliermondii* was isolated in barks and not in any plant parts. This finding agrees with Xiao *et al*. (2018) who indicated that some fungal

endophytes are host specific because they have adapted to a specific tissue microenvironment. Similarly, Kaul *et al*. 2013 and Mishra *et al*. (2012) indicated that the endophytes isolated from medicinal plants depends on the season of sample collection and the tissue used. This might have contributed to the difference in the endophytes isolated from each plant tissue.

According to Khalil *et al*. (2020), many endophytic fungal endophytes are from genera *Penicillim* and *Aspergillus*. In this study, these genera were represented with *Aspergillus niger* and *Penicillium chrysogenum*. In addition, *Chaetomium globosum* is one of the most studied endophyte(Khalil *et al*., 2020) due to its unique metabolites (Bai *et al*., 2015) and this was evident in this study as well.

5.1.5 Antimicrobial activity of the fungal endophytes

The Gram-positive bacteria were most inhibited test organisms as compared to Gram negative. The significant difference between the test organisms could be due to the cell wall composition of the bacteria and the polarity of the extracts. The finding agrees with a previous study by Malhadas *et al*. (2017) and Chi *et al*. (2019), who reported that fungal endophytes isolated from medicinal plants showed higher inhibition in Gram-positive bacteria more than gram-negative bacteria because of their difference in cell wall composition. Additionally, Borquaye *et al*. (2016) reported that the polarity of extracts used to screen for antimicrobial contributes to the difference in inhibition between one test organism and another. However, none of the fungal extracts from the fungal endophytes in this study inhibited *Candida albicans,* and this can be attributed to the amounts of the

bioactive metabolites. The active compounds could have been in low amounts and needed further purification to inhibit this test organism.

In the disk diffusion method, extracts from isolates closely related to *Ampelomycetes* sp, *Coniothrium* sp, and *Fusarium solani* showed the highest antimicrobial activity. These extracts' inhibition could be because of the bioactive metabolites present in the extracts produced by these fungal endophytes. The GC-MS analysis of the fungi extracts showed Benzyl alcohol, 1-propanol,2-methyl, Propanoic acid anhydride, and phenol,4-methyl as the common bioactive metabolites produced by the three fungi. However, the difference in their antimicrobial activity could be due to the unique metabolites produced by each isolate. For instance, Acetic acid butyl esters were produced by isolate MR26 closely related to *Ampelomyces* sp, Bezaldehyde 4-hydroxy produced by isolate MR20 closely related to *Clonostachys rosea,* and isolate MR29 closely related to *Fusarium solani* produced Acetic acid,1-methylprophyl ester. The bioactive metabolites could have inhibited the test organisms either singly or synergistically.

In well diffusion method, there was a significant difference between the endophyte extracts with those from isolates closely related to *Chaetomium globosum, Aporospora terricola, Ampelomycetes,* and *Clonostachys rosea* showing the highest antimicrobial activity when compared to other isolates. The inhibition could be attributed to the inhibitory action of benzaldehyde,4-hydroxy-, ethanone,1-(2,5-dimethoxyphenyl)-, cyclohexanone and benzothiazole metabolites produced by these fungi. According to Kanjana *et al*. (2019), Vásquez *et al.* (2019*),* Nguyen *et al*. (2019), and Morsy *et al*. (2020) compounds

benzaldehyde,4-hydroxy-, ethanone,1-(2,5-dimethoxyphenyl)-, cyclohexanone, and Benzothiazole have significant antimicrobial activity against both bacteria and fungi. Additionally, the inhibition action of isolates MR19, MR26 and MR21 closely related to *Aporospora terricola, Ampelomyces sp,* and *Clonostachys rosea* extracts could be attributed to fumaric acid, benzoic acid, n-hexadecanoic acid, produced by the three fungal endophytes. Fumaric acid and benzoic acid inhibit bacteria's growth, either singly or synergistically (Peh *et al*., 2020).

In this study, some fungal endophytes showed moderate antimicrobial activity against the test organisms. Isolate MR23 closely related to *Meyerozyma guilliermondii* showed moderate antimicrobial activity in both well and disk diffusion methods. It also inhibited both the Gram-positive and Gram-negative bacteria. Similarly, Joel and Bhimba, (2013) reported that crude extracts from *Meyerozma guilliermondii* inhibit both Gram-negative and Gram-positive bacteria. The inhibition can be attributed to acetic acid, propanoic acid, benzeneacetic acid, acetic acid,2-methylpropyl ester produced by this fungus and has been reported to have antimicrobial properties. This is in agreement with previous studies by Kumar *et al*. (2017), who reported that organic acids inhibit bacteria due to their ability to easily pass through the cell membrane and disrupt other cell functions.

In this study, isolate MR25 closely related to *Phyllosticta citricarpa* showed inhibitory abilities both in disk diffusion and well diffusion methods. The inhibition by extracts from this organism can be attributed to dibutyl phthalate produced by this fungus. Previously, Shobi and Viswanathan, (2018) reported that the compound has significant antibacterial

activity, although in this case, it was a bioactive metabolite from the medicinal plant *Begonia malabarica*. Additionally, isolate MR25 closely related to *Phyllosticta citricapa* isolated in this study produced fumaric acid, and it could have also contributed to the inhibition of the test organism. This is in support of findings by Tango *et al*. (2015), who reported that fumaric acid inactivates both Gram-positive and Gram-negative bacteria.

According to Zhai *et al*. (2016), *Clonostachy rosea* is a fungal endophyte with potential antimicrobial activity. In this study, isolate MR21 closely related to *Clonostachy rosea* inhibited both Gram positive and Gram-negative bacteria in disk diffusion method. The inhibition could be attributed to the metabolites benzaldehyde,2-hydroxy, 1,2-benzenedicarboxylic acid, bis(2-methylpropyl) ester, pentanoic acid, and octadecanoic acid produced by this fungus. Elsewhere, these compounds had been reported to have significant antimicrobial activities Mohamad *et al*., (2018). The bioactive metabolites from bacterial endophytes inhibited *Bacillus subtilis, Staphylococcus aureus,* and *E.coli*. Alternatively, these metabolites inhibit bacteria synergistically (Stefanović, 2018).

Isolate MR10 closely related to *Penicillium chrysogenum* isolated in this study showed antibacterial activity. The inhibition by extracts from this organism can be attributed to compounds 1-butanol, 3-methyl-, acetate, and benzenepropanoic acid. According to Elgorban *et al*. (2019) these compounds produced by *Alternaria* endophytic fungi have antimicrobial activity against *Staphylococcus aureus* and *E. coli.* In this study, the extracts from isolate MR10 closely related to *Penicillium chrysogenum* did not inhibit *E. coli*, which could be due to these metabolites' concentration. It could have been in low amounts that

could not inhibit the *E. coli*. When compared with isolate MR6 closely related to *Aspergillus niger* found within the same subclade, it showed antimicrobial activity both in disk diffusion and well diffusion methods. The inhibition could be attributed to phthalic anhydride, aziridine, and 2-methyl- and squalene metabolites produced by this fungus. In previous studies done by Musa *et al*. (2020), phthalic anhydride extracted from mahogany seed oil showed antibacterial activity against *Staphylococcus aureus* and *E.coli*. Additionally, Gorinstein *et al*. (2018) reported that squalene from plant sources has antibacterial and antifungal properties. This could be similar to squalene from endophytic sources too. Moreover, Aziridine,2-methyl has also been reported to have antimicrobial properties (Rostovskii *et al*., 2020).

Isolate MR14 closely related to *Lasiodiplodia theobromae* endophytic fungi showed antimicrobial activity in this study in disk diffusion method. In previous studies by Salvatore, *et al*. (2020), secondary metabolites from *Lasiodiplodia theobromae* have a wide range of biological activities such as antibacterial and antifungal. The inhibition shown by extracts from this organism can be linked to 1,2-benzenedicarboxylic acid,bis(2-methylpropyl) ester, benzaldehyde,4-hydroxy-, camphene and naphthalene. This supports findings by Chaithra *et al*. (2020), who reported that 1,2-benzenedicarboxylic acid,bis(2-methylpropyl) ester, and benzaldehyde,4-hydroxy from *Lasiodiplodia theobromae* endophyte isolated from cocoa had antimicrobial activity. Additionally, Zeng *et al*. (2020) reported that camphene a pure compound of oleoresine and an essential oil from *liquidambar formosana* inhibited *Bacillus subtilis, Staphylococcus aureus,* and

Pseudomonas aeruginosa. Furthermore, Makar *et al*. (2019) reported that Naphthalene and its derivatives have antimicrobial activity.

Apart from being plant endophytes some fungal endophytes isolated in this study have been previously reported as grass endophytes. One of them is *Leptosphaeria spegazzinii* (Orlandelli *et al*., 2012, Uzayisenga *et al*., 2020). Despite being a grass endophyte, in this study it was isolated from plant sources and it showed antimicrobial activity in both well and disk diffusion methods. The inhibition could be due to the action of camphene, naphthalene, benzeneacetic acid, 1-haxadecanoic acid and 1-octadecanol. These compounds have been previously reported to have antimicrobial properties (Kumar *et al*., 2017, Chatterjee *et al*., 2018, and Peh *et al*., 2020).

The significant difference between the *Fusarium* species could be attributed to either the mycotoxins, non-mycotoxins, or both. Moreover, the mycotoxins and non-mycotoxins have been reported to have antimicrobial properties against a wide range of human and plant pathogens. In this study, each *Fusarium* species produced one or more unique metabolites. For instance, some *Fusarium* species produced methyl vinyl ketone, methane, isocyanato, and Ethanone,1-(2-hydroxyphenyl)-, (MR3, and MR7) while others produced acetophenone,2-chloro Phenol, 4,4'-(1-methylethylidene)bis (MR11,MR18 MR20). The difference in the active compounds between the *Fusarium* species could have contributed to the antimicrobial activity difference. Others such as MR18, MR20, MR23 produced Maleic hydrazide. According to Khan *et al*., (2017), maleic hydrazides and other hydrazides derivatives have antibacterial properties. Therefore, these finding shows that organisms can

be from the same genus, but their metabolite profiles can be different. The metabolites are characterized in diverse chemical classes.

All the isolated fungal endophytes produced compounds in alkane chemical class (Pentane, Dodecane, Hexadecane, Octadecane). These compounds might have contributed to the inhibition reported in one way or another. This is in agreement with Begum *et al*., (2016), who reported that the compounds in chemical class alkanes extracted from *Paracoccus pantotrophus* had antibacterial properties. Compounds in the chemical class of long-chain fatty acids such as 1-Tetradecanol, 1-Hexadecanol, 1-Octadecanol are known to have antimicrobial properties (Chatterjee *et al*., 2018). Therefore, they might have contributed to the inhibition of the test organisms reported in this study.

There was positive interaction between the test organism and the fungal extracts in the determination of the zones of inhibition may be as a result of the extracts active compounds. Each isolate produced active compounds that have been reported previously to have antimicrobial activity. The significant interaction with the test organism maybe due to the antimicrobial potency of one active compound present in the extracts or all the active compounds in the extracts worked together in combination to inhibit the test organisms. Additionally, the permeability of the test organisms cell membrane might have contributed to the positive interaction reported in this study

5.2 Conclusions

i. The study concluded that Cherangani forest has diverse medicinal plants. The medicinal plants cut across many plant families including; *Cucurbitaceae, Euphorbiaceae, Lamiaceae, Oleceae, Canellaceae, Mimosaceae, Cyperaceae,* among others. These plants have different growth forms, including trees, shrubs, herbs, and climbers. The medicinal plants are used to treat human diseases such as gastrointestinal, respiratory, and skin while the plant parts commonly used are; roots, leaves, and barks.

ii. Medicinal plants in Cherangani forest harbor diverse fungal endophytes. A total of 172 fungal endophytes were isolated from *Croton macrostachyrus* and *Zehneria scabra* hosts. The fungal endophytes isolated from these two plants were from twelve different genera including; *Fusarium* (Isolates MR3, MR7, MR13, MR18, MR20 and MR29), *Aspergillus* (MR6), *Penicillium* (MR10*), Acremonium* (MR11), *Lasiodiplodia* (MR14), *Aporospora* (MR19*), Clonostachy (*MR21*), Meyerozyma* (MR23), *Chaetomium* (MR22*), Phyllosticta* (MR25), *Ampelomyces* (MR26), and *Leptospaeria* (MR28).

iii. Extracts from isolates closely related to *Ampelomyces* sp. (MR26), *Chaetomium globosum* (MR22), *Leptospaheria spegazzanii* (MR28), and *Fusarium solani* (MR29) showed broad antimicrobial activity in disk diffusion and well diffusion methods. *Zehneria scabra* stem and root had fungal endophytes with highest antimicrobial activity as compared to the endophytes from *Croton macrostachyrus* host.

iv. The bioactive metabolites obtained from ethyl acetate extracts of the isolated fungal endophytes in this study were distributed across different chemical classes including alkane, long and short chain fatty acids and monoterpenoids. These metabolites are different from one fungal endophyte to another. The fungal endophytes can be from similar host, similar genus and species but their metabolite profiles are different.

5.3 Recommendations

The study recommends the following;

i. Medicinal plants documented in this study should be further investigated on their potential to host fungal endophytes.

ii. Use of *Croton macrostachyrus* and *Zehneria scabra* to isolate fungal endophytic with significant antimicrobial activity and bioactive metabolites which can help in the production of medically important compounds

iii. Fungal endophytes closely related to *Ampelomycetes sp*. (MR26), *Chaetomium globosum* (MR22), *Leptosphaeria spegazinii* (MR28), and *Fusarium solani* (MR29) which exhibited broad antimicrobial activity to be used in isolation of potential active antimicrobial compounds.

iv. Advanced screening and isolation of specific active compounds in the fungal endophytes extracts to help profile the active compound in each isolate.

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APPENDICES

Appendix 1: Data Collection Questionnaires

KENYATTA UNIVERSITY
SCHOOL OFPURE AND APPLIED SCIENCES

DEPARTMET OF BIOCHEMISTRY, BIOTECHNOLOGY AND MICROBIOLOGY.

Data collection Questionnaire

Questionnaire on acquisition of medicinal plants in Cherangani, Elgeyo-marakwet, Kenya

Section 1: Information on respondents

Name
…….
Residence……
…...

Age (Years)

Code	Age bracket	Specific age	
0	20-30		
1	31-40		
2	41-50		
3	51-60		
4	Above 60		

Sex

Code	Gender	
0	Male	
1	Female	

Level of education

Code	Level of education	
0	N/A	
1	Primary	
2	Secondary	
3	College	
4	Others	

Duration of practice (Years)

Code	Duration of practice	
0	0-10	
1	11-15	
2	Above 15	

Section 2: Information on medicinal plants used

Type of disease treated

Code	Type of disease treated	
0	Stomach related infections	
1	Respiratory	
2	Skin infections	
3	Others	

Common plants used (local name)

Code	Plants used (local name)	Scientific name	
0			
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			
11			
12			

Parts of the plant frequently used

Code	Parts of plant used	
0	Leaves	
1	Stem/Barks	
2	Roots	
3	Leaves and barks	
4	Barks and roots	
5	All (leaves, backs and roots)	

Availability of the plants

Code	Response	**Source of the plants**
0	Yes	0. Nearby forests 1. Family farms
1	No	0. Outsourcing outside the County

Accompanied side effects

Code	Any side effect	Specific side effects
0	Yes	0 1 2 3
1	No	

Accompanied verbal instructions

0	Yes	0.Once a day 1.Twice a day 2.Others
1	No	

Section 3: Information on plant and recipes used in treatment of common diseases.
Method of preparation

0	Boiling	
1	Raw	
2	Both boiling and raw	
3	Others	

Solvent used

0	Water	
1	Others	

Method of administration

0	Oral	
1	Swabs	
2	Others	

Patients response after use.

0	Good	
1	Fair	
2	Poor	

Source of medicinal plants

Nearby forests		
Family farms		
Outsourcing outside the county		

Section 3: Researcher declaration

i) The information collected will be used for the described research purpose and it will not be used for any hidden intention.

ii) I will not under any obligation tamper with the information provided by the respondents.

iii) I will not at any time intimidate the respondents to give information for this study. Each respondent who will participate in this study will have given his/her consent.

Thank you for participating.

Appendix 2:Dichotomous key used in identification of the selected Medicinal plants

Description: The two plants are dicotyledons

1. Plant aquatic, floating or submerged…… go to 2
2. Plant not floating nor submerged……..go to 8
8. Perianth of two of two distinct whorls, differing in shape, size and colour…..go to 9

   Plant
9. Petals free from one another right to the base…… go to 10
10. Ovary superior…… Group D

    Ovary inferior…… go to 11
11. Carpels or styles or both joined together, or ovary of only one carpel… go to 12
12. Ovary 2 to many celled……go to Group C

Group D:

1. Plant not parasitic…… Go to 2
2. Sepals more than 2…… Go to 3
3. Leaves alternate……Go to 7

7. Plant with tendrils……Cucurbitaceae

Group C

1. Sepals four; petal four; stamen six; ovary two celled; fruit opening……

   Cruciferrae

   Combination of characters not as above……o to 2
2. Stamen not opposite the petals…… go to 3
3. Leaves opposite or whorled…… go to 4

4. Flowers not spurred…… go to 5
5. Leaves not gland-dotted…… go to 6
6. Leaves simple…… go to 7

   Flowers with the petals and stamens attached to the calyx-tube; parts usually in fours or sixes; ovary two to six celled; fruit a capsule……
7. A combination of the above…… go to 8
8. Plant woody often shrub or tree…… go to 9
9. Flowers unisexual; ovary 2-3 celled,styles 2 or 3, has a fruit capsule often breaking to several parts …….. Euphorbiaceae

Cucurbitacea

1.Leaves simple or compound…… Simple……. go to 2

2. Flowers white or yellow…… yellow…… go to 3

Unisexual male and female flowers…….. go to 4

4. Petals joined……. go to 5

5. Number of stamens……1-5……go to 6

6. Ovary 2 or 3 celled…… Zehneria

Common names……Not found

Local name (Marakwet)…….. Cheserya

Scientific name…….Zehneria scabra

Appendix 3:Consensus sequences of the active isolates

>MR6

GGGGGGACTGCGGAAGGATCATTACCGAGTGCGGGTCCTTTGGGCCCAACCTCCCCATCCGTGTC
TATTGTACCCTGTTGCTTCCGGCGGGCCCGCCGCTTTGTCGGCCGCCGGGGGGGCCGCCCTCTtgCC
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CGAAATGCGATAACTAATGTGAATTGCAGAATTCAGTGAATCATCGAGTCTTTGAACGCACATTG
CGCCCCCTGGTATTCCGGGGGGCATGCCTGTCCGAGCGTCATTGCTGCCCTCAAGCCCGGCTTGT
GTGTTGGGTCGCCGTCCCCCTCTCCGGGGGGACGGGCCCGAAAGGCAGCGGCGGCACCGCGTCC
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TTTCCAACCATTCTTTCCAGGTtGACCTCGGATCAGGTAGGGATACCCGCTGAACTTAAGCATATC
TAAA

>MR3

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TTCTATAATGTTTCTTCTGAGTAAAACAAGCAAATAAATTAAAACTTTCAACAACGGATCTCTTGG
CTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAA
TCATCGAATCTTTGAACGCACATTGCGCCCGCCAGTATTCTGGCGGGCATGCCTGTTCGAGCGTC
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TGGAGAGCGGCGCGGCCACGCCGTAAAAACACCCAACTTTTCTGAAGTTGACCTCGAATCAGGTA
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>MR7

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ATACCCGCTGAACTTAAGCATA

>MR8

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TAATGGTTTTCGGTAAGGGGTGAATGCGGAGGGATCATTACCGAGTTATACAACTCATCAACCCT
GTGAACATTACCTTAAAACGTTTTGCTTTCCGGCGGGAACAGACGGCCCTGTAACAACGGGCCGC
CYCCGCCAGAGGACCCCTAACTCTGTTTTTATAATGTTTTTCTGAGTAAACAAGCAAATAAATTA
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ATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCGCCAGTATTCT
GGCGGGCATGCCTGTTCGAGCGTCATTACAACCCTCAGGCCCCCGGGCCTGGCGTTGGGGATCGG
CGGAAGCCCCCTGTGGGCACACGCCGTCCCTCAAATACAGTGGCGGTCCCGCCGCAGCTTCCATT
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>MR10

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TGCCATCAAGCACCCGGGCTTGTGTGTTGGGCGATACCCGTCATCCGATCCCGGGGGACGGGCCC
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>MR11

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>MR13

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>MR14

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>MR18

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>MR19

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TCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCCTTGGTATTCCATGGGGCATGCCTGTTC
GAGCGTCATCTACACCCTCAAGCTCTGCTTGGTGTTGGGCGTCTGTCCCCGCCTCCGCGCGCGGAC
TCGCCCCAAATCCATTGGCAGCGTCCCCTCGCCCCCCTCTCGCGCAGCACGAATGCGCATGACGA
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>MR20

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>MR21

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>MR22

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GTACTGAATAAGTCAAAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGC
GAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGC
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CGTAGTAGCATACATCTCGCTCTGGTCGCGCCGCGGGTTCCGGCCGTTAAACCACCTTTTTAACCC
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AAA

>MR23

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T

>MR25

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>MR26

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>MR28

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>MR29

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Appendix 4: Identity of the isolated fungal endophytes from Zehneria scabra and Croton macrostachyrus obtained from Cherangani forest

Identity of the isolated fungal endophytes isolated from *Zehneria scabra* and *Croton macrostachyrus*.

Isolate	Molecular identity	Accession numbers	Genera	Isolation source	%Similarity index
MR3	*Fusarium solani*	MW509848	*Fusarium*	*Zehneria scabra* leave	99.63
MR6	*Aspergillus niger*	MW509847	*Aspergillus*	*Zehneria scabra* root	97.79
MR7	*Fusarium solani*	MW509849	*Fusarium*	*Croton macrostachyrus* leave	99.44
MR8	*Fusarium solani*	MW509850	*Fusarium*	*Croton macrostachyrus* leave	96.41
MR10	*Penicillium chrysogenum*	MW509851	*Penicillium*	*Zehneria scabra* stem	90.73
MR11	*Acremonium sp.*	MW509852	*Acremonium*	*Croton macrostachyrus* leave	99.46
MR13	*Fusarium oxysporum*	MW509853	*Fusarium*	*Croton macrostachyrus* bark	94.57
MR14	*Lasiodiplodia theobromae*	MW509854	*Lasiodiplodia*	*Zehneria scabra* stem	96.67
MR18	*Fusarium solani*	MW509855	*Fusarium*	*Croton macrostachyrus* bark	100
MR19	*Aporospora terricolla*	MW509856	*Aporospora*	*Croton macrostachyrus* bark	98.17
MR20	*Fusarium solani*	MW509857	*Fusarium*	*Zehneria scabra* root	99.46
MR21	*Clonostachy rosea*	MW509858	*Clonostachy*	*Croton macrostachyrus* root	99.27
MR22	*Chaetomium globosum*	MW509859	*Chaetomium*	*Zehneria scabra root*	98.96
MR23	*Meyerozyma guillermondii*	MW509860	*Meyerozyma*	*Croton macrostachyrus* leave	93.35
MR25	*Phyllosticta citricapa*	MW467317	*Phyllosticta*	*Zehneria scabra* leave	100

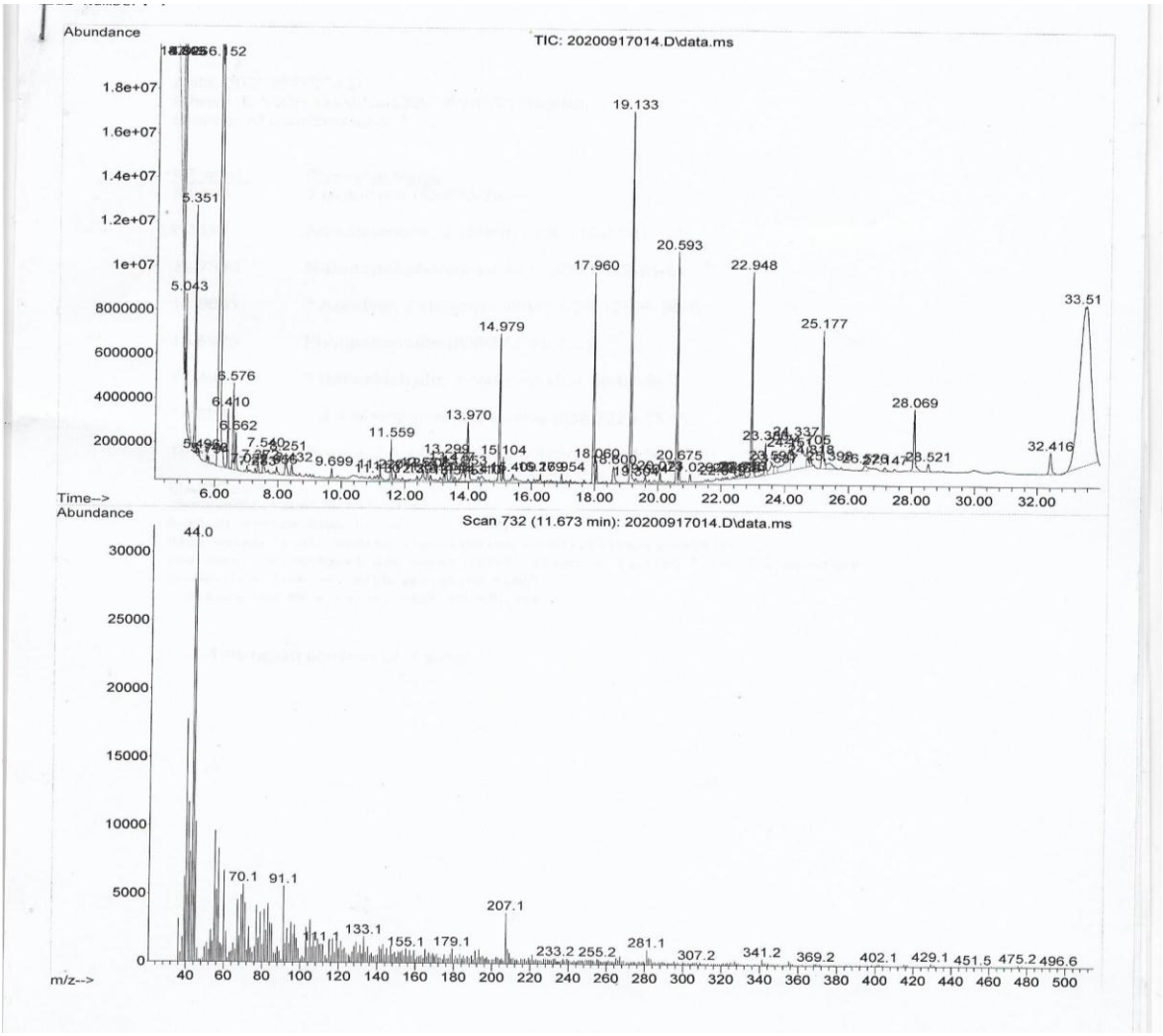
MR26	*Ampelomyces sp*	MW509861	*Ampelomyces*	*Croton macrostachyrus* bark	99.41
MR28	*Leptospheria spegazzanii*	MW509862	*Leptosphaeria*	*Zehneria scabra* stem	98.24
MR29	*Fusarium solani*	MW509863	*Fusarium*	*Zehneria scabra* root	99.64

The accession numbers of these isolate are found at

https://www.ncbi.nlm.nih.gov/nuccore/MW467317

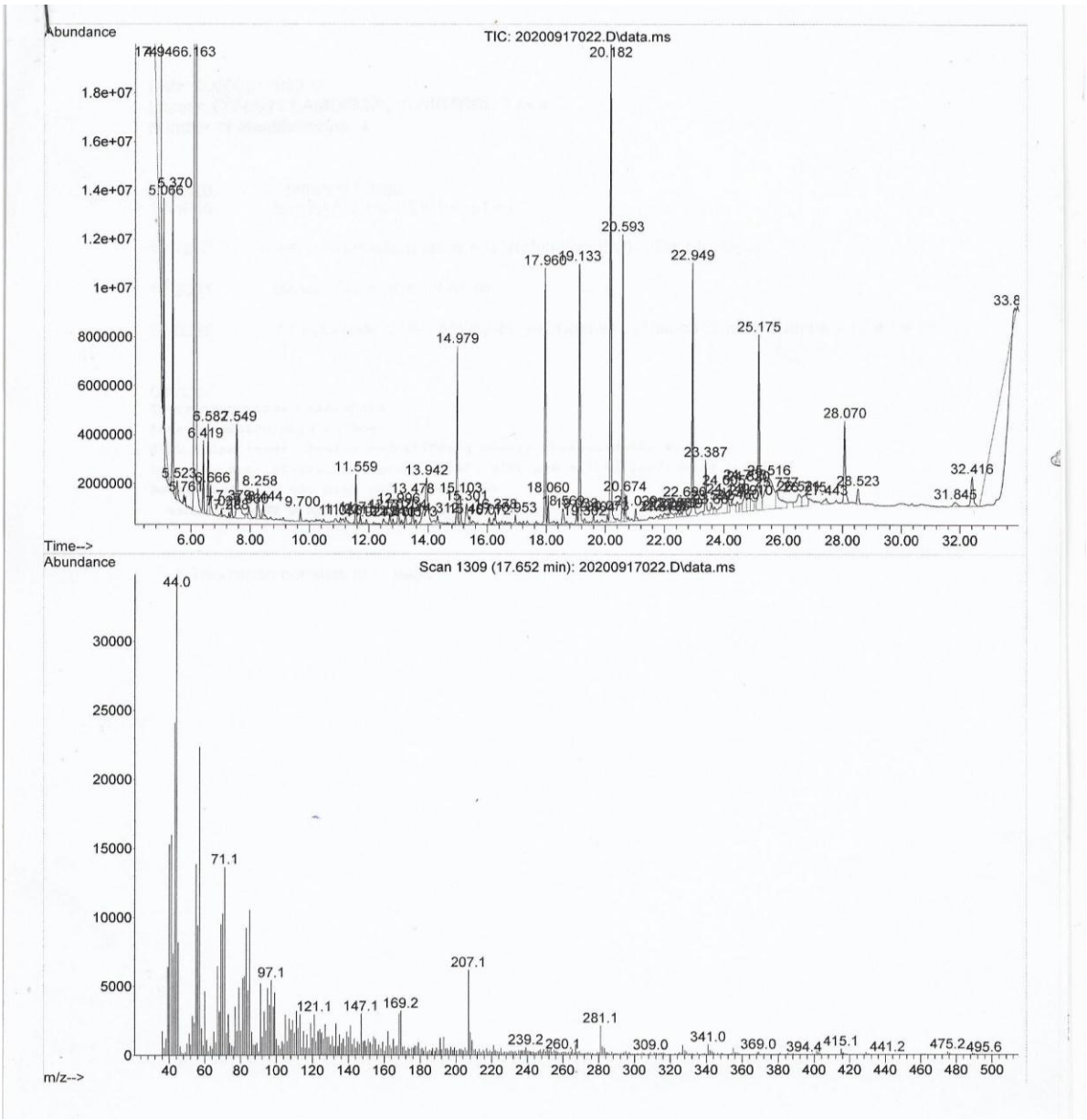
https://www.ncbi.nlm.nih.gov/nuccore/?term=MW509847:MW509863[accn

Appendix 5: Chromatogram peaks of the compounds identified in the ethyl acetate extracts



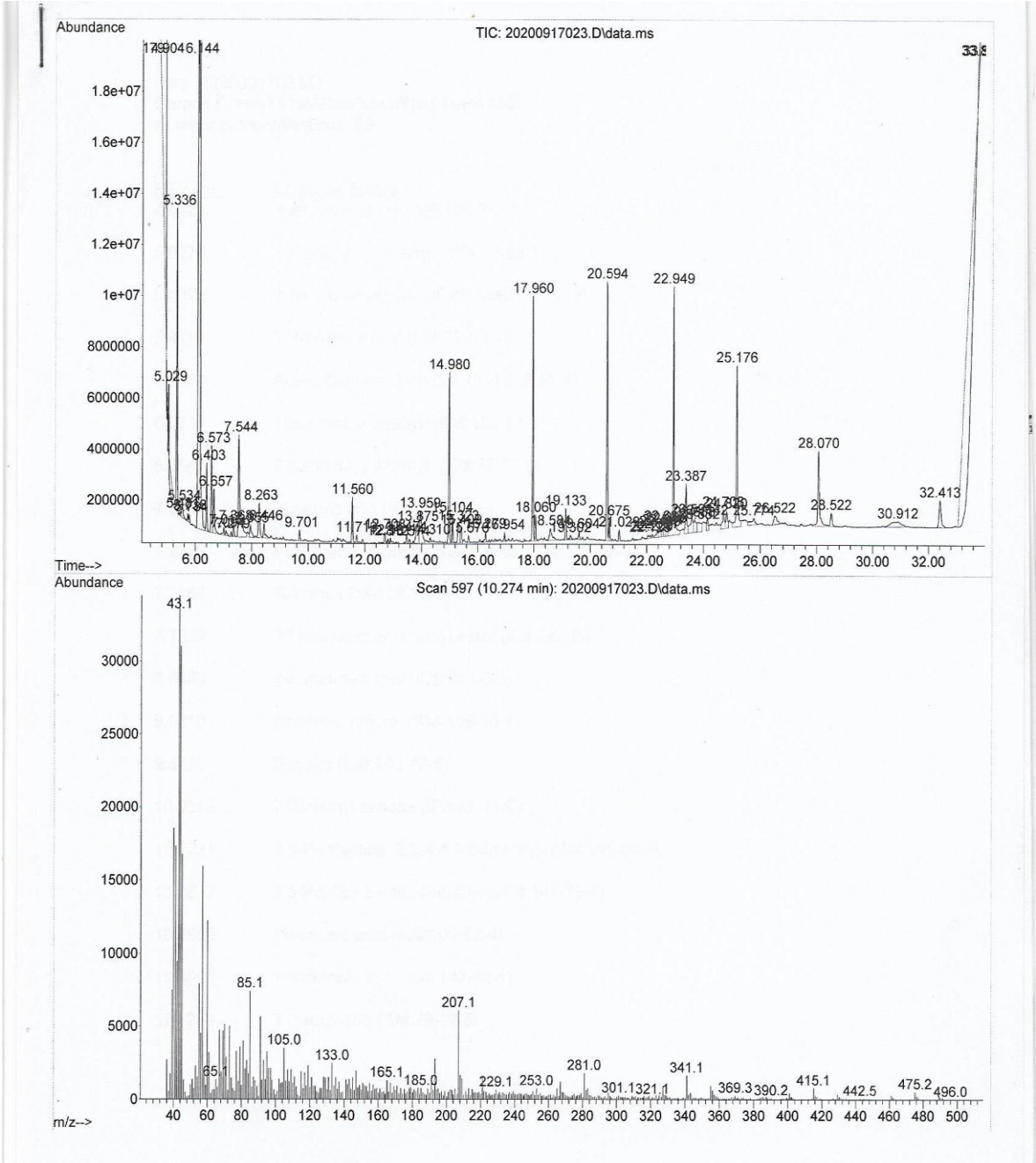
Chromatogram peak of compounds identified in ethyl acetate extracts obtained from isolate MR8

Closely related to *Fusarium solani.*



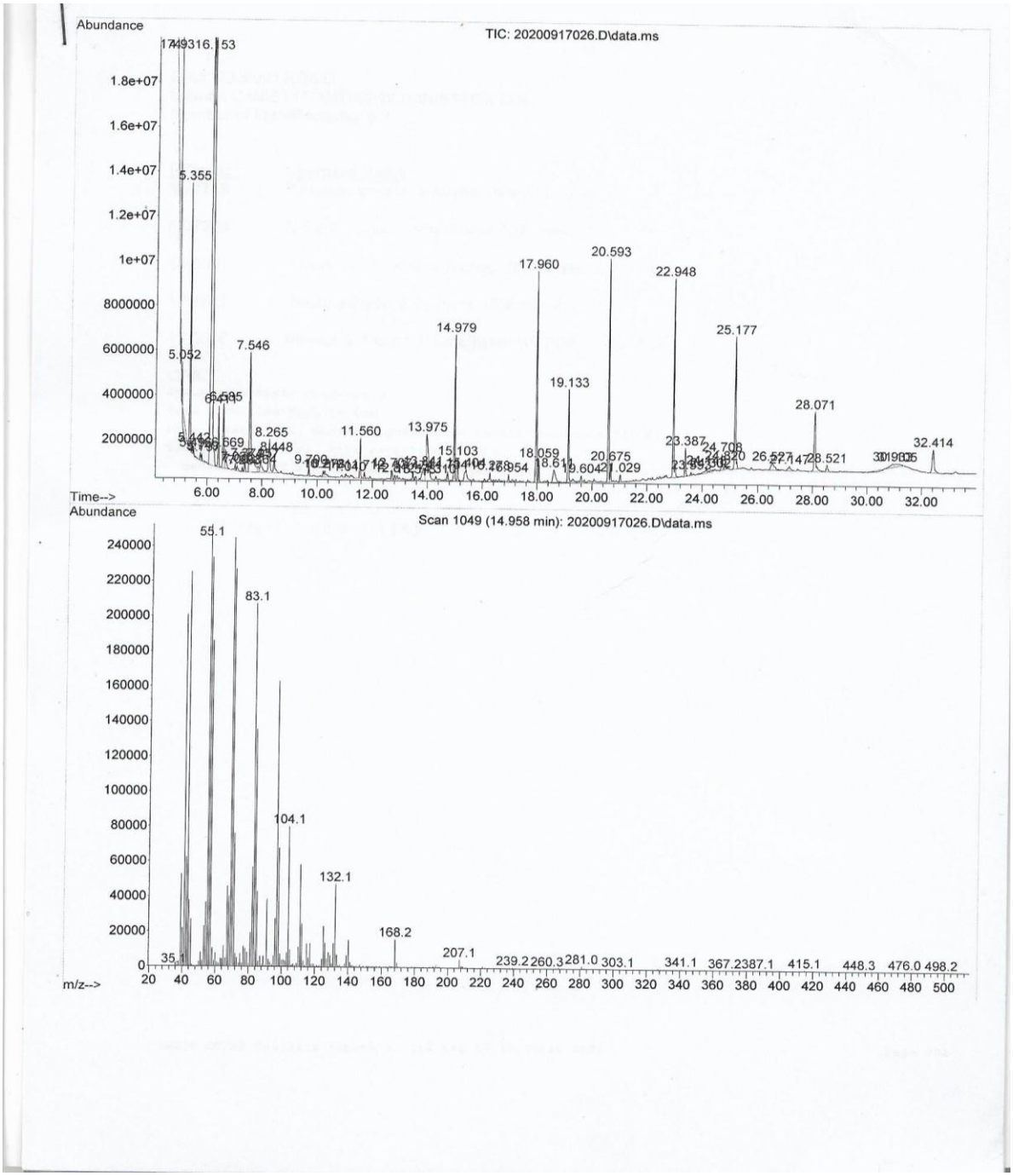
Chromatogram peak of compounds identified in ethyl acetate extracts obtained from isolate MR19

closely related to *Aporospora terricola.*



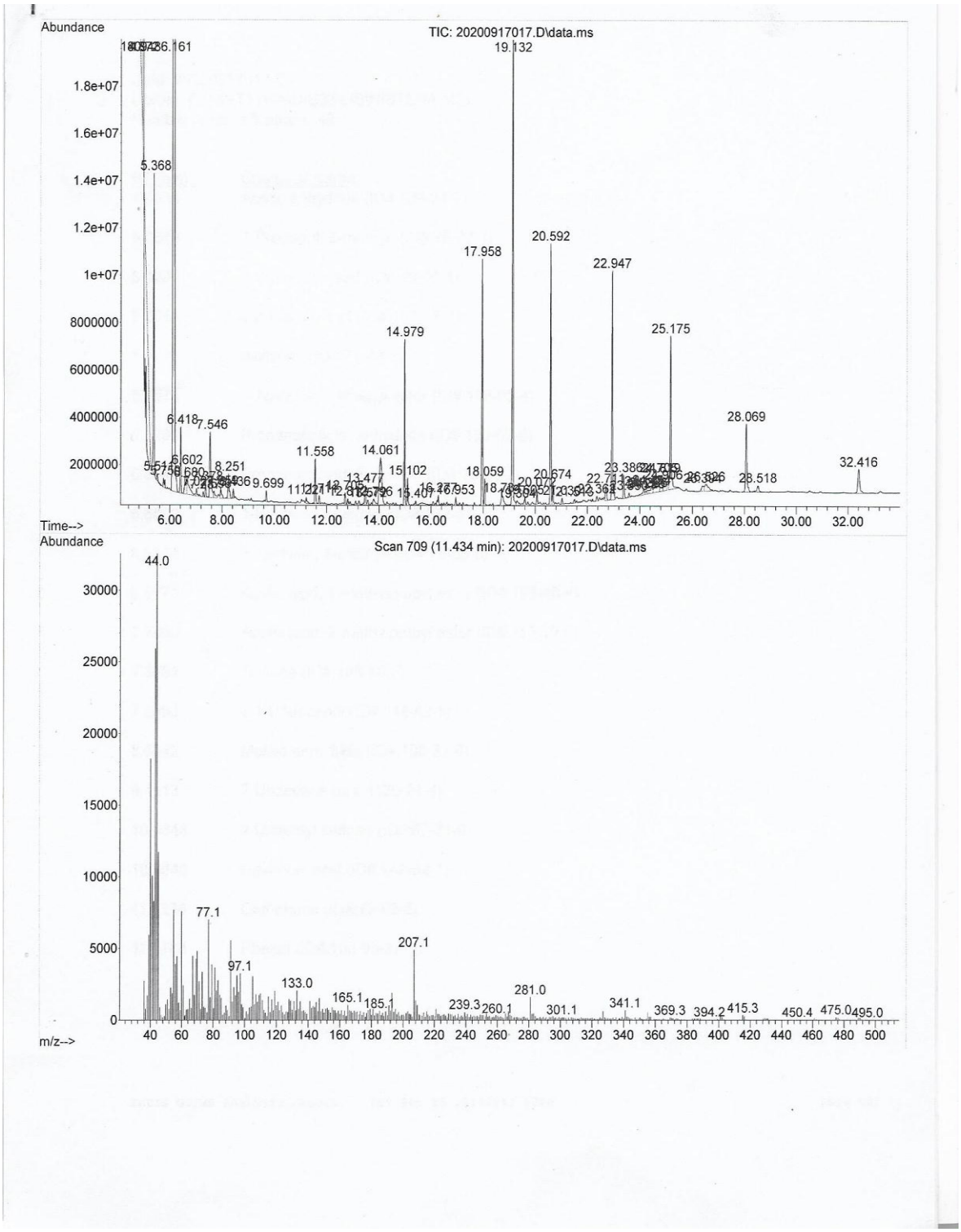
Chromatogram peak of compounds identified in ethyl acetate extracts obtained from isolate MR23

closely related to *Fusarium solani.*



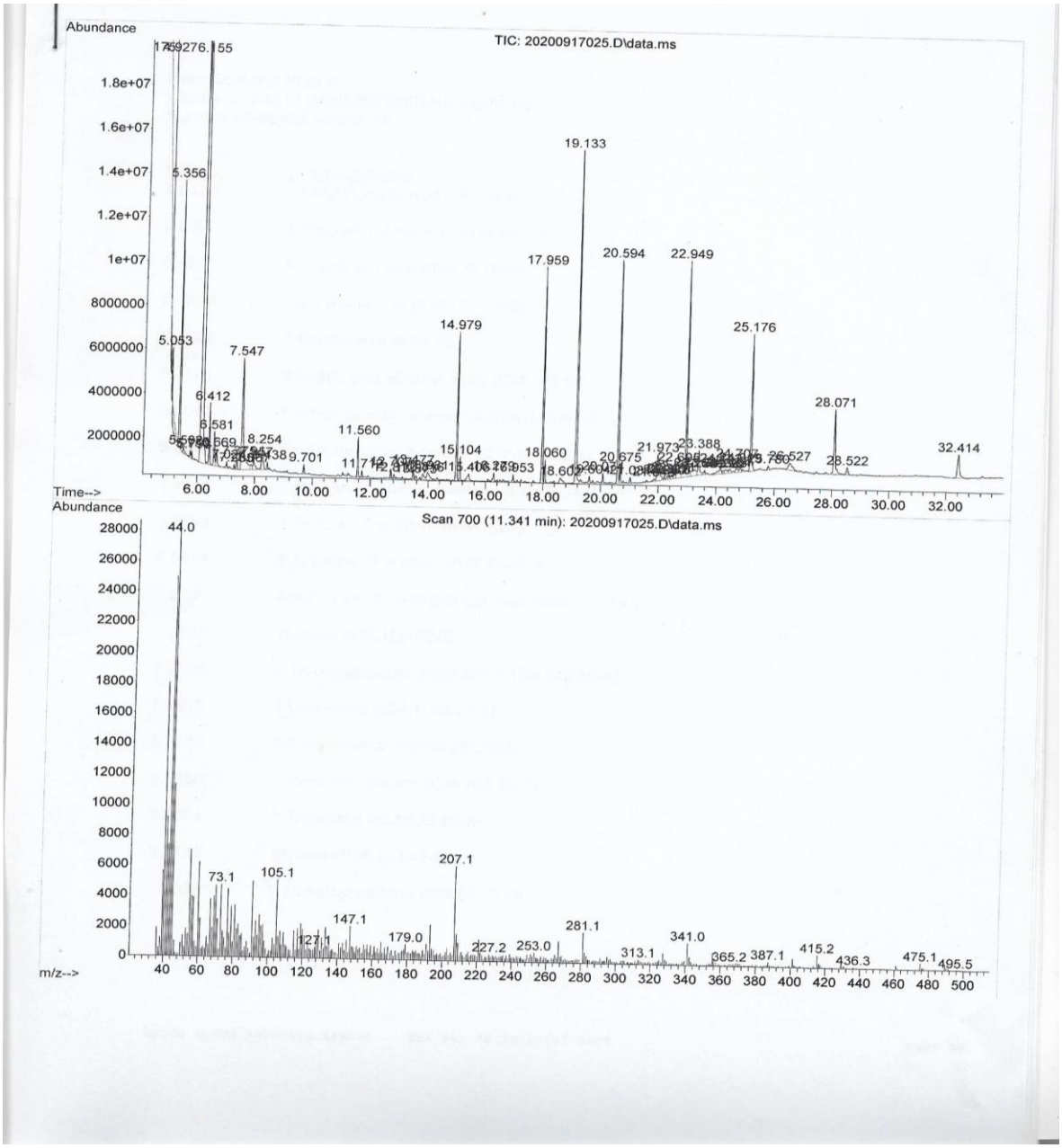
Chromatogram peak of compounds identified in ethyl acetate extracts obtained from isolate MR23

closely related to *Meyerozyma guillermondii.*



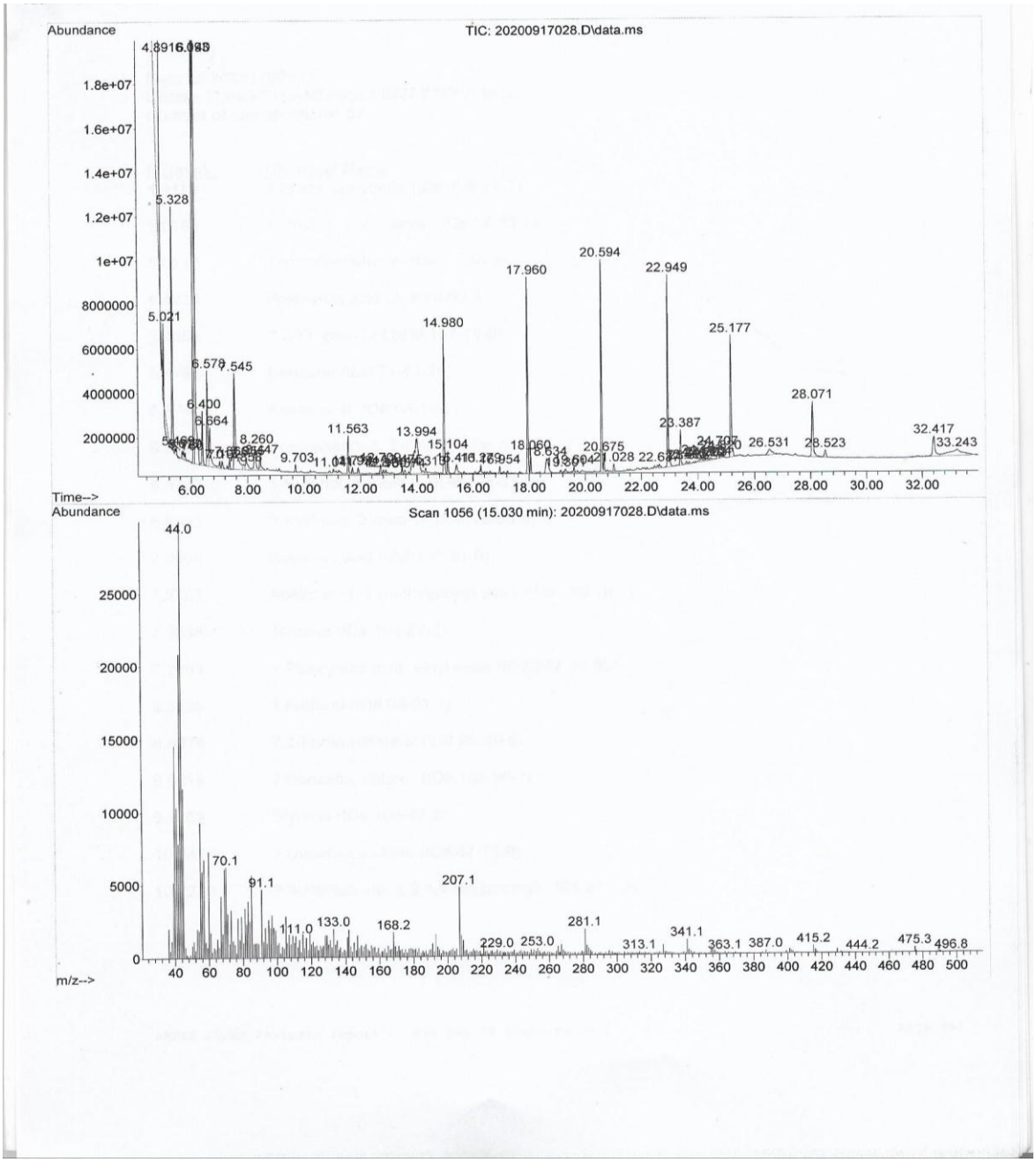
Chromatogram peak of compounds identified in ethyl acetate extracts obtained from isolate MR18

Closely related to *Fusarium solani.*



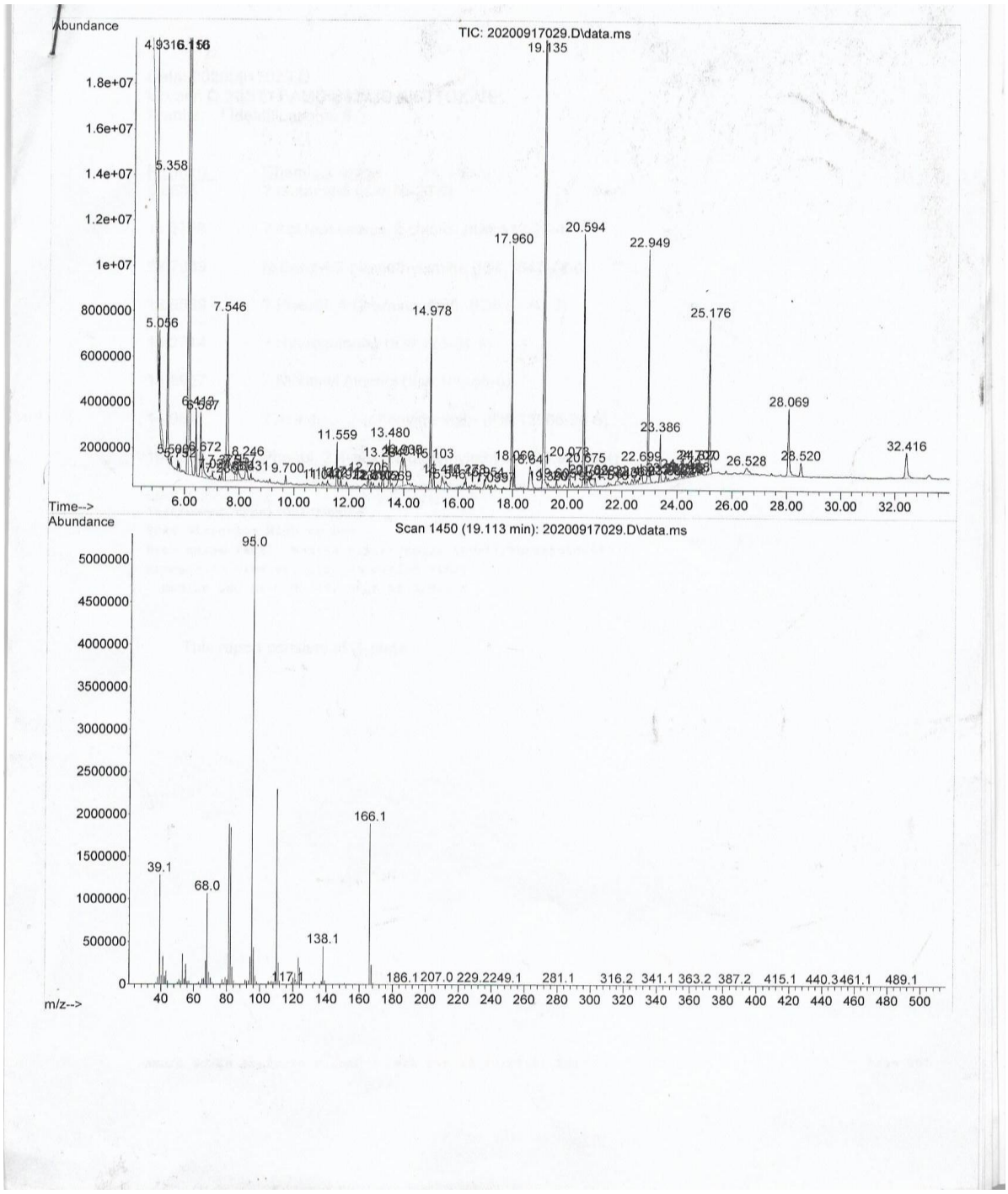
Chromatogram peak of compounds identified in ethyl acetate extracts obtained from isolate MR22

closely related to *Chaetomium globosum.*



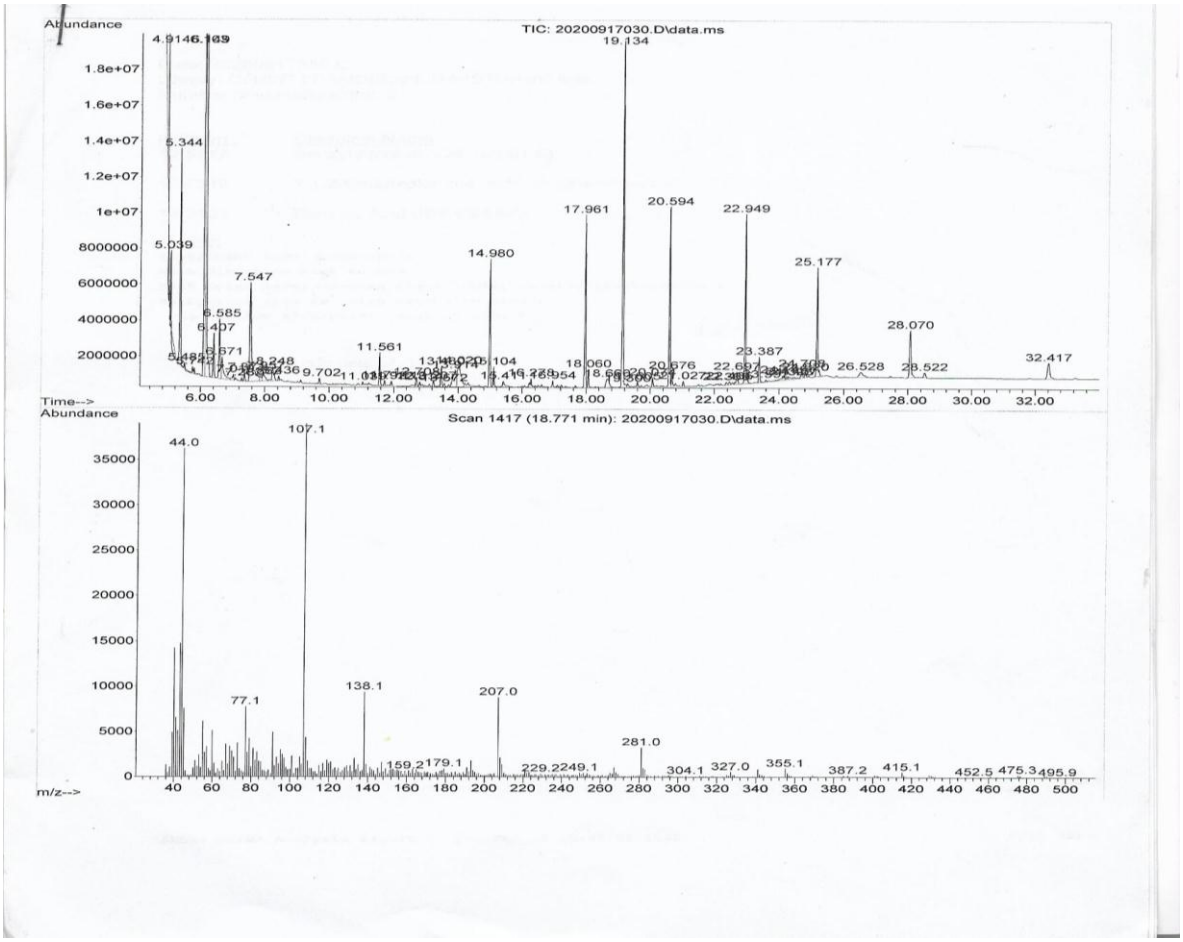
Chromatogram peak of compounds identified in ethyl acetate extracts obtained from isolate MR25

closely related to *Phyllosticta citricarpa.*



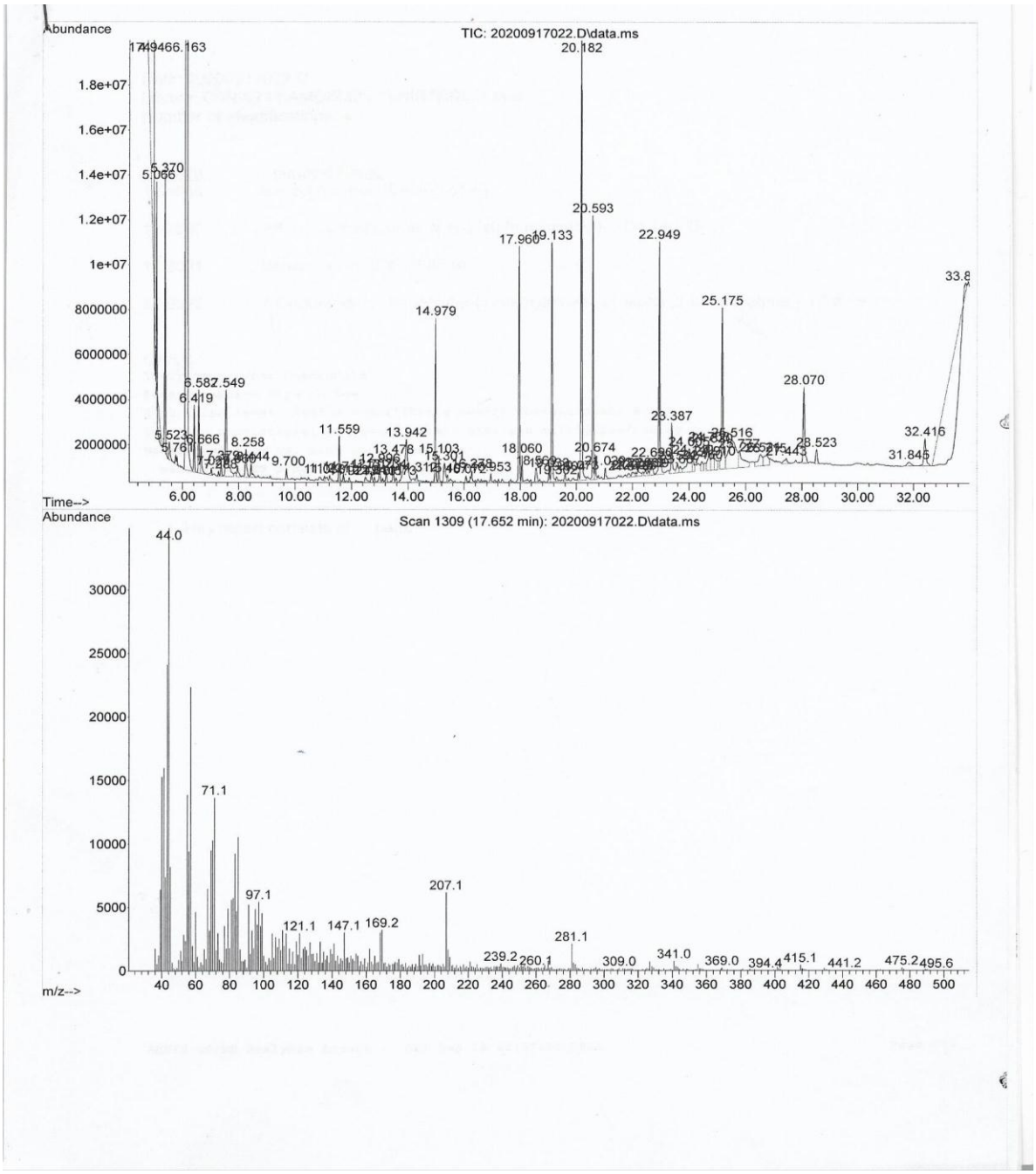
Chromatogram peak of compounds identified in ethyl acetate extracts obtained from isolate MR26

closely related *Ampelomyces sp.*



Chromatogram peak of compounds identified in ethyl acetate extracts obtained from isolate MR28

closely related to *Leptosphaeria spegazzinni.*



Chromatogram peak of compounds identified in ethyl acetate extracts obtained from isolate MR29

closely related to *Fusarium solani*.

Appendix 6: Grant of permission to collect Sample letter

COUNTY GOVERNMENT OF ELGEYO MARAKWET
OFFICE OF THE COUNTY SECRETARY

All correspondence to be Addressed to; County Secretary

P.O BOX 220 - 30700, ITEN
TEL: 05342277
Email: emcounty2013@gmail.com

Your Ref. .....
Our Ref: EMC/ADM/69/439

DATE: 20th November, 2020

Mutai Mourine
P.O. BOX 43844- 00100
NAIROBI.

RE: PERMISSION TO CONDUCT RESEARCH IN ELGEYO MARAKWET COUNTY – MARAKWET WEST SUB COUNTY.

Mutai Mourine a student at Kenyatta University wishes to conduct a research project on **"ISOLATION AND CHARACTERIZATION OF ANTIMICROBIAL FUNGAL FROM SELECTED MEDICINAL PLANTS IN CHERANGANI FOREST"** Elgeyo Marakwet County Marakwet West Sub County.

She has been granted permission to go ahead with research project within Elgeyo Marakwet County – Marakwet West Sub County . This is therefore, to request all institutions and individuals within our County to accord her the necessary assistance

Thank you.

THE COUNTY SECRETARY
ELGEYO MARAKWET COUNTY
P. O. Box 220-30700, ITEN
Email: info@elgeyomarakwet.go.ke

PAUL CHEMMUTTUT
COUNTY SECRETARY/HEAD OF COUNTY PUBLIC SERVICE

C.C

H.E GOVERNOR
COUNTY COMMISSIONER

Appendix 7: Research license

REPUBLIC OF KENYA

NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Ref No: **449277**

Date of Issue: **17/February/2021**

RESEARCH LICENSE

This is to Certify that Miss.. Mourine Jerono Mutai of Kenyatta University, has been licensed to conduct research in Elgeyo-Marakwet on the topic: Isolation and characterization of antimicrobial fungal endophytes from Cherangani Forest, Kenya for the period ending : 17/February/2022.

License No: **NACOSTI/P/21/8952**

449277

Applicant Identification Number

Director General
NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Verification QR Code

NOTE: This is a computer generated License. To verify the authenticity of this document, Scan the QR Code using QR scanner application.