ANTIFUNGAL BIOACTIVITY OF SELECTED MEDICINAL PLANTS USED IN TREATMENT OF FUNGAL INFECTIONS IN THE LAKE VICTORIA BASIN, KENYA

BY

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DECLARATION

This is my original work and has not been presented for a degree in any other University or Institution.

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Signature Date 11/8/2009

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DEDICATION

To my mum Margarate, my late Dad John, my husband Benard, my son Alvin, my Uncle Gordon, Aunts Martha and Cecilia, grandma Risper , my siblings and other members of my family.

The Late Prof. George M. Siboe was one of my supervisors in this study. In the month of Feb 2009, Prof., together with the other two supervisors signed my thesis for submission at the Board of Postgraduate studies. Like my other two supervisors, Prof, was excited that at last this work was coming to a completion, little did we know that prof, was not to sign the final copy of this thesis after defence. Just two months after I had submitted my thesis, Prof. Siboe succumbed to cancer he had struggled with for years. The science in this thesis is just part of how his profound knowledge in research goes on; because it is in the hearts and minds of those who knew him. This thesis is therefore dedicated to him as well, for his invaluable contnbutions, but never lived to see the final end of it. May his soul rest in eternal peace.

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ABBREVIATIONS AND SYMBOLS

ATCC: American Type Culture Collection

HIV/AIDS: Human Immunodeficiency Virus/Acquired Immune Deficiency

IUCEA: InterUniversity Council of East Africa

MTT: Methylthiazolyltetrazolium Chloride

Pa: Pascal

SDA. Sabouraud dextrose agar

S.T.D: Sexually Transmitted Disease

S.T.I: Sexually Transmitted Infections

T.B : Tuberculosis

TLC. Thin Layer Chromatography

UV: Ultra Violet

WHO: World Health Organization

MeOH. Methanol

CH_2CL_2: Dichloromethane

Opportunistic fungal infections are common in immunocompromised patients especially those with HTV/AIDS. The Lake Victoria Basin has some of the highest prevalence rates of HIV/AIDS hence high prevalence of these fungal infections. At least 10% of HIV/AIDS patients die of opportunistic fungal infections.

Various herbs/herbal preparations claimed to treat fungal infections were collected and tested for their antifungal activity. Out of 25 samples collected, six plants were selected for bioactivity analysis based on preliminary results. Extractions were done using hot water, cold water and organic solvents Dichloromethane (CH_2CL_2)/Methanol (MeOH) in the ratio 1:1. Antifungal activity was tested on a yeast fungus, *Candida albicans* and a filamentous fungus, *Aspergillus niger* using disc diffusion technique. Dichloromethane and Methanol (1:1) extracts of *Toddalia asiatica* (root), *Rhamnus staddo* (root) , *Momordica foetida* (shoot), *Podocarpus falcatus* (bark*),,Aloe sp* (secculent leaves), *Gladiolus dalenii* (bulb) showed activity against one or both of the test organisms. *P. falcatus* showed the highest activity (77.77% mean inhibition) against *A.niger* while *M. foetida* showed the highest activity (77.78% mean inhibition) against *C. albicans. G. dalenii* extracts inhibited spore production in *A. niger* up to day 5. Commercially used antifungal drugs, Ketaconazole and Gnseofulvin (Cosmos Pharmaceuticals) were used as standards.

Compounds responsible for the activity were identified using silica gel Thin Layer Chromatography (TLC) and appropriate spraying agents. Alkaloids and flavanoids were the major antifungal components reported. Results of this study indicate the possibility of developing plant-based drugs, from ethnomedicinal surveys, to help in longterm

management of Aspergillosis and Candidiasis, which are the frequent complications encountered in HTV-AIDS patients.

Key words Ethnomedicine, Antifungal, *Candida albicans, Aspergillus niger.*

CHAPTER ONE

1.0 INTRODUCTION

Traditional medicine is the sum total of all knowledge and practices , whether explicable or not, used in the diagnosis, prevention and elimination of physical, mental or social imbalance and relying exclusively on practical experience and observation handed down from generation to generation whether verbally or in writing (WHO 1978). Traditional medicine has been practiced since time immemorial by almost all cultures as a source of medicine, (DaSilva and Horareau, 1999). This involves use of animal substances, mineral substances and plant parts. Amongst the three, plants have been by far the most widely utilized.

In developing countries, especially among rural populations, traditional medicine remains a vital health resource in the treatment of a wide range of diseases such as skin infections, gastrointestinal, respiratory, and gynaecological among others (Palombo, 2006). This is mainly because most of the herbal medicines are easily available, affordable and effective with little or no side effects.

Human Immunodeficiency Virus/; Acquired Immune Deficiency Syndrome (HIV/AIDS) remains a global problem, with worst hit part of the World being Sub-Saharan Afnca. The HIV is known to fight the immune system of an individual. Once the immune system is suppressed, the presence of microbes and pathogens in everyday environment cause other diseases commonly referred to as opportunistic diseases or infections (UNAIDS Technical update, 1998). Lake Victoria Basin has some of the highest HIV/AIDS prevalence in the country, bringing with it a wide range of opportunistic fungal infections, which kills at least 10% of HIV/AIDS patients (Saag, 1997).The conventional management of fungal

related problems has been effected through a few pharmaceutical agents. The challenge however, is that these agents are usually expensive and hence beyond the pocket of most rural inhabitants who also face high poverty levels, some have adverse side effects thus reduces compliance by the patients, and finally some patients develop resistance to the drugs. It is therefore imperative that other sources of affordable and efficient antifungal agents be sought for, for long term management of these opportunistic fungal infections. One area for this search is in herbal medicines.

Herbal medicines are efficient in the treatment of fungal related infections in the Lake Victoria Basin, (Olembo *et al.,* 1995; Kokwaro, 1993) and they constitute a great economic and strategic value for the people in this area. However, the development of these potentially useful drug plants has been impeded by poor documentation coupled with little or no scientific validation of the herbal drugs, their safety and efficacy. According to Ngetich (2005), the use of ethnomedicinal leads to identify medicinal plants is a very slow process because herbalists maintain the secrecy of I . f their knowledge regarding it as a personal property. In order to realize the full potential of these medicinal plants, it is therefore necessary to scientifically verify the claims by the herbalists and make a reliable scientific documentation.

This study was designed to identify and screen medicinal plants claimed to treat fungal infections in the Lake Victoria basin with respect to *Candida albicans* and *Aspergillus niger*, and thereafter identify the compounds responsible for activity.

1.1 LITERATURE REVIEW

1.2 Traditional medicine

The use of traditional medicine has been incorporated into primary health care in countries such as China, India, Japan, Pakistan, Sri Lanka and Thailand. In China for example, about 40% of the total medicinal consumption is accounted for by traditional medicines while in Japan; herbal medicinal preparations are in higher demand than mainstream pharmaceutical products, (DaSilva andHorareau, 1999).

In Africa, the use of traditional medicine is important and prevalent in many societies (Kokwaro, 1983; Bussman, 2006). About 80% of rural populations in Africa use traditional medicine mostly plant preparations for their primary healthcare and have no available alternative. This is due to the high rate of population growth and difficulties in maintaining the existing levels of modem

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medicine coverage (WHO 2002-2005; Moshi 2005). About 20% of patients, who seek conventional medical care, first consult traditional healers (Dejong 1991). Traditional medicine therefore is a significant source of healthcare for significant number of Africans, (Dejong 1991). The overall number of drag plants used is large, and includes both indigenous and introduced plants (Kokwaro 1993; Fowler 2006).

Some of the plants used in African traditional medicine have been investigated as sources of antibiotics, anti-tumour agents and other useful substances (Kokwaro, 1983; DaSilva and Horareau, 1999). Dejong (1991) reported that although a wide range of traditional healers is active in Africa, information about their number and activities is scarce and only a small percentage of the plants recorded in traditional medicine have been scientifically investigated. For example,

several plants used for direct or specific treatment of particular diseases are yet to be investigated to determine their chemical composition and efficacy. Use of modem chemical analyses would therefore produce rewarding results in detecting the medicinal values of those plants reported in the treatment of various diseases (Kokwaro, 1993; Kariba, 2000).

Kenya, like many other African countnes has a long history of plant, animal and mineral based traditional medicine use, as vital primary healthcare (Kokwaro 1988). Although traditional medicine was the main healthcare system before the advent of colonialism, it remains less developed despite the fact that the practitioners are able to manage a wide range of disease conditions (Kokwaro 1988; Mukiama 2005). All ethnic groups in Kenya use different plant species for the treatment of both human and livestock diseases. (Olembo *etal.,* 1995 ; Githinji and Kokwaro, 1993; Agnew and Agnew, 1994; Kariba, 2000; Ng'etich, 2005; Lukhoba *et al.,* 2006; Bussman, 2006; Owuor and Kisangau, 2006).

Communities in the Lake Victoria Basin, like other communities in Kenya practice traditional medicine to a large extent. The diseases dealt with are mainly skin related problems, gastrointestinal diseases, sexually transmitted diseases, malaria, wounds, eye infections, measles, snake bites, diarrhoea, fever, asthma, and typhoid among others (Kokwaro 1993; 1988; Githinji and Kokwaro, 1993; Olembo *et al.,* 1995; Mukiama, 2005; Owour and Kisangau, 2006; Lukhoba *etal,* 2006; Otieno *etal,* 2007).

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1.3 Limitations in traditional medicine

Although traditional medicine is an excellent source of medicinal drugs as reported by Kubo and Taniguchi, (1993); De Smert, (1997) ; Fabricant and Farnsworth, (2001), that screening of plants used in traditional medicine showed a much higher probability of finding active extracts and is a key approach used to search for drugs, it also has some limiting factors. One factor is herbalist error. Sometimes the herbalists information are not reliable especially when the plants used are very similar in appearance and also where the number of diseases treated are too many to be remembered by the inheriting doctor. Another factor bringing herbal medicine into disrepute is the desire among some of its practitioners to become wealthy by pretending to know the remedy for every disease their patients may complain about (Kokwaro, 1993). Some herbalists may deliberately give erroneous information for fear of revealing secretly guarded information that may lead to losing their market (Yineger *et al.,* 2008). Unlike in the Asian situation, a significant part of traditional medicine in Kenya remains secret. A recent study in herbal medicine showed that most herbalists maintained the secrecy of their knowledge, and to most of them herbal knowledge is a personal property (Nge'tich, 2005), in some cases oaths are taken during passing of the knowledge so that it is kept secret and confidential (Kokwaro, 1993).

Lastly, a lot of vital information may be lost whenever a knowledgeable medicine man dies without revealing his knowledge to another. There is also therefore a real possibility that we may be losing out on the most effective medicinal plants simply because we have no records of their use in traditional medicine.

1.4 Utility and demand for medicinal plants products

There has been a high unparalleled global growth in the plant-derived medicinally useful formulations, drugs and healthcare products estimated at more than 60% of all medicaments (Wakdikar, 2004). Besides, the world market for plant derived chemical-pharmaceuticals, fragrances, flavour and colour ingredient alone exceeds several billon dollars per year. It is estimated that global trade in medicinal plants is USD 800 million per year (DaSilva and Hoareau, 1999: Wakdikar, 2004). Europe imports an estimate of about 400, 000 tons of medicinal plants with an average market value of USD 1 billion from Africa and Asia. There is an enormous market for crude herbal medicines (De Smert, 1997). Medicinal plants therefore continue to be remarkable source of new biologically active compounds which are playing an increasingly significant role in commercial development of new drugs ((Moshi, 2005; Balandrin *et at.,* 1985).

While the potential in traditional medicine is much acknowledged, little has been done in African countnes to formerly recognize, commercialize and incorporate the drug plants well utilized and already proven safe for use, despite the fact that this practice forms a significant part of traditional rural healthcare (Moshi, 2005 ; Mukiama, 2005 ; Dejong, 1991). Among about 250,000 existing higher plants which contain the highest percentage of bioactive compounds, only about 6% of the plants have been screened for biological activity, only about 15% have been evaluated phytochemically and only a handful of drug plants have successfully passed the test of commercial screening (Wakdikar, 2004 ; Fabricant and Farnsworth, 2001).

Although widely practiced, traditional medicine usage in Kenya is less developed. Despite the fact that it was the main healthcare system before the advent of colonialism, the introduction of

modern medicine health services led to rapid marginalization of traditional medicine and the practice was viewed as primitive (Mukiama, 2005). Efficient utilization and commercialization of this widely known and tested resource of traditional medicine would not only bring financial gain to the local users but also improve their health. In order to satisfy this growing market demand therefore, there is need for development of traditional medicines and deliberate efforts to encourage local industrial production of traditional/ herbal medicine derived drugs to compete effectively with the growing market and demand, while at the same time conserving the useful species (Wakdikar, 2004)

Interest and demand in medicinal plants as a re-emerging health aid the world over including the developed world has been fuelled by the rising costs of prescription drugs in the maintenance of personal health and well being, and the successes achieved in bio-prospecting of new plant-derived drugs, (DaSilva and Hoareau, 1999 ; Wakdikar, 2004). Unmet therapeutic needs, remarkable diversity of both chemical structures and biological activities of naturally occurring secondary metabolites are other reasons why a lot of work is being done on medicinal plants. Other reasons still could be due to development of sensitive techniques to detect, isolate, purify and structurally characterize the active constituents in medicinal plants. (Clark, 1996).

Among other techniques used in search for new antifungal compounds from higher plants is bioautography. This is a method used to localize antimicrobial activity on a chromatogram, (Horvath, *et al,* 2002). It allows the combination of bioassay *in situ* and at the same time, localization of active constituents on the Thin Layer Chromatography (TLC) plates employed for the assay. Spore producing fungi such as *Aspergillus, Cladosporium, Penicillium* and certain

bacteria are employed as target organisms in direct bioautographic procedures (Hostettmann and Marston 1994). After migration of an extract on a TLC plate, it is sprayed with the microorganism's spore suspension and incubated under humid conditions. Zones of inhibition appear where spore growth is prevented by the active constituents of the plant extract. Direct bioautography is not possible with yeasts such as *Candida albicans* due to their hyaline nature. A simple and rapid agar overlay assay has therefore been developed, referred to as bioautography agar overlay method. This technique is a hybrid of direct and contact bioautography which relies on the transfer of active compounds by diffusion process from the stationary phase in the TLC plate into the agar layer containing the microorganism. When the plate is sprayed with thiazolyl blue (3-(4,5 dimethyldiphenyl tetrazolium bromide) (MTT), an MTT formazan is produced and zones of inhibition are observed as colourless regions against a purple background (Hostettmann and Marston 1994 ; Runyoro *etal.,* 2006).

There is need therefore to establish the necessary expertise! to unearth new plant bioactive compounds as sources of herbal remedies and medicines, scientifically validate and expeditiously utilize more medicinally useful plants and hence a boost to war against poverty, one of the challenges in the Lake Victoria Basin.

1.5 Prospects and Potential significance of herbal remedies/medicine

Most of the natural products synthesized by many tropical plants such as alkaloids, flavanoids phenolic compounds, oils, resins, tannins, natural rubber, gums, waxes, dyes, flavours and fragrances are bioactive against a wide range of pathogens (Balandnn *el al,* 1985), and are good sources of new biologically active natural products. In addition, they are biodegradable and

renewable (Kubo and Taniguchi, 1993). Natural products from medicinal plants have served as a major source of drugs for centuries and accounts for half of the pharmaceuticals in use today (Clark 1996; De Smert 1997; WHO 2002-2005). Moshi (2005) reported that 90% of newly discovered pharmaceuticals are derived from natural products. Natural products derived from medicinal plants have proven to be an abundant source of biologically active compounds (Palombo, 2006; Fabricant and Farnsworth, 2001). According to Palombo (2006) there are approximately 500,000 plant species occurring worldwide, of which only 1% has been phytochemically investigated. There is therefore a great potential for discovering more novel bioactive compounds. Plant natural products therefore continue to play a central role in the discovery and development of new drugs.

1.5.1 Opportunistic fungal infections

These are infections caused by presence of fungal pathogens which occur in everyday environment but cause diseases in immunocompromised individuals. (UNAIDS Technical update, 1998; Hostettmann and Marston 1994).

Treatments with immunosuppressive drugs and the spread of HIV/AIDS have meant that diseases resulting from weaknesess in the immune systems of humans are becoming more and more prevalent (Hostettmann and Marston 1994). The infections commomnly observed in immuno-compromised hosts include candidiasis (*Candida albicans* and other species) of the oesophagus, systemic and of the mouth, cryptococcosis *(Candida neoformans)* and aspergillosis (*Aspergillus niger, A. fumigatus* and *A. Jlavus).* As there are few effective antifungal preparations currently available for the treatment of systemic mycoses, it is important to find new sources of antifungal agents (Hostettmann and Marston 1994).

People living with HIV/AIDS virus face tremendous health risks from these opportunistic fungal illnesses that compromise their way of life (UN-HABITAT, 2005). Scientific verification and validation of claimed ethnomedicinally effective herbal drugs could be of potential significance for the long term management of these infections, not only in HIV/AIDS patients but also in other conditions where these infections may arise.

The Kenyan Lake Victoria basin has some of the highest prevalence rates of HIV/AIDS in the country. In fact Nyanza and Rift Valley Provinces only, are home to 1.5million HIV-infected Kenyans (G.o.K, 2007; AMREF, 2008). This region is also among the most densely populated areas in the country, known to have the highest poverty levels, with 41% of the population earning less than one dollar per day (SIDA, 2005). In addition, Kisumu, the largest administrative center in the Lake Victoria basin of Kenya, is ranked the poorest city in Kenya with 48% of its inhabitants living below the poverty line and high HIV/AIDS prevalence challenge (UN-HABITAT 2005; East African Community Secretanat, 2004).

From the enumerated reports on HIV/AIDS, the higher the number of people living with the virus, so are those whose lives are compromised by the opportunistic fungal infections that are associated with the scourge. At least 10% deaths of HIV/AIDS patients are caused by common opportunistic infections most of which are fungal, (Saag, 1997). These include oral, oesophageal and systemic candidiasis with over 50% occurrence, Dermatophytoses have 20 - 90% occurrence, and another huge proportion of mortality caused by *Cryptococcus* yeast infections, and other mycoses caused by filamentous fungi such as *Aspergillus niger, A. fimiigatus* and *A. flavus* (Runyoro *et ah, 2006).* All these mycoses are fatal except dermatophytoses. To minimize these

fungal infections, several pharmaceutical antifungal agents are commonly used. However, they are expensive, less effective, have adverse side effects and there is increasing occurrence of resistance to the antifungal therapies (Runyoro *et al.,* 2006 ; Melody, 2006), yet these patients need frequent and long-term use of antifungal remedies for the long term management of these opportunistic infections.

1.6 Literature on the six plants selected for bioactivity analysis

1.6.1 *Toddalia asiatica* (Rutaceae)

1.6.1.1 Taxon description and distribution

A climbng shrub or liana 2-15 m; in lianas the lower tern is beset with spines on corky pyramids; branches and often undersides of leaves with hooked prickles to 5mm. leaflets elliptic or slightly obovate, base cuneate, apex obtusely acuminate, 3-8 by 1-3 cm, glabrous; margins sometimes crenulate. Flowers greenish-yellow, in axillary and terminal panicles; petals 2-3mm long. Fruit orange, round 7-1 Omm (Beentje, 1994)). Distributed along the coasts, riverine, forest margins or secondary regrowths.

1.6.1.2 Medicinal value and Phytocheniistry

The fruit is chewed as a cough remedy, root decoction employed as an emetic and purgative (Beentje, 1994)). Infusion used for malaria (Kuria *et al.,* 2001). Leaves and branches are boiled and steam used as vapour for both nasal and branchial pains, fruits and their decoctions are taken for coughs and colds. Root chewed and juice swallowed for stomachache (Kokwaro, 1993). Methanolic extracts of dried leaves showed antimutagenic activity (Nakahara *et al.,* 2002). Leaf essential oils showed weak activity against bacteria *Bacillus pumilus , B. subtilis, Escherichia coJi, Proteus vulgaris, Pseudomonas, Salmonella newport, S. pullorum, S. Stanley,Staphylococcus albus, S. aureus. Streptococcus agalactiae, Vibrio cholera* and against fungi *Rhizopus stolonifer*

however activity was reported when tested against *Klebsiella pneumoniae, Aspergillus fumigatus, A.niger* and *Microsporum gypseum.* No activity was reported when tested against *Aspergillus flavus, Fusarium oxysporum, Penicillium digitatum.* and *P.notatum* (Saxena and Sharma 1999). Unspecified part showed vasorelaxation activity (Ren, *et al.,* 2000). Ethanolic extracts of unspecified was reported to be active against *Plasmodium falciparum* (Kuria *et al,* 2001). Ethylacetate , Methanolic and dichloromethane extracts of dried roots have been reported to be active against *Plasmodium falciparum* (Oketch *et al.,* 2000). Tsai *et al,* (1998) and Saxena and Sharma., (1999) reported coumarins, isoquinoline alkaloid, quinoid, monoterpene, phenylpropanoid,, quinoline alkaloid, lignan as the main phytochemicals in this plant.

1.6.2 *Rhumnus staddo* (Rhamnaceae)

1.6.2.1 Taxon description and distribution

A shrub or atree, 1-7.5m, rarely reported as scandent; bark smooth, grey. Short shoots (many - nodded twigs) often present, occasionally thorn-tipped. Leaves (narrowly) obovate, rarely elliptic, base cuneate, apex blunt to acute, margin minutely glandular-serrate, 1-3(4.5) by 0.4-1.2(1.8)cm, glabrous or minutelt haiiy. Flowers greenish to yellow, solitary at the nodes, but when on short - shoots appearing fascicled, to 2mm long. Fruit red, turning black, sub globose, 5.-5.5mm across. Mainly found in dry upland forests (edges) or (secondary) evergreen bushland and clump bushled grassland (Beentje, 1994).

1.6.2.2 Medicinal value and Phytochemistry

Used against malaria and also venereal diseases (Beentje, 1994 ; Kokwaro, 1993). Root decotion drunk by barren women to be able to have children (Kokwaro, 1993). Infusion used to treat malaria (Kuria *el al,* 2001 ; Gakunju *et al.,* 1995). Root bark infusion used against fever (Gakunju *et al.* 1995). Taenicide activity and toxicity test has been assessed in water and hydroalcoholic extracts of dried leaves (Desta, 1995). Ethanolic extracts of unspecified parts have been reported to be active against *Plasmodium falciparum* (Kuria *et al.,* 2001). Water , Ethanolic, and butanol extracts of dired roots evaluated for implantation and urine stimulant effects was reported inactive (Desta , 1994). Chloroform exracts of dried root bark did not show any cytotoxic activity a against cell culture and *Plasmodium falciparum.*(Koch *et al.,* 2005). Quinoid, polycyclic, and flavonol are some of the phytochemicals reported in this plant (Pourveura, 1973).

1.6.3 *Podocarpus falcatus* (Podocarpaceae)

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1.6.3.1 Taxon description and distribution

Tree to 36m, evergreen, trunk to 2m across, bark plae grey or brown, flakingb in long irregulae rectangles. Leaves on the ends of bracnlets only, linear, base cuneate, apex acute, gradually tapered, 2-4 by 0.2-0.4 cm in mature trees, up to 18 by 1.6 cm in juvenile trees, with stomsta on both surfaces. Male cones 1-3, yellow brown, 10-23mm long ; female cones single, green to grey or yellow gree, later purple. Seed at maturity (fruit) ellipsoid or globose, 14-23 by 11-21 mm, with a woody shell of l-2mm thick., found mainly in upland forests, may form pure stands (Beentje, 1994).

1.6.3.2 Medicinal value and Phytochemistry

An infusion from bark drunk for treatment of stomachache and cattle diseases (Beentje, 1994). ;Kokwaro, 1993). The bark and sap of this species is used to treat chest complaints and also as a herbal remedy to treat a variety of livestock diseases including gallsickness (Abdillahi *et al,* 2008). Oils from this plant are used to cure gonorrhoea and powder from the bark is used for headaches (Pankhurst, 2000).Pet ether, hexane, dichloromethane, acetone and ethanol extracts of leaves and stem exhibited broad-spectrum antibacterial against *Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae* and a yest fungus *Candida albicans,* however weak activity was reported when tested with the aqueos extracts (Abdillahi *et al.*, 2008). Diterpene taxol has been isolated from this plant (Stahlhut *et al.,* 1998).

1.6.4 *Momordica foetida* (Curcubitaceae)

1.6.4.1 Taxon description and distribution

This is a hairy perennial climber with woody rootstock, simple or devided tendrils and spotted stems. Leaves heartshaped, fewer sexes on separate plants, yellow with black centres ; males fewer than 10 in a broad bract, each with three stamens , fruit up to 65mm long covered with soft orange bristles. Common in forest edges, cultivation and disturbed places in wetter regions 1200-3000m (Agnew and Agnew, 1994).

1.6.4.2 Medicinal value and Phytochemistry

Hot water extracts from the dried entire plant is used as an abortive (Yu and Pao , 1982). The same is used to treat otitis, bronchitis and malaria (Boily and Van, 1986; Gessler *et al.* 1994;

Hakizamungu *et al.* 1992). The fruit is used as a remedy for diabetes (Oliver-Bever, 1980) . Leaves are used as antivemn (Sevanayahgam *et al.* 1994). Methanol extracts of dried leaves of this plant tested active against *Staphylococcus aureus* but inactive against *Salmonella gal Una mm.* while those of stem tested active against *Bacillus subtilis and Mycobacterium* but inactive against *Candida albicans, Staphylococcus aureus, Salmonella gallinarum and Pseudomonas aeruginosa* (Boily and Van, 1986). Hakizamungu *et al.* (1992) reported activity of the same extracts against *Trichomonas vaginalis.* Ethanol, pet ether, ethylacetate,and water extracts of dried leaves of this plant showed activity against *Plasmodium falciparum.* (Gessler *et al* . 1994). The compounds isolated and identified from are reported as steroid and alkaloid from the dried entire plant (Olaniyi, 1975, 1980) respectively. Triterpenoids have also been reported in leaf extracts of this plant (Mulholland *et al.* 1997).

1.6.5 *Aloe sp.* (Aloaceae)

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1.6.5.1 Taxon description and distribution

Fleshy leaved perennial her or shrub. With leaves two ranked or in rosette, usually more or less tnangular or sickle- shaped armed with sharp teeth, usually with a bitter tasting yellow or brown juice when broken , and lateral simple or branching inflorescences with racemes of red or yellow flowers; bracts present, penanth more or less bilaterally symmetrical, with cylindrical or 3 angled tube and free, more or less equal lobes , stamens 6, each group of three elongating and exserted, and then retracted in turn during flowering, style and stigma simple. Fruit a capsule with many

angular or flattened black or brown seeds, usually with narrow membranous wings (Agnew and Agnew, 1994).

1.6.5.2 Medicinal value and Phytochemistry

An infusion of *Aloe* is used as antiseptic and to clear colds and influenza . It is also used to treat eye infections, boils , sores, ringworm and heamorrhoids. It is also used to treat skin complicatiosn and as a digestive aid for the stomach. It has been reported to be anti-inflammatory and accelerates wound healing. Has been reported to have unspecified antibiotic, antifungal and virucidal activity (Lawless and Allan 2000). Mono and polysaccharides, saponins, antrhaquinones are some of the phytochemicals reported in the Aloes (Lawless and Allan 2000).

1.6.6 *Gladiolus dalenii* (Iridaceae)

1.6.6.1 Taxon description and distribution <

Plants 25-40 (-60) cm. high. Corm 2.5-3.5 cm. in diameter, tunics coriaceous, fragmenting irregularly, straw-coloured, the outer layer sometimes becoming fibrous. Foliage leaves of the flowering stem usually all partly to entirely sheathing, (l-)2-3(or more), hardly differing from the cataphylls, sometimes with short blades 2-5 (-10) cm. long, often partly dry by anthesis; foliage leaves produced after flowering on separate shoots, evidently solitary, lanceolate, ultimately at least 30 cm. long, 1 cm. wide, the margins and midrib moderately thickened and hyaline; occasionally flowering stems with 1-2 long-laminate leaves. Stem unbranched, rarely branched, 3-4 mm. in diameter at the base of the spike. Spike 5-9(-12)-flowered; bracts (1.5-)2-2.8(-3.5) cm. long, green, often flushed reddish and becoming membranous above, the inner slightly shorter

than the outer. Flowers either orange-red to pink or rarely yellow to whitish, without markings; perianth-tube (1.2-)1.8-2 cm. long, widening evenly from the base, gently curving outward; tepals unequal, the upper 3 2.5-3.8 x 1.5-1.8 cm., strongly recurved distally, the dorsal horizontal or down-tilted, the upper laterals slightly narrower, directed forwards, the lower tepals curving downwards, the lower laterals 2.5 x 1.2-1.5 cm., the lowermost 3-3.5 cm. long, often as long as the upper. Filaments 8-10 mm. long, included in the upper part of the tube; anthers 0.7-1.2 cm. long. Style dividing 2-3 mm. beyond the apex of the anthers, the branches 6-8 mm. long. Capsules oblong-obovoid, 1.5-2 (-2.5) cm. long (Goldblatt, 1996). Widespread in sub-Saharan Africa, from Senegal to South Africa.

1.6.6.2 Medicinal value and Phytochemistry

Ethnomedicinal usage of *Gladiolus dalenii* to treat wounds, eye infections, ear infections, headache, dysentery, diarrhoea, stomach upset and gonorrhoea have been reported, ((Fawole *el al,* 2008 ; Yineger *et al,* 2008 ; Hutchings and Staden, 1994 ; Arnold and Gulumian, 1984). Steenkamp *et al,* (2007) reported no activity of this plant against some bacteria. Fawole *et al,* (2008) repoted that dichloromethane extracts from bulbs of this plant showed activity against *Bacillus subtilis, Staphylococcus aureus,* whereas ethanol extracts were active against *C.albicans* but inactive against *Escherichia coli.* Anti-amoebic activity of bulb extracts of this plant has also been reported by Moundipa *et al,* (2005).

It is within this background that this study was conceptualized.

1.7 Objectives of the study

1.7.1 Broad objective

This study was designed to identify and screen medicinal plants claimed to treat fungal infections in the Lake Victoria basin with respect to *Candida albicans* and *Aspergillus niger,* and thereafter identify the compounds responsible for activity.

1.7.2 Specific objectives

1. To identify the plants used to treat fungal infections in the Lake Victoria Basin.

2. To determine the antifungal activity of selected plants from the ethnomedicmal data.

3. To identify chemical compounds responsible for the; antifungal activity.

1.8 Hypothesis

This study was guided by the hypothesis that, medicinal plants used to treat fungal infections around the Lake Victoria Basin have a diverse chemical profiles which could be antifungal.

CHAPTER TWO

2.0 MATERIALS AND METHODS

2.1 Collection of ethnomedicinal data, herbal drug samples and plant specimens

Ethnomedicinal data was collected in September 2007 in Kisumu, Bungoma, Kitale, Busia, Eldoret which fall within the floral regions K3 and K5 (figure 1).

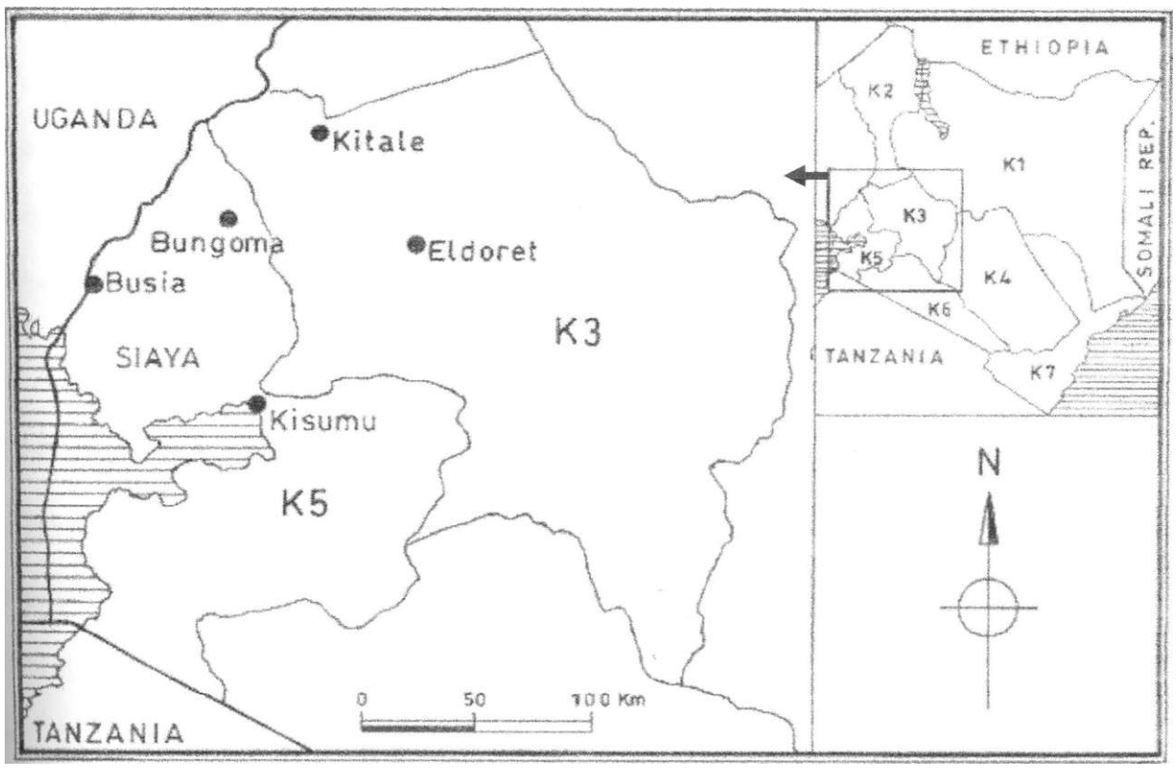

Figure 1. Floral regions of Kenya according to Flora of Tropical East Africa (FTEA) (Beentje, 1994) (right); and the study collection sites (left).

The chair persons (Leaders) were used to identify and register traditional practioners residing in and around these sites. The ethnomedicinal data were based on structured interviews that sought

answers to questions about the human ailments treated, local names of plant species, plant parts used, methods of preparation, and administration. In some cases, the interviews were facilitated by translators who were well conversant with the local language. This was done having first obtained verbal informed consent from each traditional healer. Plant samples in form of herbs/plants, herbal preparations such as powder, pastes, decoctions and concoctions were collected. The ethnomedicnal information was used to predict and identify plants with potential antifungal activity.

Voucher specimens of collected plants were carefully arranged on drying paper, tagged, pressed in a plant press and identified using keys (Agnew and Agnew ,1994; Beentje,1994) and by comparison with authentic herbarium materials, and finally mounted according to standard herbanum procedures and deposited in the Nairobi University Herbanum (NAI). Herbal preparations were kept in the refrigerator at 4°c awaiting preliminary antifungal screening.

2.2 Preliminary screening of herbs and herbal preparations for antifungal activity

Samples collected as herbs were prepared according to herbalists' instructions on their mode of preparation. Together with the herbal preparations, all the samples were subjected to preliminary antifungal tests using disc diffusion method (Serrano *et al.,* 2004). Discs were cut from Whatman filter paper No. 1 using a cock borer with a diameter of 1.2 cm and soaked in the herbal preparations. These impregnated discs were then aseptically transferred into Sabourads Dextrose Agar (SDA) plates freshly inoculated with the test organisms *Candida albicans,* a yeast fungus and *Aspergillus niger,* a filamentous fungus. The plates which were prepared in duplicates were then sealed with parafilm to avoid contamination and any possible drying up, and incubated in

humid conditions at 37°C and 25°C respectively. The antifungal activities were determined by checking the inhibition zones by simple scoring after 24, 48 and 72 hours for *C. albicans* and 3-6 days for *A.niger* (Okemo *et al.,* (2003). *C. albicans* and *A.niger* were provided by Prof G. Siboe, School of Biological Sciences, University of Nairobi.

2.3 Preparation of crude extracts

Crude extracts were made from the samples that showed high antifungal activity based on preliminary screening (Figure 2). The plant samples were air dried at room temperature, powdered, divided into three portions and extracted using hot water, cold water and organic solvents solvents Dichloromethane and Methanol in the ratio 1:1 according to standard extraction methods (Harbourne 1998). 20g of powdered plant sample was mixed thoroughly with the appropriate amount of solvent, left to stand for 24 hours, and decanted (this was repeated twice). The liquid portions were combined and filtered using a Buchner funnel. In order to obtain dry crude extracts, filtrates from organic solvents were concentrated *in vacuo* using a rotary evaporator at temperature 40° C. After evaporation, sample 330 yielded two extracts which were soluble in Dichloromethane (Ac1) and Methanol (Ac2) respectively on fractionation using a separating funnel. The two; Dichloromethane and Methanol soluble extracts were labeled and tested for antifungal activity separately. All the residues from water extracts were freeze dried. The dry extracts were stored in vials and refrigerated at 4° C prior to antifungal test.

2.4 Biological assays of the selected samples/plants

5ml Stock solution at concentration I mg/100ul were prepared for each plant extract and the two antifungal drugs and subjected to antifungal tests by disc diffusion method previously described. The set plates were incubated as indicated previously. The results were checked after time period indicated previously. Activities of the extracts were determined by measuring the zones of inhibition. Discs with solvents only were used as negative controls while those with the antifungal drugs were used as positive standards.

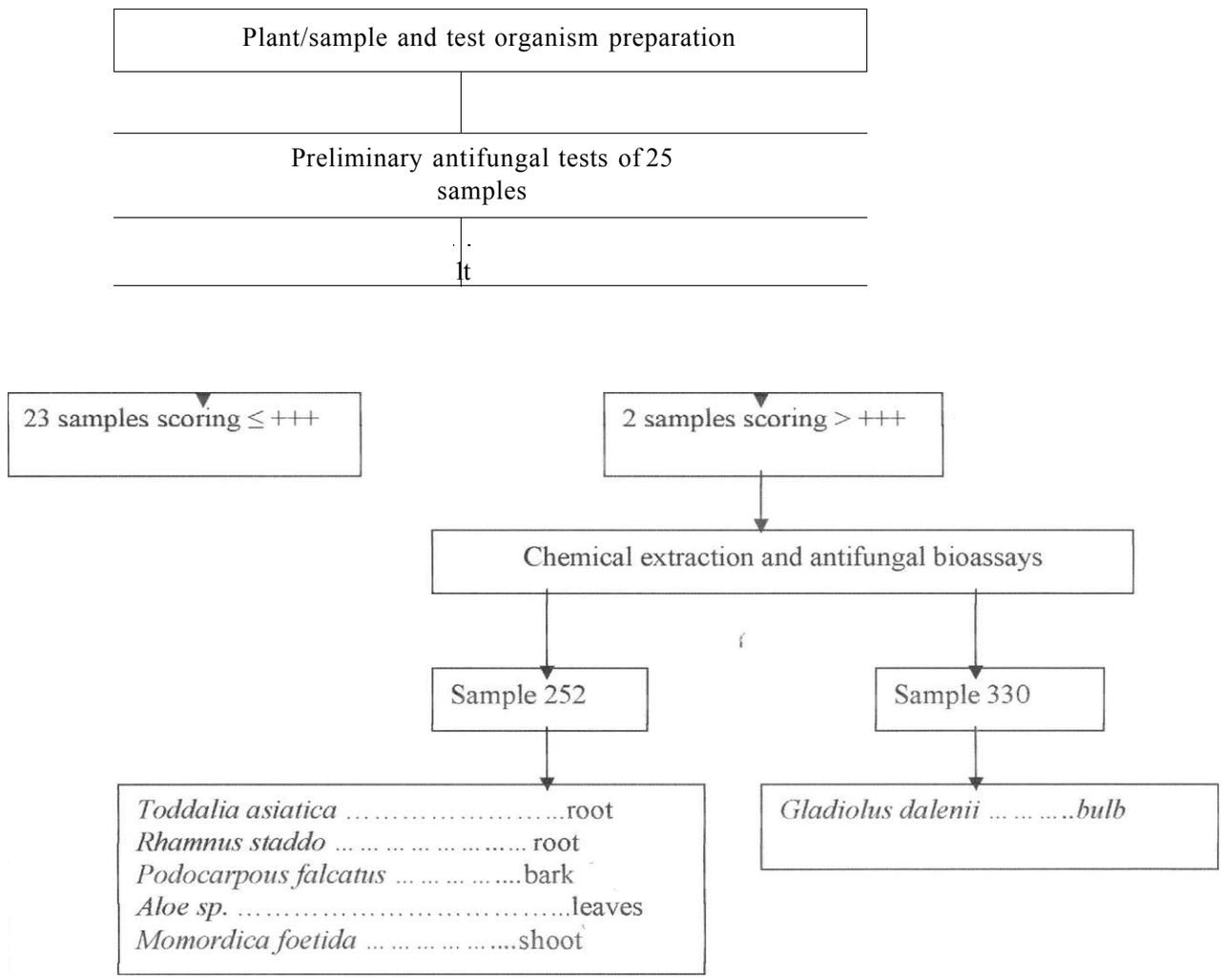

Figure 2: Selection and preparation of extracts for further antifungal bioassays

2.4.1 Direct Bioautography using *A. niger*

In order to identify the active compound/s in each extract, Thin Layer Chromatography (TLC) with prepared commercially obtained silica gel plates were developed in various organic solvent systems and ratios to separate compounds (Table 1).

Table 1. Solvent systems and ratios used to obtain best separation of compounds on a TLC

CH_2C1_2: MeOH (1:1) crude Extract	**Solvent system**	**Ratio**
Toddalia asiatica,	Hexan e/Ethy l acetate	7:3
Rhamnus staddo	Ethylacetate/Acetone	8:2
Podocarpous falcatus,	Acetone	100% {
Momordica foetida	Hexane/Acetone	7:3
P6- Combination of the above five plants	Hexan e/Ethy l acetate	7:3
Gladiolus dalenii CH2CL2 extract (Ac1)	Methanol	100%
Gladioulus dalenii MeOH extract (Ac2)	Dichloromethane	100%

The separated spots were seen visible in the UV lamp. Freshly developed TLC plates were then sprayed with previously prepared suspension of *A.niger* in Peptone media. The sprayed TLC plates were incubated under humid conditions at 25° C (Hostettmann and Marston 1994; Horvath *etal,* 2002). The observation was recorded after 3 days and the inhibition zones noted.

2.4.2 Bioautography agar overlay using *C.albicans*

2.4 .2.1 Preparation of *C. albicans* inoculum

9g of NaCl was dissolved in 700ml of distilled water and made up to 1.0 litre using water to make 0.9% NaCl. Previously cultured *C. albicans* on SDA slants was suspended into this sterile 0.9% NaCl. 14ml of the above suspension was added to 1.0 litre of freshly prepared sterile SDA media before solidification to allow fungus to grow. About 22ml of freshly prepared inoculum (culture) was poured into Petn dishes and left to solidify. TLC plates developed on the same day were carefully laid on to the inoculated medium on the petn-dishes and compounds allowed to diffuse from stationary phase in the TLC plate into in the inoculum in the petri-dish (Runyoro *et ah,* 2006; Hostettmann, and Marston, 1994).The petri dishes were then incubated at 31° C for 20 hours; after which they were sprayed with aqueous solution of thiazolyl blue (3-(4,5 dimethyldiphenyl tetrazolium bromide) (MTT) at concentration of 2.5mg/ml and then incubated further for 4 hours. The inhibition zones which appeared colourless against a purple background were noted.

2.5 Chemical analysis of selected plants using Thin Layer Chromatography plates

In this study, Dichloromethane and Methanol (1:1) crude extracts of each plant screened were tested for the presence or absence of five classes of secondary natural compounds using standard methods (Chowdhury *et al.,* 2008 ; Harbourne, 1998). The five classes of compounds tested were alkaloids, flavonoids, sapogenins, quinones, and terpenoids. The analyses were carried out as shown in Table 2.

Table 2. Thin Layer Chromatography analyses of the antifungal compounds

Phytochemicals	Spraying reagent	Appearance of the positive spot
Flavonoids	25% aqueous solution Basic lead acetate (25g of Pb in 75 ml of water)	Spots fluoresce in long-wave UV light
Alkaloids	Dragendorff ; solution a consist of 0.85g basic bismuth nitrate dissolved in a mixture of 10ml acetic acid and 40ml water; solution b consists of 8g potassium iodide in 20ml of water. Equal volumes of solutions a and b are mixed to make Dragendorff reagent. 1ml is mixed with 2ml acetic acid and 10ml water before use.	Orange
Sapogenins	Antimony chloride in concentrated hydrochlonc acid	Violet
Terpenoids	Antimony chloride in chloroform in the ratio 1:4. heated in oven at 120° C. also evaluated in Longwave UV light	Green
Quinones	Exposure to ammonia fumes	Red ,orange ,yellow, brown

(Adapted from Chowdhury *et al.,* 2008.)

2.6 Data analysis

In order to analyse data, MS Excel 2003 was used to quantify, sort data, determine % mean inhibitions, and draw bar graphs and tables. One way Analysis Of Variance (ANOVA) was used to determine the means, the standard deviations and the pvalue. Student t- test was used to determine significance of the difference of the antifungal activities of the extracts.

CHAPTER THREE

3.0 RESULTS

3.1 Ethnomedicine

Data from traditional healers in this study were collected from Western, Nyanza and Rift Valley provinces. The traditional healers involved administered treatment using concoctions, decoctions and crushed powder which are claimed to treat a wide range of illnesses such as ringworms, ulcers, malaria, typhoid, T.B and S.T.D among others. However, high degree of secrecy surrounding ethnomedicinal knowledge was evident, especially among traditional healers from Nyanza province. It was noted that herbalists in many cases used herbs as mixtures or one plant to treat one or more ailments. A total of twenty five samples were collected and screened in this study. Total number of individual plants collected in the samples amounted to thirty four distributed within twenty one botanical families. Compositae had the highest number of medicinal plants followed by Leguminosae and Labiatae respectively. Ethnomedicinal data are summarized in Table 3.

Table 3 . Ethnomedicinal information on herbal drugs collected from Lake Victoria basin

Sample No.	Plant species	Vernacular names	Plant family	Part used	Preparation method	Ailment treated	Predicted antifungal activity-	Locality of collocetion
464	*Ageratum conyzoides* L.	Namasambu (Luhya)	Compositae	Leaves	Pounded and juice applied	Mouth sores, anti-tetanus, fresh bleeding wounds	Oral candidiasis, Skin Lesions caused by *Candida*	Ndengerwa Bungoma
84	*Tithonia diversifolia* (Hemsl.) A. Gray	Mbekurahisi (Luhya)	Compositae	Leaves	Pounded fresh ,soaked in cold water and drunk	Malaria	Systemic candidiasis	Bungoma
I	*Vemonia lasiopus* O.Hoffm	Nambaa (Teso)	Compositae	Roots	Pounded fresh, boiled and drunk.	Typhoid	Systemic candidiasis	Myanga-Busia
r	*Schkuria pinnata* (Lam) Thell.	Nabuyeywe (Teso)	Compositae	Whole	**pounded fresh or boiled and liquid drunk**	**Malaria and fever**	**Systemic candidiasis**	Busia
	Senna occidentalis (L.) Link.	Enyeribebe, Ekayeywet (Teso)	Leguminosae	Leaves	**Pounded together, burned, ash soaked in water and drunk.**	**Malaria and typhoid**	**Systemic candidiasis**	Myanga-Busia
	Tithonia diver sifolia (Hemsl.) A. Gray	Tithonia	Compositae	Leaves				Myanga-Busia
l	*Abe sp.* L.	Ichichiku (Teso)	Aloaceae	Leaves	**Pounded fresh, boiled and drunk**	**Malaria, meningitis**	**Systemic candidiasis**	Myanga-Busia
1	*Tagetes m inu ta* L.	Ngukwe (Teso)	Compositae	Leaves				Tuiukuyi - Busia
r	*Schkuria pinnata* (Lam) Thell	Nabuyeywe (Teso)	Compositae	Leaves				Tuiukuyi Busia
	Conyza sumatrensis (Retz.)E.	Nalusambu (Teso)	Compositae	Leaves	**Dried and pounded together into powder, added to busaa, porridge, or hot water and drunk**	**S.T.D**	**Vaginal candidiasis**	Tuiukuyi - Busia
L	*Entada abyssinica* Steud. Ex A. Rich.	Kumukokwe (Teso)	Leguminosae	Bark				Lupida-Busia
1	*Harrisonia abyssinica* Oliv.	Sibondwe (Teso)	Simaroubaceae	Roots				Lupida-Busia
	A lb izia co riaria Welw.ex Oliv.	Kumupeli(Teso)	Leguminosae	Bark				Lupida-Busia
i	*Carisa edulis* (Forssk.) Vahl)	Kumurhwa (Teso)	Apoeynaceae	Leaves				Lupida-Busia
	Acacia hockii De Wild.	Kumunyenya (Teso)	Leguminosae	Back				Lupida-Busia
	Indigofera dendroides Jacq.	Kumukuyu (Luhya)	Leguminosae	Leaves	Pounded fresh and juice applied Boiled	Fungal diseases, masiiillingi S.T.D	Lesions caused by *Candida*	Nakhwana-Busia
r	*Hippo cratea sp.*	Ekuo (Teso)	Celastraceae	Root				Shirilila -Busia

	L.				together fresh and drunk			
	Eryihrina excels Ben th.	Ekayeywet (Teso)	Leguminosae	Root			Vaginal candidiasis	Shirilila -Busia (K5)
	Combretum molle R.Br ex G. Don	Ekuluny (Teso)	Combretaceae	Root				Shirilila -Busia (K5)
	Turraea robusta Guerke.	Etikwa (Teso)	Mcliaceae	Root	Pounded fresh, soaked in water and drunk	S.T.D		Shirilila —Busia <K5)
	Harrissonia abyssinica Oliv.	Mugende (Teso)	Simaroubaceae	Root			Vaginal candidiasis	Tulienge-Busia
	Albizia coriara Welw.ex Oliv.	Kumupeli (Teso)	Leguminosae	Root	Mixture of fresh leaves pounded, and soaked in hot water then drunk	Asthma		Tulienge-Busia
	Hoshmdia opposita Vahl.	Upwaka (Teso)	Labiatae	Leaves			Aspergillosis	Ebulamubhiri-Busia
	Pavetta crassipes L	Oohumbi (Teso)	Rubiaoeae	Leaves				Ebulamubhiri-Busia
	Plectranthus prostatas Guerke	Ang'we (Luo)	Labiateae	whole	Pounded, soaked in cold water for drinking and bathing.	Skin problems	Skin Lesions caused by *Candida*	Kisumu
	***Toddalia asiatica* (L.) Lam**	**Pili (Kalenjin)**	**Rutuceae**	**Root**	**All the plants' portion taken in equal proportions, cut into pieces, put together boiled and drunk.**	**Malaria**	**Systemic candidiasis**	**Moiben — Eldoret**
	***Podocarpous falcatus* (Thunb)R. Br.ex Mirb.**	**Bcnii (Kalenjin)**	**Podocarpaceae**	**Bark**				**Moiben-Eldoret**
	***Aloesp.* L**	**Tengebi (Kalenjin)**	**Aloaeeae**	**Succulent leaves**				**Moi ben-Eld oret**
	***Rhamnus staddo* A. Rich.**	**Blakii (Kalenjin)**	**Rhaninaceae**	**Root**				**Moiben Eldoret**
	***Momordica foetida* Schuinach.**	**Tendere (Kalenjin)**	**Cucurbitaceae**	**Shoot including fruits**				**Moiben-Eldoret**
	Harrisonia abyssinica Oliv.	Sibondwe (Luhya)	Simaroubaceae	Roots	Boiled and drunk	Fungal, ringworms	Candidiasis	Myanga -Busia
	Moringa sp. Adans.	Moringa	Moringaceae	Leaves	Dried, pounded and powder mixed with Vaseline and paste used	Skin diseases, ringworms, scabies	Lesions caused by *Candida*	Myanga -Busia
	Moringa sp. Adans.	Moringa	Moringaceae	Leaves	Dried, pounded and powder mixed with Vaseline and paste used	Skin diseases, ringworms .itching	Lesions caused by *Candida*	Myanga -Busia
	Alangifera mdica L.	Maembe (Swahili)	Anarcadiaceae	Leaves	Pounded fresh, soaked in cold water and drunk	T.B, stubborn cough and malaise	Svstemic candidiasis and Aspergillosis	Nakhwana-Busia
	Securidaca longipedunculata Fresen.	Elele (Teso)	Polygonaeeae	Roots	Pounded, dried, pinch of powder added to porridge	Cough and chest pains	filamentous fungi	Lupida -Busia

24	*Carissa edulis* (Forssk.) Vahl	Sirrhua (Luhya)	Apocynaceae	Root	Dried, pounded into powder, soaked in water and drunk	S.T.I	Vaginal candidiasis	Tulienge Busia
U	*Ricinus communis* L.	Kuresiet (Kalenjin)	Euphorbiacea	Roots	Boiled and drunk	Diarrhoea and vomiting	Systemic candidiasis	Kapchorwa-Eldoret
83	*Ximenia caffra* Sond.	Kumutili (Luhya)	Olacacea	Root	Pounded fresh or dry then applied in open wounds. Powder added to water and drunk	Ulcer, wounds, asthma	Oral and esophageal candidiasis Aspergillosis	Nangeni Busia
58	*Dichondra repens* JR. Forst & G. Forst.	Nalulanda (Luhya)	Convulvulaceac	Leaves	Pounded fresh with oil or ghee, paste used	Skin wounds, ringworms	Lesions caused by *Candida*	Bungoma
37	*Oxygonium sinuatum* (Hochst. & Steud. Ex Meisn)Dammer	Nabikumba (Luhya)	Polygonaoeae	Leaves	Pounded fresh, paste used applied	Boils	Lesions caused by *Candida*	Bungoma
80	*Gladiolus dalenii* **Van GeeL**	**Liandamuna (Luhya)**	**Iridaceae**	**Bulb**	**Dried, pounded, sieved, and sniffed to treat meningitis. A pinch of powder added to water and drunk to treat malaria and diarrhoea.**	**Meningitis malaria diarrhoea**	**Systemic candidiasis**	**Bungoma**
16	*Hyptis suaveolens (L.)* Poit.	Egopi (Teso)	Labiateae	Leaves	Pounded fresh, soaked in water for bathing	Skfn, wounds, fungal infections.	Lesions caused by Candida, and Systemic candidiasis	Kapira Busia

Of these samples, 57.1% consisted of herbal preparations as mixtures of two or more plants in various traditional preparations while the remaining 42.9% consisted of single plants, as shown in Figure 3.

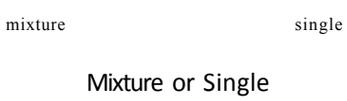

Figure 3. Herbal preparations A; as a mixture, B; as single plants

3.2 Antifungal activity of herbs/herbal preparation

Preliminary antifungal bioassays against *C. albicans,* showed 40.5% of the samples giving initial positive inhibition while 58.46% of the tested samples gave initial positive inhibition against A *niger*. Samples 252 and 330 showed the best antifungal bioactivity and were selected for further bioassay analysis. Sample 252 consisted of *Toddalia asiatica, Rhamnus sladdo, Podocarpous falcatus, Aloe* sp.and *Momordica foetida* while Sample 330 was a single plant *Gladiolus dalenii* as shown Table 2. Preliminary antifungal activity of samples 252 and 330 are shown in Figures 4 and 5 respectively.

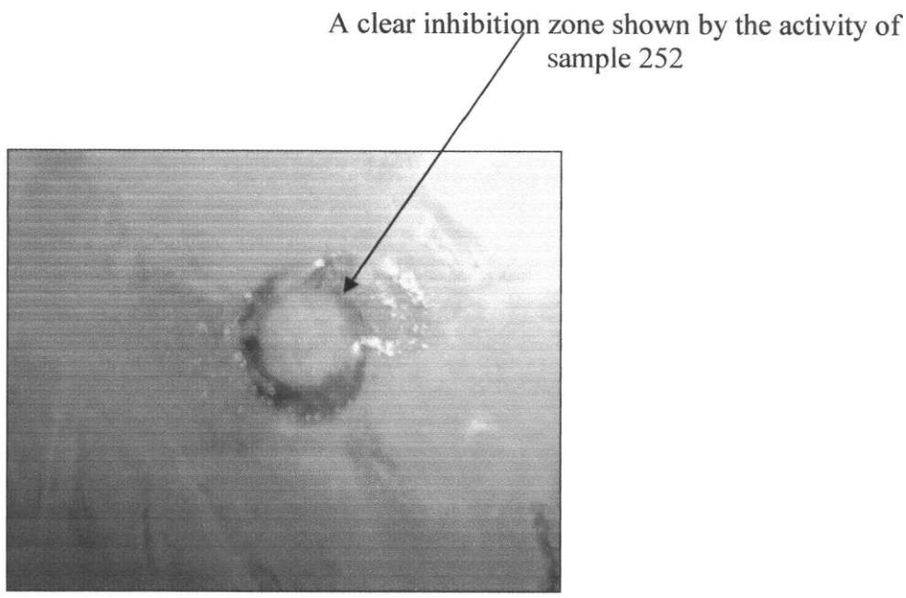

Figure 4. Antifungal activity of sample 252 in a plate of *C. albicans*

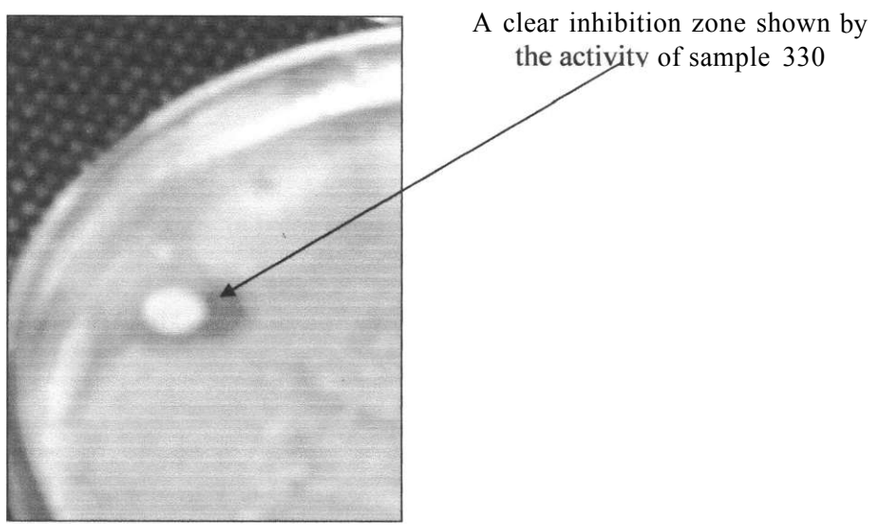

Figure 5. Antifungal activity of sample 330 in a plate *oiA.niger*

3.2.1 Further antifungal bioassays of the crude extracts of Sample 252

Crude extracts obtained from all the individual plants in sample 252 were subjected to antifungal tests against *C. albicans* and *A.niger*. Only extracts from organic solvents showed inhibition activity on one **or** both test pathogens. Water extracts did not show any antifungal activity. Organic solvents' extracts of all individual plants in sample 252 showed fungitoxic activity against both *C. albicans* and *A.niger* except *Aloe sp.* which showed no activity against *A.niger*. (Figure 6).

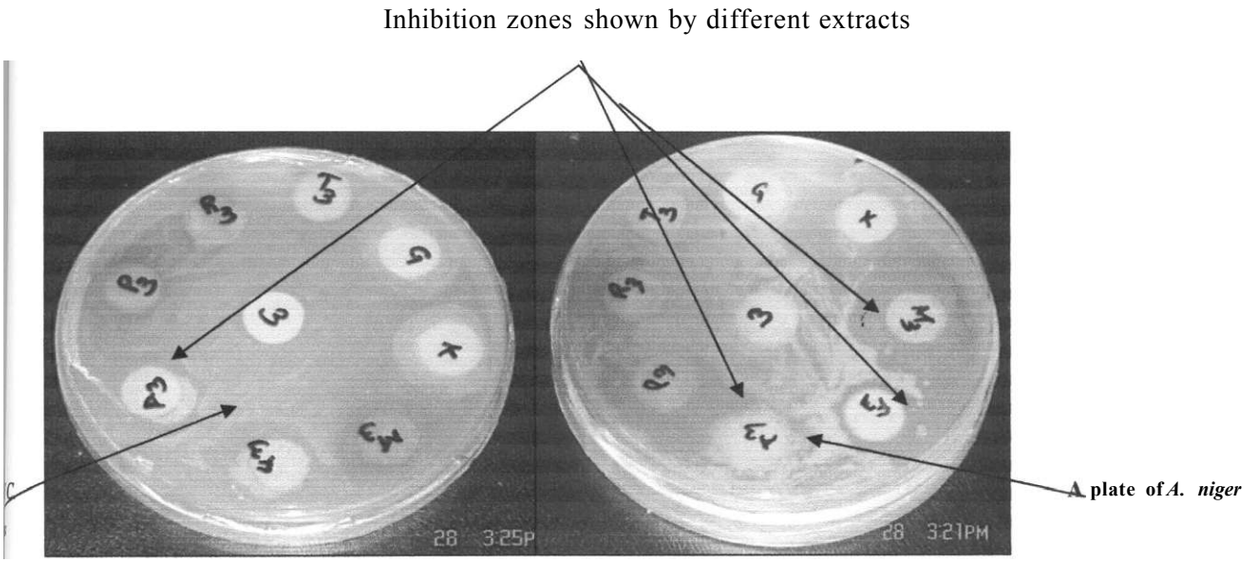

Figure 6. Antifungal activity of the crude extracts from sample 252 against *C.albicans* (Left) and *A.niger* (Right).

T3 -*Toddalia asiatica,* R3-*Rhamnus staddo,* P3- *Podocarpusfalcatus,* A3- *Aloe sp.,*
F3- *Momordica foetida,* M3- combined proportions of the five plants, K-Ketoconazole,
G-Griseofulvin, 3-control.

Dichloromethane and Methanol (1:1) extracts of *Toddalia asiatica, Rhamnus staddo, Momordica foetida, Podocarpus falcatus, Aloe sp.,* their combined proportions showed activity against one or

both of the test organisms. *Momordica foetida* showed the highest activity (77.78% mean inhibition) against *C. albicans* as shown in Figure 7.

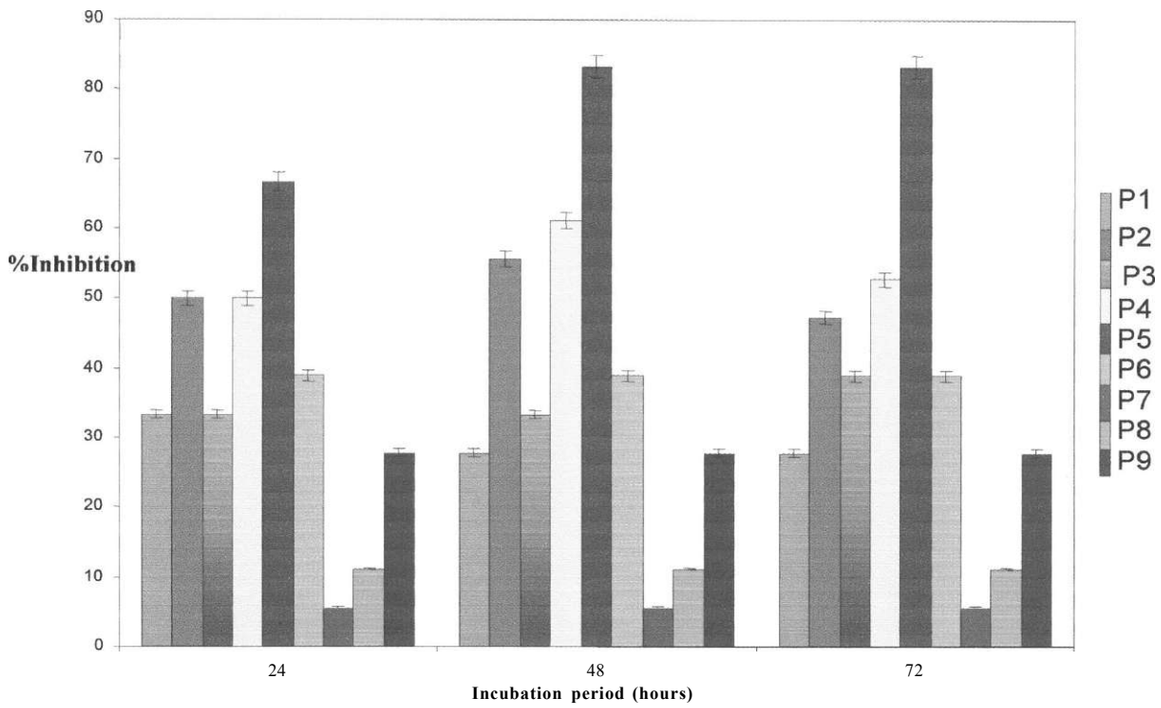

Figure 7 . Percentage inhibition of the plant extracts against *C. albicans*
PI -*Toddalia asiatica, V2-Rhamnus staddo,* P3- *Podocarpous falcatus,* P-4 *Aloe sp.,* P5- *Momordica foetida,* P6- Combination of the above five plants, P7- Griseofulvin, P8- Ketoconazole, P9- Negative Control.

Amongst the five plants together with their combined proportions, *Podocarpus falcatus* showed the highest activity (77.77% mean inhibition) against A *niger* while *Aloe sp.* Showed no activity (Figure 8). When tested against *C. albicans, Rhamnus staddo, Aloe sp., Momordica foetida,* and the combined sample were significantly more active than Ketoconazole and Griseofulvin. When tested against *A.niger, Rhamnus staddo, Podocarpous falcatus, Momordica foetida,* and combined

proportions were more active than Griseofulvin but similar in activity to Ketoconazole. Although the combined proportions had significantly similar activity to *Rhamnus staddo, Aloe sp., Momordica foetida* when tested against *C. albicans* and ; *Rhamnus staddo, Podocarpous falcatus, Momordica foetida* when tested against *A.niger,* its percentage inhibitions was much lower than for individual plants (Figures 7 and 8).

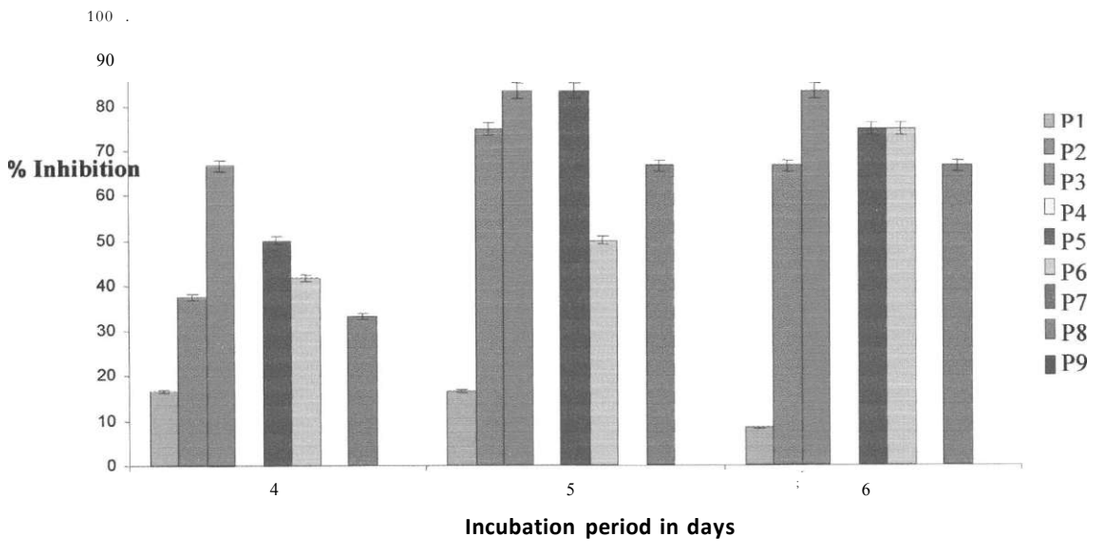

Figure 8 . Percentage inhibition of the plant extracts against *A. niger*

?\-Toddalia asiatica, P2-Rhamnus staddo, P3- *Podocarpous falcatus,* P-4 *Aloe sp.,* P5- *Momordica foetida,* P6- Combination of the above five plants, P7- Griseofulvin, P8- Ketoconazole, P9- Negative Control

3.2.2 Further antifungal bioassays of the crude extracts of Sample 330

The two extracts yielded by Sample 330, *{Gladiolus dalenii*) were labeled as Acl (CH2CI2 soluble) and Ac2 (MeOH soluble) respectively.

Both the extracts showed antifungal activity against *A. niger.* (Figure 9)

clear zone shown by the activity of Acl

A clear zone shown by the activity of Ac2

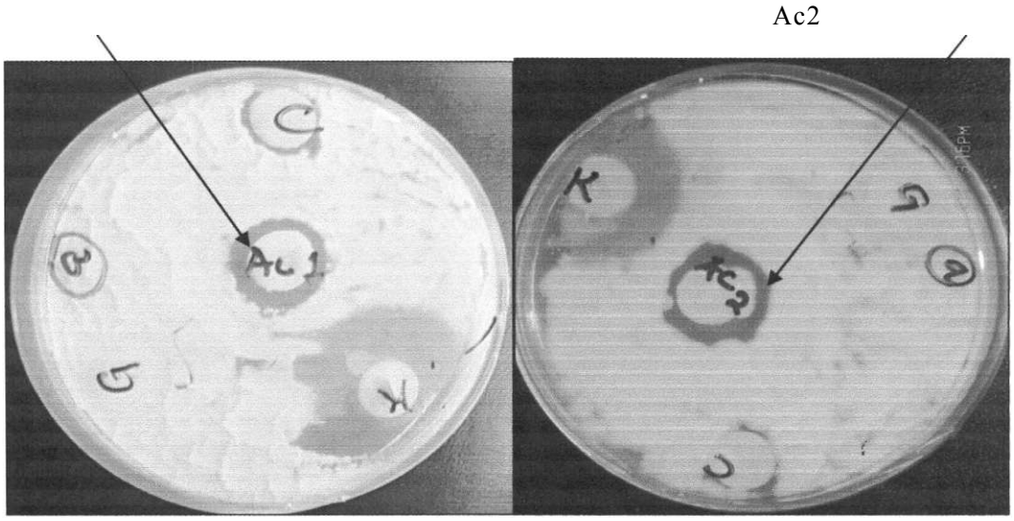

Figure 9 . Antifungal activity of the two crude extracts from *Gladiolus dalenii.* Sample 330
Acl-(CH_2Cl_2 soluble extract), Ac2- (MeOH soluble extract), G-Griseofulvin, K-Ketoconazole, C- Negative Control,

However, the activity of the two extracts, Acl and Ac2 were similar. The activity of the two extracts was also found to be similar to that of Ketoconazole but higher than that of Griseofulvin (Figure 10).

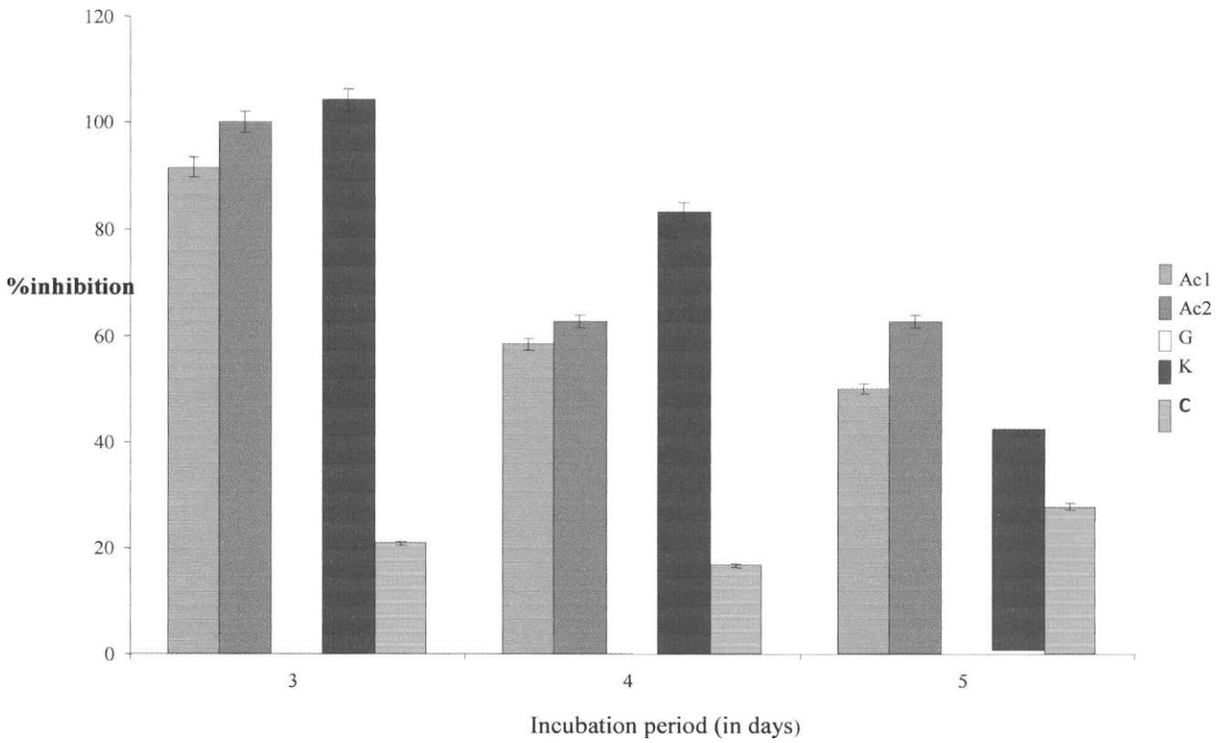

Figure 10. Percentage inhibition of the Dichioromethane and Methanol (1:1) extracts of *Gladiolus dalenii* against A *niger*

Ac1- CH_2C1_2 solube extract, Ac2- MeOH soluble extract, G-Griseofulvin, K-Ketoconazole, C- Negative Control

3.2.3 Spore inhibitory activity of *Gladioulus dalenii* (sample 330)

When testing for the antifungal activity of the two crude extracts of *Gladioulus dalenii* against *A.niger.,* an interesting observation was made; Ac1, a CH2CI2 soluble crude extract had the ability to inhibit spore production in A *niger* up to day five. Norrmal sporulation was observed in petri-dish containing methanol (MeOH) soluble crude extract (Ac2) as shown in Figure 11.Cultured young mycelia from a petri-dish containing CH2CI2 soluble extract (Ac1) showed revived normal sporulation in absence of CH2CI2 soluble extract (Ac1).

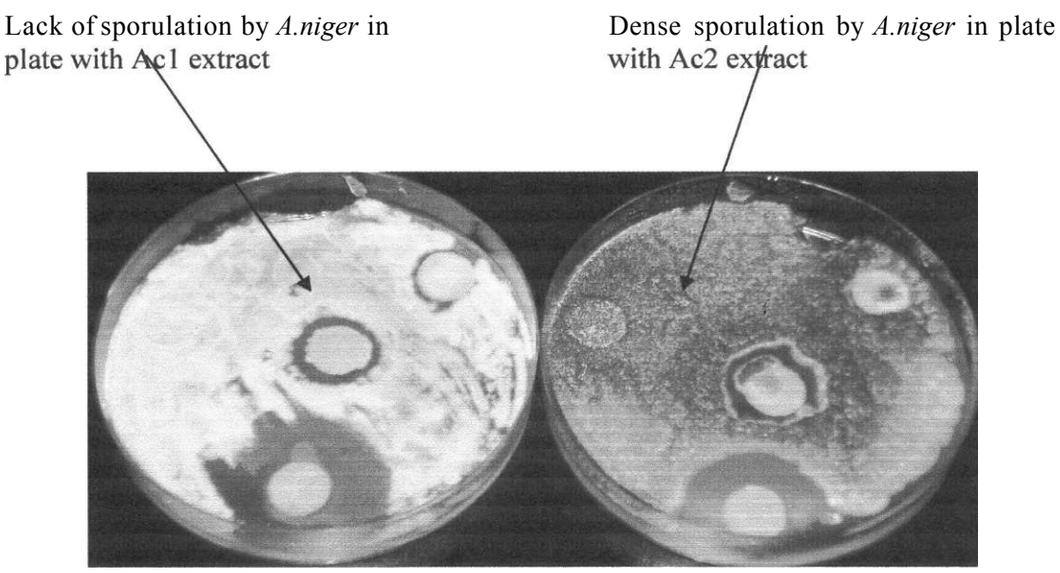

Figure 11. Spore inhibitory properties of Ac1

Ac 1 - CH2CI2 solube extract, Ac2- MeOH soluble extract

3.3 Identification of antifungal compounds

Components responsible for the antifungal activity of the plants were identified as alkaloids, flavonoids, terpenoids, quinones and sapogenins. Appearing as colourless regions against a purple background are the inhibition zones showed by the active components of individual plants of sample 252 against *C.albicans* by bioautography agar overlay (Figure 12).

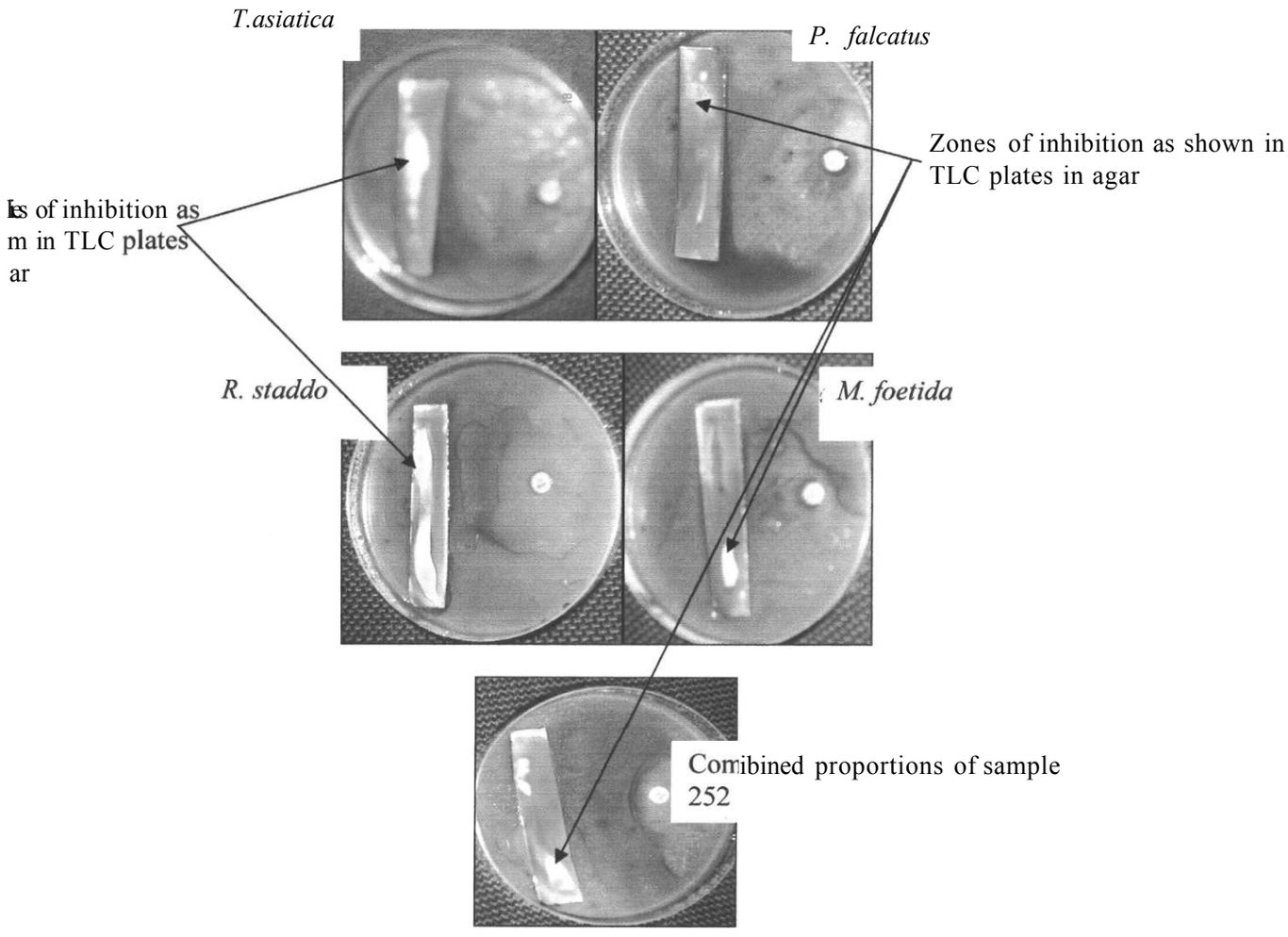

Figure 12. Inhibition zones caused by Dichloromethane/Methanol (1:1) crude extracts against *C. albicans*

Although only five groups of chemical components were successfully tested in each active extract in this study, the presence of other antifungal compounds not tested in these plants cannot be ruled out. Active groups of compounds identified in these plants are shown in Table 4.

Table 4. Active chemical components identified in the different extracts

Chemical compounds identified in the active spots in TLCs										
^Cl_2:MeOH crude Extract	ALKALOIDS		FLAV ONOIDS		SAPOGENLNS		QUINONES		TERPENOIDS	
	C.a	*An*	*C.a*	*An*	*C.a*	*An*	*C.a*	*An*	*C.a*	*An*
Toddalia asiatica.	+	+	+	+	.	+	.	.	.	.
•*Rhamnus staddo*	+?	+?	+	.	.	.	.	.	+	.
[Podocarpous falcatus,	.	+	+	+	.	.	.	.	.	.
[Momordica foetida	+	+	.	.	.	.	.	.	+	+
•Combination of the above e plants	+	+	+	+		+	1			−
1 - *Gladiolus dalenii* bCl2 extract	NT	+	NT		NT		NT	−	NT	-
b- Gladioulus dalenii tOH extract	NT	+	NT		NT		NT		NT	

C.a*. . . . *Candida albicans* , *A.n*____*Aspergillus niger, **+. . . . Present and active**
.May have been absent or present but not active, NT. . . . .Not tested

+?...may have been an artifact.

Gladiolus dalenii showed high activity against *A. niger* during preliminary tests and not C. I *albicans* . This explains why the active compounds were tested against A *niger* only.

In this study, the major antifungal compounds were found to be alkaloids and flavanoids. Plant extracts containing flavonoids and alkaloids were active against both the yeast and the filamentous fungus (Table 5).

Table 5. Active chemical components identified in different crude extracts

	ALKALOIDS	FLAVONOIDS	SAPOGENINS	QUINONES	TERPEN(
CH_2Cl_2:MeOH crude Extract					
Toddalia asiatica,	+	+	+	-	-
Rhamnus staddo	+ ?	+			+
Podocarpous falcaIns,	+	+	-	-	-
Momordica foetida	+	-	-	-	+
Gladiolus dalenii	+	-	-	-	-

+.....presence, -.....absence /inactive

+?.....May have been an artefact.

Alkaloids and flavonoids were the major chemical components identified in the crude antifungal extracts (Table 5) and were active against one or both the test pathogens as indicated in Table 4.

CHAPTER FOUR

4.0 DISCUSSION

Traditional healers from the Lake Victoria Basin were found to be rich in their indigenous knowledge on the use of ethnomedicinal plants species to manage various human ailments. However, findings of this study revealed high degree of secrecy surrounding traditional medicine by some healers. This is in agreement with reports by Yineger *et al.,* (2008), who attributed this to the fact that herbalists derive either income or compensation for the treatment they provide. Observation that most of ethnnomedicinal plants species were prepared through concoction, crushing, decoction , crushing and powdering and administered mainly through oral and dermal routes is in agreement with those reported by Boer *et al.,* (2005) and Yineger *el al,* (2008); who in addition, observed that the traditional measurements of quantities of the herbs being dispensed however lacked precision. In this study, it was observed that most medicinal plants species used by traditional healers of the study area had multiple uses, this could be due to the availability of the herbal plant or its effectiveness in the treatment of various ailments. This seems to be in agreement with reports by Kanba (2000); Okemo *et al.,* (2003); Lukhoba *et al,* (2006).

The plant family reported with the highest number of medicinal plant species was Compositae (Table 3). This was followed by Legumnosae and Labiatae. This is in agreement with results of Yineger *et al,* (2008) who reported Compositae and Labiatae as the number one and three families with highest number of medicinal plants respectively. However, results in this study contrast with his reports by showing Leguminosae as the family with the second highest number of medicinal plants rather than Gramineae.

Findings in this study showed that several herbal samples obtained from herbalists suspected/predicted to have antifungal activity tested positive against the two human pathogenic fungi *C. albicans* and *A. niger*. Preliminary bioassays gave 40.5% of the screened samples as active against the yeast fungus *C. albicans* while 58.46% of the screened samples were active against the filamentous fungus *A. niger*. This activity supports the folk therapy of infections of the skin and other microbial related diseases whose symptoms might involve Candidiasis and Aspergillosis. There is therefore a correlation between ethnomedical uses and bioactivity of the medicinal plants used in the Lake Victoria Basin. These findings are in agreement with those of Chowdhury *et al.,* 2008; Mbwambo *et al,* (2007); Fabncant and Farnsworth (2001); Kanba *et al* (2001); De Smert, (1997); Kubo and Taniguchi (1993) that screening of plants used in traditional medicine showed a much higher probability of finding active extracts and is a key approach used to search for drugs. However, it is important to note that not all ethnomedicinal data is reliable as noted by Yineger *et al,* (2008); Ng'etich (2005) and Kokwaro (1993). A correlate to findings in this study was the failure of 59.5% (against *C. albicans)* and 41.7% (against A *niger)* of samples screened to give positive results though reportedly used and predicted to treat fungal related infections. Reasons for this failure could be that some of the herbs are probably used to treat non fungal infections ; other reasons could be due to different ways of administration, methods of drug preparation by the herbalist; expiry of the drug storage period before being dispensed to patients; and other unhygienic conditions. For example, leaf extracts from *Dichondra repens* (Convolvulaceae) were not fungitoxic, although traditionally pounded leaves of this plant is mixed with solid oil/ghee and rubbed onto wounds. In this case the addition of oil/ghee could have increased the potency of the fungitoxicity of the extracts. This is in agreement with Otieno *et al,*

(2007) who reported that a crude mineral *Kadosero* supplemented to other plant extracts by herbal practitioners to treat bacterial infections showed increased bioactivity of the herbal medicine.

Traditional healers visited in the study were found to use concoctions or decoctions prepared from one or more herbal plants in a sample (in combined proportions) and therefore the activity could be expected to be synergistic. 57.1% of the samples collected were in mixtures, while only 42.9% of the samples consisted of single herbal plants in a sample (Figure 3). Sample 252 a mixture of five different plants species gave the best activity against *C. albicans* from preliminary results. The reported antifungal activity may have been synergistic. The same mixture showed high activity with A *niger* however *Aloe sp.* one of the plants in the mixture showed no activity against *A. niger* when tested singly (Figure 8). Similar case was reported by Olembo *et al,* (1995) in which *Aspilia pluriseta* used in combination with *Microglossa pyrifolia* and *Indigofera arrecta* was reported to be a good remedy for fungal infections of the skin but showed no activity when tested singly against dematophytic fungi as reported by Kariba (2000).

Claims by the traditional medicine practitioner to treat malaria using Sample 252 is supported by previous bioassay reports which have shown antimalarial activity of all the plants in the mixture except *P. falcatus.* (Muregi *etal.,* 2007; Kuna *etal,* 2001; Gessler *etal,* 1994 ; Orwa *et al,* 2008 ; Gakunju*etal,* 1995; Clarkson *etal,* 2004).

On the other hand, claims of ethnomedicinal use of bulbs of *Gladiolus dalenii* (sample 330) to treat meningitis and malaria had not been documented, however, other usage in traditional medicine to treat wounds, eye infections, ear infections, headache, dysentery, diarrhoea, stomach

upset and gonorrhoea have been reported, (Fawole *et al,* 2008 ; Yineger *et al,* 2008 ; Hutchings and Staden, 1994 ; Arnold and Gulumian, 1984).

Only organic solvent extracts Dichloromethane and Methanol (1:1) of the plants selected for further antifungal bioassays showed activity against the tested fungi. This observation is of particular interest, given that traditionally, the preparation of herbal remedy is often with water (Table 3). Fungitoxic activity of water extracts may have been too low to be detected at concentrations lmg/100|j.l. This is supported by Fawole *et al.,* (2008), Parekh and Chanda, (2007), Boer *et al,* (2005), and Kariba (2000) who found out that water extracts showed no/poor fungitoxicity than those made using organic solvents, among other reasons they reported could be that same active substances were present in water extracts but at concentrations at which bioactivity was not longer detectable, another reason could be that the active substances were soluble in organic solvents and basically not present in water extracts. This finding is also in agreement with that of Clarkson *et al,* (2004) who reported that the inactivity of water extracts may have been because they (extracts) were not prepared according to the traditional methods, which in some cases involved boiling for several hours. It is therefore worth noting that the traditional practitioners use water because that's all they have at their disposal, and success may be due to administration of the concoctions/decoctions in large quantities i.e in a basin, cups, water glasses, and in all or most cases, the treatment involves using the extracts for a long period of time, (Yineger *et al,* 2008 ; Lulekal *et al,* 2008 ; Erasto *et al,* 2005).

Some of the investigated plants in this study did not show strong antifungal activity. Active compound(s) may have been present in insufficient quantities in the crude extracts to show

activity with the dose levels employed. This is in agreement with Parekh and Chanda, (2007), who reported that alternatively, if the active principle is present in high enough quantities, there could be other constituents exerting antagonistic effects or negating the positive effects of the bioactive agents and that, the plant extracts may have been active but against other fungal pathogens which were not tested.

Inhibition data of microbial growth in the presence of organic solvents' extracts are shown. Promising anti-candida results were obtained with extracts from *Momordica foetida, Aloe. Sp, Rhamnus staddo* and the combined proportions. Same extracts except *Aloe sp.,* together with *P. falcatus, G. dalenii* gave promising anti-aspergillus results, which all to a varying extent inhibited growth of these pathogens. Other tested plant extracts also inhibited the growth of the test organisms but significantly to a lesser extent e.g *Toddalia asiatica.*

The finding of anti yeast activity of *M. foetida* seems to be in agreement with that reported by Boily and Van, (1986) who found out that methanolic extracts of leaf of this herb were active against *C. albicans*, however no reported work on the activity of this plant against *A. niger* was found. It is significant to note that this may be the first report.

Although reported as significantly active against the two fungi in this study, reports of the antifungal activity of *R. staddo* were not found, however a lot of studies on the antimalarial activity have been reported (Kuna *et al,* 2001; Muregi *et al,* 2007).

This survey showed that *P. falcatus* was active against *C. albicans.* This agrees with report by Abdillahi *et al.*, (2008) who tested the activity of leaves and stems of this plant using several organic solvents and found that they were active. Amongst the plants tested in this study, *P. falcatus* showed the highest activity against *A.niger.* However no literature reports were found on the activity of this plant against the same fungus and therefore may have been reported in this study for the first time.

Figure 7 shows the relatively high activity of *T. asiatica* extract against *C. albicans.* This is in agreement with findings reported by Duraipandiyan *et al.* (2006) and Gurib-Fakim *et al., (2005)* but contrasts with those of Rajakaruna *et al.,* (2002) and Kar *et al,* (2005) who found no activity of this plant against the same yeast fungus. The discrepancy could be due to factors such as time of collection of plant matenals, environmental factors, geographical and ecological location of the plants and extracting solvents (Runyoro *et al,* 2006).

The insignificant activity of *T. asiatica* against *A. niger* (Figure 8) is in agreement with Gurib-Fakim *et al,* (2005) who reported no activity, but constrasts with those of Saxena and Sharma (1999) who reported that essential oils from leaves of this plant was active against this fungus.

Synergy, antagonism and additive interactions were observed in Sample 252 (combined proportions). From the results, it was clear that although the activities of individual extracts of *M. foetida, R. staddo* and *Aloe sp.* against *C. albicans* were significantly similar to that of the mixture (combined proportions), the % inhibitions of these individual extracts were higher (Figure 7). On the other hand individual extracts of *T. asiatica* and *Aloe* sp. against *A.niger* were comparatively

lower than that of the mixture (combined proportion) (Figure 8). These findings are in agreement with that of Bella (2005) who reported that synergistic or antagonistic interactions between antimicrobial drugs is a reflective of relationships between their actions on cellular components, and that synergism between two antimicrobial drugs might result from (i) binding to the same target protein such that a conformational change caused by the binding of drug A enhances the binding of B, (ii) binding of drug A to a transporter causing increased uptake of B into the cell or the subcellular compartment in which it acts, (iii) formation of a complex between A and B of enhanced toxicity, (iv) stimulation by A of the conversion of B to a more active form. Converse examples could be constructed for antagonism and also when the active compounds have a common target. The individual plants may have had compounds which had antagonistic effects which made the mixture to have comparatively lower activity when tested against *C.albicans.* This finding seems to be also in line with the observation by Gathirwa *et al.,* (2008), that marked antagonism, synergy and additive interactions are observed when combinations of the drugs are assayed *in vitro.* The plants that showed lower activity than the mixture when tested individually such as *T. asiatica* and *Aloe* sp. against *A.niger* may be acting as antipyretics or immune stimulants to relieve the symptoms of the disease, rather than having direct activity as reported by Phillipson *et al,* (1993). Use of herbal remedies as mixtures of different herbs have also been reported in Chinese traditional medicine by Xiao (1983), who observed that determination of their pharmacological effects and the isolation of their active pnnciples are much more difficult than is the case of single medicinal plants owing to the interaction of various constituents.

The activity of *Gladiolus dalenii* against A. *niger* and its ability to inhibit the fungus' sporulation was an interesting finding. Steenkamp *et al,* (2007) reported no activity of this plant against some

bacteria; this was in contrary to reports by (Fawole *etal,* 2008) who found out that CH_2C1_2 bulb extracts of this plant was active against *Bacillus subtilis, Staphylococcus aureus,* while ethanol extracts were active against *C.albicans* but inactive against *Escherichia coli.* Anti-amoebic activity of bulb extracts of this plant has also been reported by Moundipa *el al,* (2005). In this -egard, the activity of this plant and its ability to inhibit sporulation in *A.niger* is reported in this study for the first time.

[t was observed that *R. staddo, Aloe sp., M. foetida*, and the combined proportions against C. *ilbicans* had higher antifungal activity than Gnseofulvm and Ketoconazole (figure 7). The same extracts except *Aloe sp.* Together with *P. falcatus,* were more active than Griseofulvin when :ested against *A.niger* (figure 8). Gnseofulvm and Ketoconazole are both commercial antifungal drugs. This suggests that extracts from *R. staddo P. falcatus, Aloe* sp. *M. foetida* as well as their combined extracts could be used successfully for the treatment of Candidiasis and / or Aspergillosis. Results showing higher activity of herbal drugs compared to commercial drugs lave been reported by Moundipa *et al,* (2005), who found higher activity of some medicinal 3lant extract compared to that of metronidazole against *Entamoeba histolytica* and Samie *et al.,* [2005) who reported higher antibactenal activity of some medicinal plants compared to :ommercially used antibiotics.

The higher effectiveness of these plant extracts and Ketoconazole drug against the filamentous fungus *A niger* compared to the yeast fungus *C albicans* may be due to differences in cell wall composition, as cell-wall synthesis is the target for various groups of drugs (Gooday 1993).Yeast fungi contain glucans and mannan proteins in their cell walls compared to chitin and glycan in the

cell walls of filamentous forms (Murrey *et al.,* as in Kariba 2000). Depending on composition, the cell wall can act as a barrier preventing drugs from reaching the site of action (Matthison 1977 as in Kanba 2000). This finding is in agreement with that Espinel-Ingroff and Flynn (1996), who reported that yeast fungus, are less susceptible to the few available antifungal agents due to their continuously new emergence.

This study reported that the major antifungal components against both the yeast and the filamentous fungus were alkaloids and flavonoids (Table 5). Although alkaloid test in *R.staddo* showed positive, this plant has been known to lack alkaloids and so this could have been as a result of artefact (Pourveura, 1973). The fact that there could have been other major antifungal compounds which were not tested in this study cannot be ruled out. The mechanisms of action of the constituents could be by inhibition of fungal cell wall, protein and amino acid, biosynthesis and electron transport chain. Edeoga *et al.,* (2005) also reported alkaloids and flavonoids as being among the most important bioactive plants constituents. He reported the presence of flavonoids and alkaloids from all the ten medicinal plants used in traditional medicine to treat various ailments among others ringworms, diarrhoea, coughs, chest pains, bronchial asthma, fever, malaria, vomiting, inflammation, sore throat, boils, sores and wound healing which were in agreement to the ailments reported in this study suspected to be as result of candidiasis and aspergillosis. This finding of alkaloids and flavonoids as major important medicinal plant phytochemicals is also in agreement with reports by Adegboye *et al.,* (2008) and Paiombo, *et al,* (2006) that they play wide range of biological activities amongst others antidiarrhoeal, anti-inflammatory, anti-allergic effects , and have analgesic properties.

The antifungal activity of the selected plants makes them potential source of antifungal agents and may be of economic importance as source of antifungal natural plant products. This study therefore is in agreement with Chowdhury *et al.,* (2008); Mbwambo *et al.,* (2007) ; Duraipandiyan *el al.,* 2006 ; Fabncant and Famsworth 2001 ; Kariba *et al,* 2001 ; De Smert, 1997 ; Kubo and Taniguchi, 1993; that the information from ethnomedicinal sources may offer potential leads to new active natural products.

CHAPTER FIVE

5.0 CONCLUSIONS AND RECOMMENDATIONS

Results of this study indicate that it is important to carry out scientific studies on medicinal plants having traditional claims of effectiveness in ethnomedicinal uses. This confirms the possibilities of developing plant-based compounds to help combat the lung problems caused by *Aspergillus niger;* diorrhoea and skin problems caused by gastrointestinal and esophageal *Candida albicans* infections, the most frequently encountered fungal infections in HIV- patients (Mbwambo *et al,* 2007).

As only *in vitro* methods were used in assessing the antifungal activity of the crude extracts, further investigation using bioassay guided fractionations are recommended to isolate and identify the pure compounds responsible for the antifungal activity and sporulation inhibitory activity of *Gladiolus dalenii.*

Though there has been no alarm on exploitation or threat against these potential plant species, they should be conserved and probably domesticated for commercial exploitation. Possibilities of cultivation in large scale for exploitation are recommended.

In this study, it was noted that many herbalists used herbs as combined proportions (as mixtures), investigations of their pharmacological effects and the isolation of the active principles of plant compounds when combined are recommended.

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APPENDICES

Appendix I:

Average % inhibitions of individual plants of sample 252 exhibited against *C. albicans* and *A.niger*.

	% mean inhibitions	
Organic solvent extracts/drugs	***C. albicans***	***A.niger***
Toddalia asiatica	29.63	13.8889
Rhamnus staddo	50.93	59.7222
Podocarpous falcatus	35.19	77.7778
Aloe sp.	54.63	0.0000
Momordica foetida	77.78	69.4444
Combination of the above five plants	38.89	55.55,56
Griseofulvin	5.56	.0000
Ketoconazole	11.11	55.5556
Control	27.78	.0000

Appendix II:

Average % inhibitions of DCM and MeOH extracts of *Gladiolus dalenii* (sample 330) exhibited against *A.niger*

Plant extracts/drugs	% mean inhibitions
Acl	69.4444
Ac2	75.0000
Griseofulvin	.0000
Ketoconazole	90.2778
Control	12.5000

Ac 1 - DCM solube extract, Ac2- MeOH soluble extract.