ANTIFUNGAL ACTIVITY, TOXICITY AND PHYTOCHEMICAL COMPOSITION OF *GLADIOLUS* AND *DIERAMA* SPECIES (IRIDACEAE) IN 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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DEDICATION

To my mother Margarate and my Late father John for their appreciation of education, my husband Benard for his endless encouragement and motivation, Bilha, Alvin and Aylene for always being there.

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List of Abbreviations and Acronyms

ANOVA: Analysis Of Variance

ATCC: American Type Culture Collection

BLT: Brine shrimp Lethality Test

CC: Column Chromatography

DCM: Dichloromethane

DMSO: Dimethyl Sulphoxide

EToAC : Ethylacetate

EUCAST: European Committee on Antimicrobial Succeptibility Testing

FAA: Formalin Acetic acid Alcohol

HIV-AIDS: Human Immunodeficiency Virus- Aquired Immune Deficiency Syndrome

LD_{50} : Lethal Dose required to kill 50% of the population of organisms under study

MeOH : Methanol

MIC: Minimum Inhibitory Concentration

ml: Mililitre

mm: Milimetre

µg: Microgram

µl: Microlitre

NMR: Nuclear Magnetic Resonance

SDA: Sabourads Dextrose Agar

TLC: Thin Layer Chromatography

UV: Ultra Violet

WHO: World Health Organization

ABSTRACT

Opportunistic infections caused by fungi have been on the rise and are a threat to the lives of the immunocompromised individuals. The few antifungal drugs available are either toxic or have adverse side effects, necessitating search for an alternative agent. This present study was conducted to determine the antifungal activity, cytotoxicity and the phytochemical composition of the Kenyan species of *Gladiolus* and *Dierama* (Iridaceae). Aerial and bulb crude aqueous and organic extracts (DCM:MeOH) were tested against *Candida albicans, Aspergillus niger* and *Cryptococcus neoformans* by disc diffusion method while cytotoxicity was determined using freshly-hatched brine shrimp (nauplii), *Artemia salina*. Semi-purified fractions of bulbs were also tested against the three fungi. Phytochemical screening, Thin Layer Chromatography and column chromatographic bioassay guided fractionation of bulbs and aerial parts were conducted using standard procedures. Anatomical features of bulbs and leaves were evaluated to establish characteristics to differentiate between the species. Organic extracts were found to be more active than aqueous crude extracts while bulbs from both extracts showed higher activity than the aerial parts. Bulbs of *Dierama cupuliflorum, Gladiolus watsonoides* and *G. goetzenii* all showed a minimum inhibitory concentration value of 0.3907mg/ml when tested against *C. albicans* implying their strong activity against this fungus. Other than *G. ukambanensis*, bulb extracts of all the other plants had weak activity (>0.625mg/ml) against *A. niger* while only bulbs of *G. watsonoides* and *G. goetzenii* showed weak activity (>0.625mg/ml) against *C. neoformans*. Semi-purified fractions from ethyl acetate, methanol or their mixtures showed the highest activity whereas fractions from hexane and dichloromethane were either inactive or had little activity. When compared to the activity of the commercially used drugs, two–way ANOVA revealed that bulbs of *D. cupuliflorum, G. watsonoides, G. goetzenii* and *G. newii* had activities not significantly different (P>0.05) to that of amphotericin B at concentrations 5mg/100µl, 2.5mg/100µl and

1.25mg/100µl when tested against *C. albicans*. *G. watsonoides* and *G. goetzenii* bulbs had activity significantly higher ($F_{,8,72}$=4.56, P<0.05) than nystatin at 5mg/100µl. when tested against *C. neoformans.* All the drugs had significantly higher activity than the plant extracts when tested against *A. niger.* Cytotoxic activities revealed that all the bulb extracts were toxic to the newly hatched shrimps with LD_{50} value <500µg/ml except those of *G. ukambanensis* which showed weak toxicity with LD_{50} value >500µg/ml. Similarly, all aerial extracts were toxic except those of *G. ukambanensis* which were non toxic with LD_{50} value >1000µg/ml. The phytochemicals screened such as alkaloids, flavonoids, saponins, terpenoids, glycosides, anthraquinones, tannins were detected in the bulbs than in the aerials. Bulbs of *D .cupuliflorum* and *G. watsonoides* tested positive for seven of the eight tested major classes of phytochemicals followed by *G. goetzenii* and *G. newii* in that order. *G. ukambanensis* had only three of the tested phytochemicals. There was a linear correlation between bioactivity, cytotoxicity and richness of phytochemicals. Anatomical features of bulbs were found to be different and the description was done based on the tunic texture and colour and stack formation of the bulbs. These may be of value in identification of these plants during dry periods when the aerial parts are absent. This study has shown the potential of these plants as source of leads for antifungal drug development towards combating emerging antifungal resistance to conventional drugs. Further purification and bioassay analyses of these plants are recommended to ascertain the antifungal and cytotoxic principles. Due to their possible economic value conservation measures may be necessary to control their exploitation.

Key words: *Gladiolus, Dierama*, Opportunistic fungi, Phytochemicals, Drug development

1.0 CHAPTER ONE. INTRODUCTION

Plants have been used to treat a wide range of diseases throughout the history of human beings and this practice continues to date. Despite the development and growth in conventional medicine, 80% of the third world population still rely on medicinal plants as the answer to their health problems (WHO, 2002). This is mainly because most of these herbals are available, affordable and have little or no side effects (Samejo *et al.,* 2013; Thippeswamy *et al.,* 2012) . Phytochemical investigation has proved that indeed plants posses a wide range of secondary metabolites, many of which are bioactive against various pathogens, while other compounds have disease preventive properties. Still, others are important in food preservation, as alternative and natural medicine (Khoshkholgh-Pahlaviani *et al.,* 2013). Drugs derived from plants may be used in their crude form or as semi-synthetic derivatives that manage various ailments. Some naturally medicinal compounds include, ephedrine (bronchodilator), colchicines (antigout), morphine (analgesic), and artemisinin (antimalarial) among others (Salim *et al.,* 2008; Barlunas and Kinghorn, 2005) .

Natural products therefore continue to play a crucial role in drug development as they account for almost 50% of new chemical entities in drug discovery and hence providing a starting point for new synthetic drugs. Infact, natural products are sources to about 90% of newly discovered pharmaceuiticals (Moshi, 2005; WHO, 2002). In addition to this, they may also be used as templates for synthetic modification in drug development. Although for many years, research has been geared towards discovering novel natural products with pharmaceutical significance, only 1% of the approximately 500,000 plants species occurring world wide have been phytochemically screened (Palombo, 2006). According to Lodonkar *et al.,* (2013) and Wakdikar (2004), only 15% of the existing 250,000 higher plants have been evaluated phytochemically.

A lot of this research has been done on plants that have ethnomedicinal significance; Many other plants in the wild without ethnomedicinal knowledge remain unresearched yet they too may have natural products with potential significance for drug developement. Lack of ethnomedicinal reports on these plants can be attributed to lack of their usage. Man discovered medicinal and food plants through trial, error, and experience. Therefore, accessibility, availability and distribution may have prevented investigations on such plants (Agnew, 2013; Kokwaro, 2009). Among such plants, are some species of *Gladiolus* and *Dierama* in the family Iridaceae.

Gladioli are flowery plants of great economic importance and are mainly cultivated for ornamental (Riaz *et al.,* 2007) and floricultural purposes (Bose *et al.,* 2003;Saifiula and Ahmed, 2001; Ranjan, 2008). In addition, some species of Gladioli have various ethnomedicinal uses such as in the treatment of a variety of diseases including hypertension (Mensah *et al.,* 2009). *Gladiolus dalenii* is used to treat gonorrhea, earache, wounds and eye diseases among others (Yineger *et al.,* 2008 ; Arnold and Gulumian, 1984).

In Kenya, there are four species of *Gladiolus,* namely: *G. watsonoides, G. ukambanensis, G. goetzenii and G. dalenii syn. G. newii*; which are mainly distinguished by morphological features of aerial parts (Agnew and Agnew 1994). However, identification based on these aerial parts is only possible during the rainy season. During the dry season, the aerial parts wither away while corms from which new aerial parts regenerate remain underground. Therefore, new features may be required for identification and classification during dry seasons.

Among these Gladioli, only *G. dalenii* has been reported to have ethnomedicinal value (Yineger *et al.*, 2008; Arnold and Gulumian, 1984*).* So far, bioassays have shown that this

species is active against amoeba (Moundipa *et al.,* 2005); the bacteria, *Bacillus subtilis* and *Staphylococcus aureus* and the fungus *Candida albicans* (Fawole *et al.*, 2008). Another bioactivity study on the Kenyan *G. dalenii* also reported moderate activity against *Aspergilus niger* (Odhiambo *et al.,* 2010). Although these studies revealed that this species is active against some fungal species, there is no similar information about the remaining Kenyan Gladioli species. Taxonomy involves classification whose achievement is based on similarities of various characters. Based on this fact, features of a particular species are likely to be similar to those of other related species even with little or no studies. Therefore with the known ethnomedicinal, biological and phytochemical composition of *G. newii*, other *Gladiolus* and *Dierama* species which have no such information were investigated.

1.1 Justification

Opportunistic fungal infections caused by *Candida* spp., *Aspergillus* spp. and *Cryptococcus* spp. are a major challenge to immunocompromised individuals (Vandeputte *et al.,* 2012). Infections by these opportunistic fungi are on the rise due to growing numbers of individuals with compromised immune systems as a result of HIV/AIDS, cancer, organ transplant and other surgical procedures amongst others (Vandeputte *et al* 2012; Pfaller, 2012). They have up to 90% occurrences in such individuals and are fatal if untreated (Butts & Krysan 2012; Runyoro *et al*., 2006). There are antifungal drugs used to treat fungal infections, however, they are limited in number (Vandeputte *et al.,* 2012), most of them are unaffordable, have poor bioavailability, interact with other drugs, toxic to various body organs or have adverse side effects leading to non compliance which in turn results into rapid development of resistance (Pfaller, 2012; Erika, 2001).

1.2 Problem statement

The above mentioned factors necessitate the search for an alternative, potent, safe and affordable antifungal agents for drug development, probably from plants. This study was conducted to determine the antifungal activity, toxicity and phytochemical composition of species of the Kenyan *Gladiolus* and *Dierama.*

1.3 Broad Objective of the study

This study was designed to determine the in *vitro* antifungal activity, toxicity, phytochemical components and to establish additional anatomical features that can be used to identify *Gladiolus* and *Dierama* species.

1.3.1 Specific objectives

1. To determine the antifungal activity of aqueous, organic and fractionated extracts from Kenyan *Gladiolus* and *Dierama* species;
2. To determine the toxicity of crude and fractionated extracts against the Brine shrimp nauplii;
3. To screen for selected classes of phytochemicals present in the crude and fractionated extracts;
4. To establish anatomical features for species identification and classification

1.4 Hypothesis

Gladiolus and *Dierama* species have elaborate chemical compounds with potential antifungal activities and several distinguishing anatomical features.

2.0 CHAPTER TWO. LITERATURE REVIEW

2.1 History of plant usage as medicine

Plants have been used by humanity since ancient times for various reasons. These include usage as food, clothing, shelter, arrow poisoning for hunting, protection during war, preparation of stimulant drinks, ritual purposes and as medicines (Salim *et al.,* 2008; Philipson, 2001). In the ancient times, medicines were used in their crude form in drinks as tea, porridge, beer, in powders forms for use as decoction or concoction, or with carriers such body jelly, and water for bathing (Kokwaro 2009;Yineger *et al.,* 2008; Barlunas and Kinghorns, 2005). The isolation of active compounds from medicinal plants began in the early 1800s with morphine and quinine being amongst the earliest compounds isolated (Salim *et al*. 2008; Barlunas and Kinghorns, 2005). Phytochemical screening, bioassay guided fractionation, isolation and characterization of bioactive compounds from plants has led to drug discovery and is a continuing process to date in new drug development.

2.2 Description and of the family Iridaceae Juss.

The family Iridaceae, commonly called the Iris family, belongs to the Kingdom Plantae, Division Magnoliophyta (flowering plants), order Liliales and class Liliopsida (monocotyledons) (Goldblatt & Manning, 2000). The family consists of about 60 genera and 1,500 species found in both temperate and tropical climates hence has a worldwide distribution. Plants of this family are shrubs of 20-100 cm tall. They are easily recognized vegetatively by the way their leaves are oriented edgewise to the stem. Leaves are simple, alternate and overlapping at the base in two rows. Flowers are structurally simple and highly modified in three distinct petal-like styles, corolla consists of three petals and 3 stamens which are all attached at the top of the ovary. The fruit is capsule. Some members of this family are herbs growing from rhizomes, bulbs or corms with narrow basal leaves, showy flower clusters at the tips of long stalks (Goldblatt and Manning, 2000). Amongst the many

genera found in this family only nine are found in Kenya. These are *Aristea, Romulea, Moraea, Dietes, Hesperantha, Oenostachys, Freesia, Dierama* and *Gladiolus* (Agnew, 2013). Most members of this family are important economically as medicine and as ornamental plants (Munyemana, 2013).

2.2.1 Description, ethnobotany, biological activity and phytochemistry of the genus *Gladiolus* L. (Iridaceae)

Gladiolus is a cormous genus with about 260 species and is the second largest after the genus *Iris* within Iridaceae (Ameh *et al.,* 2011). Of these species 250 are native to South Africa and tropical Africa. About 10 species are native to Eurasia. The species vary from very small to spectacular giant flower spikes. These plants are erect with attractive showy flowers. They are perennial herbs that are semi-hardy in temperate climates. They grow from rounded, symmetrical corms that are enveloped in several layers of brownish fibrous tunics.

They have unbranched stems with 1-9 narrow sword shaped longitudinally grooved leaves, enclosed in a sheath. The fragrant flower spikes are large and one-sided with bisexual flowers, each subtended by 2 leathery green bracts. The 3 lobed stigmas, sepals and the petals are almost identical in appearance and are termed as sepals. They are united at the base into a tube shaped structure. The dorsal tepal is the largest, arching over the three stamens. The outer three tepals are narrower. The perianth, funnel shaped with the stamens attached to its base, many seeded capsule, seeds flattened and arrow shaped anthers at the base.

The flowers are variously coloured as follows: white or cream with red centres, orange to red, uniformly bright red, yellow or orange with streaks or markings (Agnew, 2013;Ochekpe *et al.,* 2009;Agnew and Agnew, 1994).

Gladioli, have been used in ethnomedicine, food, as ornaments, and in flouriculture industry amongst others.

2.2.1.1 Use in ornamental and floriculture industry

Gladiolus spp. have showy flowers of various colours and shapes. These attributes make them stand out over other flowers. They are extensively used for decorations as cut flowers due to their nature and beauty (Ahmad and Siddique, 2005; Saifiula and Ahmad, 2001; Sharma *et al.,* 1988). They have a large number of cultivars, they are widely cultivated commercially for ornamental purposes as well as aesthetics and social functions (Ranjan, 2008; Riaz *et al.,* 2007; Bose *et al*., 2003). They are among some of the most important cut-flowers in the international floriculture trade. In Pakistan, they are among most important floricultural crops in cut flower trade (Burkill, 1985).

2.2.1.2 Use in ethnomedicine and food

Gladiolus spp. have been utilized by a number of various cultures to manage medical ailments various ailments. The parts most utilized are the corms and the flowers used to treat ailments such as skin infections, gut, urinogenital system, upper respiratory tracts, gonorrhea, dysentery, constipation, meningitis and malaria.The mode of preparation includes pounding fresh corms with water and/or other herbal plants or mixed with food before administration, or sniffing of the powdered corms, burnt corms are inhaled to treat colds and headaches. Decoction of the corms is also used as a relief of rheumatic pains, dysmenorrhoea, headaches, remedy for impotency, hemorrhoids, a booster for patients' immunity, epilepsy, convulsions (Ameh *et al.,* 2010; Odhiambo *et al.,* 2010;Yineger *et al.,* 2008;Tadesee, 1994; Burkill, 1985;Hutchings and Staden, 1994). Some of these plants are used to treat sterility in women, treatment of women with endometriosis, and making medicine to facilitate placenta passage, chest ailments, earaches, wounds, eyes, asthma , intestinal parasites, hypertension, clean

teeth, relieve gastric upsets, gonorrhea, remedy for snake bite, sores in the tongue and to calm patients with mental disorders (Munyemana, 2013; Mensah *et al.,* 2009; Arnold and Gulumian, 1984; Adjanohoun *et al.,* 1991; Gonzalez-Tejero *et al.,* 1995; Defour, 1994). Flower petals are crushed, rubbed on nails to strenghthen them while infusion from petals also used to provide a soothing wash for tired feet.

In food, corms are used to prepare a non-alcoholic drink made from millet or sorghum. Corms are also edible, and eaten raw or cooked, also by wild animals as food , used in soup, mixed with other plant parts to be used as purge, boiled and leached in water before consumption (Delfeld and Delfeld, 2007). Flowers are also used as food, raw or cooked, added to salads or used as boiled vegetables.

Amongst the Kenyan Gladioli only *Gladiolus dalenii* syn *G.newii* has reports on ethnobotany.

2.2.1.3 Biological activity of *Gladiolus* spp.

Various bioassay tests have been conducted on some *Gladiolus* spp. The dichloromethane extracts of bulb of *G.dalenii* are reported to have antibacterial activity against *Bacillus subtilis, Staphylococcus aureus,* while ethanol extracts of the plant showed antifungal activity against *C. albicans* and *A. niger* (Odhiambo *et al.,* 2010; Fawole *et al.,* 2008) . Anti-amoebic activity has also been reported by Moundipa *et al.,* (2005). Other *Gladiolus* spp. such as *G. gregasius* are active against *A. niger*, *Candida* spp. and *Psuedomonas aeruginosa* (Ameh *et al.,* 2011; Nguedia *et al.,* 2004).

Again, amongst the species studied in this research, only *G. dalenii* syn *G. newii* has reports on biological assays.

2.2.1.4 Phytochemistry

The family Iridaceae has a wide range of chemical compounds such as flavonoids, isoflavones, anthocyanins, quinonoid, xanthone, saponins, sterols, the *O*-glycosides of the flavonols quercetin, kaempferol, flavones , C-glycosides, nonprotein aminoacids and biflavones have been reported (Harborne and Williams, 2000; Goldblatt, 1990). The unusual free amino acids carboxyphenylalanine and carboxyphenyglycine have distinctive distribution in this family. G-glutamylpeptides have also been reported in some members of this family (Goldblatt, 1990). In the genus *Gladiolus,* various compounds have been reported in various parts, however amongst the species studied here, only *G. dalenii* has phytochemical reports. Corms of *G. dalenii* have been reported to have alkaloids (Odhiambo *et al.,* 2010; Burkill, 1985) anthraquinones, saponins, tannins, amino acids, reducing sugars, proteins, iridoids, terpenoids coumarins, flavonoids and carbohydrates amongst others (Munyemana, 2013; Ameh *et al.,* 2011; Ameh *et al.,* 2010; Ngamga *et al.,* 2007) . Other *Gladiolus* spp such as *G. gregasius* was reported by Assob *et al.*, (2011) and Nguedia *et al.,* (2004) to have saponins, glycosides, steroids, alkaloids, triterpenes and phenols. *G. imbricatus* contain flavonones, quinines and carbohydrates (Krvavych *et al.,* 2014). *G. gandavensis* have anthraquinones (Wang *et al.,* 2003). *G. segetum, G. atroviolaceus* and *G. illyricus* have been reported to have flavonoids, anthocyanidins, ascorbic acid, fatty acids, saponins, lipid, steroid, quinoid, chromone, cinnamic acid, benzoic acid, furfural, glycosides (Mohamed, 2005; Wubert *et al.,* 1996; Ali *et al.,* 1989; Viladomat *et al.,* 1986; Ali *et al.,* 1985).

2.2.2 Description of the *Gladiolus* species studied

The *Gladiolus* species studied in this research included *G.watsonoides*, *G. ukambanensis* syn *G. candidus*, *G. goetzenii* syn *G. dalenii* ssp. *andongensis*, *G. newii* syn *G. dalenii* ssp. *dalenii* were investigated.

2.2.2.1 *Gladiolus watsonoides* Baker

A conspicuous erect herb with hairless leafy stems ending in a spike of up to 15 uniformly bright glossy red flowers. It is found in wet soils in bamboo alpine zones 2400-4200m.Specific places include Aberdares, Mount Kenya (Agnew 2013 ; Agnew and Agnew 1994) (Figure 1).

Figure 1: A photograph of *G.watsonoides* showing the aerial parts with glossy red flowers and brownish bulbs : Source (photo taken in 2011 by Judith Odhiambo)

2.2.2.2 *Gladiolus ukambanensis* Baker

Flowers of this herb are pure white, fragrant up to 6 on each stem each up to 15 cm long with long perianth tube. Commonly found in grasslands with black cotton soils at medium altitudes of 100-2100m above sea level. Found in Aberdares, Narok, Rift Valley, Nanyuki, Embu, Machakos, Nairobi, Kajiado and Konza area along Nairobi-Mombasa road. *Gladiolus candidus* (Rendle) Goldblatt is asynonym for this plant (Agnew 2013; Agnew and Agnew 1994) (Figure 2).

Figure 2: A photograph of *G.ukambanensis* showing the aerial parts with white flowers and creamish bulbs: Source (Photo taken in 2012 Judith Odhiambo)

2.2.2.3 *Gladiolus goetzenii* Baker

Flowering and leafy stems are separate. Flowers bright orange with markings (Figure 3). Common in grasslands from savanna edges to montane forests in altitude zone between 1200-3100m a bove sea level. Found in Mount Elgon,Tinderet hills, Mau, Loita, Mount Kenya, Kitale, Mumias, Kisii, Narok, Nanyuki, Embu, Machakos, Nairobi, Kajiado, Cherenganyi, Laikipia district, Olkalau- Nyahururu . This is synonym to *Gladiolus dalenii* Van Geel subsp *andongensis* (Baker) (Agnew 2013; Agnew and Agnew 1994; Goldblatt 1996), (Figure 3).

Figure 3: A photograph of *G.goetzenii* showing the bright orange flowers with markings and brownish bulbs: Source (Photo taken in 2012 by Judith Odhiambo)

2.2.2.4 *Gladiolus newii* Baker

Flowers yellow orange without markings (Figure 4). Flowering and leafy stems not separate. Common in grasslands from savanna edges to montane forests in altitude zone between 1200-3100m a bove sea level. Found in Mount Elgon,Tinderet hills, Mau, Loita, Mount Kenya,

Kitale, Mumias, Kisii, Narok, Nanyuki, Embu, Machakos, Nairobi, Kajiado, Cherenganyi, Laikipia district, Olkalau- Nyahururu . *G.newii* is synonym to *Gladiolus dalenii* Van Geel subsp. *dalenii* Van Geel (Agnew 2013 ; Agnew and Agnew 1994 ; Goldblatt 1996) (Figure 4).

Figure 4: A photograph of *G.newii* showing the aerials with yellow orange flowers and yellowish bulbs: Source (photo taken in 2011by Judith Odhiambo)

2.2.3 Description of the genus *Dierama* K. Koch

This genus consists of evergreen perennial herbs with large corms. Aerial parts persist for several years, corms have coarse fibrous tunics. Leaves are several with the lower 2-3 sheathing the stem base (cataphylls). These often dry and become fibrous. The foliage leaves are linear with many conspicuous parallel veination often without a mid rib. Stems slender with branched inflorescence consisting of spikes which are few to many, erect to pendulous,

terminal on the main axis. Flowers are usually pink to red, yellow or white or purple. Style branches simple exerted from the perianth tube are simple, short. Stamens alternate with 3 entire stigmas. Capsules globose, seeds small and often shiny.

The genus consists of up to about 44 species extending from the Eastern Cape province of South Africa through East Tropical Africa to Ethiopia. Some species of this genus include *Dierama adelphicum* , *D. ambiguum, D.atrum, D.cooperi, D.dessimile, D.cupuliflorum* among others. *Dierama cupuliflorum* however is the only species found in Kenya (Agnew 2013; Goldblatt, 1996 ; Agnew & Agnew 1994; Rundall & Goldblatt, 1991).

2.2.3.1 Use as ornamental

Dierama spp. commonly called the Angel's fishing rod have graceful, arching flower and bell shaped blooms that makes it attractive as an ornamental flower. They are mainly planted in borders or gravel gardens with other ornamental grasses (Royal Horticultural Society, 2009). No reports were found on the usage of these plants in ethnomedicine. Similarly, no reports were found on biological activity as well as phytochemical composition.

2.2.4 *Dierama cupuliflorum* Klatt.

This is a robust tufted perennial herb with delicate blue or purple flowers hanging on long thin stalks. Flowers about 25mm in diameter. Commonly found in high altitude zones of undisturbed grassland between 2400-3900m above the sea level such as in areas like Mount Elgon, Cherenganyi, Mau, Aberdares, Mount Kenya (Agnew ; 2013 Agnew and Agnew 1994). (Figure 5).

Figure 5: A photograph of *D.cupuliflorum* showing the aerials with purple flowers and brownish bulbs: Source (photo taken in 2011 by Judith Odhiambo)

2.3 Opportunistic fungal infections

Opportunistic fungal infections remain threat to individuals with compromised immune system (Roemer and Krysan, 2014). Infections caused by *Candida albicans, Aspergilus fumigatus, Aspergilus niger*, *Cryptococcus neoformans, Histoplasma capsulatum, penicillium marneffei* and other dematorphytes do complicate and compromise the lives of such patients (Butts and Krysan, 2012; Torkarski *et al*., 2012; Walsh *et al.,* 2008).

Increasing incidence of Infections by *Candida, Aspergillus* and *Cryptococcus* is due to growing number of immunocompromised cases related to HIV/AIDS, cancer, old age, diabetes, organ transplants, cystic fibrosis and other surgical procedures (Vandeputte *et al* 2012; Herbrecht*, et al.,* 1992). Among these, HIV/AIDS is the main predisposition (Duffalo, 2006 ; Saag, 1997). It is not surprising that with the few available antifungal agents, the mortality rates and the occurence of invasive infections with these three most common opportunistic human fungal pathogens (Herbrecht *et al.,* 1992) are still high. Oral, oesophageal and systemic candidiasis caused by *C. albicans* have up to 50% occurrence, *Aspergillus* with 50-90% occurrence, and *Cryptococcus neoformans* with 20-70% occurrence (Butts and Krysan, 2012). These infections are fatal if left untreated (Vandeputte *et al.,* 2012; Runyoro *et al.,* 2006).

2.3.1 Candidiasis

Mucocutaneous candidiasis is the most common fungal infection amongst immunocompromised patients and is caused by *C. albicans* (Duffalo, 2006). Other *Candida* species such as *C. tropicalis, C. kruseii, C. glabrata* and *C. parapilosis* are less frequently associated with the disease. These organisms are part of the normal flora in the gastrointestinal tract but may cause disease when they overgrow in the tract. Candidiasis presents itself in three forms as follows: oropharyngeal, esophageal, and vulvovaginal disease. The occurrence of candidiasis is common, up to 90%, in advanced HIV cases (Vandeputte *et al.,* 2012). Symptoms of oropharyngeal candidiasis include mouth swellings or painful lesions which may be psuedomembranous and makes it difficult when opening the mouth. Psuedomembranous candidiasis commonly called the thrush presents itself as creamy white exudative on the tongue. Erythmatous candidiasis presents itself as red plaques on mucosal surfaces. Vaginal candidiasis presents itself with vaginal discharge , vulvar rush and

vaginal pruritus (Duffalo, 2006). Presence of characteristic psuedohyphae and spherical budding yeast confirm the diagnosis of candidiasis.

Treatment of candidiasis depends on the form and severity. Various antifungal agents used include fluconazole, ketoconazole, itraconazole, voriconazole; polyenes such as nystatin, amphotericin B; Candins such as caspofungin, micafungin and anidulafungin. However, all these antifungal agents have adverse side effects such as gastrointestinal irritation, hepatitis, endocrine system malfunction, liver abnormalities, skin rashes, diarrhoea, poor bioavailability, occurrence of a relapse which require a full course of the retreatment (Duffalo, 2006). Ketoconazole which is the antifungal agent used in treatment of systemic candidiasis is poorly absorbed in basic environments and has other adverse side effects such as gastrointestinal irritation , liver function abnormalities, vomiting and nausea (Dismukes, 2000).

2.3.2 Aspergillosis

Infections by *Aspergillus* spp. have become life threatening in individuals with weak immune systems, especially those with advanced HIV/AIDS infection (Duffalo, 2006) and those who have undergone lung transplant (Walsh *et al.,* 2008). *Aspergillus* molds are found in the soil, or decaying matter, and in certain dried foods. Invasive fungal infections by *Aspergillus* spp. occurs more frequently in the immunocompromised individuals and is currently the leading cause of death of such patients in the United states (Kulkarni, 2013).

The organisms responsible for causing aspergillosis include *Aspergillus fumigatus*, *A. flavus, A. terreus, A. niger, A. nidulans, A. ustus, A. glaucus* and *A. lentulus*. Fungal spores enter in the lungs and sinuses whereby in individuals with weak immune systems, the spores spread

throughout the lungs and other parts of the body leading to three forms of aspergillosis (Kulkarni, 2013; Walsh *et al.,* 2008). The first form is hypersensitivity pneumonitis which is an allergic reaction to the fungal spores and has symptoms such as shortness of breath and coughing: the second form presents itself like asthma with symptoms including coughing, wheezing and shortness of breath. In this form, affected individuals do not improve with normal asthma treatment this condition is called allergic bronchopulmonary aspergillosis. The third form is invasive aspergillosis and is a life threatening illness whose symptoms include fever that does not improve with antibiotics, chest pain, difficulty in breathing and dry cough with blood (Kulkarni, 2013). Other symptoms in severe cases include ulcerations in mouth and chest wall, bleeding from the nose, seizures or stroke like symptoms, an indication that the infection has spread to the brain (Walsh *et al.,* 2008). Diagnosis include sampling a tissue biopsy from the lungs, examination of sputum or taking cultures from breathing tubes- air ways (Kulkarni, 2013).

Treatment of aspergillosis includes the use of antifungal drugs such as amphotericin B, voriconazole, itraconazole, caspofungin, micafungin, anidulafungin, posaconazole and steroids in allergic reactions (Walsh *et al.,*2008; Dismukes 2000) Surgery may be recommended to remove fungal balls (aspergilloma) if infection does not improve with drugs. These drugs however have adverse side effects such as chills, vomiting, renal impairment, hepatotoxicity, visual hallucinations, skin rash, and the chances of abuse is high because some of them are to be taken for several months and some species have also showed resistance (Donnely and Maertens, 2013).

2.3.3 Cryptococcosis

Cryptococcosis is a global invasive mycosis associated with significant morbidity and mortality (Perfect *et al.,* 2010). It is mainly caused by *Cryptococcus neoformans;* an

encapsulated round to oval yeast organism commonly associated with immunocompromised patients or immune system dysfunction (Jain *et al.,* 2013). The fungus is found in soils contaminated with decaying pigeon and chicken droppings. The organism is inhaled and finds its way in the host through the respiratory path. This results in invasion and infection to the respiratory system. The organism then spreads to extra-pulmonary tissues specifically the brain tissue where it causes meningitis (Duffalo, 2006). Cryptococcal infection has been to reported to be less often in Europe and United states but appear to be higher in Africa and South East Asia, a report which correlates with HIV prevalence (Perfect *et al.,* 2010; Duffalo, 2006).This infection is the leading cause of death in AIDS patients especially in the developing countries (WHO, 2011; French *et al.,* 2002).

Cryptococcus infects the central nervous system presenting as meningitis. Symptoms of this disease include headache, altered mental status, photophobia, blurred vision, nausea and vomiting and fever ; acute cases may be cerebral edema and elevated intercranial pressure which occurs in more than 50% of patients with cryptococcal meningitis leading to morbidity and mortality. Diagnosis is possible by examination of cerebrospinal fluid for an encapsulated yeast using Indian ink (Duffalo, 2006).

Treatment of cryptococcal meningitis involves combination of antifungal drugs such as amphotericin B and flucytosine (Butts and Krysan, 2012; Perfect *et al.,* 2010). A long period of therapy is necessary to avoid a relapse. Side effects of treatment include liver abnormalities, skin rashes, nausea, vomiting, gastrointestinal intolerance, rapid development of resistance and bone murrow suppression (Duffalo, 2006 ; Dismukes, 2000) .

2.4 Natural products and Drug Development

Natural products in medical chemistry, also known as secondary metabolites from plants are organic compounds (Wink, 2011). Although such compounds are produced by plants as a defense mechanism, they are known to have curative activities against several ailments in man (Zohra *et al.,* 2012). Plants however produce the vast majority of natural products used for a wide variety of purposes such as supplements, food preservatives (Khoshkholgh-Pahlaviani *et al.,* 2013) cosmetics and natural therapies (Samejo *et al.,* 2013). Natural products are considered to be biodegradable and renewable hence environmentally safe (Dave and Ledwani, 2012; Kubo and Taniguchi, 1993) and with low incidences of adverse side effects coupled with their reduced costs (Doughari, 2006). Natural products from plants remain vital in drug discovery where they can be used directly as drugs or serve as leads to new drugs by providing chemical entities (Barlunas and Kinghorns, 2005).

The initial stage of drug discovery involves various fields including botany, ethnobotany and phytochemistry. A number of plant derived compounds have been used as drugs either in their original or semi-synthetic form in management of various ailments such as ephedrine (bronchodilator), colchicine (anti-gout), morphine (analgesic), artemisinin (antimalarial), (Salim *et al*. 2008; Philipson, 2001). According to Barlunas and Kinghorns (2005), natural products account for about 50% of new chemical entities in drug discovery and provide a starting point for new synthetic compounds. Other than the usage of derived natural compounds as drugs, these compounds may also be used as templates for synthetic modification in drug discovery .

WHO estimates that 80% of the rural population in Africa, Asia and Latin America relies on traditional medicine for their primary health care to manage various ailments, due to their affordability, accessibility and cultural values (Sawadogo *et al.,* 2012; WHO, 2002). And the curative properties of these medicinal plants is as a result of the natural products that they contain (Ishaq *et al.,* 2014).

Natural products such as alkaloids, flavonoids, tannins, resins, glycosides, and oils among others have been reported to be bioactive against a vast range of pathogens and have therapeutic activities as antibacterials, antivirals, anti-oxidants, antihelminthes and antifungals among others (Khoshkholgh-Pahlaviani *et al.,* 2013). Moshi, (2005) reported that plants´ natural products are source to about 90% of the newly discovered pharmaceuticals in current use. According to Palombo (2006), 1% of the 500,000 plants species occurring worldwide have been phytochemically investigated, while according to Wakdikar (2004) and Fabricant & Farnsworth (2001), amongst 250,000 existing higher plants, 6% have been screened for bioactivity and only 15 % have been phytochemically evaluated. Therefore, a lot more needs to be done to unearth natural products with potential anti-infective agents.

In most cases plants that have reports of traditional usage are the ones that are subjected to bioassay tests and phytochemical investigation and eventually contribute to modern therapy (Siqueira *et al.,* 2011). In deed, some plants, may have no reports on traditional usage because they are not being used but possess bioactivity and hence may be source of natural products vital in drug development.

2.5 Secondary metabolites of medicinal plants

Secondary metabolites occur in plants in a high structural diversity (Wink, 2011). The major classes of secondary plant products include tannins, glycosides, flavonoids, alkaloids,

terpenoids, steroids, quinones, saponins amongst others and are significant in drug discovery (Zohra *et al.,* 2012; Bart and Pilz, 2011).

2.5.1 Flavonoids

Flavonoids are a group of oxygen containing aromatic anti-oxidant compounds with more than one benzene ring in their structure (Wink, 2011). More than 4000 flavonoids have been reported some of which are pigments in higher plants. Quecertin and kaempferol are among the common flavonoids and are found in about 70% of plants. Other groups of flavonoids include flavones, flavans, dihidroflavons, flavonols, calchones, catechins and anthocyanidins among others. Other than being anti-oxidants, anticancer (Yao *et al.,* 2004), flavonoids also have antimalarial and antifungal activities (Musila *et al.,* 2013; Otang *et al.,* 2012). The molecular structure of quercertin is given here in (Figure 6).

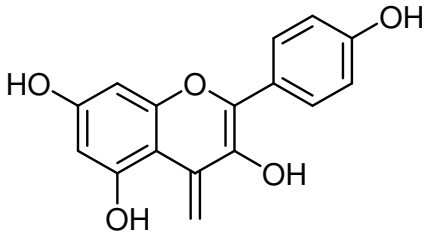


Figure 6: Molecular structure of quecertin (adapted from Wink, 2011)

2.5.2 Alkaloids

Alkaloids are organic compounds with basic chemical properties containing at least one nitrogen atom in a heterocyclic ring . They are among the largest group of phytochemicals known and about 21,000 are known. These nitrogenous compounds are alkaline in nature turning red litmus paper blue (Wink, 2011). They are widely distributed in angiosperms especially in the families liliaceae and solanaceae (Wink, 2011). Majority exist as solids, while some as oils. They include cocaine, morphine, hordenin and narcotine among others .

They may be poisonous, addictive or medicinal (Kokwaro, 2009; Woolley, 2001). Other biological activities reported include anticancer, antiviral, antimalarial (Musila *et al.,* 2013; Woolley, 2001), as well as antibacterial and antifungal (Doughari, 2006). A molecular structure of hordenin is shown in Figure 7.

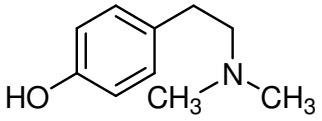


Figure 7: Molecular structure of hordenin (adapted from Wink, 2011)

2.5.3 Terpenoids

Terpenoids are any class of hydrocarbons that consist of terpenes attached to an oxygen-binding group. They are widely found in plants and can form cyclic structures such as sterols. They exist as oils, commonly found in essential oils and resins. Most of them interact with biomembranes and membrane proteins (Wink, 2011) , hence they have broad inhibitory effects against the natural enemies of plants such as bacteria, viruses, fungi, insects and even vertebrates (Cheng *et al.,* 2007). They mainly occur in tissues such as bark, leaf, fruit, root, seed and are classified as mono-, di-, tri- and sesqui-terpenoids depending on the number of carbon atoms (Cheng *et al.,* 2007). Sesquiterpenes are common in the families asteraceae and other families such as those of apiaceae, magnoliacae, lauraceae and even ferns (Wink, 2011). Diterpenes are quite toxic and are found in euphorbiaceae, ericaceae and thymelaceae. Common terpenoids include menthol, camphor, eugenol, thujone, sterols and thymol amongst others. They have been utilized in drug, food, perfumery industries and also have insecticidal, anti-inflamatory and cytotoxic, analgesic, antihelminthic activities (Bhargava *et al.,* 2013). Figure 8 is a molecular structure of a mono-terpene, thymol (Wink, 2011).

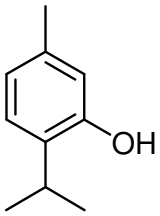


Figure 8: Molecular structure of thymol (adapted from Wink, 2011)

2.5.4 Tannins

Tannins are polyphenolic compounds that bind and precipitate proteins . Mainly from plant origin, they are widely distributed in the plant flora (Harborne, 2002). They are mainly found in bark, root, stem and outer layers of plant tissues. They form complexes with proteins to form insoluble complexes (Hassanpour *et al.,* 2011). In plants, they play a role of protection against herbivory and attacks by insects (Hassanpour *et al.,* 2011). Tannins posess antidiarhoeareal and antimicrobial activities (Samejo *et al.,* 2013; Siqueira *et al.,* 2011) as well as antimicrobial and antihelmithic activities (Hassanpour *et al.,* 2011). Example tannins include catechins,(Figure 9) according to Wink, (2011).

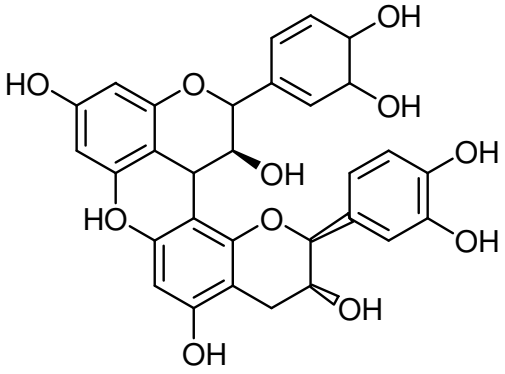


Figure 9: Molecular structure of catechin(adapted from Wink, 2011)

2.5.5 Steroids

Steroids are compounds containing a 17-carbon 4-ring system. They include sterols, numerous hormones and glycoside. They have also been used as arrow poisons. Examples of

plant steroids include diosgenin (Figure 10) and cevadin (Doughari, 2006). Some of them possess antibacterial activity and also important due their relationship with sex hormones (Samejo *et al.,* 2013).

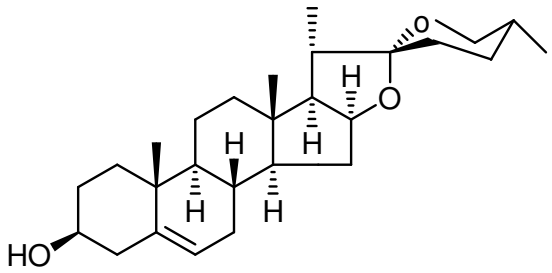


Figure 10: Molecular structure of diosgenin (adapted from Raju and Rao, 2012)

2.5.6 Saponins

Saponins are glycosides of triterpenes or steroids. Steroid saponins are common in monocots and less in dicot families (Wink, 2011). The term saponin was derived from *Saponarria vaccaria,* a plant which was once used as soap. These compounds possess soaplike behavior in water by producing foam. They are widely distributed and posses various biological activities (Sparg *et al.,* 2004) . A part from plants, some marine organisms and insects also produce saponins (Thakur *et al.,* 2011). Some biological activities reported include antifungal, antileshmania, antimalarial, antitumour and antiviral amongst others (Samejo *et al.,* 2013; Thakur *et al.,* 2011). Solanine, an example of saponin is illustrated (Figure 11).

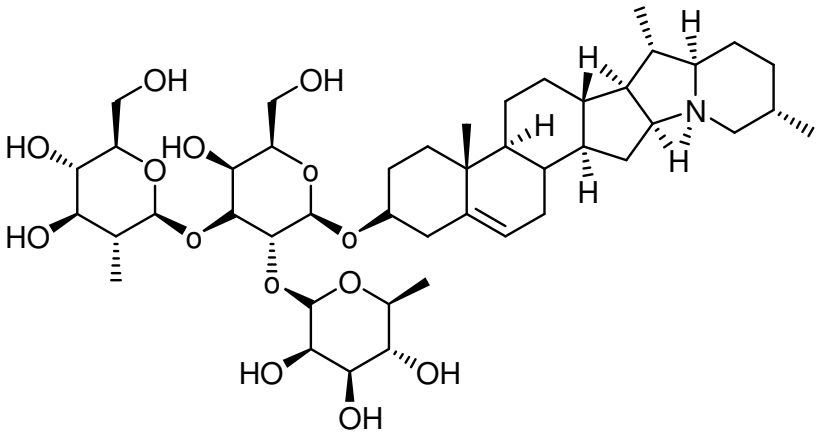


Figure 11: A structure of solanine, a saponin, drawn from, http://en.wikipedia.org/wiki/Saponin, 2014

2.5.7 Glycosides

Glycosides are molecules in which a sugar group is bound to a non-sugar group by a nitrogen or oxygen atom, and yield sugar after undergoing hydrolysis (Houghton, 2013).They are colourless crystalline carbon, hydrogen and oxygen containing, water soluble phytochemicals found in the cell sap. They have been used in treatment of skin diseases and as flavouring agents in pharmaceutical preparations. They also posses antiprotozaon activity, amygdalin is anticancer as well as a cough suppressant. Cyanogenic glycosides have been reported in a bout 2000 plant species including members of the families rosaceae, fabaceae, poaceae, euphorbiaceae, lamiaceae, sapindaceae and ranunculaceae amongst others (Wink, 2011). However foodstuffs with cyanogenic glycosides are potentially poisons (Doughari, 2006) an example being, prunasin (Figure 12).

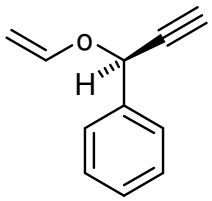


Figure 12: Molecular structure of prunasin (adapted fromWink, 2011)

2.5.8 Anthraquinones

Anthraquinones are yellow crystalline ketone $C_{14}H_8O_2$ often derived from an anthracene. They occur in plants such those in the families polygonaceae, rhamnaceae, fabaceae, liliaceae and rubiaceae amongst others (Wink, 2011). Other than occurrence in higher plants, they are also found in fungi, lichens and insects where they serve as the basic skeletons of their pigments. They have a wide range of applications in food, cosmetics, dye and pharmaceutical industries due to their therapeutic and pharmacological properties (Dave and Ledwani, 2012). They have been reported to have antifungal, antioxidant and laxative activities (Dave and Ledwani 2012; Yen *et al.,* 2000). Figure 13 shows a structure of an anthraquinone.

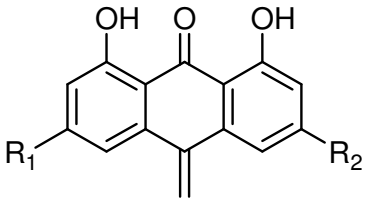


Figure 13: Molecular structure of an anthraquinone (adapted from Wink, 2011)

2.6 Antifungal Drugs Used to Treat Opportunistic Fungal Infections

Fungal infections are managed/treated by four main classes of drugs namely polyenes, pyrimidines (antimetabolites), candins and the azoles (Vandeputte *et al.,* 2012; Duffalo, 2006 ; Ernst 2001; Dismukes 2000). Polyenes include amphotericin B and nystatin. Nystatin is toxic when used parenterally and is therefore used only in *Candida* infections that involves the skin, oral mucosa and gastrointestinal tract (Duffalo, 2006; Ernst,2001).

Although Amphotericin B is the most appropriate drug for many invasive or life threatening fungal infections, as it has a broad spectrum activity against many fungal pathogens and moulds; its use is however limited especially in cases of invasive aspergillosis in severely immunocompromised patients, due the toxic effects elicited with up to 80% of patients experiencing renal impairment, nausea, vomiting, fevers, chills (Duffalo, 2006; Dismukes, 2000).

Candin agents include micafungin, caspofungin, anidulafungin; all these have poor bioavailability and hence have limited usage (Duffalo, 2006). Amongst the three in this category, caspofungin is the most used in treating invasive candidiasis and invasive aspergillosis in individuals infected with HIV (Pfaller, 2012), however presence of other co-medications affects its effectiveness, many fungal pathogens have also developed resistance against this class of antifungals drugs (Ernst, 2001).

Flucytosine, a pyrimidine for is used in combination with other antifungal drugs to obtain better results as it has limited spectrum of activity (Butts and Krysan, 2012; Duffalo, 2006). Pathogens rapidly develop resistance against this drug when used alone. Adverse side effects include gastrointestinal intolerance, skin rash, liver dysfunction and bone murrow suppression (Vandeputte *et al.,* 2012; Dismuskes, 2000).

The azoles are the most commonly used antifungal drugs in clinical practice (Vandeputte *et al.,* 2012). They include ketoconazole, voriconazole, itraconazole and fluconazole. They are used mainly as an alternative to amphotericin B: this is because of their relative broad spectrum activity against common opportunistic fungal infections, limited toxicity and high bioavailability generally. However these drugs frequently interact with coadministered drugs which may results in decreased concentration leading to decreased absorption or metabolism. Azole – drug interaction may also lead toxicity of the coadministered drug as the azoles have the ability to increase plasma concentration of other drugs by altering the hepatic metabolism (Dismukes, 2000). The other limitation is the emergence of resistance by the fungal organisms (Vandeputte *et al.,* 2012). Ketoconazole is poorly absorbed in the absence of acidic environments and has multiple drug interactions. Other adverse effects include the gastrointestinal irritation, nausea, vomiting and liver function abnormalities (Vandeputte *et al.,* 2012). Fluconazole is well absorbed without the requirement of acidity, it is well tolerated and is bioavailable. However, resistance by the organisms and interactions with various drugs are major concerns about this drug. It is ineffective against certain *Aspergillus* spp. and has limited activity against certain *Candida* spp. Other side effects include: nausea, vomiting, or diarhoea (Pfaller, 2012; Duffalo, 2006; Ernst, 2001).

Voriconazole which is structurally similar to fluconazole has broad spectrum activity against many fungal pathogens (Duffalo, 2006). It is much more potent than fluconazole against many *Candida* species. It is highly bioavailable, however its absorption is impaired in presence of food, various drug interactions exist and other side effects include liver abnormalities, skin rashes, photosensitivity, and visual disturbances (Ernst, 2001).

In view of the above mentioned adverse side effects, multidrug resistance by the organisms, morbidity and mortality caused by the yeasts and moulds implies that the search for safe, effective and new improved antifungal agents with new structures, probably from plants is of paramount importance. This study on the Kenyan *Gladiolus* and *Dierama* species (Iridaceae) was conducted to contribute towards this goal.

2.7 Techniques Used in Bioassays and Natural Products Studies

Several techniques are employed in studying the bioactivities of phytochemicals from a given plant. The process begins by first drying the plants samples at room temperature to enable its prolonged storage time. Solvent extraction then follows which requires the use of various solvents both polar and nonpolar (Bart and Pilz, 2011). This is in order to, as much as possible, exhaust extraction of constituents including those with biological activity. The extracts are filtered and concentrated in *vacuo* (Harborne, 2002).The bioactive component has to be separated from the crude extracts through bioassay guided fractionation (Bart and Pilz ,2011). Bioassay-guided fractionation from plant extracts is done closely with other chromatographic separation techniques which eventually lead to the isolation of biologically active molecules whose chemical structures can be determined by spectroscopic techniques (Philipson, 2001).

2.7.1 Chromatography

Chromatography is an analytical method used for separation, identification and determination of chemical components from mixtures. Chromatography as a technique involves two phases, mobile and stationary phase. TLC is used to show profiles of mixtures of components hence utilized in determination of approximate number of components, classes of compounds in a mixture, the level of purification and to monitor the progress of column chromatography It

consists of three steps; spotting, development and visualization (Harborne, 2002; Smith and Feinberg, 1962). Column chromatography is used as apurification technique as it isolates desired compounds from the mixture, the mobile phase flows down through a column by gravity. Compounds are isolated based on the polarities of the solvents used (Harborne, 2002).

2.7.2 Bioactivity and Disc diffusion technique

Bioactivity screening provides relevant information about the biological effects of natural products in different organisms. They are conducted in order to find new lead compounds of pharmaceutical relevance, to confirm ethnomedicinal utilization of plants and to develop phytomedicines for use as herbal medicine (Ellof, 2004). Some challenges facing bioassay screening is the incomplete solubility of some fractions even in the solvent originally used to extract them which may lead to loss of some compounds or activity (Weller, 2012; Ellof, 2004). Among the popular methods used in bioassay activity is disc diffusion method.

2.7.2.1 Disc Diffusion Technique and Minimum Inhibitory Concentrations

Many tests on antimicrobial activity are conducted using disk diffusion method. It is one of the common methods used to date to determine the activities of drugs and plant extracts. Although an ancient method, it is still accepted by the European Committee on Antimicrobial Susceptibility Testing (EUCAST). It is used for testing susceptibilty of a majority of fungal and bacterial pathogens (Atul, 2014; EUCAST, 2013).

Discs, (5-6mm diameter) may be commercially acquired or prepared from Whatman filter paper No. 1. These are impregnated with a specific amount of the extract using a micropipette and carefully and aseptically placed firmly to the surface of the inoculated and dried agar plate. According to EUCAST (2013) guidelines, the number of discs in a plate should be limited to avoid overlapping zones of inhibition. The plates are then covered with parafilm

and incubated at appropriate temperatures depending on the organisms. During the incubation, the chemical ingredients diffuse from the disc and spread outwards into the agar (Hudzicki, 2013), causing inhibition to growth of an organism which manifests as a ring of inhibition a round the disc, If it is succeptible to the diffusing elements. At the end of the incubation period, a complete zone of inhibition is visible to the naked eye (EUCAST, 2013). The zone of inhibition is then determined by measuring its diameter using a transparent ruler or a caliper in milimitres (Dzomba and Muchanyereyi, 2012 ; Selvamohan *et al.,* 2012). Other than being used in bioactivity tests, this techniques has also been used to determine the Minimum Inhibitory Concentrations (Palazzo *et al.,* 2007).

MIC is the lowest concentration of an antimicrobial agent or a drug that is able to inhibit any visible antimicrobial growth after an incubation period (Andrews, 2001). According to Kariba (2000), MIC can be determined by impregnating the sterile discs with various serially diluted concentrations of the reconstituted sample. It is regarded as the most basic laboratory measurement used to monitor activity of antimicrobial agents or extracts against microorganisms (Sen and Batra, 2012). MIC values are used to classify bioactive plants as strongly, moderately, weakly or not active against a specific microorganism (Tangarife-Castano *et al.,* (2011). This information is important in the selection of bioactive plants which deserve further analysis (Awuoafack *et al.,* 2013). For instance plants with MIC range between 0.1- 0.625mg/ml implies they are strongly active while those with MIC values above 0.625mg/ml are considered to have weak activity and therefore do not deserve further investigation (Awuoafack *et al.,* 2013). However, according to Tangarife- Castano *et al*., (2011) and Tsuzuki *et al.,* (2007), plants with MIC range up to 0.5mg/ml are strongly active, those with range between 0.6-1.5mg/ml are moderately active while those with the MIC value above 1.6mg/ml are weakly active. Classification of plants studied here based on their activities against the microorganisms tested was done based on the MIC value ranges

according to Awuoafack *et al.,* (2013) ; Tangarife- Castano *et al*., (2011) and Tsuzuki *et al.,* (2007).

2.7.2.2 Cytotoxicity and Brine shrimp Lethality Test (BLT)

Toxicity studies are vital in determining the safety of a test substance and to characterize the possible toxic effects it may produce (Arome and Chinedu, 2014). The guiding principles of toxicity testing are to observe the effects of the test substances on experimental organisms (Arome and Chinedu, 2014).

Many preliminary assessment of cytotoxicity in plant extracts are done using the nauplii of brine shrimp (*Artemia* spp.). They offer a simple tool to guide in screening and fractionation of biologically active extracts (Carballo *et al.,* 2002). It has become popular since the only biological response to monitor is lethality (Montainher *et al.,* 2002).

The eggs are commercially available and are hatched for the larvae to be used. Hatching involves preparation of artificial sea water by obtaining a solution from commercial sea salt in distilled water. This artificial sea water is poured into a tank which is partitioned using a plastic divider with several holes near the base (Ul-Haq *et al.,* 2012) . One side of the compartment is darkened using alluminium foil while the other is illuminated using a 60 watt lamp. Brine shrimp eggs are then sprinkled into the darkened compartment. The hatching takes place between 24 to 48 hours. Since they are phototropic, the nauplii move to the illuminated compartment through the holes in the partition (Manmoon and Azam, 2011). These are collected using a pipette and used in tests conducted at different concentrations in vials. Observations are made after 24 hours (Musila *et al.,* 2013).

2.7.3 Phytochemical screening

Phytochemical studies are conducted to determine the type of compounds responsible for the antiinfective effects (Abdollahzadeh *et al.,* 2011). Although these secondary metabolites produced by plants are for their own defence a against attacks by various pathogenic microorganisms, they have been reported to be vital in drug discovery and development as they are the ones that exert the biological activities (Filgueiras *et al.,* 2011). The major chemical classes of compounds screened in plants have been reported to include flavonoids, alkaloids, terpenoids, steroids, tannins, saponins, glycosides, anthraquinones amongst others (Ishaq *et al.,* 2014; Samejo *et al.,* 2013). The qualitative screening is based on stepwise addition of specific chemical reagents to powdered plant material or extract resulting into visual colour change and or formation of a precipitate (Dib *et al.,* 2013).

A summary of specific reagents and visual colour reactions observed during the screening of different secondary metabolites is given in (Table 1) .

Table 1: Qualitative phytochemical tests and expected output

Phytochemical	Chemical reagents	Visual colour change
Flavonoids	Ethylacetate and 50% ammonia	Yellow colouration
Alkaloids	2% suphuric acid and Drangedorff´s reagent	Orange red precipitate
Saponins	Distilled water	Formation of a stable froth
Terpenoids	Chloroform and concentrated sulphuric acid	A reddish brown colouration at the interphase
Glycosides	Distilled water, concentrated hydrochloric acid, ammonia solution, Benedicts' reagent.	Reddish to brown precipitate
Tannins	Distilled water and Ferric chloride	Dark or dirty green precipitate
Steroids	Acetic anhydride , concentrated sulphuric acid	Violet to blue or green colour
	Concentrated sulphuric acid.	Effervescence followed by a Clear reddish brown colour at the interphase
Anthraquinones	10% hydrochloric acid, chloroform, 10% ammonia	Rose pink colour

According to Dabai *et al.,* (2013), Dushimemaria *et al.,* (2012) and Mushi *et al.,* (2012).

2.8 Plant taxonomy and anatomy

Plant taxonomy is an aspect of plant systematic that is concerned with the principles, procedures, rules and regulations of classification (http://www.unaab.edu.ng). It mainly has two aims; to identify all kinds of plants and to arrange the plants in a classification scheme that shows their true relationships. In order to achieve these, taxonomists utilize the methods and resources of major fields of botanical investigation such as morphology to enable understanding of form and structure (http://www.unaab.edu.ng) . Anatomical characters have been used as indices in taxonomical studies for more than a hundred years and have been significant in the revision of certain plant families like Myrtaceae and Ericaceae and the part utilized were the internal leaf structure (Al-Edany & Al-Saadi, 2012;Watson, 1963) , which mainly consists of cuticle ,sclerenchyma, xylem, phloem, bundle sheath and mesophyll cells (Figure 14).

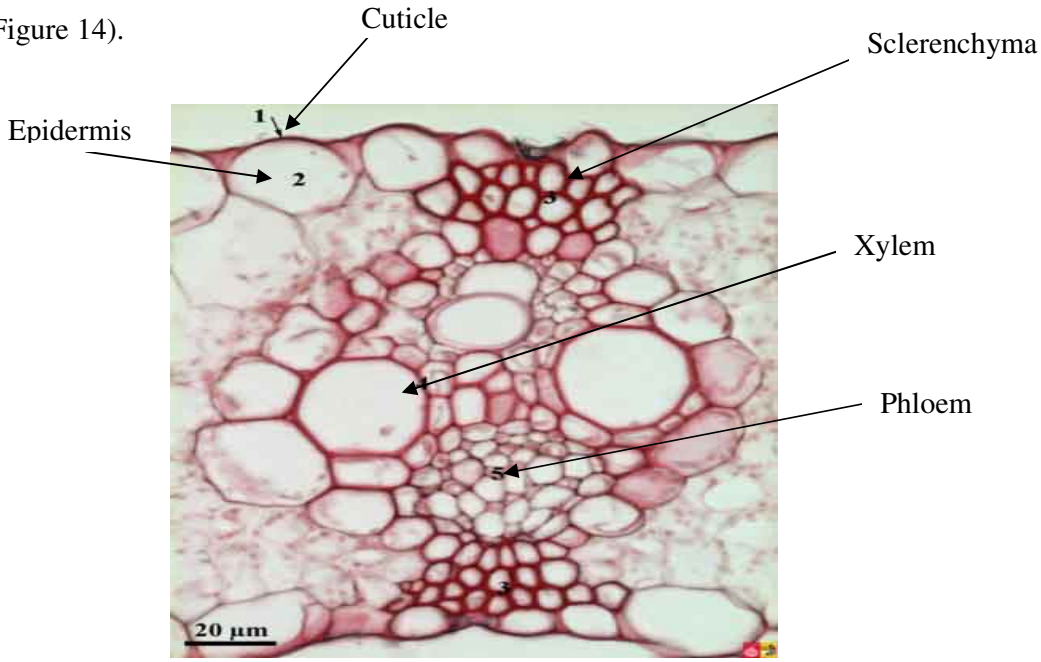


Figure 14: Generalised anatomical structure of the cross-section of a monocot leaf (Adapted from **http://www.vcbio.science.ru.nl/**)

3.0 CHAPTER THREE. MATERIALS AND METHODS

3.1 Collection and identification of plant material

Dierama cupuliflorum and *Gladiolus watsonoides* were collected in August 2011 from Aberdares National park, through Wandare route towards Satima. *Gladiolus newii* was collected from Mt. Elgon in September, 2011 while *Gladiolus goetzenii* and *Gladiolus ukambanensis* were collected from Kinamba in Laikipia and Konza along Nairobi-Mombasa highway respectively in May 2012. All the plants were collected between the years 2011 and 2012. Entire plants were collected and identification done using keys (Agnew 2013;Agnew & Agnew 1994 ; Beentje , 1994) and by comparing with authentic herbarium materials at the University of Nairobi Herbarium. Voucher specimens were deposited at the University of Nairobi herbarium with numbers as follows; *G. watsonoides* No. 901, *D. cupuliflorum* No. 913, *G. newii* No. 967, *G. ukambanensis* No. 968 and *G. goetzenii* No. 969.

The plant parts were separated into aerials and bulbs. The aerial parts were cut into pieces while the corms were blended using a blender to hasten drying and dried under room temperature. The dried plant materials were then ground into powder and kept in dry conditions awaiting extraction.

3.1.1 Preparation of crude aqueous and organic extracts

The air dried ground samples were divided in two portions and extracted with water and organic solvents ($CHCl_2$: MeoH in the ratio 1:1), using standard methods (Harbourne, 2002; Harbourne, 1998). A 30g portion of each powdered plant part was soaked with appropriate amounts of solvents left to stand for 24 hours and filtered. This process was repeated twice. The filtrates obtained from organic solvents extraction were concentrated/dried using a rotary evaporator, while those from water were freeze dried. The dried crude extracts were stored

in clearly labeled vials in refrigerator at 4ºC for antifungal tests, cytotoxic activity tests and phytochemical screening.

3.2 Determination of antifungal activity

Antifungal activity of the crude extracts and fractions was determined against pure cultures of the three most common opportunistic fungi; *Candida albicans* (ATCC MYA-2876/SC5314), *Aspergillus niger* (ATCC 16404) and *Cryptococcus neoformans* (ATCC 90113).

C. albicans was kindly donated by Ted White of Seattle Biomedical Research Institution, USA, *C. neoformans* was a gift from Prof. James Fraser of University of Queensland, Australia while *A. niger* was a culture collection of the Late Prof. George Siboe, School of Biological Sciences, Univesity of Nairobi.

All the fungi were first sub-cultured in Sabourads Dextrose Agar growth media (SDA). Broth suspensions of the organisms were prepared using distilled water and peptone media. These were kept ready awaiting the test with crude extracts. Stock solution of each sample was prepared by dissolving 0.5g of dried crude extract in 10ml of water (in the case of water extracts) or DMSO (in the case of the organic solvent extracts). This was at first serially diluted such that there were five different concentration levels in which the tests were done. These were 5mg/100µl, 2.5mg/100µl, 1.25mg/100µl, 0.625mg/100µl, 0.3125mg/100µl. Samples which showed activity with the lowest concentration set were further tested at much lower concentrations at 0.15625mg/100µl, 0.07813mg/100µl and 0.03902mg/100µl. The concentrations used in this study were arrived at after several optimizations/ range finding. The antifungal tests were done using disc diffusion method according to European Committee on Antimicrobial Succeptibility Testing guidelines (EUCAST, 2013) and Serrano *et al.,* (2004). Commercially prepared sterile discs (Hi media) impregnated with 100 µl of the rest extract solution of sample at each concentration, were aseptically transferred into petri

dishes with about 25ml Saborauds Dextrose Agar (SDA) growth media, freshly innocultaed with the test organism. DMSO and water which were used to re-dissolve the crude extracts, served as the negative controls while Ketoconazole, Amphotericin B and Nystatin served as the positive controls. The antifungal drugs were bought from counter and had expiry dates of 2017 at the time of usage, all were in tablet form. The plates were prepared in replicates, sealed with parafilm to avoid contamination and incubated at 37ºC for *Candida albicans* and *Cryptococcus neoformans* while those of *Aspergillus niger* were incubated at 25ºC. The antifungal activities were evaluated by measuring the diameter of the zones of inhibition using a transparent ruler after 24 hours, 48 hours and 72 hours (Kumari & Gupta, 2014;Dzomba & Muchanyereyi 2012;Ameh *et al.,* 2011;Okemo *et al.,* 2003). Fractions obtained from bulbs after fractionation were tested for antifungal activity using the same procedure.

3.2.1 Determination Minimum Inhibitory Concentration (MIC)

As explained in section 3.2, the sterile disc were impregnated with crude extracts at different concentrations of 5mg/100µl, 2.5mg/100µl, 1.25mg/100µl, 0.625mg/100µl, 0.3125mg/100µl, 0.15625mg/100µl, 0.07813mg/100µl and 0.03902mg/100µl. The lowest concentration at which the growth of the test organisms was Inhibited was considered to be the minimum inhibitory concentration (Palazzo *et al.,* 2007;Kariba 2000).

3.3 Brine shrimp cytotoxicity test

Cytotoxicity of the crude extracts was tested using freshly hatched brine shrimp larvae (nauplii). Artificial sea water was first prepared by dissolving 10g of the salt in 500ml of distilled water. This was added to the culture tank which had two partitions. One part was covered with dark polythene paper while the other was left exposed. The partition had holes through which the nauplii moved to the other exposed side. About 1g of brine shrimp eggs

was added to the darkened portion and exposed to a 60 watt lamp to provide warmth necessary for hatching to take place. The observation was made after 24 hours. Successful hatching was confirmed by the presence of visible several actively moving nauplii in the exposed side of the culture tank (Musila *et al.,* 2013;Ul-Haq *et al.,* 2012).

Stock solutions were prepared by dissolving 3mg of each extract in 3ml of DMSO in the case of organic extracts and water in the case of water extracts. The stock solution (1000ppm) was serially diluted to prepare a servier of concentrations as 500ppm, 100ppm, 10ppm and 5ppm. 10 live nauplii were transferred using a micro pipette into vials with these set concentrations. This experiment was set in triplicates (three vials per concentration per sample). Water and DMSO served as the negative controls while rotenone served as the positive control. The observations were done after 24 hours. Cytotoxic activity was determined by recording the number of dead nauplii (immobile) in each vial. This was then used to calculate the mortality mean and LD_{50} values. The active fractions were also tested fot cytotoxicity following the same procedure.

3.4 Thin Layer Chromatography analysis

In order to conduct fractionation using column chromatography, Thin Layer Chromatography (TLC) was first employed to determine the presence of compounds and to select the appropriate solvent system for use in column chromatography. Crude organic extracts from bulb species of *Gladiolus* and *Dierama* were re-dissolved in the extraction solvent. A minimum amount of the re-dissolved extracts were first spotted on TLC plate (6cm by 3cm) and developed (Harborne, 2002; Harborne, 1998). Each spotted TLC plate was slowly lowered in a TLC tank with appropriate solvent system (the mobile phase) and covered to ensure the solvents are not lost by evaporation. As the mobile phase slowly moved up the

plate, different components of the crude extracts travelled at different rates and the mixture separated into different compounds seen as spots.

The developed plate was removed from the tank, left to dry for 5 minutes and observed under UV light (254 and 366nm) and then exposed to iodine (Harborne, 2002)

3.5 Column chromatography for bioassay guided fractionation

After the TLC analyses, the plant samples (from five plants) were subjected to fractionation using column chromatography. The powdered bulbs, 1.627 kg of *D. cupuliflorum,* 423.5g of *G. watsonoides,* 1.325kg of *G. newii,* 470g of *G. ukambanensis* and 332g of *G. goetzenii* were first extracted using $CHCl_2$: MeoH in the ratio 1:1 and dried as previously described. The dry crude extracts were adsorbed in silica gel, air dried in a foil and packed in a column as follows:

A 1m long column was first washed with detergent and rinsed in water severally. The second rinsing was done using the organic solvents acetone and methanol to remove water. The clean column was then tightened on a stand. Cotton wool was stacked to the bottom end. Silica gel, 300g was made slurry using hexane and poured into the column using a funnel. This was left to settle for about 30 minutes and the adsorbed plant extract carefully loaded on the column just above the silica gel. Cotton wool was placed above the adsorbed sample to avoid disturbance during elution (Harborne, 2002).

The following eluents were used in the order of increasing polarity from 100% hexane, hexane & dichloromethane (ratio 1:1), 100% dichloromethane, dichloromethane & ethyl acetate (ratio 1:1), 100% ethyl acetate, ethyl acetate & methanol (ratio 1:1) and finally 100% methanol. This resulted into seven fractions for each plant sample. The fractions were concentrated to dryness by rotary evaporator and kept at 4ºC awaiting bioassay tests. The fractions obtained were subjected to bioassays and phytochemical analysis.

3.6 Qualitative phytochemical analyses

Chemical tests were conducted on organic extracts and powdered plant materials of both aerial and bulb parts according to Dabai *et al.,* 2013; Dushimemaria *et al.,* 2012; and Mushi *et al.,* 2012. Selected active fractions from column chromatography were also tested for the presence of alkaloids, flavanoids, saponins, tannins, glycosides, steroids, anthraquinones and terpenoids.

3.6.1 Screening procedure

3.6.1.1 Test for saponins

Distilled water (5ml) was added to the powdered plant material (0.5g) in a boiling tube. The mixture was shaken and heated in water bath for 2min. The presence of a stable froth indicated the presence of saponins (Dushimemaria *et al.,* 2012).

3.6.1.2 Test for steroids

Two methods were used to determine the presence of steroids in the plant extracts. I) The powdered plant material (0.5g) was mixed with acetic anhydride (2ml) in a boiling tube and then cooled in ice for five minutes , concentrated sulphuric acid (2ml) was added slowly along the wall of the test tube. Colour change from violet, to blue, to green was an indicative of the presence of steroids (Dushimemaria *et al.,* 2012). II) concentrated sulphuric acid was slowly added to 2g of plant extract . Effervescence followed by appearance of a clear reddish brown colour at the interface was an indication of a steroidal ring (Dabai *et al.,* 2013).

3.6.1.3 Test for tannins

Distilled water (10ml) was added to powdered plant material (0.5g) in a test tube. This was boiled for 3min and filtered using Whatman filter paper No. 1. Ferric chloride (0.2g) was

added and the mixture observed for dark or dirty green precipitate which indicated the presence of tannins (Dushimemaria *et al.,* 2012).

3.6.1.4 Test for terpenoids

A portion of powdered plant material (0.5g) was added in a boiling tube and chloroform (2ml) carefully added, concentrated sulphuric acid (3ml) was added drop wise. Presence of a reddish brown colouration at the interface showed positive presence of terpenoids (Mushi *et al.,* 2012;Dushimemaria *et al.,* 2012).

3.6.1.5 Test for glycosides

A portion of the ground plant material (0.5g) was added to a boiling tube. Distilled water (10ml) was added and stirred. This was filtered and a portion of the filtrate (2ml) was hydrolyzed with few drops of concentrated hydrochloric acid; a few drops of ammonia solution was then added to the mixture. Five drops of this solution was put aside in a separate test tube and then 2 ml of benedicts reagent added and boiled. Reddish to brown precipitate was an indicative of the presence of glycosides (Mushi *et al.,* 2012).

3.6.1.6 Test for flavonoids

A portion of the extract (0.5g) was heated with 10 ml ethyl acetate over a steam bath for 3 min, the mixture was filtered and 4 ml of the filtrate was shaken with 1ml dilute ammonia (50%) . A yellow colouration which indicated the presence of flavonoids (Mushi *et al.,* 2012).

3.6.1.7 Test for alkaloids

A portion of the powdered plants parts (0.5g) was added into a boiling tube . A 5ml portion of 2% sulphuric acid was added, mixed and filtered . Few drops of Drangedorff´s reagent was added to the filtrate. An orange red precipitate indicated the presence of alkaloids (Dushimemaria *et al.,* 2012).

3.6.1.8 Test for anthraquinones

A 1g portion of powdered plant was placed in a boiling tube and boiled with 2 ml portion of 10% hydrochloric acid for 5min. The mixture was filtered and the filtrate cooled.The filtrate was partitioned against equal volume of chloroform and the chloroform layer transferred into a clean test tube. Equal volume of 10% ammonia solution was added into the chloroform layer, shaken and allowed to separate. The separated aqueuos layer was observed for a delicate rose pink colour which showed the presence of anthraquinones (Dabai *et al.,* 2013).

3.7 Preparation of slides from leaves and bulbs plant parts for micro-anatomy analysis

Leaves and bulbs of each of the five plants freshly collected from the field were fixed by dipping in formalin acetic acid alcohol (FAA) to preserve cells and tissues in original form. These plant specimens were then dehydrated to remove water. This was done gradually and progressively in a series of alcohol and water solutions of increasing strength as follows; 70% ethanol (5min), 80% ethanol (5min), 100% ethanol (10min) (Rolls, 2011). The specimens were then dipped into a clearing agent xylene to make them more translucent. Each specimen was then embedded with paraffin wax to provide support during sectioning. During embedding, specimens were picked with forceps and transferred into a molten paraffin wax. The paraffin wax was given time to homogeneously solidify with each specimens within the wax (Rolls, 2011).The embedded specimens were sectioned into a series of thin slices by a cutting apparatus called a microtome. Carefully, the thin sections were transferred into surface of warm water (42°C) to enable them flatten. The sections were then picked from the water (mounted) using clean microscope slides and labelled. They were left overnight to dry (Rolls, 2011). They were then dipped xylene for five minutes and 100% ethanol to clear any traces of water and paraffin. The slides were put in Safranin stain bath for two hours, water was ran over the slides to remove excess stain and dipped again in 100% ethanol to remove water. Fast green stain was applied for 10 seconds , then slides dipped in xylene with drops of

100% ethanol for 10 seconds and then dipped in xylene again for 1 minute to make the cells transparent (Rolls, 2011). These prepared slides were then photographed using an Olympus microscope a waiting interpretation.

3.8 Data analysis

In order to analyze data on antifungal activity, Microsoft Excel 2007 was used to quantify, sort data, determine the mean inhibitions and draw bar graphs using the computed means. Using SPSS Version 16 (Statistical Package for the Social Sciences), a preliminary Analysis Of Variance (ANOVA) was done to determine whether there were significant differences in the activity among the various concentrations. Once significant differences were identified, further (Post hoc) ANOVA was carried out using Dunnett's test to compare each concentration with the positive controls to find out whether there were significant differences between each concentration and the positive control (Dunnett's test is a recommended post hoc ANOVA test when we have control groups in an experiment). In order to determine the LD_{50} values from the cytotoxicity tests, the raw toxicity data was analyzed using Finney's Probit analysis method with the help of Biostat 2009 statistical program.

4.0 CHAPTER FOUR. RESULTS

4.1 Antifungal activity of crude extracts

Crude extracts from the five plants showed antifungal activity at various concentrations at varied levels. Crude organic extracts showed more activity while crude aqueuos extracts showed little or no activity against the tested fungi. Similarly, crude organic extracts from bulbs were more active compared to the activity of the aerial parts. In general, bulbs of *D. cupuliflorum* and *G. watsonoides* were the most active followed by those of *G.goetzenii* and *G. newii* in that order. *G. ukambanensis* showed the least activity.

Amongst the three tested opportunistic fungi, *C.albicans* was the most susceptible followed by *A. niger* while *C. neoformans* was quite unsusceptible to the extracts.

Of the three antifungal drugs used as controls, Amphotericin B was the most active control drug against all the three fungi, its activity was however comparable or higher in some instances than those of the crude extracts. On the other hand, the activity of Nystatin was lower or not different from some crude extracts. Ketoconazole had no activity against *C. albicans* and *C. neoformans*.

4.1.1 Antifungal activity of aerial organic extracts on *C. albicans*

All extracts from aerial parts of *Gladiolus* and *Dierama* were active (Figure 15) against *C. albicans* at 5mg/100µl; the highest concentration used in this study. Within the first 24 hours, extracts from all plants were active with a mean inhibition zone above 8mm. This activity decreased to below 8mm in all plants´ extracts except *D. cupuliflorum*. Amphotericin B had the highest activity with inhibition zone of 20mm and above followed by nystatin (above 10mm). A two-way ANOVA for the five extracts and three controls revealed no significant difference between the activity of aerials of *D. cupuliflorum* and Amphotericin B ($P>0.05$) . The activity of aerial parts *D. cupuliflorum* was significantly higher than that of Nystatin

($F_{,8,72}$ =809.003, P<0.05). All the other plants had lesser activities than both Nystatin and Amphotericin B.

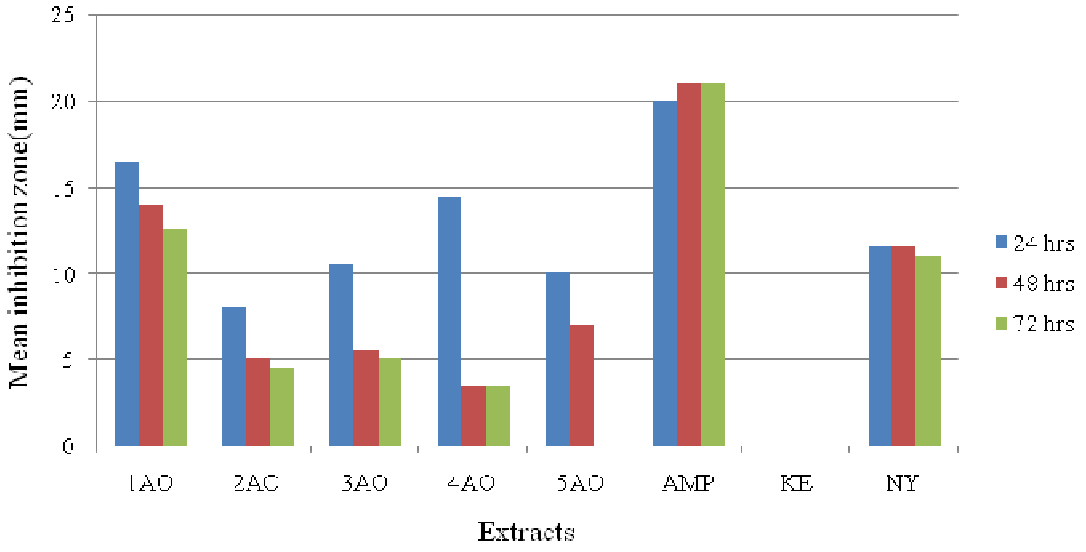


Figure 15: Activity of crude aerial organic extracts on *C.albicans* at 5mg/100µl. AMP; amphotericin B, KE: Ketoconazole Ny: Nystatin. *1:D.cupuliflorum 2:G. watsonoides 3:G. newii 4:G. ukambanensis 5:G. goetzenii,* AO: Aerial organic extract

A similar pattern was observed over same period at a lower concentration of 2.5mg/100µl (Figure 16). However, the activities of all plant extracts were significantly lower than that of amphotericin B. Aerial crude extracts from *D. cupuliflorum* and *G. watsonoides* had activity not significantly different to that of nystatin ($F_{,8,72}$ =113.04, P>0.05); while the other plants had activities much lower than that of nystatin and amphotericin B.

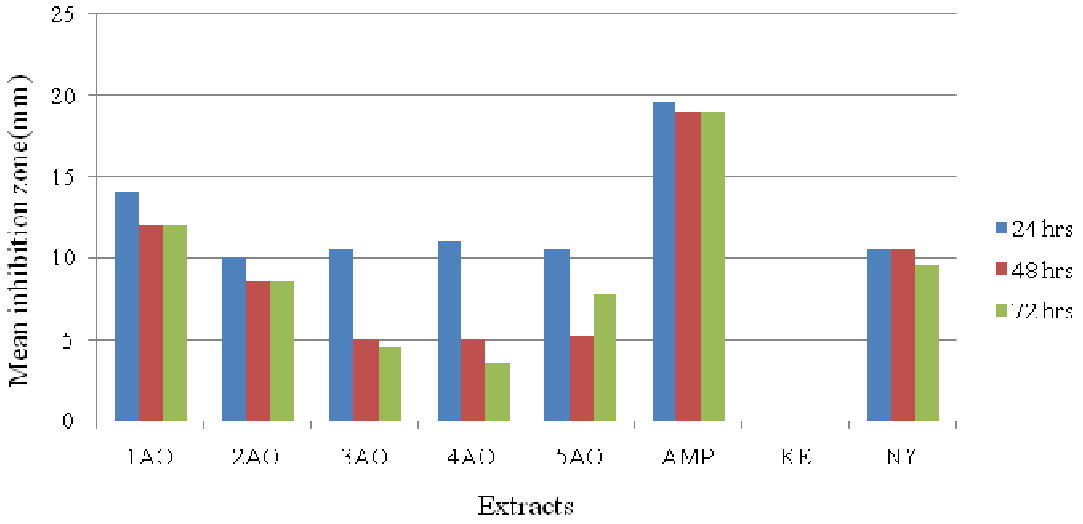


Figure 16: Activity of crude aerial organic extracts on *C.albicans* at 2.5mg/100µl. AMP; amphotericin B , KE: Ketoconazole Ny: Nystatin. 1:*D. cupuliflorum* 2:*G. watsonoides* 3:*G. newii* 4:*Gl. ukamabanensis* 5: *G. goetzenii*, AO: Aerial organic extract.

There was a decrease in activity as the concentrations decreased as observed at a much lower concentration of 0.625mg/100µl (Figure 17). Most extracts had mean inhibition zones slightly higher than 8mm, and were not different from that of nystatin ($F_{,8,72}$ =36.33, P>0.05). The activity of amphotericin B remained significantly different from the other extracts and drugs.

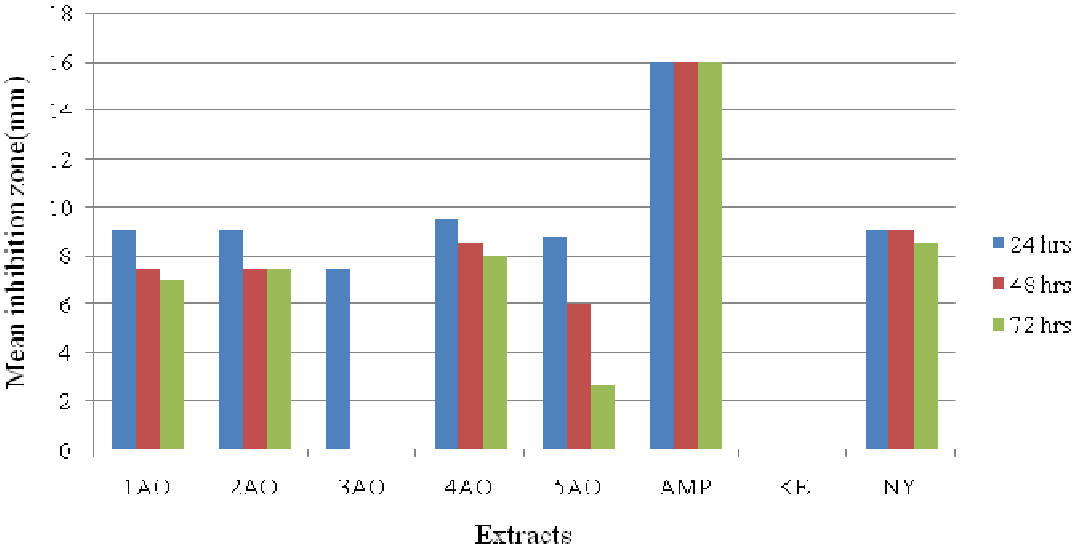


Figure 17: Activity of crude aerial organic extracts on *C.albicans* at 0.625mg/100µl. AMP; amphotericin B , KE: Ketoconazole Ny: Nystatin. *1:D. cupuliflorum 2:G. watsonoides 3:G. newii 4:G. ukamabanensis* 5: *G. goetzenii,* AO: Aerial organic extract

At a much lower concentration (0.15625mg/100µl), only *D. cupuliflorum* and *G. watsonoides* were weakly active against *C. albicans* as illustrated in Figure 18 below. For each extract, this decline in activity with concentration was found to be significantly different at $F_{,8,72}$ = 36.33,P<0.05.

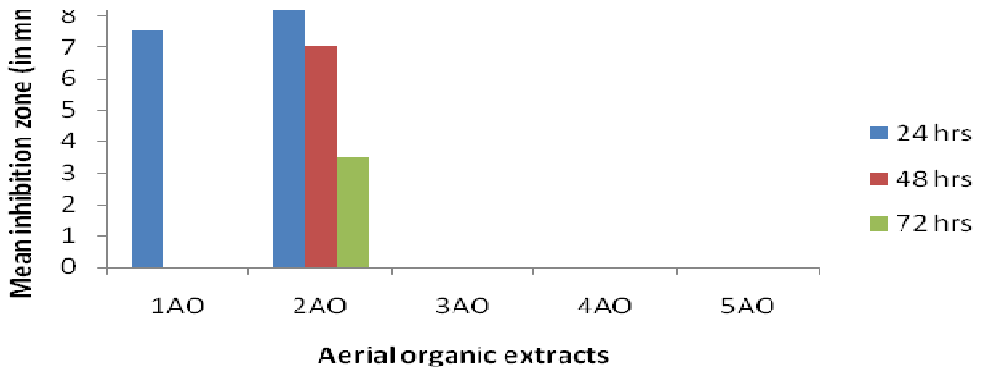


Figure 18 : Activity of crude aerial organic extracts on *C. albicans* at 0.15625mg/100µl. 1:*D. cupuliflorum* 2:*G. watsonoides* 3:*G. newii* 4:*G. ukambanensis* 5: *G. goetzenii*, AO: Aerial organic extract.

4.1.1.1 Minimum Inhibitory Concentration of the crude organic aerial extracts on *C. albicans*

Extracts from different plants lost activity at different concentrations. The minimum inhibitory concentration for each plant is presented in Table 2. Extracts from *G. watsonoides* had the lowest MIC followed by that of *D. cupuliflorum* while extracts from *G.newii* had the highest MIC value.

Table 2: Minimum Inhibitory Concentration values of Aerial organic extracts on *C. albicans*

Crude aerial organic extracts	MIC (mg/ml)
D.cupuliflorum	1.5625
G.watsonoides	0.7813
G. newii	6.25
G.ukambanensis	3.125
G.goetzenii	3. 125

This implies that the organic extracts from aerials of *G. watsonoides* were the most active against *C. albicans* while those of *G. newii* were the least active.

4.1.2 Antifungal activity of bulb organic extracts on *C. albicans*

Organic extracts from bulbs were also active against *C. albicans*. At 5mg/100µl, all the bulb organic extracts had activity with inhibition zones measuring at least 20mm, except *G. ukambanensis* which had a lower activity with inhibition zone of 10 mm (Figure 19).

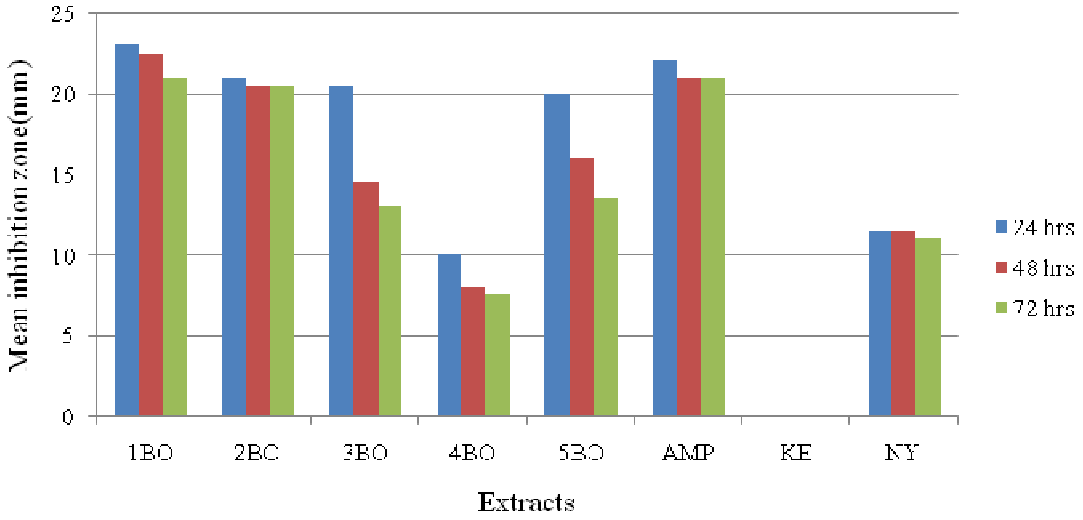


Figure 19: Activity of crude bulb organic extracts on *C.albicans* at 5mg/100µl AMP; amphotericin B, KE: Ketoconazole Ny: Nystatin. 1*:D. cupuliflorum* 2*:G. watsonoides* 3*:G. newii* 4*:G. ukamabanensis* 5*: G. goetzenii,* BO: Bulb organic extracts

At concentrations of 5mg/100µl, 1.25mg/100µl and 2.5mg/100µl, Two-way ANOVA revealed that bulb extracts from *D. cupuliflorum, G. watsonoides*, *G. newii*, and *G. goetzenii* had activities which were not significantly different as that of amphotericin B ($P>0.05$). The activities were however significantly higher than that of nystatin (($F_{,8,72=}234.42$, $P<0.05$). Extract from *G. ukambanensis* had activity lower than amphotericin B but its activity was not different from that of nystatin (Figure 20).

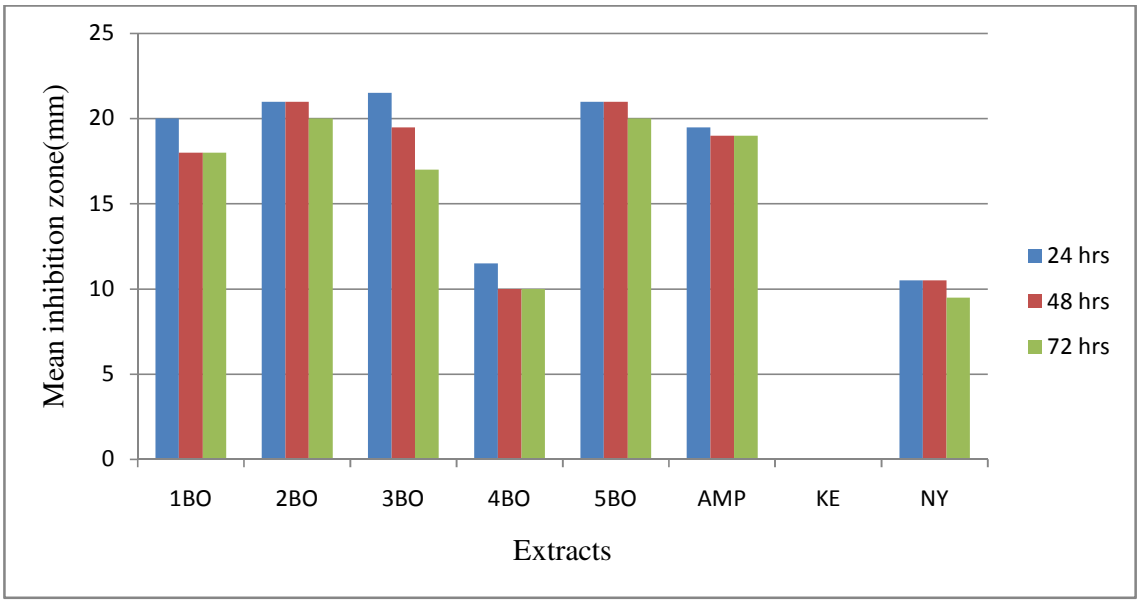


Figure 20: Activity of crude bulb organic extracts on *C. albicans* at 2.5mg/100µl AMP; amphotericin B , KE: Ketoconazole Ny:Nystatin. 1*:D. cupuliflorum* 2*:G. watsonoides* 3*:G. newii* 4*:G. ukamabanensis 5:G. goetzenii,* BO: Bulb organic extracts

At 0.625mg/100µl, the activity of all the five plant extracts reduced significantly $F_{,8,72=}15.64$, $P<0.05$) relative to that of amphotericin B. At this concentration, however, the activity of extracts from *D.cupuliflorum, G.watsonoides , G.newii, G.goetzenii* was still significantly higher than that of nystatin ($F_{,8,72}=15.64$, $P<0.05$). The activity of *G.ukambanensis* was still not different from that of nystatin (Figure 21).

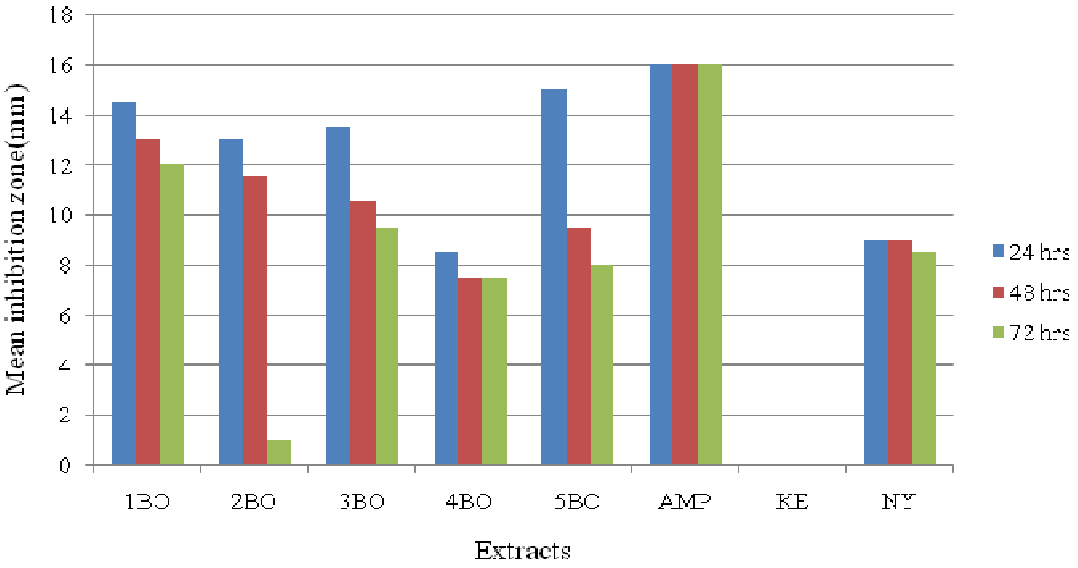


Figure 21: Activity of crude bulb organic extracts against *C.albicans* at 0.625mg/100µl. AMP;amphotericin B, KE:Ketoconazole, Ny:Nystatin. 1:*D. cupuliflorum* 2:*G.watsonoides* 3:*G. newii* 4:*G. ukamabanensis* 5:*G. goetzenii,* BO: Bulb organic extracts

At a much lower concentration (0.3125mg/100µl), Two-ANOVA revealed no significant differences in activities between plant extracts and nystatin ($P>0.05$) except for the activity of *G. goetzenii* after day one which was higher than that of nystatin. The activity of amphotericin B remained siginificantly higher than those of plant extracts ($F_{8,72}= 145.34$, $P<0.05$) (Figure 22).

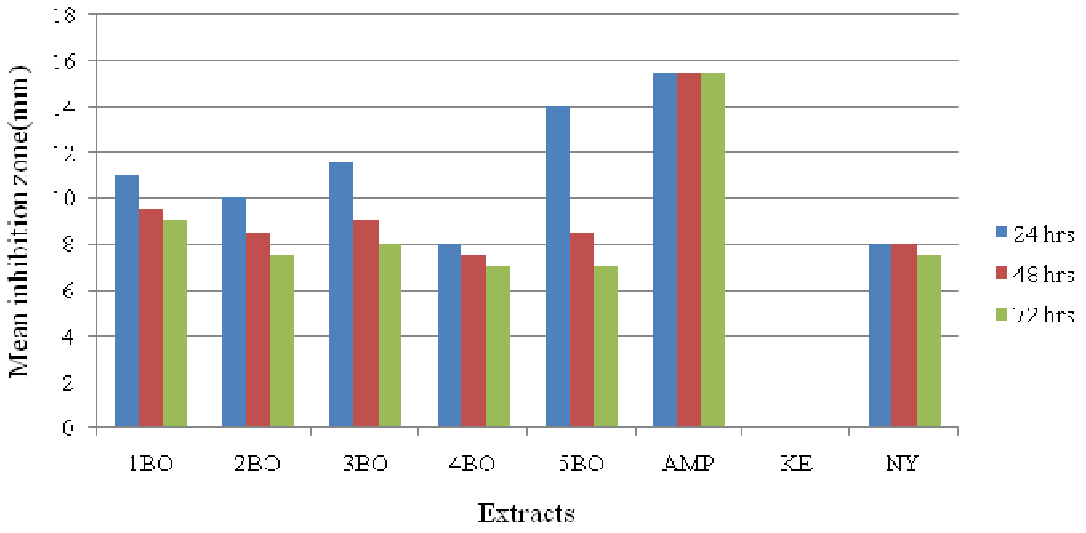


Figure 22: Activity of crude bulb organic extracts on *C.albicans* at 0.3125mg/100µl; AMP; amphotericin B ,KE:Ketoconazole, Ny:Nystatin. *1:D. cupuliflorum 2:G. watsonoides 3:G. newii 4:G. ukamabanensis 5: G. goetzenii,* BO: Bulb organic extracts

4.1.2.1 Minimum Inhibitory Concentration of crude organic bulb extracts on *C. albicans*

Based on the above outlined data, the MIC values for each of these extracts was determined and is presented in Table 3. *D. cupuliflorum, G. watsonoides* and *G. goetzenii* had the least MIC values followed by *G. ukambanensis* whereas *G.newii* had the highest value when tested against *C. albicans*. This implied that bulbs of *D. cupuliflorum, G. watsonoides* and *G. goetzenii* were the most active while *G.newii* bulbs had the least activity.

Table 3: The Minimum Inhibitory Concentration values of Bulb organic extracts against *C. albicans*

Crude extracts	MIC (in mg/ml)
D. cupuliflorum	0.3907
G. watsonoides	0.3907
G. newii	1.5625
G.ukambanensis	0.7813
G. goetzenii	0.3907

Worth noting here is that MIC values of bulbs extracts are much lower than that of aerial extracts, an indication that extracts from bulbs were more active than those of the aerials as shown in Figure 23.

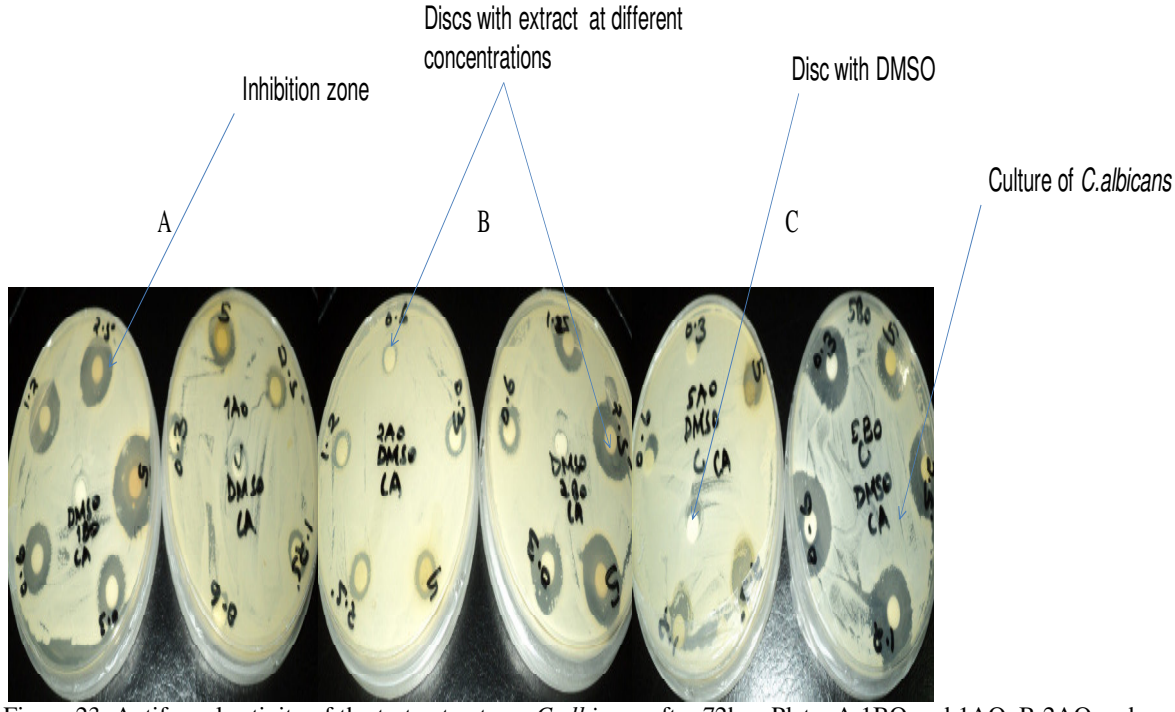


Figure 23: Antifungal activity of the test extracts on *C.albicans* after 72hrs. Plate A:1BO and 1AO, B:2AO and 2BO and C: 5AO and 5BO. 1:*Dierama cupuliflorum* 2: *Gladiolus watsonoides* 5: *G.goetzenii,* AO: Aerial Organic extracts, BO: Bulb Organic extracts, CA: *C.albicans*, DMSO: Dimethyl suphoxide

4.1.3 Antifungal activity of aerial organic extracts on *A. niger*

This was only observed at 24. The test drugs, including ketoconazole were active after 48 hours. At 5mg/100µl, the highest concentrations tested, only *G. newii* showed some ctivity which was far below 8mm . The extracts from the aerial parts of all the plants were generally less active against *A. niger* than was the case with *C. albicans.* Therefore, the MIC values for the extracts were not determined against *A. niger.*

4.1.4 Antifungal activity of Bulb organic extracts on *A. niger*

Bulb extracts showed more activity tested on *A. niger* than aerial parts. Extracts from all plants, except *G. ukambanensis* were active (at 5mg/100µl) against *A. niger* with zones above 8mm. This activity was however lost after 24 hours and also the activity decreased with the

decreasing concentration. Only extracts from *D. cupuliflorum* and *G. newii* had activity with mean inhibition zones above 8mm at 2.5mg/100µl. The activity of each plant extract was significantly different (($F_{,8,72}$=235.34,P<0.05)) from each other and signifcantly lower than that of control drugs, which were consistently active up to 48 hours (Figure 24).

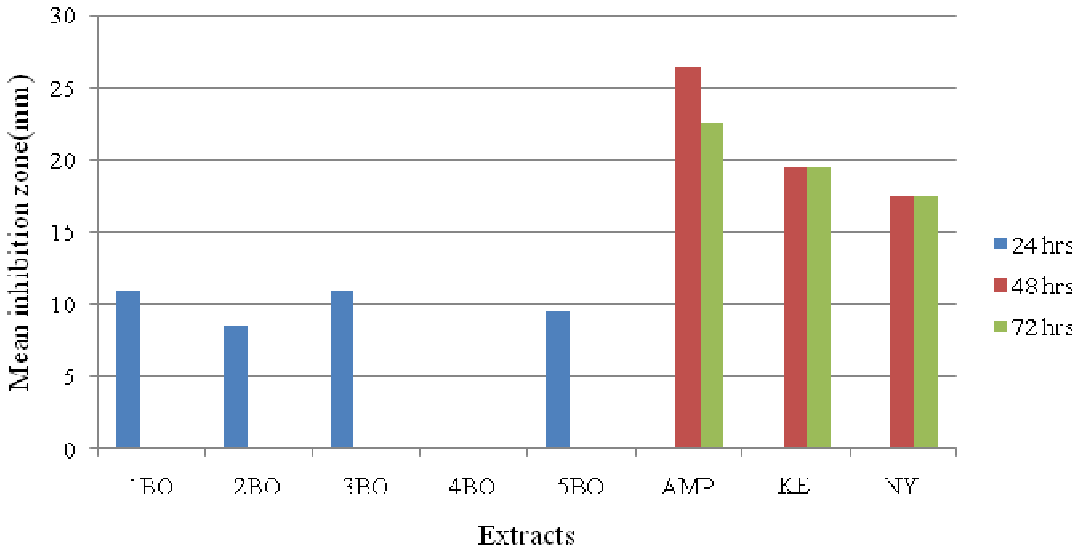


Figure 24: Antifungal activity of crude bulb organic extracts against *A.niger* at 5mg/100µl. AMP: Amphotericin B, KE:Ketoconazole Ny:Nystatin. *1:D. cupuliflorum 2:G. watsonoides 3:G. newii 4:G. ukamabanensis 5: G. goetzenii*, BO:Bulb organic extracts

4.1.4.1 Minimum Inhibitory Concentrations of crude bulb organic extracts on *A. niger*

Determination of MIC for the active plants gave a value of 6.25 mg/ml, (Table 4).

Table 4: Minimum Inhibitory Concentration values of bulb organic extracts on *A.niger*

Crude extract	MIC (mg/ml)
D. cupuliflorum	6.25
G. watsonoides	6.25
G. newii	6.25
G. ukambanensis	NA
G. goetzenii	6.25

4.1.5 Antifungal activity of crude aerial extracts on *C. neoformans*

A test on *C. neoformans* revealed that amphotericin B and nystatin inhibited growth throughout the study period (Figure 25). Ketoconazole (not shown) was not active against this fungi. Other than *G. ukambanensis,* which had weak activity at (5mg/100µl) at 24 hour, all the other extracts displayed no activity against this fungus. It was therefore not possible to determine MIC values.

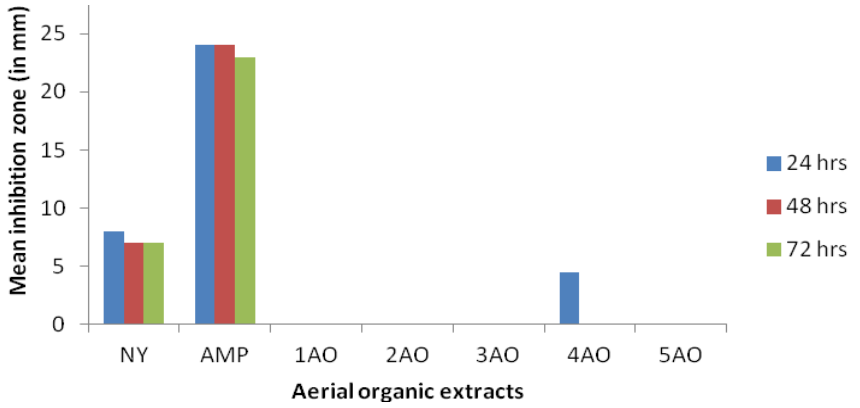


Figure 25: Activity of crude aerial organic extracts against *C.neoformans* at 5mg/100µl., AMP:Amphotericin B,Ny:Nystatin. *1:D. cupuliflorum 2:G. watsonoides 3:G. newii 4:G.ukamabanensis 5: G. goetzenii*, AO: Aerial organic extracts

4.1.6 Antifungal activity of bulb organic extracts on *C. neoformans*

Similar antifungal tests on *C. neoformans* revealed that amphotericin B and nystatin inhibited growth throughout the study period (Figure 26). Nystatin had low activity while ketoconazole was not active at all against this fungus. Only the bulb extracts of *G. watsonoides* and *G. goetzenii* were active against *C. neoformans* at a concentration of 5mg/100µl. The activities of extracts from *G.watsonoides* and *G.goetzenii* were significantly higher than that of nystatin ($F_{,8,72}$=4.56, P<0.05) The activity of *G.watsonoides* extracts was lost after 48hours

of study. Amphotericin B was significantly higher ($P<0.05$) than those of the two active plants.

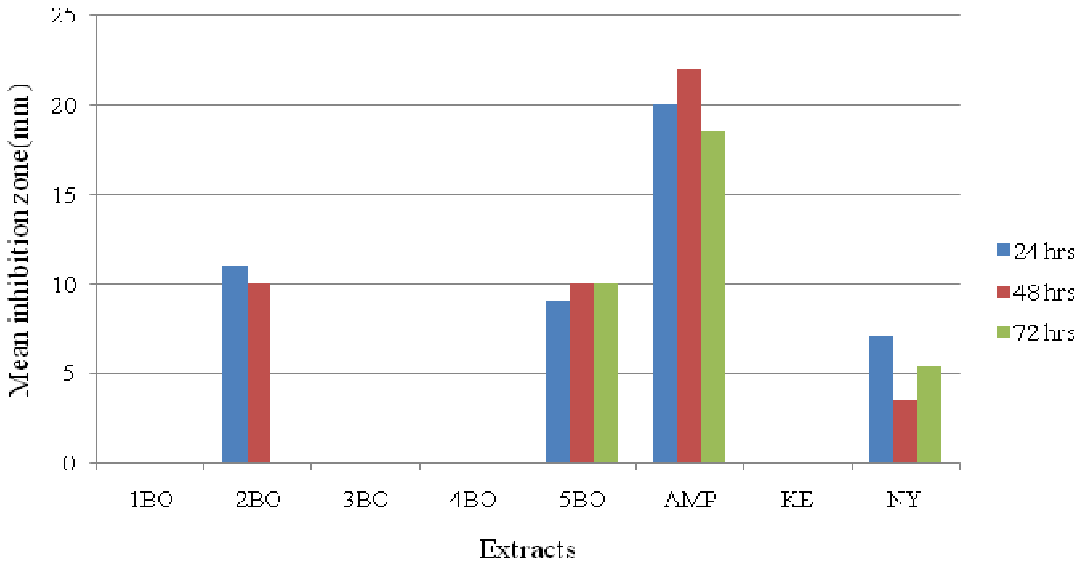


Figure 26: Antifungal activity of crude bulb organic extracts against *C.neoformans* at 5mg/100µl. AMP:Amphotericin B,KE:Ketoconazole Ny:Nystatin. 1:*D. cupuliflorum* 2:*G. watsonoides* 3:*G. newii* 4:*G. ukambanensis* 5:*G. goetzenii*, BO: Bulb organic extracts

In general, crude organic extracts were more active against *C.albicans* than *A.niger* and *C. neoformans* (Figure 27). The varied sensitivity may be as a result of the cell wall composition and different active secondary metabolites.

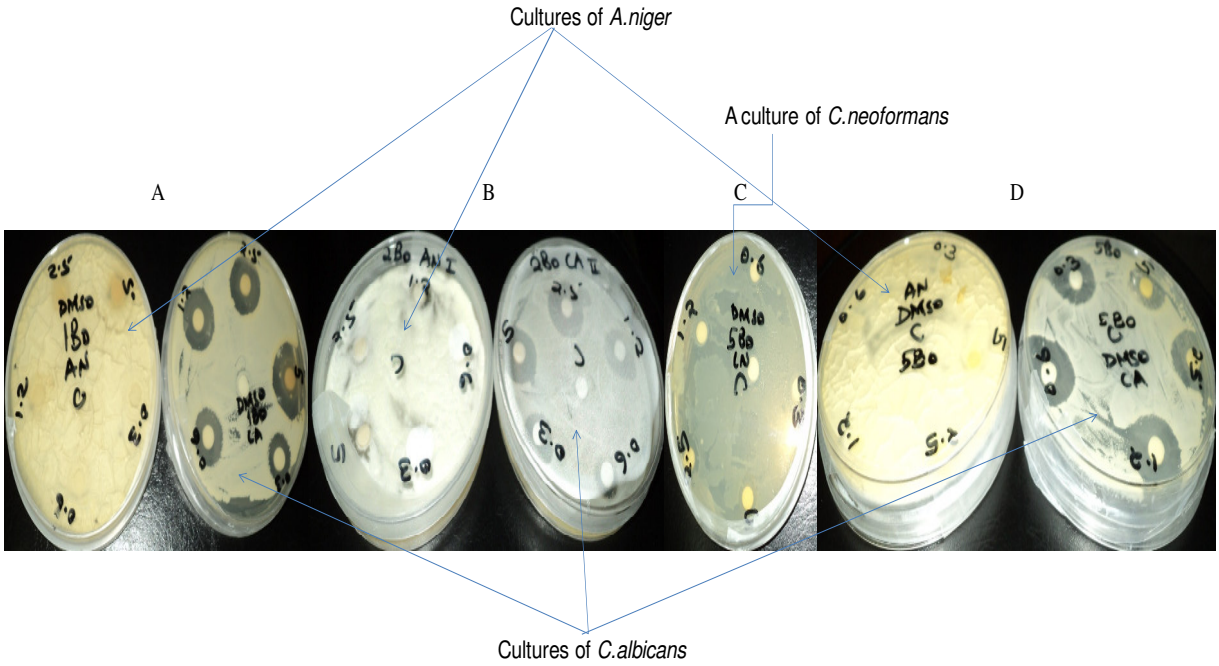


Figure 27: Plates of different fungal cultures showing differences in sensitivities to different extracts . A: B: D:Plates of *A. niger* and *C. albicans* in presence of 1BO; 2BO;5BO respectively, C:A plate of *C. neoformans* in presence of 5BO. 1:*D. cupuliflorum* 2: *G. watsonoides* 5: *G. goetzenii*; AN: *A. niger*, CA: *C. albicans*, CN: *C. neoformans*

4.2 Toxicity of the crude extracts

To determine the safety of crude extracts, cytotoxicity tests were conducted using brine shrimps (*Artemia salina*) nauplii . Both water and organic solvent extracts were subjected to this test at concentrations of 500, 100, 10 and 5 ppm. LD_{50} values were calculated for each plant extract and are presented in subsequent subsections.

4.2.1 Toxicity of Aerial Extracts

The only plant whose aerial water extracts were not toxic to the shrimps was *G.ukambanensis* with LD_{50} value above1000µg/ml. Those from *D. cupuliflorum, G. newii* and *G. goetzenii* had LD_{50} values between 100-500µg/ml implying that they were moderately toxic. *G. watsonoides* had LD_{50} values less than 100µg/ml, an indication that aerials of this plant were strongly toxic to the shrimps (Table 5).

Table 5: Toxicity of aerial water and organic extracts on Brine shrimp larvae

Plant name	LD_{50} (µg/ml)	
	Aq	Org
D. cupulliflorum	391.4	27.5
G. watsonoides	57.5	2.7
G. newii	126.1	20.4
G. ukambanensis	1205.9	7.1
G. goetzenii	209.0	23.7

Key: Aq:Aqueous extracts, Org:Organic extracts

Similar toxicity tests using aerial organic extracts using the same concentrations gave LD_{50} values less than 100µg/ml for all the plants, meaning that all organic extracts had strong toxic activity to the brine shrimp larvae (Figure 28).

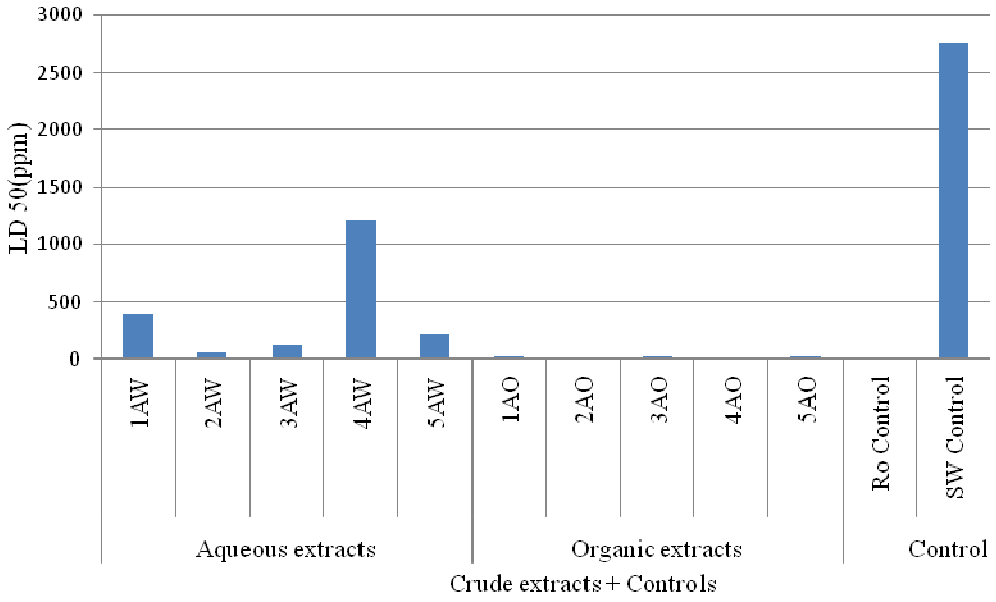


Figure 28 : Toxicity of crude aerial extracts from water and organic solvents against nauplii: R_O: Rotenone,SW:Sea water *1:D. cupuliflorum 2:G. watsonoides 3:G. newii 4:G. ukambanensis 5: G. goetzenii*, AO: Aerial organic extracts BO: Bulb organic extracts

4.2.2 Toxicity of bulb extracts

Toxicity tests conducted on the bulb extracts revealed that they had strong cytotoxic activity to the freshly hatched shrimps as shown by the LD_{50} values (Table 6). Other than water extracts of *G. ukambanensis* which had an LD_{50} value more than 500µg/ml (weakly toxic), all the other extracts had LD_{50} values of less than 100µg/ml.

Table 6: Toxicity of bulb water and organic extracts on Brine shrimp larvae

Plant name	LD_{50} (µg/ml)	
	Aq	Org
D. cupulliflorum	31.8	0.8
G. watsonoides	27.5	6.5
G. newii	9.3	3.1
G. ukambanensis	697.7	9.0
G. goetzenii	54.2	7.5

Key: Aq:water extracts, Org:Organic extracts

Again, the LD_{50} values of all organic solvent extracts were less than that of water extracts. It is therefore clear from this data that organic solvent extracts had much higher cytotoxic activity compared to the water extracts (Figure 29).

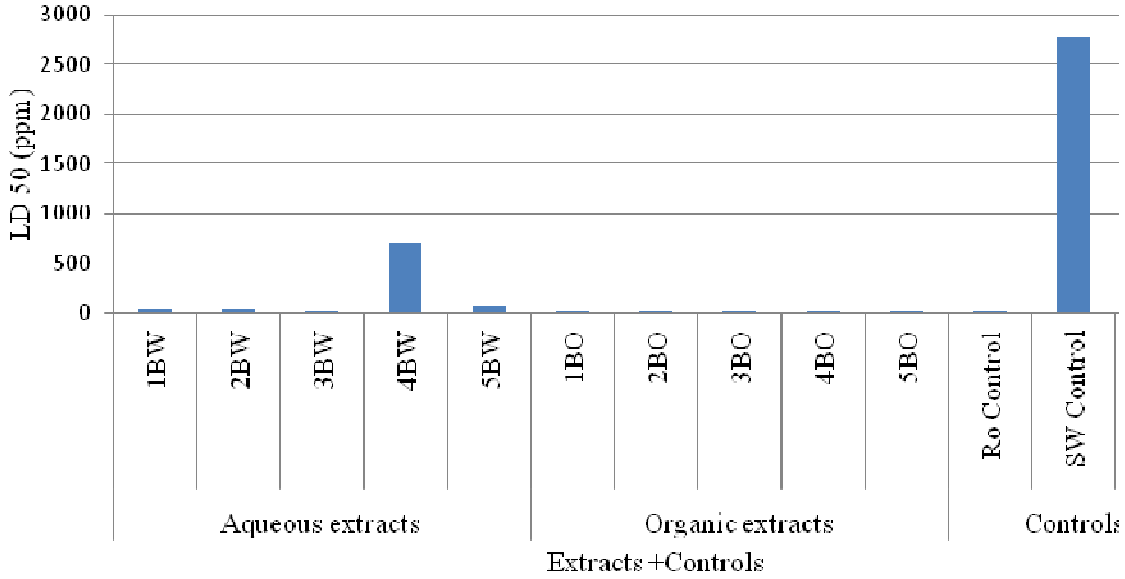


Figure 29: Toxicity of bulb extracts from water and organic solvents against brine shrimp larvae: R_O: Rotenone:SW: Sea water *1:D. cupuliflorum 2:G.watsonoides 3:G. newii 4:G. ukambanensis 5: G. goetzenii*, AO: Aerial organic extracts BO: Bulb organic extracts

4.3 Antifungal activity of fractions from bioassay guided fractionation

Based on the data in the preceding sections, bulb organic extracts of all the five plants were subjected to fractionation using column chromatography to determine if the inhibitory active component (s) could be isolated. Seven fractions were collected from each of the bulb organic extract and antifungal activity carried out at 1mg/100µl over a period of 72 hours.

4.3.1 Activity of fractions against *Candida albicans*

Dierama cupuliflorum ethylacetate:methanol fraction was the most active with inhibition zone measuring >15mm followed by the methanol fraction, dichloromethane: hexane fraction, ethyl acetate fraction, hexane fraction and dichloromethane: ethylacetate fraction in that order (Figure 30). The dichloromethane fraction did not show any activity. These activities were significantly ($F_{,8,72}$=143.49, P<0.05) lower than that of the crude extract.

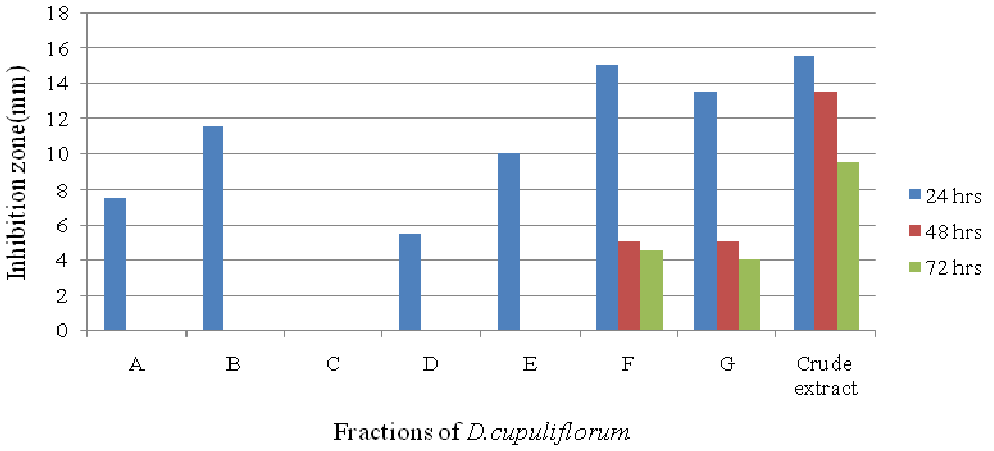


Figure 30: Activity of semi-purified bulb *D.cupuliflorum* fractions from organic solvents against *C.albicans*, A-100% Hexane; B-DCM:Hexane (ratio 1:1); C-100% DCM; D-DCM:EtOAc (1:1); E-100% EtOAc; F-EtOAc:MeOH (ratio 1:1) ;G-100% MeOH.

All except DCM and Hexane:DCM fractions were active against *C. albicans* (Figure 31). Fractions collected using ethyl acetate, ethyl acetate : methanol and methanol had high activity with inhibition zones measuring >15mm while those obtained using dichloromethane and dichloromethane : ethylacetate had low activity wth inhibition zones measuring about 11mm. The activities of the fractions was however significantly lower than that of the crude extract ($F_{,8,72}$=143.49, P<0.05) . Fractions obtained using hexane and dichloromethane: hexane were inactive.

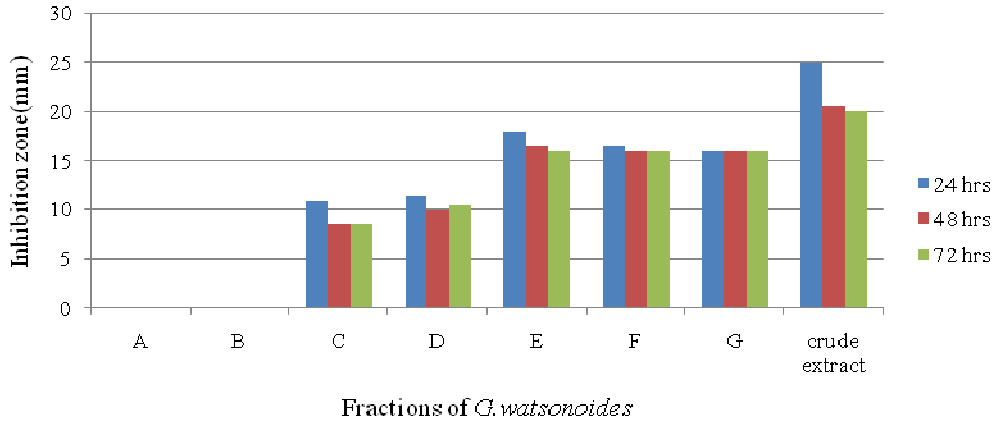


Figure 31: Activity of semi-purified bulb *G.watsonoides* fractions from organic solvents against *C.albicans,* A-100% Hexane; B-DCM:Hexane (ratio 1:1); C-100% DCM; D-DCM:EtOAc (1:1) ; E-100% EtOAc ;F- EtoAc :MeOH (ratio 1:1) ;G-100% MeOH.

All fractions from *G. newii* were active against *C. albicans*. Ethyl acetate and methanol fractions showed the highest activity with inhibition zones measuring > 13mm. For this plant, two-way ANOVA revealed that the methanol fraction had activity which was not significantly different from that of the crude extract (P >0.05). The other active fractions had activities significantly lower than that of the crude extract ($F_{,8,72}$=28.91,P <0.05) (Figure 32).

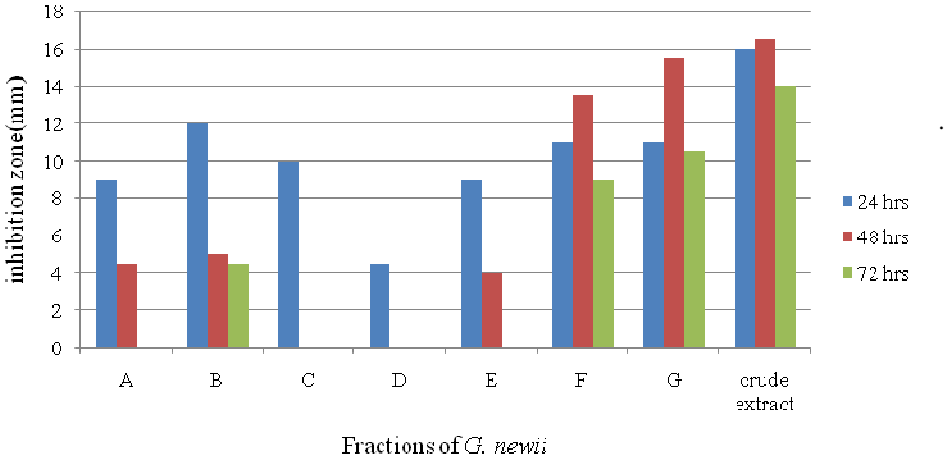


Figure 32: Activity of semi-purified bulbs of *G.newii* fractions from organic solvents against *C.albicans*, A-100% Hexane; B-DCM : Hexane (ratio 1:1); C-100% DCM; D-DCM : EtOAc (1:1) ; E-100% EtOAc ; F-EtOAc : MeOH (ratio 1:1) ; G-100% MeOH

For *G. ukambanensis,* two fractions extracted by: hexane and dichloromethane : hexane were not active while the rest showed a much lower activity with inhibition zones of 5-10mm. However the activity of the active fractions (dichloromethane, dichloromethane : ethylacetate, ethylacetate, ethylacetate : methanol and methanol) was significantly lower than that of the control ($F_{,8,72}$=43.56, P<0.05 (Figure 33).

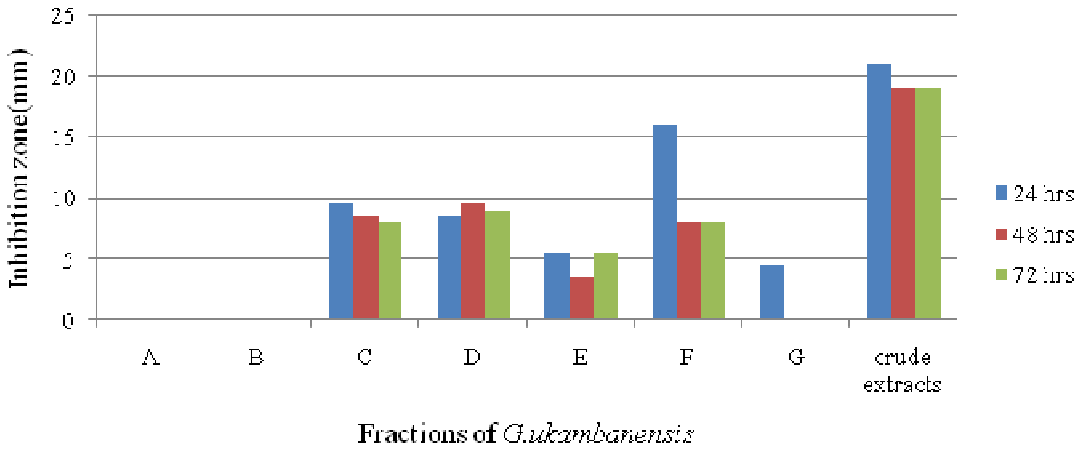


Figure 33 : Activity of semi-purified bulbs of *G.ukambanensis* fractions from organic solvents against *C.albicans*, A-100% Hexane; B-DCM: Hexane (ratio 1:1);C-100% DCM;D-DCM:EtOAc (1:1) ;E-100% EtOAc ; F- EtOAc :MeOH (ratio 1:1) ;G-100% MeOH.

The *G. goetzenii* ethyl acetate fraction was the most ctive (inhibition zone of 17mm) followed by the dichloromethane : ethylacetate fraction (Figure 34). Analysis by two-way ANOVA showed the activity of the ethy lacetate fraction to have no significant difference from that of the crude extract (P >0.05). Fractions extracted using dichloromethane : Hexane, methanol and ethyl acetate had moderate activity. The dichloromethane fraction had lower activity (inhibition zone of 8mm) while the hexane fraction was not active at all. .

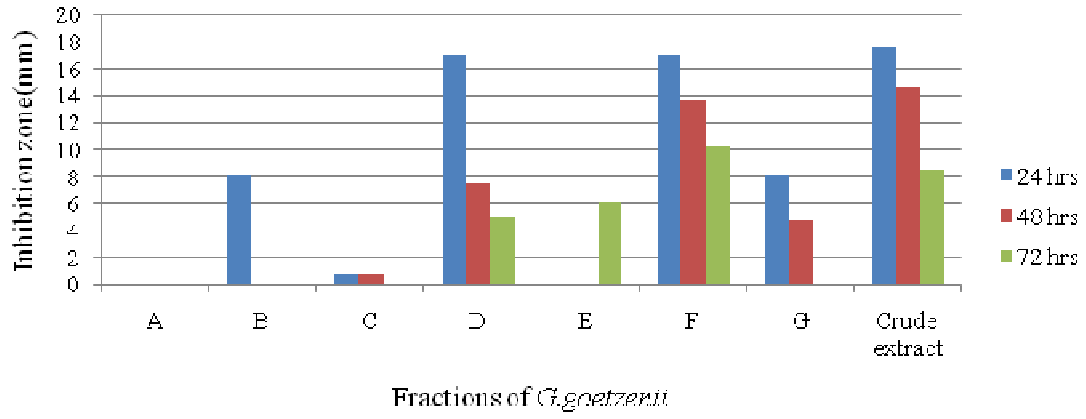


Figure 34: Activity of semi- purified bulbs of *G. goetzenii* fractions from organic solvents against *C.albicans*, A-100% Hexane;B-DCM:Hexane (ratio 1:1);C-100% DCM; D-DCM:EtOAc (1:1) ;E-100% EtOAc ;F- EtOAc : MeOH (ratio 1:1);G-100% MeOH.

4.3.2 Activity of fractions against *A. niger*

Weak activity (with inhibition zone less than 7mm) was noted in the crude and fractions of *D.cupuliflorum* when tested against *A.niger* (Figure 35).

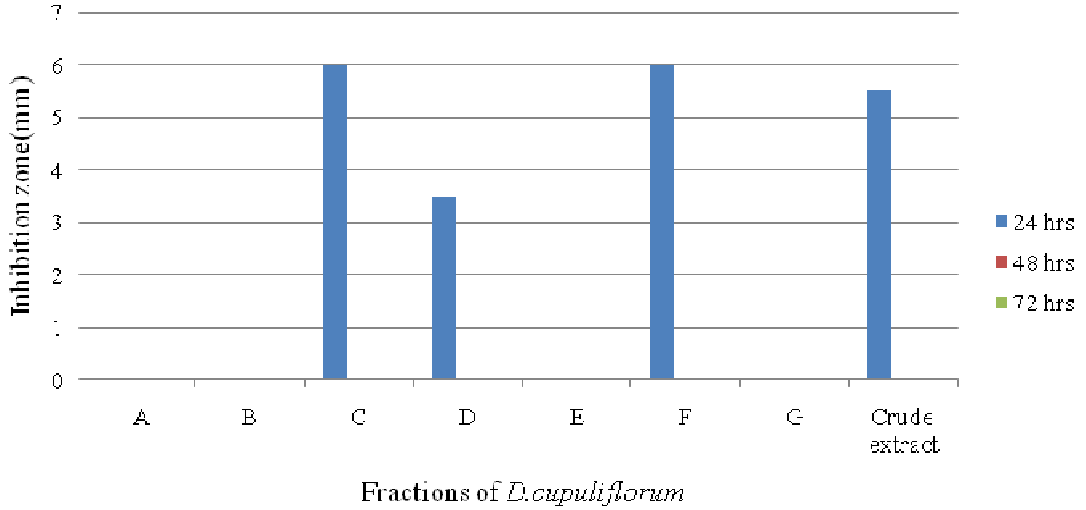


Figure 35: Activity of semi-purified bulbs of *D.cupuliflorum* fractions from organic solvents against *C.albicans*, A-100% Hexane;B-DCM:Hexane (ratio 1:1);C-100% DCM;D-DCM:EtOAc (1:1);E-100% EtoAc ;F- EtOAc : MeOH (ratio 1:1);G-100% MeOH.

Only the ethyl acetate and methanol fractions of *G. watsonoides* showed activity (with inhibition zones 9mm and 8.5mm respectively) against *A. niger* (Figure 36). The activity of these fractions were however significantly lower than that of the crude extracts ($F_{,8,72}$=87.12, P< 0.05).

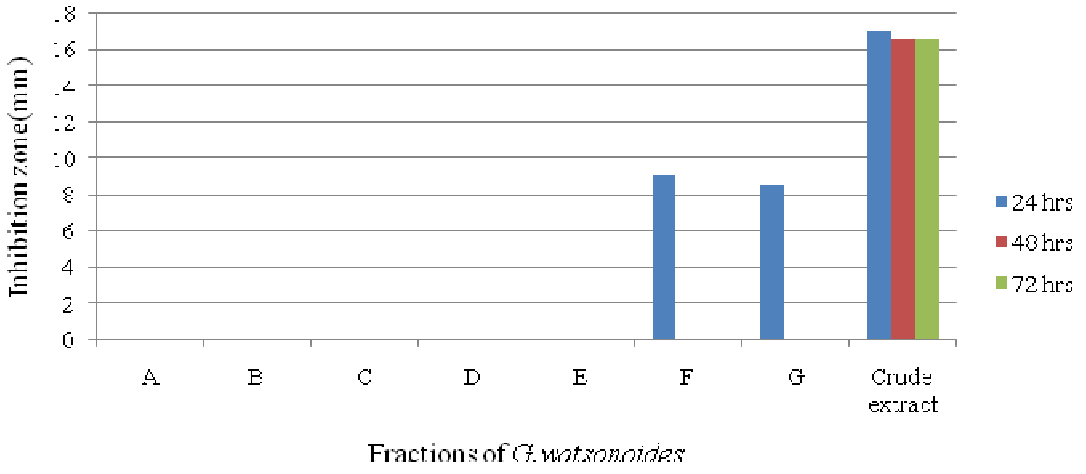


Figure 36: Activity of semi-purified bulbs of *G.watsonoides* fractions from organic solvents against *A.niger*, A-100% Hexane;B-DCM:Hexane (ratio 1:1);C-100% DCM;D-DCM: EtOAc (1:1) ;E-100% EtOAc ;F- EtOAc : MeoH (ratio 1:1) ;G-100% MeOH.

The dichloromethane, dichloromethane : ethyl acetate, ethy lacetate, ethyl acetate : methanol, and methanol fractions from *G. newii*, showed activity (with inhibition zones ranging between 9mm and 13mm) against *A. niger* (Figure 37). Again the ethyl acetate : methanol and methanol fractions had the highest activity with an inhibition zone of 13mm. The activity of the crude extract was however lower than that of the methanol fraction.

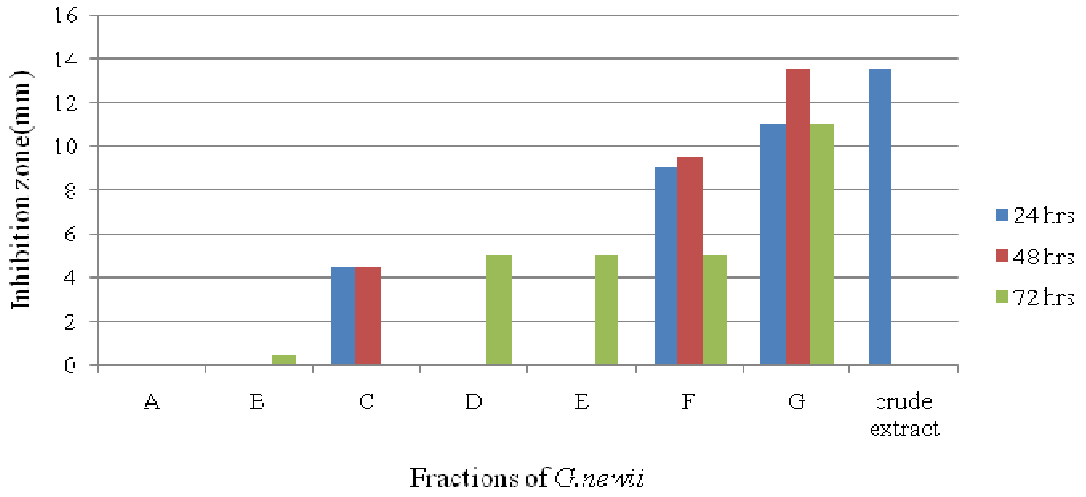


Figure 37: Activity of semi-purified bulbs of *G.newii* fractions from organic solvents against *A.niger*, A-100% Hexane;B-DCM : Hexane (ratio 1:1);C-100% DCM;D-DCM:EtOAc (1:1);E-100% EtOAc ;F- EtOAc:MeOH (ratio 1:1);G-100% MeOH.

Extracts from *G. goetzenii* had slight activity (with inhibition zones between 7mm and 9mm) against *A. niger* as displayed in Figure 38. The ethyl acetate: methanol and methanol fractions had the highest activity, slightly above 8 mm.

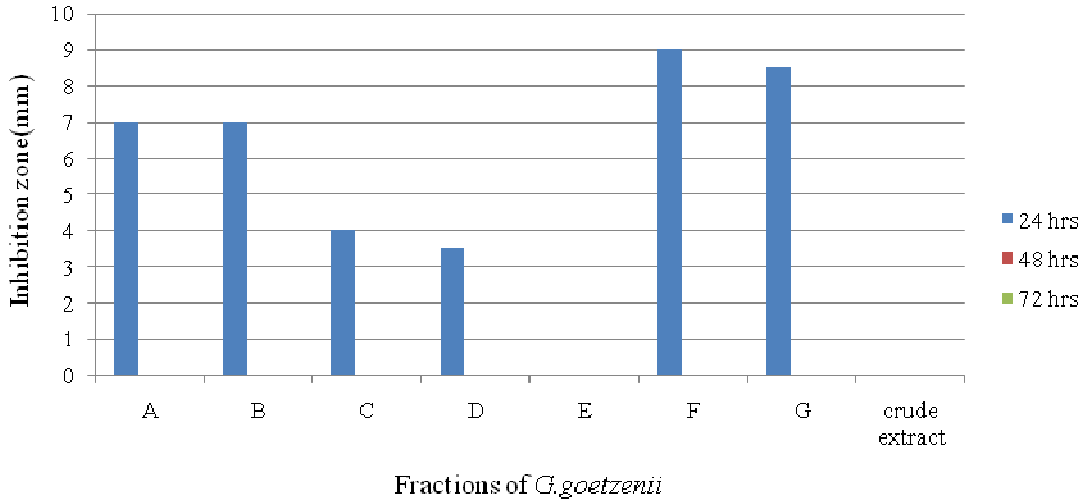


Figure 38 : Activity of semi-purified bulbs of *G.goetzenii* fractions from organic solvents against *A.niger*, A-100% Hexane;B-DCM : Hexane (ratio 1:1);C-100% DCM;D-DCM:EtOAc (1:1) ;E-100% EtOAc ;F- EtOAc : MeOH (ratio 1:1) ;G-100% MeOH.

G. ukambanensis fractions showed no activity against *A. niger*. This result is quite consistent with that in section 4.1.4 in which even the crude extracts of *G. ukambanensis* showed no activity against *A. niger* . Similarly no fraction from any of these five plants showed any activity against *C. neoformans,* contrary to the result in section 4.1.6. The tested concentration may have been too low to exert any activity.

In an attempt to identify the compounds, further purification of fractions from *Dierama* and *Gladiolus* species by TLC gave spots of relatively pure compounds, however NMR analyses did not provide sufficient information for characterization of the active compounds .

4.4 Toxic activity of the active fractions

In order to determine the toxicity of the active extracts , the most active fractions : ethylacetate, ethylacetate & methanol and methanol were subjected to toxicity test using brine shrimps larvae as previously stated. The data obtained was consistent with the previous data on the crude extracts. Other than the methanol fraction from *G. ukambanensis*, all the other bulb fractions had LD_{50} values less than 500µg/ml , an indication that they generally had toxic activity. In this plant *G. ukambanensis*, only bioactives extracted using ethyl acetate may have caused the cytotoxic effects in its bulbs.

4.5 Phytochemical screening of crude extracts and active fractions

4.5.1 Screening of crude extracts

In order to identify some classes of the secondary metabolites in these plants, qualitative chemical tests were conducted on the organic solvent extracts and powdered plant materials obtained from the five plants. Identification was based on a characteristic colour change of precipitate or foam development (Table 1). Alkaloids, flavanoids, saponins, tannins, glycosides, anthraquinones and terpenoids were detected as illustrated in Figure 39.

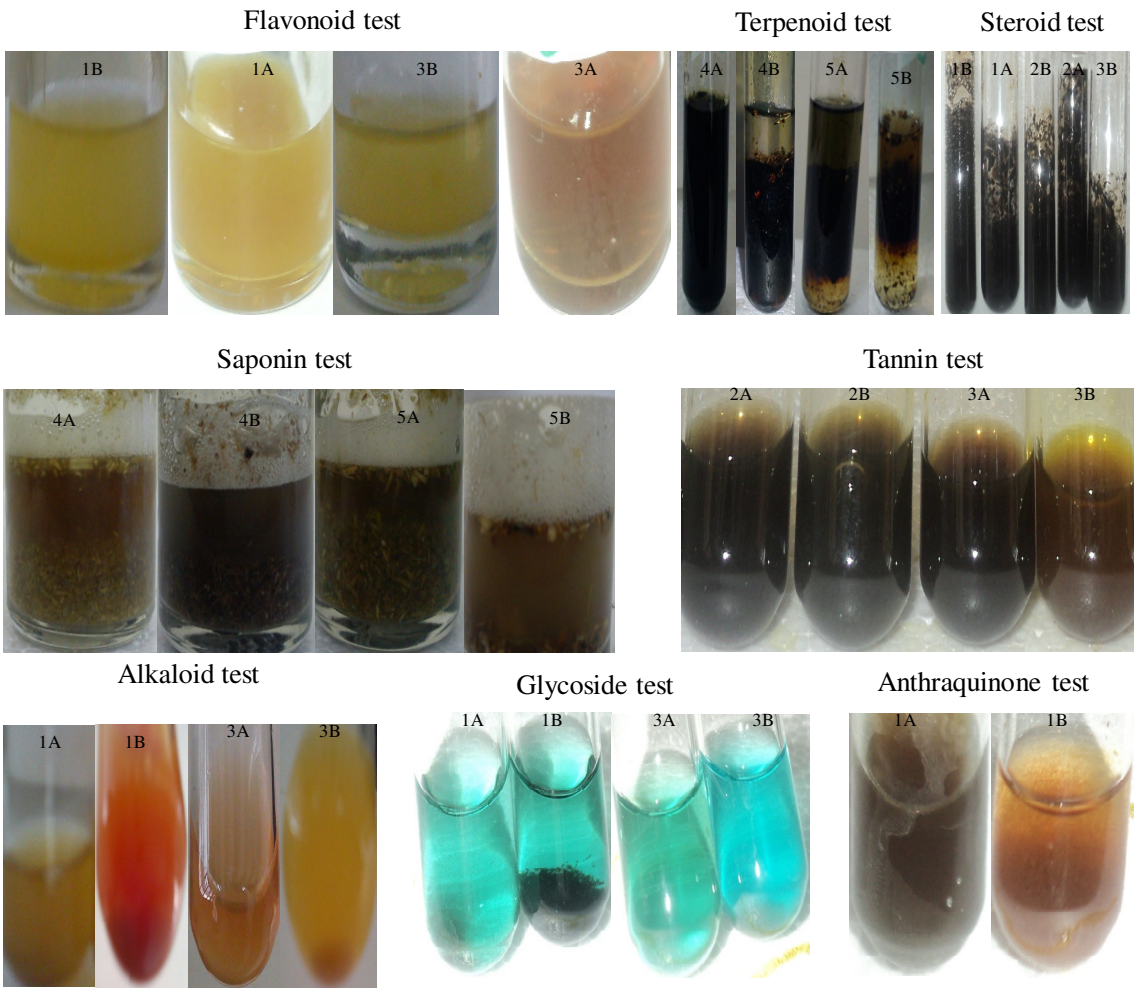


Figure 39: A representive photographs of phytochemical tests with the visual colour changes 1:*D.Cupuliflorum*, 2: *Gladiolus watsonoides*, 3: *G.newii*, 4: *G.ukambanensis*, 5: *G.goetzenii* A: Aerial part, B: Bulb part .Yellow colouration implies presence of flavonoids; orange red precipitate for alkaloids; stable froth for saponins ;a reddish brown colouration at the interphase for terpenoids; dark green precipitate for tannins, brown precipitate for glycosides; rose pink colour for anthraquinones; green colour for steroids.

The intensity of each attribute was assumed to be proportional to available amounts and was thus used to estimate relative abundances (Misra *et al.,* 2011;Prabhu *et al.,* 2011). Strong presence was recorded as ``+++´´; moderate presence as ``++´´; weak presence as ``+´´ and not detected as`` –´´. More phytochemicals were detected in the bulbs than the aerial parts Tannins and Saponins were detected in all plants at different abundances (Table 6). Flavonoids were detected in all the plants except the aerial parts of *G. newii*.

Table 7: Relative abundance of detected phytochemicals in the crude extracts

Plant Part	Flavonoids	Saponins	Alkaloids	Terpenes	Steroids	Tannins	Glycosides	Anthraquinones
1A	+++	+++	+	+	-	+++	++	+
2A	++	+++	+	+	-	+++	-	-
3A	-	+	+	+	-	+++	-	-
4A	+	++	-	-	-	+++	-	-
5A	+	++	-	++	-	+++	-	-
1B	+++	+++	+++	+++	-	+++	+++	++
2B	++	+++	++	+++	-	+++	++	+
3B	++	+++	++	+++	-	++	+	-
4B	+++	+++	-	-	-	+	-	-
5B	+	+++	++	+++	-	++	++	-

Key :1-*D. cupuliflorum,* 2-*G.watsonoides*, 3- *G.newii*, 4- *G. ukambanensis,* 5-*G. goetzenii*, B-Bulbs, A-Aerials ; +++ strongly present,++ moderately present, + weakly present, - not detected.

Alkaloids were present in all the plants except in the extracts of *G. ukambanensis* and aerials of *G. goetzenii.* All the plants showed the presence of terpenoids, except *G. ukambanensis.* Similarly, all the plants showed the presence of glycosides either in bulbs or aerial parts, except in *G. ukambanensis*. Only *D. cupuliflorum* and *G. watsonoides* showed the presence of anthraquinones. *D.cupuliflorum* and *G.watsonoides* showed presence of all the tested phytochemicals, except steroids. *G. ukambanensis* however showed the presence of flavonoids, saponins and tannins, only (Table 7).

4.5.2 Screening of bioactive fractions

In order to name the classes of compounds that may have been responsible for the observed activity, the extracts from the bulbs of the five plants were fractionated and tested for phytochemicals. Fractions eluted with ethyl acetate, methanol or their mixtures which had the highest activity were investigated. As in the previous case, qualitative tests were done on the extracts from these fractions, colour change, precipitate formation and were recorded as indicators for the presence or absence of phytochemicals according to Misra *et al.,* (2011)

and Prabhu *et al.,* (2011) (Table 8). Phytochemicals which were not detected in the crude extracts were not tested in the active fractions and are recorded as N/A (Not applicable) . Other than activity these tests were also done based on the availability of extracts.

Table 8: Relative abundances of detected Phytochemicals in the active fractions in bulbs of *Gladiolus* and *Dierama* species

Plant Part	Flavonoids	Saponins	Alkaloids	Terpenes	Tannins	Glycosides	Anthraquinones
1B :F	+++	++	+	+++	+++	++	+
2B :E	++	+++	-	+++	+++	-	-
2B :F	-	++	-	+++	+	-	-
2B :G	-	++	++	+++	++	-	-
3B :F	++	+++	-	+++	+++	-	N/A
3B :G	-	-	++	+++	-	-	N/A
4B:F	+	++	-	N/A	+	N/A	N/A
5B :F	-	+		+++	-	-	++
5B :G	-	++		+++	-	-	++

Key :1-*D. cupuliflorum*, 2-*G. watsonoides*, 3- *G. newii*, 4- *G. ukambanensis,* 5-*G.goetzenii*, B-Bulbs ; E – Ethy lacetate fraction (100%) ; F- Ethyl acetate : Methanol fraction (1:1) ; G- Methanol fraction (100%) ; +++ strongly present,++ moderately present, + weakly present, - not detected ; N/A Not applicable .

As previously recorded, screened fraction of *D. cupuliflorum* showed positive response for all tested phytochemicals (Appendix vii). Those of *G. watsonoides* showed the presence of phytochemicals except glycosides and anthraquinones similar to those of *G. newii. G. ukambanensis* had flavonoids, saponins and tannins whereas *G. goetzenii* had saponins, terpenoids and alkaloids present in one or more of the tested fractions (Table 8). This data is quite consistent with that on the phytochemicals found in the crude extracts.

4.6 Anatomical differences of cells

To identify more morphological features that can be used in the taxonomy of these plants, the physical features of bulbs and; structural features of leaves and bulbs were evaluated

Bulbs of these plants had noticeable shape and tunic colour differences among them. *D.cupuliflorum* had a main bulb with up to 3 slightly smaller bulbs stacking from the main bulb (Figure 40;DC). The tunics were papery to fibrous and brown in colour. The

morphology was similar to that of *G. watsonoides*. However *G. watsonoides* had fewer (1-2) smaller bulbs stacking from the main bulb (Figure 40; GW). The *G. watsonoides* main bulbs appeared generally larger than those of *D.cupuliflorum* but tunics were yellow brown and fibrous, just like those of *D. cupuliflorum*.

For *G.newii* the bulb was made up mainly of two bulb stacks. The lower bulb was smaller and had a different colour (yellow) from the main one (Figure 40;GN). The tunics looked papery and creamish in colour. The *G. goetzenii* had two bulbs, a main one and a minor one very small in size stacked together (Figure 40;GG). Bulbs had dark brownish papery tunic. The bulbs of *G. ukambanensis* had two stacks (of similar size) which were only visible after the removal of the fibrous tunics. The tunics had several layers with the outer layer being brown while the inner one was creamish in colour (Figure 40;GU) The tunics of *G. ukambanensis* looked more fibrous than those of the other plants.

Figure 40: Bulbs of the the different species of *Gladiolus* and *Dierama cupuliflorum*.DC: *D.cupuliflorum,* GW: *Gladiolus watsonoides,* GN: *G.newii,* GU:G.*ukambanensis,* GG: *G.goetzenii*

At anatomical level, there were no obvious differences among cells from leaves of the different plants species, (Figure 41A). Similarly, bulbs of all the plants generally had parenchyma and collenchymas cells within which were visible starch granules.

There were however, a few differences as well. Cells of *G.ukambanensis* bulbs looked smaller than those of the other plants Figure 41B . *D. cupuliflorum* and *G.watsonoides* had numerous and visible vascular bundles compared to *G. goetzenii.* Vascular bundles of the other plants were not visible.

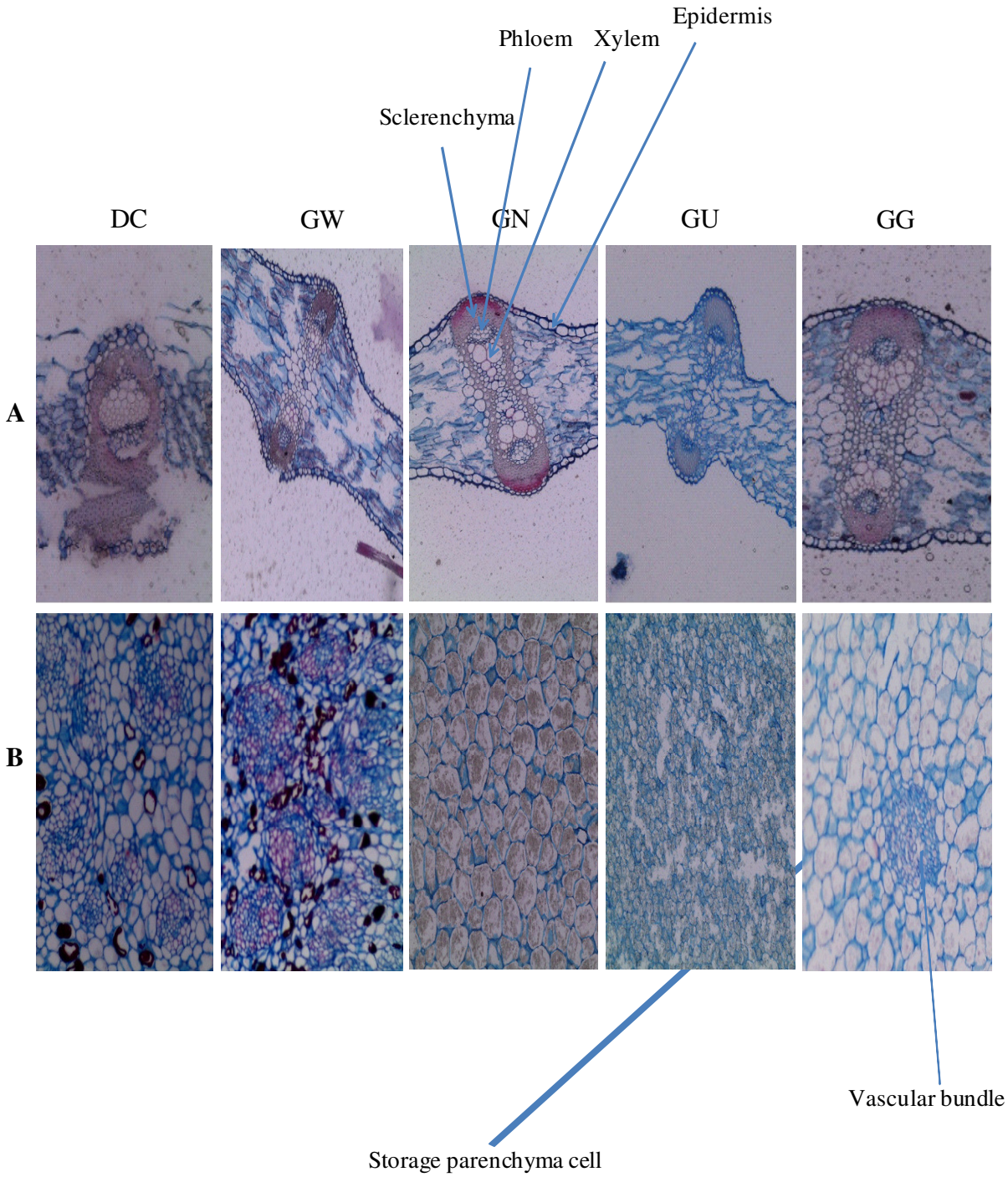


Figure 41: A photograph showing cross section of the internal structure of A:Leaves and B:Bulbs. DC-*D. Cupuliflorum,* GW-*G. watsonoides,*GN-*G. newii*,GU-G. *ukambanensis*,GG-*G. goetzenii* at Mag×100.

5.0 CHAPTER FIVE. DISCUSSION

5.1 Antifungal activity

This study revealed a wide range of antifungal activity of crude extracts of test plants at various concentrations. According to Bhalodia & Shukla (2011) plant extracts eliciting inhibition zones measuring above 8 mm were considered to be active. When tested against *C. albicans*; at concentration 2.5mg/100µl, the aerial extracts of all the five plants had activity with inhibition zone measuring of at least 10 mm . At a lower concentration of 0.625 mg/100µl, all the extracts of aerial parts were active producing inhibition zones measuring at least 8.8 mm except those of *G. newii.* At lower concentration of 0.3125 mg/100µl, all these extracts were active, except those of *G. newii* and *G. goetzenii*.

The trend was similar for bulbs; at a concentration of 2.5 mg/100µl, all the extracts from the bulbs of the five plants had activity with inhibition zones measuring between 11.5 mm-21.5 mm. At a lower concentration of 0.625 mg/100µl all these extracts were active with up to 15 mm zones of inhibition. At a much lower concentrations of 0.3125 mg/100µl, all the extracts were still active except those of *G. ukambanensis* which were only marginally active. When tested against *A. niger,* except *G. ukambanensis*, the extracts of the bulbs of all the plants had activity with inhibition zone measuring between 8.5 mm to 11 mm at 5 mg/100µl. At a lower concentration of 0.625 mg/100µl , only the bulbs of *G. newii* showed activity against this fungus with inhibition zone of 10 mm.

At 5 mg/100µl, only the bulbs of *G. watsonoides* and *G. gotzenii* had activity with inhibiton zones measuring 11 mm and 10 mm respectively when tested against *C.neoformans.*

The only plant with previous reports on antifungal activity against *C. albicans* (Fawole *et al.,* 2008) and *A.niger* (Odhiambo *et al.,* 2010 is *G. newii* syn. *G. dalenii*, The finding in this study on the activity of this plant against the two fungi is therefore in agreement with other

reports. Fawole *et al.,* (2008) also reported its activities against *Bacillus subtilis* and *Staphylococcus aureus*. Anti-amoebic activity has also been reported by Moundipa *et al.,* (2005). Other *Gladiolus spp.* such as *G. gregasius* have been reported to show activity against *A. niger*; *Candida* spp. *Psuedomonas aeruginosa,* amongst other bacteria (Ameh *et al.,* 2010;Nguedia *et al.,* 2004).

The anticandida activities of *D. cupuliflorum, G. watsonoides, G. ukambanenis, G. goetzenii*; the antiaspergillus activities of *D. cupuliflorum, G. watsonoides*, *G.goetzenii;* and the anti-cryptococcus activities of *G. watsonoides* and *G. goetzenii* are documented here for the first time.

Herbal preparations are usually made using water, alcohol or other solvents which extract the active components for easy consumption by the users (Yineger *et al.,* 2008;Kokwaro, 2009). Though water is the most commonly used solvent in the preparation of herbal concoctions and decoctions (Kokwaro, 2009), however, water extracts in this study had very little inhibitory effect on the test fungi. Contrary to this, crude extracts obtained using organic solvents were generally more active than water extracts at the same concentrations. There are two possible explainations to this observation; the bioactive elements may have been present in water at lower concentrations which could not inhibit antimicrobial growth (Boer *et al.,* 2005) or the bioactive elements were soluble in the organic solvents only and therefore not present in water extracts (Fawole *et al*.,2008). This finding was found to be in agreement with other previous research studies using different plant species (Talibi *et al.,* 2013;Singh *et al.,* 2013;Selvamohan *et al.,* 2012;Sen & Batra, 2012;Parekh & Chanda, 2007; Kariba, 2000)

Secondary metabolites from plants are by-and-large responsible for the curative properties with broad spectrum bioactivity (Ishaq *et al.,* 2014;Ramamoorthy *et al.,* 2012). The abundance of phytochemicals in a given part of the plant correlates with high bioactivity (Singh *et al.,* 2013). In this study, extracts from bulbs showed strong antifungal activity compared to those from the aerial parts of *Gladiolus* and *Dierama* species (Figure 23), an indication that bulbs probably have a variety of active phytochemicals which may have been present in high quantities as compared to the aerial parts. Data from the phytochemical analyses revealed an array of chemical classes and comparative abundances in the bulbs than in the aerial parts (Table 7). This finding is in agreement with various similar studies which reported more activity of extracts from underground parts compared to extracts from aerial parts; *Adiantum capillus* (Singh *et al.,* 2013), *Terminalia mollis* (Moshi *et al.,* 2006), *Carica papaya* (Anibijuwon & Udeze, 2009), *Halodule pinifolia* (Vijayakumar & Amirthanathn, 2014), *Clibadium sylvestre* and *Derris amazonica* (Filgueiras *et al.,* 2011), *Alstonia scholaris* (Misra *et al.,* 2011) and *Terminalia brownii* (Mbwambo *et al.,* 2007). It appears then that bulbs of these plants could be used for storage or as biosynthetic sites for the phytochemicals.

In this study, *C. albicans* was the most susceptible to the extracts followed by *A. niger* while *C. neoformans* was the most resistant (Figure 27) . For instance, at concentration 0.3125 to 5mg/100µl, bulb organic extracts from all the five plants were active against *C. albicans* . At concentration 5mg/100µl, all the organic bulb extracts showed activity against *A.niger* except those of *G. ukambanensis* (Figure 24), whereas at 2.5mg/100µl, only bulb extracts of *D. cupuliflorum* and *G. newii* were active against the same fungus. When tested against against *C. neoformans*, only bulb extracts of *G. watsonoides* and *G. goetzenii* were active at 5mg/100µl (Figure 26). This finding is in agreement with those of Nguedia *et al.,* (2004) and Bhalodia & Shukla, (2011) in which *C. albicans* was reported as the most sensitive amongst the tested microorganisms which included *A. niger*. Dzoyem *et al.,* (2007) and Abu-Mejdad,

(2014) reported more activity of extracts against *C.albicans* than both *A. niger* and *C. neoformans.* Although reports by Abdullah *et al.,* (2013), Dulger & Hacioglu, (2008) and Moshi *et al.,* (2006) also showed that *C. albicans* was the most sensitive organisms, their findings on the sensitivity of *A.niger* and *C.neoformans* is contrary to findings of this study. To them *C. neoformans* was more sensitive than *A. niger.* Other reports contrary to this finding include those by Mathur *et al.,* 2011, Ameh *et al.,* 2010, Odhiambo *et al.,* 2009 in which *A.niger* was more sensitive than *C.albicans*. According to Doughari & Nuya (2008), both *A.niger* and *C.neoformans* were more sensitive than *C.albicans*. These disparities in susceptibilities may partly be attributed to differences due to extraction solvents and concentrations of phytochemical compounds.

Amphotericin B, Nystatin and Ketoconazole are commercial antifungal drugs used in the management of Candidiasis, Cryptococcosis and Aspergillosis in the immunocompromised (IC) individuals. Amphotericin B, which is considered the 'gold standard', a drug of choice for treatment of these infections (Doughari & Nuya, 2008) was consistently active against all the three opportunistic fungi. Nystatin was also active but at lower levels. Ketoconazole was however active only against *A. niger*. Of importance is the observation that at certain concentrations, the activity of some of these plants was not significantly different to that of amphotericin B and in some instances higher than nystatin. For instance, at a concentration of 5 mg/100µl, 2.5 mg/100µl and 1.25 mg/100µl, the bulb extracts of all plants, except *G. ukambanensis,* had activities not significantly different from that of amphotericin B, when tested against *C. albicans*. The extracts of aerial parts of *D. cupuliflorum* also displayed an activity not different (at 5mg/100µl) to this drug. Similarly, at a much lower concentration of 0.3125mg/100µl all bulb extracts of the five plants had activities not different to those of nystatin against the same fungus. Again, at 5 mg/100µl and 2.5 mg/100µl, the activities of *G. watsonoides* and *G. goetzenii* bulb extracts were higher ($P<0.05$) than that of nystatin when

tested against *C. neoformans*. This finding is in agreement with other research reports which have also shown the activity of extracts to be either higher or not different to that of commercial antifungal drug fluconazole (Adamu *et al.,* 2012; Correa-Royero *et al.,* 2010), griseofulvin (Ali-Shtayeh & Ghdeib, 1999), ketoconazole (Nino *et al.,* 2012; Odhiambo *et al.,* 2009), amphotericin B (Sadeghi-Nejad *et al.,* 2010) and nystatin (Bakhshi *et al.,* 2012; Khoshkholgh-Pahlaviani *et al.,* 2013). Therefore, it is possible that extracts from the investigated plants have active antifungal compounds which could be potentially exploited to the level of some of the commercial drugs.

Although amphotericin B remains the most appropriate drug for many invasive and life threatening fungal infections, due to its broad spectrum activity, its usage is limited due to many adverse side effects such as nephrotoxicity, neurotoxicity, hepatotoxicity and gastrointestinal intolerance mongst others (Thippeswamy *et al.,* 2012 ; Tangarife-Castano *et al.,* 2011;Dismukes, 2000). Nystatin which is also used to treat opportunistic fungal infections also has its side effects such as skin rashes, diarrhoea, vomiting and high levels of resistance (Duffalo, 2006;Dismukes, 2000) .

The inactivity ketoconazole to the test organsms; *C. albicans* and *C. neoformans* was unexpected (Figure 18 and Figure 26). This observation may have been attributed to the lower potency or efficacy of the drug or resistance developed by these organisms to the drug. However other reports exist where commercially used drugs showed no activity against the tested fungi (Doughari & Nuya 2008; Dulger & Hacioglu 2008).

With the continuously rising cases of HIV, organ transplants, surgical procedures, cancers and diabetes, the number of individuals suffering from fungal infections is on the rise (Vandeputte *et al.,* 2012). Natural products from plants have been found to be rich sources of

new, safe, effective and biodegradable leads for drug development (Thippeswamy *et al.,* 2012) and they can also be used as medicines in form of extracts or as pure compounds (Ishaq *et al.,* 2014). Therefore, plants studied in this research may offer a potential source of leads to antifungal drug development.

The Minimum Inhibitory Concentrations (MIC), is the lowest concentration at inhibition of microbial growth is visible is used to monitor activity of antimicrobial agents against micro-organisms (Sen & Batra 2012). MIC values have been used to classify plants as strongly bioactive; moderately bioactive; weakly bioactive and non bioactive. According to Tangarife-Castano *et al.,* (2011) and Tsuzuki *et al.,* (2007), classification of MICs of plants extracts in relation to antimicrobial activity is provided as follows; extracts with MIC values upto 0.5 mg/ml are considered to have strong activity, those extracts with MIC values between 0.6-1.5 mg/ml are considered to have moderate activity while those with MIC values above 1.6 mg/ml are considered to have weak activity, however according to Awuoafack *et al.,* (2013) extracts with MIC values ranging between 0.1mg - 0.625 mg/ml are considered to have strong activity while those with MIC values above 0.625 mg/ml are considered to have weak activity. Based on the first classification (Tangarife-Castano *et al.,* 2011;Tsuzuki *et al.,* 2007) on analysis of MIC and bioactivity, the aerial parts of plants *D. cupuliflorum* and *G. watsonoides* with MIC values of 1.5625 mg/ml and 0.7813 mg/ml respectively, had moderate antifungal activity, while aerial parts of *G. newii, G. ukambanensis* and *G. goetzenii* with MIC values of 6.25 mg/ml, 3.125 mg/ml and 3.125 mg/ml respectively had weak activity against *C. albicans*. However, according Awuoafack *et al.,* (2013) on analysis of MIC and bioactivity, the aerials of all the plants studied here showed weak activity when tested against *C.albicans* (Table 2). On the other hand, the bulbs of *D.cupuliflorum*, *G.watsonoides* and *G.goetzenii* all with MIC value of 0.3907 mg/ml are considered to have

strong antifungal activity while those of *G.newii* and *G.ukambanensis* with MIC values of 1.5625 mg/ml and 0.7813 mg/ml respectively had moderate activity acoording to Tangarife-Castano *et al.,* (2011) and Tsuzuki *et al.,* (2007). According to Awuoafack *et al.,* (2013) the bulbs of plants *D. cupuliflorum*, *G. watsonoides* and *G. goetzenii* are considered to have had strong activity while those of *G.newii* and *G. ukambanensis* had weak activity when tested against *C. albicans*. Although there are minor disparities among the two schools of thought (Tangarife-Castano *et al.,* 2011 & Tsuzuki *et al.,* 2007 and Awuoafack *et al.,* 2013) in the MIC classification in regard to activity, all their analysis are in agreement in support of the inclusion of the bulbs of *D. cupuliflorum, G. watsonoides* and *G. goetzenii* in the category of strongly active antifungal plants against *C. albicans.* MIC values of all extracts were above 1.6 mg/ml when tested against *A. niger* and *C. neoformans* suggesting that the extracts are weakly active against these two fungi. The generalized reduced activity against these fungi as compared to *C. albicans* may have been due to the differences in cell wall composition and site of action by the extracts (Espinel-Ingroff & Flynn, 1996;Gooday, 1993) . Further research on the site of action of the extracts on these organisms would be worthwhile.

Bioactivity guided fractionation using ethyl acetate, methanol, hexane and dichloromethane revealed that fractions obtained using ethyl acetate, methanol or their mixture possesed the highest antifungal activity. Given that these two solvents are the most polar, the high antifungal activity may suggest that these bulbs may have had high concentrations of polar bioactive compounds which were readily solube in these solvents (Adongo *et al.,* 2012). This findings of high activity of ethyl acetate and methanol fractions is consistent with other reports, though on different plants species (Ishaq *et al.,* 2014; Awuoafack *et al.,* 2013; Mohamed *et al.,* 2010; Ezae *et al.,* 2008). The weak activities displayed by fractions obtained using other solvents may have been due relative polarity or that some non-polar compounds

are slightly marginally active at best (Mills-Robertson *et al.,* 2012). For plants studied here, ethyl acetate and methanol seem to be the most appropriate eluents as a follow up in order to isolate and identify the active compounds.

Generally when tested against *C. albicans* bulbs fractions of all the plants were active with strong activity noted with fractions eluted with ethyl acetate, methanol and their mixtures. The inhibition zones measuring between 9.5 -17 mm. When tested against *A. niger* the same fractions showed activity with inhibition zones between 8.5-13 mm. Activities of bulb fractions from the bulb extracts of *D. cupuliflorum, G. watsonoides*, and *G. ukambanensis* were significantly lower ($P<0.05$) than the corresponding crude extracts. On the other hand, those fractions from *G. goetzenii* and *G. newii* had activity not significantly different to that of the crude extracts when tested against *C. albicans*. When tested against *A. niger*, the activity of the fractions of the bulb extracts of *G. watsonoides* were significantly lower ($P<0.05$) than the crude, while those from *G. newii* and *G. goetzenii* had better activity than the crude extracts.

As indicated above, most crude extracts had higher activities than their semi-purified fractions. This may have been due synergistic or additive effects of the bioactive elements in the crude extracts as compared to separated fractions. Reports on higher activity of the crude extracts than the fractions have also been recorded by Kuete *et al.,* (2009) and Ezea *et al.,* (2008). Comparable activities of some crude extracts and fractions has also been reported by Martins *et al.,* (2013).

The results that *G. goetzenii* and *G. newii* fractions had better activity than those of the crude extract when tested against *A. niger* is interesting . The lower activity of the crude extracts may have been due to the antagonistic effects of the bioactive compounds present which may

have been reduced in the semi-purified fractions making the fractions to have better activity. Although these finding appears contrary to the previous reports by Kuete *et al.,* (2009) and Ezea *et al.,* (2008) on the efficiency of the crude extracts, an observation that fractions may have activity better than the crude extracts have been reported by Awuoafack *et al.,* (2013) ; Mills-Robertson *et al.,* (2012) ; Kuete *et al.,* (2011) ; Mahlke *et al.,* (2009) and Mansouri *et al.,* (2001). This present study has revealed that it would be better to use crude extracts of *D. cupuliflorum, G. watsonoides*, and *G. ukambanensis* in the management of infections from these fungi. In the latter case, the ethyl acetate and methanol fractions of *G. newii* and *G. goetzenii* should be further purified for better activity and identification of the active compounds.

5.2 Toxic activity of water and organic extracts

To evaluate the toxicity of these extracts, brine shrimp lethality tests (BLT) was done using brine shrimp nauplii. According to Nguta *et al.,* (2012) , toxicity can be termed strong when LD_{50} values range between 0-100µg/ml; moderate when the range is between 101-500µg/ml weak when the range is between 501-1000 µg/ml and; non-toxic when the range is >1000 µg/ml. All organic solvent extracts had LD_{50} <100µg/ml meaning that they had strong toxic activity according this scale. Similarly, water extracts from bulbs and aerial parts had toxic activities at varied levels, except *G. ukambanensis*. The bulb water extracts of *G. ukambanensis* were weakly toxic (LD_{50} of 698µg/ml), while water extracts of its aerial parts were nontoxic to the freshly hatched (LD_{50} of 1205µg/ml) to the freshly hatched shrimps (Table 5). Apparently, the most toxic extracts/plants had the highest number of phytochemicals and were also the most active extracts/plants. Therefore, a linear relationship between toxicity and antifungal activity, with high concentration of phytochemicals can be inferred from this study. Similar correlation has also been reported on work done on *Derris*

trifoliata (Manmoon & Azam, 2011), *Swietenia mahagoni* (Sahgal *et al.,* 2010), *Artemisia dubia* (Ul-Haq *et al.,* 2012) *Terminalia mollis* (Moshi *et al.,* 2006), *Lantana camara, Euphorbia hirta* and *Chromolaena odorata* (Olowa & Nuneza, 2013), *Terminalia brownie* (Mbwambo *et al.,* 2007), *Clerodendrum paniculatum* (Praveen *et al.,* 2012) and *Everniastrum cirrhatum* (Ramamoorthy *et al.,* 2012).

There are two possible explanations for this concurrent display of antifungal activity and toxicity. Extracts from these plants may have elaborate compounds, which are either cytotoxic, antifungal or both. This observation was also made by Bastos *et al.,* (2009), who after purifying and screening *Zeyheria tuberculosa* extracts, found out that some compounds were toxic, some antimicrobial, while others showed both toxic and antimicrobial activity. Alternatively, the active compounds may have showed toxicity due to high concentration. Similar observations were also made by Montainher *et al.,* (2002). According to them, ´toxicology is pharmacology at a higher dosage´, thus some toxic components applied at reduced dosage results into useful pharmacological significance. In this case cytotoxicity may be reduced by using low doses. According to Nguta *et al.,* (2012) , Ramamoorthy *et al.,* (2012) and Moshi *et al.,* (2006), extracts with strong toxic activities may also be useful as anticancer or antitumour as well as pesticidal.

In the current study, it was only possible to do partial fractionation. And when the active fractions were tested for toxicity, they showed similar toxic activities as the corresponding crude extracts (Appendix vi). The compounds with antifungal activity may also be responsible for the toxicity or the fractions may contain antifungal as one set and toxic compounds as another set. Pure compounds were not isolated due to technical challenges, which made it difficult to ascertain if the active compounds were also toxic. While it is

possible to use crude herbal preparations which have both toxic and beneficial effects (Ved *et al.,* 2010), its however better to minimize side effects by separating beneficial active compounds from toxic ones. Future studies should therefore focus on the purification and characterization of pure compounds to ascertain the antifungal and the toxic ones.

5.3 Phytochemical Screening

Phytochemical screening based on the formation of foam, precipitate and specific colour (Dib *et al.,* 2013), revealed diverse classes of chemical compounds. Both the aerial and the bulb showed the presence of alkaloids, flavonoids, saponins, tannins, glycosides, anthraquinones and terpenoids. The two most bioactive plants, *D. cupuliflorum* and *G. watsonoides* were rich in the tested phytochemicals whereas, *G. ukambanensis* which exhibited the least bioactivity had fewer classes namely; flavonoids, saponins and tannins. Similarly, the bulb extracts showed the presence of more classes of secondary metabolites with higher concentrations than were detected in the aerial parts (Table 7). Again, this trend correlated well with the observed high antifungal and cytotoxic activities. The presence of higher concentrations of secondary metabolites in the underground parts of the plants which contributed to high bioactivity than the aerials has also been reported for other plants (Vijayakumar & Amirthanathn, 2014; Singh *et al.,* 2013; Filgueiras *et al.,* 2011; Misra *et al.,* 2011; Anibijuwon & Udeze, 2009; Mbwambo *et al.,* 2007 and Moshi *et al.,* 2006).

In the plants investigated here, saponins were found to be abundantly present. Although previous reports indicate that saponins are widely distributed amongst dicotyledon members (Thakur *et al.,* 2011), *Gladiolu*s and *Dierama* species were found to be rich in this secondary metabolite yet, they belong to the monocotyledon family Iridaceae. The detected saponins could have contributed to the antifungal activities of these plants since a wide range of

biological activities have been reported on saponins (Sparg *et al.,* 2004). These include antifungal activity (incuding anti: *Candida, Aspergillus* and *Cryptococcus*; antiprotozoan, antioxidant, insecticidal, among others (Yang *et al.,* 2006). The mechanism of action appears to be by destruction of the membranes of pathogens (Mert-Turk, 2006). They also have showed cytotoxic effects on some cancer cells (Ogu *et al.,* 2012; Thakur *et al.,* 2011; Saha *et al.,* 2010; Francis *et al.,* 2002).

These plants, especially the aerial parts were found to be rich in tannins. Most plants with high amounts of tannins have been found to be usefull in the treatement of wounds and intestinal disorders including systemic candidiasis (Sheh-Hong *et al.,* 2011; Doss *et al,* 2009; Sanches *et al.,* 2005; Latte & Kolodziei, 2000). In particular, they have been reported to inhibit the growth of *C. neoformans* (Ishida *et al.,* 2009) and *C. albicans* by either inhibiting extracellular microbial enzymes or by weakening the cell wall (Ogu *et al.,* 2012; Abdollahzadeh *et al.,* 2011). In this study, their contribution to the observed antifungal activities is however in doubt since they were more abundantly present in extracts of the aerial parts which were less active compared to the bulb extracts.

Flavonoids occur widely in the plant kingdom and have wide range of biological activities, the carbonyl group being involved by forming complexes (Okoh-Esene *et al.,* 2011; Hernandez *et al.,* 2000). These secondary metabolites have been reported to posses, antifungal, antiviral, antibacterial activity and antitumour activities among others (Cushnie & Lamb, 2011; Orhan *et al.,* 2010;Saravanakumar *et al.,* 2009; Cushnie & Lamb, 2005; Taleb-Contini *et al.,* 2003). Their activity as antimicrobial agents is believed to be due to their ability to complex with fungal cell wall leading to the disruption of fungal membranes (Arif *et al.,* 2011). Flavonoids from *Conyza* species have been reported to posses inhibitory

activity against *C. albicans* and *C. neoformans* (Ruiz *et al.,* 2012). In the current study, extracts which were rich in flavonoids were cytotoxic and also are more active against *C. albicans,* than the other two fungi, and therefore these metabolite may have played a role in these activities.

Terpenoids was present in all the studied plants except in *G. ukambanensis* (Table 7). Terpenoids posses a range of biological activities beneficial to humans such as antifungal, antibacterial antiviral, antioxidant and antiprotozoa activities (Ogu *et al.,* 2012; Marcos-Arias *et al.,* 2011; Galluci *et al.,* 2010; Silva & Fernandes, 2010; Abad *et al.,* 2007; Grassmann, 2005; Aharoni *et al.,* 2005). In particular, they have been found to inhibit the growth of several *Candida* species via cell wall damage and membrane disruption (Martinez *et al.,* 2014; Pemmaraju *et al.,* 2013, Zore *et al.,* 2011; Rao *et al.,* 2010). Terpenoids from other plants such as *Cuminum cyminum, Ziziphora clinopodioides, Nigella sativa* and *Hyptis suaveolens* have also been reported to have activity against *Aspergillus* species (Khosravi *et al.,* 2011; Moreira *et al.,* 2010; Silva & Fernandes, 2010). Terpenoids may have contributed to the observed bioactivity of these plants except in *G. ukambanensis* where these were not detected.

In this study, strong presence of alkaloids were detected in the bulbs of *D. cupuliflorum,* lower amounts in the bulbs of *G. watsonoides, G. newii, G. goetzenii* and trace amounts in the aerial parts of *D. cupuliflorum, G. watsonoides, G. newii* syn *G. dalenii.* Detection of alkaloids has also been reported in *G.newii* (Burkill, 1985). This class of secondary metabolites has been associated with various biological activities such as antimicrobial, antimalarial, antihelminthic, anticancer among others (Souto *et al.,* 2011;Kumar *et al.,* 2009; Igbinosa *et al.,* 2009; Roberts & Wink, 1998). More importantly, alkaloids from *G. newii*

were reported to be active against *A. niger* (Odhiambo *et al.,* 2010). In agreement with this report, the results of the current study also found *G. newii* to be the most active against *A. niger*, impliying that alkaloids could have contributed to the observed antifungal activity.

All the bulb extracts, except those of *G. ukambanensis* showed the presence of glycosides (Table 7), while the majority of extracts of the aerial parts tested negative. Glycosides have been reported to posses antimicrobial and antioxidant properties (Nenaah, 2013; Ogu *et al.,* 2012; Kouam *et al.,* 2011; Ogunleye & Ibitoye, 2003). Zhao *et al.,* (2011) and Engel *et al.,* (2011) have reported them to have antifungal activities against *C. albicans* and *A. niger.* However, based on their distribution in the studied plants, its unlikely that they contributed much to the observed bioactivities.

Anthraquinones were detected in *D. cupuliflorum* and *G. watsonoides* and this finding is in agreement with other studies which reported the presence of this class in other species of *Gladiolus* such as *G. gandavensis* and *G. psittacinus* (Ameh *et al.,* 2011; Ameh *et al.,* 2010). Anthraquinones have showed antimicrobial and cytotoxic activities (Ali *et al.,* 2000).

There was no much difference between the phytochemicals present in the crude extracts and the active bulb fractions (Table 8). It is therefore possible that these fractions had the most of the phytochemicals present in these plants compared to the other lesser active fractions.

It is worth pointing out that there are few reports on the occurrence of phytochemicals in the studied plants (Munyemana, 2013; Ameh *et al.,* 2010; Odhiambo *et al.,* 2010; Ngamga *et al.,* 2007; Burkill 1985) compared to the reports on other species of *Gladiolu*s such as *G. segetum, G. gregasius, G. atroviolaceus, G. gandavensis. G. illyricus,G. primulinus, G.*

quartinianus and *G. imbricatus* (Krvavych *et al.,* 2014; Assob *et al.*, 2011; Ameh *et al.,* 2011 ; Ameh *et al.,* 2010; Mohammed 2005; Nguedia *et al.,* 2004 ; Wang' *et al.,* 2003; Wubert *et al.,* 1996;Ali *et al.,*1989; Viladomat *et al.,*1986 and Ali *et al.,* 1985). This study has therefore contributed to the scientific knowledge of the phytochemical composition of these plants (Appendix viii & ix).

5.4 Morphological Features of *Gladiolus* and *Dierama* sp.

The taxonomic description of the studied plants is mainly based on the morphological differences in aerial parts, particularly the flower features (Agnew, 2013; Agnew & Agnew, 1994). There is little information on the structural features of bulbs and cells that can be used for taxonomic purposes. A close look at structural features of cells from different plants did not reveal any obvious difference. However, bulbs had additional features that could be of taxonomic value (Figure 40). Bulbs of all plants analyzed in this study looked different just like their flowers. Bulbs of *D. cupuliflorum* have up to three stacks which were almost similar in size, while tunics were papery to fibrous and brown in colour. *G. watsonoides* had fewer and smaller stacks than *D. cupuliflorum*. The main bulb was larger than the rest. Its tunics were yellow brown and papery to fibrous, similar to those of *D. cupuliflorum*. *G. newii* had bulbs which consisted of two stacks, the lower bulb was smaller and yellow in colouration, the larger bulb had papery tunics which were creamish in colour. Similarly, *G. goetzenii* had two stacks of bulbs, a main one and a minor one which was relatively very small compared to the main bulb. The tunics were papery and dark brown in colour. Bulbs of *G. ukambanensis* were generally smaller in size with two stacks which were visible only after the removal of the fibrous tunics. Tunics consisted of several layers, the outer one was brown in colour while the inner one was creamish in colour. The physical features described on the bulbs here, may be useful in the idenfication of different *Gladiolus* species and *Dierama cupuliflorum,* where aerial parts are not available.

6.0 CHAPTER SIX. CONCLUSION AND RECOMMENDATIONS

6.1 CONCLUSION

The extracts of the bulbs of *D. cupuliflorum, G. watsonoides* and *G. goetzenii* were strongly active (based on the MIC values) against *C.albicans* while the bulb extracts of of *G. newii* and *G. ukambanensis* and the extracts of aerial parts of all plants had weak activity against this fungus. Other than *G. ukambanensis* which had no activity against *A. niger*, bulb extracts of all the other plants had weak activities. On the other hand, all bulb extracts were inactive against *C. neoformans,* except *G.watsonoides* and *G.goetzenii* which had weak activities. Data presented here generally showed that bulb extracts of *Gladiolus* and *Dierama species* were more active against the tested fungi than aerial extracts while organic extracts were more active than water extracts.

In bioassay guided fractionation of the active bulb organic extracts, the fractions eluted with ethyl acetate and methanol were the most active against *C. albicans* and *A. niger,* while fractions obtained using hexane were the least active. Other than *G. goetzenii* and *G. newii,* the semi-purified fractions showed more activity than their crude extracts. In all the other plant extracts , the fractions had activities either lower or with no significant difference to that of the crude extracts.

The level of activity of extracts and fractions parallels the presence of phytochemicals (flavonoids, saponins, alkaloids, terpenoids, steroids, tannins, glycosides and anthraquinones). Thus the bulb extracts containing more phytochemicals were more active than the extracts of aerial parts. Similarly, *D. cupuliflorum* and *G. watsonoides* which showed the presence of higher amounts and variety of phytochemicals were the most active followed by *G. goetzenii* and *G. newii* in that order. *G. ukambanensis* which had the fewest

phytochemicals, was the least active. Of these phytochemicals, tannins and saponins were present in all plants at varying amounts while flavonoids were detected in all plants, except in the aerial parts of *G. newii*. Similarly,alkaloids were detected in all plants, except *G. ukambanensis* and the aerial parts of *G. goetzenii,* while terpenoids and glycosides were present in either bulbs or aerials of all the plants, except *G. ukambanensis.* This information clearly demonstrates that the observed antifungal activity was associated with the presence of the secondary metabolites.

The antifungal activity levels of the bulb organic extracts of *D. cupuliflorum, G. watsonoides G. goetzenii* and *G. newii* were not significantly different to that of amphotericin B at a concentration of 1.25 mg/100µl and higher, while the bulb extracts of these plants had higher activity than nystatin and ketoconazole at this concentration when tested against *C. albicans.* When tested against *C. neoformans*, *G. watsonoides* and *G. goetzenii,* the bulb organic extracts were significantly more active than nystatin at 5 mg/100µl. On *A.niger,* however, the test drugs had significantly higher activity than the plant extracts. This study has proved that these plants are significant as source of leads to antifungal drug development.

All organic extracts showed cytotoxic activity to freshly hatched shrimps (LD_{50} values <100µg/ml), and hence the antifungal and cytotoxicity levels of pure compounds from these plants have to be established before their application can be established.

Anatomical analyses of cells of these plants did not reveal obvious differences, however the morphology of bulbs were noted to be different. The bulbs of *D. cupuliflorum* had three stacks which were almost similar in size. On the other hand, *G. watsonoides* had fewer and smaller stacks than *D. cupuliflorum*. Its main bulb was larger than the rest. Bulbs for *G.*

newii had two stacks, the lower bulb was smaller and yellow in colour. Similarly, the bulbs of *G. goetzenii* had two stacks, a main one and a minor one which was relatively smaller compared to the main bulb. The bulbs of *G. ukambanensis* were generally smaller in size with two stacks which were only visible after the removal of the fibrous tunics. These additional features which can be used to identify these plants in the abscene of floral features had not been described before. Therefore to a small extent, this study has also contributed to the taxonomic knowledge of these plants.

6.2 RECOMMENDATIONS

This work has laid a foundation on the study of medicinal and economic value of the investigated plants. To improve on these findings, more work is required in the following areas:

1. Isolation and structure elucidation of the constituents is recommended as this is key in the determination of the bioactive elements and possible significance in chemotaxonomy.

2. The toxicity of the extracts and pure compounds should be investigated *in vitro* and i*n vivo*. This may reveal the likely effect on humans.

3. This study focused on only antifungal activities. Many medicinal plants are used for treatment of multiple diseases. Other than *G.newii*, reports on biological activity of these plants on various organisms is lacking. It would be prudent to find out whether these plants are also active against other microbial pathogenic microorganisms.

4. Phytochemical data and bulb morphology showed some similarities between *G. watsonoides* and *D. cupuliflorum*. Chemical profiling using unique pure compounds and molecular technology may help to establish and refine phylogenetic relationships among these plants.

5. Literature reports indicate that in many parts of the world, other species of *Gladiolus* and *Dierama* are used in horticulture (Ranjan, 2008) and ethnomedicine (Ameh *et al.,* 2011). Other than *G. newii* which has ethnomedicinal use, all the others have not been

put to such use. Domestication, cultivation and conservation of the studied plants for floriculture and medicinal use would be of economic value.

REFERENCES

Abad J.M., Ansuategui M., Bermejo P., (2007). Active antifungal substances from natural resources. *Arkivoc* 7: 116-145

Abdollahzadeh S., Mashouf., Mortazavi H., Moghaddan M.H., Roozbahani N., Vahedi M., (2011). Antibacterial and antifungal activities of *Punica granatum* peel extracts against oral pathogens. *Journal of Dentistry* 1 (8) : 1-6

Abdullah K., Soliman M., Mohamed M., (2013) Succeptibility of *Candida albicans* clinical isolates to some plant extracts in Saudi Arabia. *Zagazig University Medical Journal* 5 (19) : 294-299

Abu-Mejdad A., J., M., N., (2014). Antifungal activity of some plant extracts against two yeast isolates *in vitro*. *Research Journal of Pharmaceutical, Biological and Chemical sciences* 2 (5) : 1992-1998

Adamu M., Naidoo V., Eloff N.J., (2012). Some Southern African plant species used to treat helminth infections in ethnoveterinary medicine have excellent antifungal activities. *BMC Complementary and Alternative Medicine* 12: 213

Adjanohoun, E., Ahyi, M. R. A., Ake-Assi, L., Elewude, J. A., Dramane, K., Fadoju, S.O., Gbile, Z. O., Goudole, E.,. Johnson, C. L .A , Keita, A., Morakinyo, O. , Ojewole, J. A. O. , Olatunji, A. O. and Sofowora, E. A. (1991) Traditional medicine and pharmacopoeia. Contribution to ethnobotanical floristic studies in Western Nigeria, Pub. Organization of African Unity, Scientific Technical and Research Commission Lagos, Nigeria. 420p

Adongo J.O., Omolo O.J., Njue A.W., Matofari J.W., (2012). Antimicrobial activity of the root extracts of *Tylosema fassoglensis* Schweinf. Torre & Hillc (Caesalpiniaceae). *Science Journal of Microbiology* 2012

Agnew A.D.Q., (2013) Upland Kenya wild flowers and Ferns, 3rd Ed . East Africa Natural History Society, page 379.

Agnew, A.D.Q., Agnew, S., (1994). Upland Kenya wild flowers, 2nd Ed. East Africa Natural History Society

Aharoni A., Jongsma A., Bouwmeester J.H., (2005). Volatile science? Metabolic engineering of terpenoids in plants. *Trends in plant science* (10) 12 : 594-602.

Ahmad M.J & Siddique W., (2005). Effect of spike clipping on corm and cormel yield of *Gladiolus* . *Sarhad Journal of Agriculture*. 21 (3):335-338

Ali A.M., Ismail N.H., Mackeen M.M., Yazan L.S., Mohamed S.M., Ho A.S., Lajis N.H., (2000). Antiviral,cytotoxic and antimicrobial activities of anthraquinones isolated fro roots of *Morinda elliptica*. *Pharmaceutical Biology* 38 (4) : 298-301

Ali, A.A., Abdalla O.M., Steglich W. (1989). Anthraquinone derivatives from *Gladiolus Segetum. Phytochemistry* 28 (1): 281-282

Ali A.A., El-Shanawany M.A., Mesbah M.K., Ross S.A., (1985). Pharmacognostical study of *Gladiolus segetum* Ker-Gawl, cultivated in Egypt. *Bulletin of Pharmaceutical Sciences Assuit University* 8(1):102-126

Ali-Shtayeh & Ghdeib S.I., (1999). Antifungal activity of plant extracts against dermatophytes. *Mycoses* 42 : 665-672

Al-Edany Y.T., & Al-Saadi M.A.A.S., (2012). Taxonomic significance of anatomical characters in some species of the family myrtaceae. *American Journal of plant sciences* 3: 572-581

Ameh S., Obodozie O., Olorunfemi P., Okoliko I., Ochepke N., (2011). Potentials of corms as antimicrobial agents in food processing and traditional medicine. *Journal of Microbiology and Antimicrobials* 3 (1): 8-12

Ameh S.J., Ochepke N.A., Okoliko E.I., Olorunfemi P.O., (2010). Basis for ethnomedicinal use of corm (Family: Iridaceae) in West Africa. *Asian Journal of Experimental Biological Sciences* 1 (4) : 902-906

Anatomy, Taxonomy and Physiology of Agricultural plants http://www.unaab.edu.ng, accessed on 8/08/2014

Andrews J.M., (2001). Determination of minimum inhibitory concentrations. *The Journal of Antimicrobial Chemotherapy* 48(1):5-16

Anibijuwon I.I., & Udeze A.O., (2009). Antimicrobial activity of *Carica papaya* (paw paw leaf) on some pathogenic organisms of clinical origin from South-Western Nigeria. *Ethnobotanical leaflets.* 13: 850-864

Arif T., Mandal T.K., Dabur R., (2011). Natural products: Anti-fungal agents derived from plants.*Opportunity, challenge and Scope of Natural Products in Medicinal Chemistry* 283-311

Arnold H.J., & Gulumian M., (1984). Pharmacopoeia of traditional medicine in Venda. *Journal of Ethnopharmacology* 12: 35-74.

Arome D. & Chinedu E., (2014). The importance of toxicity testing. *Journal of Pharmaceutical and Biosciences* 4 (2013): 146-148

Assob C.N.J., Kamga F.L.H., Nsagha S.D., Njunda L.A., Nde F.P., Asongalem E.A., Njouendou J.A., Sandjon B., Penlap B.V., (2011) Antimicrobial and toxicological activities of five medicinal plant species from Cameroon traditional medicine. *BMC Complementary and alternative medicine* 11:70

Atul T., (2014). Study of some essential oil to show antifungal activity with special reference to *Abutilon indicum* and *Lantana camara* . *Research Journal of Pharmaceutical Sciences* 3 (2):1-2

Awuoafack D.M., McGaw J.L., Gottfried S., Mbouangouere R., Tane P., Spiteller M., Eloff N.J., (2013). Antimicrobial activity and cytotoxicity of the ethanol extract, fractions and

eight compounds isolated from *Eriosema robustum* (Fabaceae). *BMC Complementary and Alternative* 13:289

Bakhshi M., Taheri J., Shabestari B.S., Tanik A., Pahlevan R., (2012). Comparison of therapeutic effect of aqueous extract of garlic and nystatin mouth wash in denture stomatitis. *Gerodontology* 29: 680-684

Barlunas J.M., & Kinghorn D.A., (2005) Drug discovery from medicinal plants. *Life Sciences* 78 : 431-441

Bart H., & Pliz S., (2011). Industrial scale natural products extraction, www.beck.de accessed on 7/3/2014

Bhargava D., Agrawal D., Agrawal O., (2013). Repellent activity of essential oil and leaf extract of *Lantana camara* L. in laboratory condition. *International Journal of Theoritical and Applied Sciences* 5: 170-174

Basic anatomy (2013)
http://www.vcbio.science.ru.nl/en/virtuallessons/leaf/basicanatomy/#blad Missouri State University; accessed on 8/8/2014

Beentje, H.J., (1994). Kenya trees, shrubs and Lianas. National Museums of Kenya. Nairobi

Burkill, H.M., (1985). The useful plants of West Tropical Africa. Royal Botanic Gardens, Kew 2

Butts A., & Krysan J.D., (2012). Antifungal Drug Discovery: Something old and something new. *Plos pathogen* 8:9

Bhalodia R.N., & Shukla V.J., (2011). Antibacterial and antifungal activities from leaf extracts of Cassia fistula I. An ethnomedicinal plant. *Journal of Advanced Pharmaceutical Technology & Research* 2 (2):104-109

Boer, J.H., Kool, A., Mziray, W.R., Herdberg I., Levenfors J.J., (2005). Antifungal and antibacterial activity of some herbal remedies from Tanzania. *Journal of Ethnopharmacology* 96 :461-469

Bose, T.K., L.P. Yadav, P. Pal, V.A. Parthasarathy, Das P., (2003). *Commercial Flowers* 2**,**Naya Udyog, Kolkata, India

Carballo J.L., Hernandez-Indal L. Z., Perez P., Garcia-Gravalos D.M., (2002). A comparison between two brine shrimp assays to detect *in vitro* cytotoxicity in marine natural products. *BMC Biotechnology 2002* (2):17

Cheng A., Lou Y., Mao B.Y., Lu S., Wang J.L., Chen Y.X., (2007). Plant Terpenoids: Biosynthesis and Ecological functions. *Journal of Integrative Plant Biology* 49(2):179-186

Correa-Royero J., Tangarife V., Duran C., Stashenko E., Mesa-Arango A., (2010). In vitro antifungal activity and cytotoxic effect of essential oils and extracts of medicinal and

aromatic plants against *Candida krusei* and *Aspergillus fumigatus .Brazilian Journal of Pharmacognosy* 20 (5): 734-741
Cushnie T.P.T. & Lamb J.A., (2011). Antimicrobial activity of flavonoids. *International Journal of Antimicrobial Agents* 38 (2) : 99-107

Cushnie T.P.T., & Lamb J.A., (2005). Antimicrobial activity of flavonoids. *International Journal of Antimicrobial Agents* 26 : 343-356

Dabai T.J., Zuru A.A., Hassan L.G., Itodo A.U., Wara S.H., (2013) Phytochemical screening of crude extracts of *Amblygocarpus* and *Sclerocarya birrea. International Journal of Traditional and Natural Medicines* 2 (1): 1-8

Dave H., & Ledwani L.A., (2012). A review on anthraquinones isolated from *Cassia* species and their applications. *Indian Journal of Natural Products and Resources* 3: 291-319

Defour G., (1994). Plantes medicinales traditionnelles au Kivu (Republic du Zaire). Documentation du Sous-Reseau prelude.

Delfeld M., & Delfeld N., (2007). Plants for use. http://plants for use.com, accessed on 9/6/2014.

Dib A.E.M., Allali H., Bendiabdellah A., Melliani M., Tabti B., (2013). Antimicrobial activity and phytochemical screening of *Arbutus unedo* L. *Journal of Saudi Chemical Society* 17:381-385

Dismukes E.W., (2000). Introduction to antifungal drugs. *Clinical Infectious Diseases* 30: 653-657

Donnely J., & Maertens J., (2013). The End of the Road for Empirical antifungal treatement.*The Lancet* 13: 470-472

Doss A., Mubarack M.H., Dhanabalan R., (2009). Antibacterial activity of tannins from the leaves of *Solanum trilobotum* Linn. *Indian Journal of Science and Technology* 2(2):41-43

Doughari J.H., & Nuya S.P., (2008). In vitro antifungal activity of *Deterium microcarpum* . *Pakistan Journal of Medical Sciences* 24(1): 91-95

Doughari J.H., (2006). Antimicrobial activity of *Tamarindus indica* Linn. *Tropical Journal of Pharmaceutical Research* 5 (2): 597-603

Dulger B., & Hacioglu N., (2008). Antifungal activity of endemic *Salvia tigrina* in Turkey. *Tropical Journal of Pharmaceutical Research* 7 (3): 1051-1054

Duffalo L.M., (2006). Fungal opportunistic infections in HIV disease. *Journal of Pharmacy practice* 19 (3): 17-30

Dushimemaria F., Mumbengegwi R.D., Preeze D.I., Bock R.,(2012).Qualitative phytochemical screening and in in vitro antimicrobial effects of plant extracts of *Searsia tenuinervis. Journal of Research in Microbes* 1(2) : 088-095

Dzomba P., & Muchanyereyi N., (2012). Potential antimicrobial plant extracts based therapeutics from *Temnocalyx obovatus* roots. *European Journal of Medicinal Plants* 2 (3): 209-215

Dzoyem P.J., Tangmouo G.J., Lontsi D., Etoa X.F., Lohoue P., (2007). In vitro antifungal activity of extract and Plumbagin from the stem bark of Diospyros crassiflora Hiern (Ebenaceae) *Phytotherapy Research* 21 : 671-674

Ellof J.N., (2004). Quantification of the bioactivity of plant extracts during screening and bioassay guided fractionation . *Phytomedicine* 11: 370-371

Engel R., Ghani I., Montenegro D., Thomas M., Klaritch-Vrana B., Castano A., Friedman L., Leb J., Rothman L., Lee H., Capodiffero C., Ambinder D., Cere E., Awad C., Sheikh F., Rizzo J., Nisbett L., Testani E., Melkonian K., (2011). Polycationic Glycosides. *Molecules* 2011 (16):1508-1518

Ernts E.J., (2001). Investigational antifungal agents. *Pharmacotherapy* 21(s): 165-174

Espinel-Ingroff A.,& Flynn T., (1996). Antifungal activity of the new triazole voriconazole against established, new and emerging yeast pathogens: a comparative study. *Antimicrobial Agents and Chemotherapy,* 114 : 15-18

European Committee on Antimicrobial Susceptibility Testing (2013). Eucast disk diffusion method for antimicrobial susceptibility testing. European Society of Clinical Microbiology and Infectious diseases.

Ezea C.S., Inyanya I.S., Odukoya O., Okoye C.B.F.,(2008). Antimicrobial evaluation of crude methanolic extracts and fractions of *Prosopis Africana* stem bark. *Planta medica* 74.

Fabricant S.D., & Farnsworth N.R., (2001). The value of plants used in Traditional Medicine for Drug Discovery. Reviews in Environmental health*, Environmental Health Perspectives* 109 (1): 69-75.

Fawole, O.A., Finnie, J.F., Van Staden J., (2008). Antimicrobial activity and mutagenic effects of twelve traditional medicinal plants used to treat ailments related to the gastro-intestinal tract in South Africa. *South African Journal of Botany* 10.1016

Filgueiras C.C., Farias S.R.P., Cardoso G.M., Vendramin D.J., Rasmos S.L.M.E., Cantos O.R.F., (2011). Bioactivity of aqueous extracts of *Clibadium sylvestre* (Aubl.) Baill. and *Derris amazonica* Killip on the aphid Myzus persicae (Sulzer, 1776)(Hemiptera: Aphididae). *Cienc Agrotec Larvras*. 35 (6): 1059-1066

Francis G., Kerem Z., Makkar S.P.H., Becker K., (2002). The biological action of saponins in animal systems: A review. *British Journal of Nutrition* 88:587-605

French N., Gray K., Watera C., Nakiyingi J., Lugada E., Moore M., Lalloo D., Whitworth J.A., Gilks C.F., (2002). Cryptococcal infection in a cohort of HIV-1-infected Ugandan adults. *AIDS* 16(7):1031-1038

Galluci N., Oliva M., Carezzano E., Zygallo J., Demo M., (2010). Terpenes antimicrobial activity against slime producing and non-producing staphylococci. *Molecular Medicinal Chemistry* 21:132-136

Gooday G.W., (1993). Cell envelope diversity and dynamics in yeasts and filamentous fungi. *Journal of Applied Bacteriology Symposium Supplement* 74: 12-20

Gonzalez-Tejero M.R., Molero-Mesa J., Casares-Porcel M., Lirola M.J.M., (1995). *Journal of Ethnopharmacology* 45 (3):157-163

Goldblatt P., (1996) Flora of Tropical East Africa 1.

Goldblatt, P., (1990). Phylogeny and Classification of Iridaceae. *Annals of Missouri Botanical garden* 77 (4):607-627 .

Goldblatt P., and Mannington J. C., (2000). The Iris family: Natural History and Classification. Timber press.

Grassman J., (2005). Terpenoids as plant antioxidants. *Vitamins and Hormones Journal* 72 : 505-53

Harborne, J. B. (2002) Phytochemical Methods: *A Guide to Modern Techniques of Plant Analysis*. Chapman & Hall, London. 295.

Harborne, J. B. (1998) *Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis*. Chapman & Hall, London. 295.

Harborne J.B & Williams A.C., (2000). Advances in flavonoid research since 1992. *Phytochemistry* 55(2000): 481-504

Hassanpour S., Maheri-Sis N., Eshratkhah B., Mehmandar B.F., (2011). Plants and secondary metabolites (Tannins): A review . *International Journal of Forest, Soil and Erosion* 1(1):47-53

Herbrecht R., Waller J., Dufour P., Koenig H., Lioure B., Marcellin L., Oberling F., (1992). Rare opportunistic fungal diseases in patients with organ or bone marrow transplantation. *Agressologie* 33 (2s): 77-80

Hernandez E.N., Tereschuk M.L., Abdala L.R., (2000). Antimicrobial activity of flavonoids in medicinal plants from Tafi´ del Valley (Tucuma´ n , Argentina). *Journal of Ethnopharmacology* 73(2000):317-322

Houghton, M., (2013). The American Heritage Dictionary of English, 5th edition. Houghton Mifflin Harcourt Publishing Company, America.

Hudzicki J., (2013). Kirby-Bauer Disk Diffusion Succeptibility Test Protocol. *American Society of Microbiology*

http://www.merriam-webster.com/dictionary/anthraquinone, accessed on 12/10/2014

Hutchings, A., & Staden J.V., (1994). Plants used for stress related ailments in traditional Zulu, Xhosa, and Sotho medicine. Part 1: plants used for headaches. *Journal of Ethnopharmacology* 43: 89-124

Igbinosa O.O., Igbinosa E.O., Aiyegoro O.A., (2009). Antimicrobial activity and phytochemical screening of stem bark extracts from Jatropha curcas (Linn). *African Journal of Pharmacy and Pharmacology* 3 (2) : 058-062

Ishaq S.M., Hussain M.M., Afridi S.M., Khattak M.A.G., Ahmad S., Shakirullah., (2014). In Vitro Phytochemical, Antibacterial and Antifungal activities of leaf, stem and root extracts of *Adiantum capillus veneris.The ScientificWorld Journal.*1-7

Ishida K., Rozental S., Mello P.C.J., Nakamura V.C., (2009). Activity of tannins from *Stryphnodendron adstringens* on *Cryptococcus neoformans* : effects on growth, capsule size and pigmentation.*Annals of clinical Microbiology and Antimicrobials* 8 (29) : 8-29

Jain D., Najjar M., Azher Q., Bachuwa G., (2013). Cryptococcal sterna osteomyelitis in a healthy woman: A review of *Cryptococcus neoformans*. *BMJ case rep* 2013

Kariba M. R., (2000). Antifungal activity of extracts from selected Kenyan medicinal plants. Ph.D thesis, University of Nairobi, Nairobi, Kenya.

Khoshkholgh-Pahlaviani R.M., Massiba R.A., Issazadeh K., Bidarigh S., Giahi M., Ramtin M., (2013). Evaluation of Antifungal Activity of Methanol Extracts of Acacia (*Anagalis arvensis*) leaves and Nystatin against *Candida albicans* in vitro. *Zahedan Journal of Research in Medical Sciences* 15 (8) : 39-41

Khosravi A.R., Minooeianhaghighi M.H., Shokri H., Emami S.A., Alavi S.M., Asili J., (2011). The potential inhibitory effect of *Cuminum Cyminum, Ziziphora clinopodioides* and *Nigella sativa* essential oils on the growth of *Aspergillus fumigatus* and *Aspergillus flavus. Brazilian Journal of Microbiology* 42 : 216-224

Kokwaro J.O., (2009). Medicinal plants of East Africa. East Africa 3rd .Ed. Literature Bureau, Nairobi

Kouam J., Mabeku B.K.L., Kuiate R.J., Tiabou T.A., Fomum T.Z., (2011). Antimicrobial glycosides and derivatives from roots of *Picralima nitida*. *International Journal of Chemistry* 3(2):23-31

Krvaych A.S., Konechna R.T., Petrina R.O., Kyrka M.S., Zayarnuk N.L., Gulko R.M., Stadnytska N.E., Novikov V.P., (2014). Phytochemical research of plant extracts and use *in vitro* culture in order to preserve rare wild species of *Gladiolus imbricatus*. *Research Journal of Pharmaceutical, Biological and Chemical Sciences* 5(1):245

Kubo, I. & Taniguchi M., (1993). Ethnobotanical drug discovery based on medicinemen's trial in the African Savanna; screening of East African plants for antimicrobial activity II. *Journal of natural products* 56 (9):1539-1546.

Kuete V., Kamga J., Sandjo P.L., Ngameni B., Poumale H.M.P., Ambassa P., Ngadjui T. B., (2011). Antimicrobial activities of the methanol extracts, fractions and compounds from *Ficus polita* Vahl (Moraceae).*BMC Complementary and Alternative Medicine* 11 (6)

Kuete V., Nana F., Ngameni B., Mbaveng A.T., Keumedjio F., Ngadjui B.T., (2009). Antimicrobial activity of the crude extract, fractions and compounds from stem bark of Ficus ovate (Moraceae). *Journal of Ethnopharmacology* 124 (3) : 556-561

Kulkarni S.H., (2013).American Thoracic Society, Patient information Series, Aspergillosis; fungal disease series 4. *American Journal of Respiratory and Critical care medicine* ; 186:1-2.

Kumar P., Sharma B., Bakshi N., (2009). Biological activities of alkaloids from *Solanum dulcamara* L. *Natural Product Research* 23 (8): 719-723

Kumari K., & Gupta S., (2014). Antifungal potentiality of *Polyalyhia longifolia* leaf extracts. *International Journal of Research in Pure and Applied Microbiology* 4 (1):7-9

Latte K.P., & Kolodziej H., (2000). Antifungal effects of hydrolysable tannins and related compounds on dermatophytes, mould fungi and yeasts. *Zeitschrift für Naturforschung C* 55 (5-6) : 467-472

Lodonkar R.L., Kattegouga U.M., Shivsharanappa K., Hanchinalmath J.V., (2013). Phytochemical screening and in vitro antimicrobial activity of *Typha angustifolia* Linn leaves extract against pathogenic gram negative micro organisms. *Journal of Pharmacy Research* 6: 280-283

Mahlke D.J., Boligon A.A., Machado M.M., Spader B.T., Alves H.S., Canto-Dorow T., Athayde L.M., (2009). In vitro antimicrobial and antioxidant activities of a crude extract and fractions from *Buddleja thyrsoides* Lam. Leaves. *Quim Nova* 32 (2): 277-281

Manmoon A.S., & Azam G.M., (2011). Diuretic activity and brine shrimp toxicity of *Derris trifoliata* Lour. *International Journal of Pharmacy and Life sciences* 2(9):1029-1032

Mansouri S., Foroumadi A., Ghaneie T., Najar G.A., (2001). Antibacterial activity of the crude extracts and fractionated constituents of *Myrtus communis*. *Pharmaceutical Biology* 39(5):399-401

Marcos-Arias C., Eraso Elena., Madariaga L., Quindos G., (2011). In vitro activities of natural products against oral *Candida* isolates from denture wearers. *BMC Complementary and Alternative Medicine* 11: 119

Mathur A., Singh R., Yousuf S., Bhardwaj A., Verma K.S., Babu P., Gupta V., Prasad G.B.K.S., Dua V.K., (2011). Antifungal activity of some plant extracts against clinical pathogens. *Advances in Applied Science Research* 2 (2):260-264

Martins S., Amorim E.L.C., Sobrinho P.J.S.T., Saraiva M.A., Pisciottano C.N.M., Aguilar N.C.,Teixeira A.J., Mussato I.S., (2013). Antibacterial activity of crude methanolic extrct and fractions obtained from *Larrea tridentata* leaves. *Industrial Crops and Products* 41:306-311

Martinez A., Rojas N., Garcia L., Gonzalez F., Dominiquez M., Catalan A., (2014) In vitro activity of terpenes against *Candida albicans* and ultrastructural alterations. *Journal of Oral Surgery, Oral Medicine, Oral pathology Oral Radiology.* Accepted Manuscript in press.

Mbwambo, Z.H., Moshi, M.J., Masimba P.J., Kapingu, M.C., Nondo, R.S.O., (2007). Antimicrobial activity and brine shrimp toxicity of extracts of *Terminalia brownii* roots and stem. *BMC Complimentary and Alternative Medicine*, **7**: 9

Mensah J.K. , Okoli R I., Turay ,A. A. ,Ogie-Odia E.A.,(2009) .Phytochemical Analysis of Medicinal Plants Used for the Management of Hypertension by Esan people of Edo State, Nigeria. *Ethnobotanical leaflets 13:* 1273-1287

Mert-Turk F., (2006). Saponins versus plant fungal pathogens. *Journal of Cell and Molecular Biology* 5: 13-17

Mills-Robertson F. C., Tay K.C.S., Duker-Eshun G., Walana W., Badu K., (2012). In Vitro antimicrobial activity of ethanolic fractions of *Cryptolepis sanguinolenta*. *Annals of Clinical Microbiology* 11: 16

Misra S.C., Pratyush K., Lipin D.M.S., James J., Veettil T.K.A., Thankamani V., (2011). Acomparative study of phytochemical screening and antebacterial activity of roots of *Alstonia scholaris* with the roots, leaves and stem barks. *International Journal of Research in Phytochemistry and Pharmacology* 1 (2): 77-82

Mohamed S.S., Hansi H.P.D., Kavitha T., (2010). Antimicrobial activity and phytochemical analysis of selected Indian folk medicinal plants. *International Journal of Pharma Sciences and Research* 1(10):430-434

Mohamed M.K., (2005). Chemical constituents of *Gladiolus segetum* Ker-Gawl. *Bulletin of Pharmaceutical sciences* 28(1):71-78

Moundipa P.F., Melanie K.G., Bilong B., Bruchhau I.,(2005). In vitro amoebicidal activity of some medicinal plants of Bamun Region (Cameroon)..*African Journal of Traditional, Complementary and Alternative Medicines* 2 (2): 113-121

Moreira P.C.A., Lima O.E., Wanderley A.P., Carmo S.E.,Souza L.E., (2010). Chemical Composition and antifungal activity of *Hyptis suaveolens* (L.) Poit leaves essential oil against *Aspergillus* species . *Brazilian Journal of Microbiology* 41:28-33

Moshi J.M., Mbwambo H.Z., Kapingu C.M., Mhozya H.V., Marwa C., (2006). Antimicrobial and Brine shrimp lethality of extracts of *Terminalia mollis* Laws. *African Journal of Traditional, Complementary and Alternative Medicines* 3 (3): 59-69

Moshi M.J., (2005). Current and future prospects of integrating traditional and medicine alternative medicine in the management of diseases in Tanzania. *Tanzania Health Research Bulletin* 7(3): 159:67

Montanher P.B.A., Pizzolatti G.M., Brighente C.M.I., (2002). An application of the Brine shrimp Bioassay for general screening of Brazilian Medicinal plants. *Acta farm, Bonaerense* 21(3):175-178

Munyemana F., (2013). Qualitative phytochemical screening and antimicrobial activity evaluation of bulb extracts of *Gladiolus psittacinus* Hook (Iridaceae). *International Network Environmental Management conflicts Santa Catarina – Brasil,*2 (1): 14-31

Mushi F.N., Mbwambo Z.H., Innocent E., Tewtrkul S., (2012) Antibacterial, anti-HIV-1 protease and cytotoxic activities of aqueous ethanolic extracts from *Combretum adegonium* Steud. Ex A. Rich (combretaceae). *BMC complementary & Alternative medicine* 12 : 163

Musila M.F., Dossaji S.F., Nguta J.M., Lukhoba C.W., Munyao J.M., (2013). In vivo antimalarial activity , toxicity and phytochemical screening of selected antimalarial plants. *Journal of Ethnopharmacology* 2013(146): 557-561.

Nenaah G., (2013). Antimicrobial activity of *Calotropis procera* Ait.(Asclepiadaceae) and isolation of four flavonoid glycosides as the active constituents. *World Journal of Microbiology and Biotechnology* 29 (7): 1255-1262

Ngamga D., Awouafack M.D., Tane P., Bezabih M., Abegaz B.M., (2007). Two new anthraquinones from *Gladiolus psittascinus*. *Biochemical Systematics and Ecology* 35:709-713

Nguedia A.C., Etoa X.F., Beng P.V., Lontsi D., Kuete V., Moyou S.R., (2004). Anti-candidal property and acute toxicity of *Gladiolus gregasius* Baker (Iridaceae). *Pharm Med Trad Africa*13:149-159

Nguta J.M., Mbaria J.M., Gakuya D.W., Gathumbi P.K., Kabasa J.D., Kiama S.G., (2012). Evaluation of acute toxicity of crude plant extracts from Kenyan Biodiversity using Brine shrimp, *Artemia salina* L.(Artemiidae). *The open conference proceedings Journal* 3:30-34

Nino J. Mosquera M.O., Correa M.Y., (2012). Antibacterial and antifungal activities of crude plant extracts from Colombian biodiversity. *International Journal of Tropical Biology* 60 (4):1535-1542

Njume C., Jide A.A., Ndip N.R., (2011). Aqueous and organic solvent extracts of selected South African Medicinal Plants Posses Antimicrobial activity against Drug resistant strains of Helicobacter pylori: Inhibitory and bacteriocidal potential .*International Journal of Molecular Sciences* 12:5652-5665

Ochepke N.A., Ameh S., Okoliko I., Olorunfemi., (2009). Antimicrobial and phytochemical properties of aqueous extracts of *Gladiolus* corm (Iridaceae) from Benue State of Nigeria. *Journal of Pharmacy and Bioresources* 6(2):65-70

Odhiambo J., Dossaji S., Lukhoba W., Yenesew A., (2014). Antifungal activity, brine shrimp cytotoxicity and phytochemical screening of *Gladiolus watsonoides* Baker (Iridaceae). *Journal of Pharmacy Research* 8 (9): 1218-1222

Odhiambo J., Dossaji S., Lukhoba W., Yenesew A., (2014). Phytochemical screening of *Dierama cupuliflorum* Klatt. (iridaceae). *Journal of Pharmacy Research* 8 (4): 589-592

Odhiambo, J.A., Siboe, G.M., Lukhoba, C.W., and Dossaji, S.F., (2010). Antifungal activity of crude extracts of *Gladiolus dalenii* Van Geel (Iridaceae). *African Journal of Traditional Complementary* and *Alternative Medicine* 7 (1): 53 - 58

Odhiambo J.A., Siboe G.M., Lukhoba C.W., Dossaji S.F., (2009). Antifungal activity of crude extracts of selected Medicinal plants used in combinations in Lake Victoria Basin, Kenya. *Plant Product Research Journal* 13 : 35-43

Ogu G.I., Tanimowo W.O., Nwachukwu U.P., Igere B.E., (2012). Antimicrobial and phytochemical evaluation of the leaf, stem bark and root extracts of *Cyathula prostrata* (L) Blume against some human pathogens. *Journal of Intercultural Ethnopharmacology* 1 (1):35-43

Ogunleye D.S., & Ibitoye S.F., (2003). Studies of antimicrobial activity and chemical constituents of *Ximenia americana*. *Tropical Journal of Pharmaceutical Research* 2(2):239-241

Okemo P.O., Bais P.H.,Vivanco M.J., (2003). In vitro activities of *Maesa lanceolata* extracts against fungal plant pathogens. *Fitoterapia* 74 : 312-316

Okoh-Esene, Uchechukwu R., Husseini S.J., Asuquo T.S.,(2011). Proximate and phytochemical analysis of leaf, stem and root of *Euginia uniflora* (Surinam or Pitanga Cherry).*Journal of Natural Products and Plant Resources* 1(4): 1-4

Olowa F.L., & Nuneza M.O., (2013). Brine shrimp lethality assay of the ethanolic extracts of three selected species of medicinal plants from Iligan City, Philippines. *International Research Journal of Biological Sciences* 2(11):74-77

Orhan D.D., Ozcelik B., Ozgen S., Ergun F., (2010). Antibacterial, antifungal and antiviral activities of some flavonoids. *Microbiological Research* 165: 496-504

Otang W.M, Grierson D.S, Ndip R.N, (2012). Phytochemical studies and antioxidant activity of two South African medicinal plants traditionally used for the management of opportunistic fungal infections in HIV/AIDS patient. *BMC Complementary and Alternative Medicine*, 12:43

Palazo I.C., Rheder A., Darini A.L., (2007). Quantitative disk diffusion as a convenient method for determining minimum inhibitory concentrations of oxallin for staphylococci strains. *Journal of Microbiological methods* 71(3):186-190

Palombo, E.A (2006). Phytochemicals from Traditional Medicinal Plants used in the Treatment of Diarrhoea: Modes of Action and Effects on Intestinal Function. *Phytotherapy Research* 20: 717–724.

Parekh J., & Chanda S., (2007). Antibacterial and phytochemical studies on twelve species of Indian medicinal plants . *African Journal of Biomedical Research* 10:175-181

Pemmaraju S.C., Pruthi P.A., Prasad R., Pruthi V., (2013). *Candida albicans* biofilm inhibition by synergistic action of terpenes and fluconazole. *Indian Journal of Experimental Biology* 51:1032-1037

Perfect R.J., Dismukes E.W., Dromer F., Goldman L. D., Graybill R.J., Hamill J.R., Harrison S.T., Larsen A.R., Lortholary O., Nguyen M., Pappas G.P., Powderly G.W., Singh N., Sobel D.J., Sorrell C.T., (2010). Clinical practice Guidelines for the Management of Cryptococcal Disease: 2010 update by the Infectious Diseases Society of America. *Clinical Infectious Diseases* 2010 (50): 291-322

Pfaller A.M., (2012). Antifungal Drug Resistance; Mechanisms, Epidermiology and Consequences of treatment. *The American Journal of Medicine* 125 (s):3-13

Phillipson D.J., (2001). Phytochemistry and Medicinal plants. *Phytochemistry* 56 (2001):237-243

Prabhu K., Karar P.K., Hemalatha S., Ponnudurai K., (2011). A comparative preliminary phytochemical screening on the leaves, stems and the roots of three *Viburnum* Linn. Species. *Der Pharmacia Sinica* 2 (3):81-93

Praveen M., Radha K., Hari K.R., Padmaja V., Mathew A., Kumar A.P., (2012). Preliminary phytochemical , antimicrobial and toxicity studies on *Clerodendrum paniculatum* Linn Leaves.

Raju J., & Rao V. C., (2012). Diosgenin, steroid saponin constituent of Yams and Fenugreek: Emerging evidence for applications in medicine, Bioactive compounds in phytomedicine, Iraj R., Ed. ISBN:978-953-307-805-2, In Tech, Shangai. *Hygeia Journal for Drugs and medicines* 4(1):41-50

Ramamoorthy T.K.P., Shetty L.H.R., Devidas S., Mudduraj T.V., Vinayaka S.K., (2012). Antifungal and cytotoxic activity of Everniastrum cirrhatum (Fr.) Hale. *Chiang Mai Journal of Science* 39(1):76-83

Rao A., Zhang Y., Muend S., Rao R., (2010). Mechanism of antifungal activity of terpenoid phenols resembles calcium stress and inhibition of the TOR Pathway. *Antimicrobial Agents and Chemotherapy* 54(12):5062-5069

Ranjan p., Bhat, K.V., Misra R. L, Singh, S.K., Ranjan, J.K., (2008). Genetic relationships of gladiolus cultivars inferred from fluorescence based AFLP markers. *Scientia Horticulture* 562-567.

Riaz T., Khan S.N., Javaid A., (2007). Scenario of *Gladiolus* production in Punjab, Pakistan. *Pakistan Journal of Botany* 39 (7): 2389-2393.

Roberts M.F., & Wink M.,(1998). Alkaloids: Biochemistry, Ecology and Medicinal Applications pp 1-8. Plenum Press. New York and London.

Roemer T., & Krysan J. D., (2014). Antifungal Drug Development: Challenges, unmet clinical needs and new approaches. *Cold spring Harbor Pespectives in medicine* 2014 (4)

Rolls G., (2011). An Introduction to specimen preparation. Leica Biosystems, Wetlzar, Germany.

Royal Horticultural Society (RHS), (2009).

Rudall & Goldblatt P., (1991) *Botanical Journal of Linnean Society* 106: 329–345

Ruiz M.G.M., Richard-Greenblatt M., Juarez N.Z., Av-Gay Y., Bach H., Hernandez R.L., (2012). Antimicrobial, anti-inflammatory, antiparasitic and cytotoxic activities of *Laennecia confuse*. *The Scientific world Journal*

Runyoro D., Matee M., Ngassapa O., Joseph C., Mbwambo Z.,(2006).Screening of Tanzanian medicinal plants for anti-candida activity.*BMC Complementary and Alternative Medicines* 30; (1) 6

Sadeghi-Nejad B., Shiravi F., Ghanbari S., Alinejadi M., (2010). Antifungal activity of *Satureja khuzestanica* (Jamzad) leaves extracts. *Jundishapur Journal of Microbiology* 3 (1):36-40

Saag M.S.,(1997).Cryptococcosis and other fungal infections (Histoplasmosis, coccidioidomycosis) pp. 327-342 in the Management of AIDS. Eds Merle, A.; Sande, M.D; Paul, A.; Volberding, M.DW.D Saunders Company. London.

Safiullah, & Ahmed, M.J., (2001), "Evaluation of exotic caltivars of *Gladiolu*s under Rawalakot conditions," *Sarhad Journal of Agriculture* 17(2):171-174.

Saha S., Walia S., Kumar J., Parmar B.S., (2010). Structure-biological activity relationships in triterpenic saponins: the relative activity of protobassic acid and its derivatives against plant pathogenic fungi. *Pest Management Science* 66:825-831

Sahgal G., Ramanathan S., Sasidharan S., Mordi N.M., Ismail S., Mansor M.S., (2010). Brine shrimp lethality and acute toxicity studies on *Swietenia mahagoni* (Linn.) Jacq. Seed methanolic extract. *Pharmacognosy Research* 2(4):215-220

Salim A.A., Chin Y-W, Kinghorn A.D., (2008) Drug discovery from plants In: Bioactive Molecules and Medicinal plants, Ramawat KG, Merillon JM, eds. *Springer* : 1-4.

Samejo Q.M., Sumbul A., Shah S., Memon B.S.,(2013). Phytochemical screening of *Tamarix dioica* Roxb. Ex Roch. *Journal of Pharmacy Research* (7): 181-183

Sanches C.C.A., Lopes C.G., Nakamura V.C., Filho D.P.B., De mello P.C.J., (2005). Antioxidant and antifungal activities of extracts and condensed tannins from *Stryphnodendron obovatum* Benth. *Brazilian Journal of Pharmaceutical Sciences* 41(1)

Saravanakumar A., Venkateshwaran K., Vanitha J., Ganesh M., Vasudevan M., Sivakumar T., (2009). Evaluation of antibacterial activity, phenol and flavonoid contents of *Thespesia populnea* flower extracts. *Pakistan Journal of Pharmaceutical Sciences* 22(3):282-286

Sawadogo R.W., Schumacher M., Teiten H.M., Dicato M., Diederich M.,(2012) Traditional West African Pharmaopeia, plants and derived compounds for cancer therapy. *Biochemical pharmacology* 84 : 1225-1240

Selvamohan T., Ramadas V., Kishore S.S.S.,(2012)Antimicrobial activity of selected medicinal plants against some selected human pathogenic bacteria. *Advances in Applied Science Research* 3 (5):3374-3381

Sen A. & Batra A., (2012). Evaluation of activity of different solvent extracts of medicinal plant: *Melia azedarach* L. International Journal of Current Pharmaceutical Research 4 (2):67-73

Serrano M.C., Ramírez, M., Morilla D., Valverde A., Chávez M., Espinel-Ingroff A., Claro, R., Fernández A., Almeida C., Martín-Mazuelos E., (2004). A comparative study of the disc diffusion method with the broth microdilution and Etest methods for voriconazole susceptibility testing of *Aspergillus* spp. *Journal of Antimicobial Chemotherapy* 53 :739-742

Shakeri A., Hazeri N., Vlizadeh J., Ghasemi A., Tavallaei Z.F., (2012). Phytochemical screening , Antimicrobial and Antioxidant activities of *Anabasis aphylla* L. extracts. *Kragujevac Journal of Science* 34: 71-78

Sharma, S.C., Sharga, J.C., Bhutani, and Ohri, D., (1988), *Gladiolus* .Bulletin economic botany information service, National Botanical Research Institute, Lucknow, India.

Sheh-Hong L., Kassim J., Ibrahim D., Sulaiman S., (2011). Antimicrobial and antioxidant activities of condensed tannin from *Rhizophora apiculata* barks. *Journal of chemical and pharmaceutical Research* 3(4):436-444

Silva N.C.C., & Fernandes J.A., (2010). Biological properties of medicinal plants: a review of their antimicrobial activity. *Journal of Venomous Animals and Toxins including Tropical diseases* 16 (3):402-413

Singh R.A., Bajaj K.V., Sekhawatt S.P., Singh K., (2013). Phytochemical estimation and antimicrobial activity of aqueous and methanol extract of *Ocimum sanctum* L. *Journal of natural products and plant resource* 3(1):51-58

Siqueira Q.F.C., Cabral V.L.D., Sobrinho P.S.J.T., Amorim C.L.E., Mello G.J., Araujo S.A.T., Albuquerque P.U., (2011). Levels of tannins and flavonoids in medicinal plants. Evaluating bioprospecting strategies. *Evidence-Based Complimentary and Alternative medicine* 2012.

Smith I. & Feinberg J.G., (1965). Paper & Thin Layer Chromatography and Electrophoresis. A teaching Manual, 2nd Edition.Bell and Bain Ltd, London, England.

Souto A. L., Tavares F.J., Da Silva, S.M., Diniz M.F.F.M., Athayde-Filho F.P., Filho B.M.J., (2011). Anti-inflammatory activity of alkaloids: An update from 2000 to 2010. *Molecules* 16:8515-8534

Sparg S.G., Light M.E., Staden J.V., (2004). Biological activities and distribution of plant saponins. *Journal of Ethnopharmacology* 94 (2): 219-243

Tadesse D., (1994). Traditional use of some medicinal plants in Ethiopia ; proceedings of the 13th plenary meeting of AETFAT, Zombia, Malawi 1: 273-293

Taleb-Contini S.H., Salvador M.J., Watanabe E., Ito I.Y., Oliveira D. C.R., (2003). Antimicrobial activity of flavonoids and steroids isolated from two *Chromolaena* species . *Brazilian Journal of Pharmaceutical Sciences* 39 (4)

Talibi I., Karim., Askarne L., Boubaker H., Boudyach E.I.H., Msanda F., Saadi B., Aoumar A.A.B., (2013). Antifungal activity of aqueous and organic extracts of eight aromatic and medicinal plants against *Geotrichum candidum*, causal agent of citrus sour rot. *International Journal of Agronomy and Plant Production* 4 (S): 3510-3521

Tangarife-Castano V., Correa-Royero J., Zapata- Londono B., Duran C., Stanshenko E., Messa-Arango A.C.,(2011). Anti-*candida albicans* activity, cytotoxicity and interaction with antifungal drugs of essential oils and extracts from aromatic and medicinal plants. *Infectio* 15 (3): 160-167

Thakur M., Melzig F.M., Fuchs S., Weng A., (2011). Chemistry and Pharmacology of saponins; special focus on cytotoxic properties. Botanics: *Targets and Therapy* 1: 19-29

Thippeswamy S., Mohana C.D., Manjunath K., (2012). Screening in vitro antifungal activity of some Indian medicinal plants against *Candida albicans* and *Cryptococcus neoformans. International Journal of Current Research* 4 (3) : 037-042

Torkarski C, Hummert S, Mech F, ThiloFigge M, Germrodt S, Shroeter A, Schuster S, (2012). Agent-based modelling approach of immune defence against spores of opportunistic human pathogenic fungi. *Frontiers in Microbiology* 3: 1-13

Tsuzuki J.,Svidzinski T.I.E., Shinobu C.S., Silva L.F.A., Rodrigues-Filho E., Cortez D.A.G., Ferreira I.C.P., (2007). Antifungal activity of the extracts and saponins from *Sapindus saponaria. Annals of Brazilian academy of Sciences* 79 (4): 577-583

Ul-Haq I., Mannan A., Ahmed I., Hussain I., Jamil A.M., Mirza B., (2012). Antibacterial activity and brine shrimp toxicity of *Artemisia dubia* extract. *Pakistan Journal of Botany* 44(4):1487-1490

Vandeputte P., Ferrari S., Coste T.A., (2012). Antifungal resistance and new strategies to control fungal infections. *International Journal of Microbiology* 2012

Ved H.C., More S.N., Bharate S.S., Bharate S.B., (2010) Cytotoxicity, screening of selected Indian medicinal plants using Brine-shrimp lethality bioassay.*Advances in Natural and Applied Sciences 4(3):389-395*

Vijayakumar S., & Amirthanathn A., (2014). Bioactivity of sea grass against the malarial fever mosquito *Culex quinquefasciatus*. *Asian Pacific Journal of Tropical Disease* 4 (4):287-291

Viladomat F., Codina C., Llabres M.J., Bastida J., (1986). Alkaloid screening of plants of Catalonia (Spain) III. *International Journal of crude drug research* 23: 105-117

Wakdikar S., (2004) Global health care challenge: Indian experiences and new prescriptions. *Journal of Biotechnology* 7 :3

Walsh J.T., Annaissie J.E., Denning W.D.,Herbrecht R., Kontoyiannis P.D., Burik V.J., Winghard R.J.,Patterson F.T., (2008).Treatment of Aspergillosis: Clinical practice, Guidelines of the infectious Diseases Society of America. *Clinical InfectiousDiseases :* 46 (3): 327-360

Wang, D.Y., Ye Q., Li B.G., Zhang G.L., (2003). A new anthraquinone from *Gladiolus gandavensis. Natural products Research* 17(5): 365-368

Watson L., (1963). The Taxonomic significance of certain anatomical observations on Ericaceae, *Calluna* and *Cassiope.* Department of Botany. Manchester University

Weller G.M.,(2012). A unifying review of Bioassay-Guided fractionation , effect directed analysis and related techniques. *Sensors* (12):9181-9209

Wink M., (2011).Occurrence and function of natural products in plants in Phytochemistry and Pharmacognosy, (Ed) Pezzuto J.M, & Kato J.M.

WHO report, (2011). Rapid Advice, Diagnosis, Prevention and Management of Cryptococcal Disease in HIV-infected Adults, Adolescents and Children. World Health Organization, HIV/AIDS programme; 1-44.

WHO report, (2002).Traditional Medicine Strategy 2002-2005.World Health Organization; 1-74.

Woolley G.J., (2001). Plant alkaloids. *Ecyclopedia of life sciences, Nature publishing group* www.el.net accessed on 12/11/13

Wubert J., Oster U., Rudiger W., (1996) Inhibitors of chlorophyll biosynthesis from bulbs of *Gladiolus spp. Znaturforsch ser c* 51 (7/8): 548-557

Yang C., Zhang Y., Jacob M.R., Khan S.I., Zhang Y., Li X., (2006). Antifungal activity of C-27 steroidal saponins. *Antimicrobial Agents and Chemotherapy* 50(5):1710

Yao L.H., Jiang Y.M., Shi J., Tomas-Barberan F.A., Datta N., Singanusong R., Chen S.S., (2004). Flavonoids in food and their health benefits. *Plant Foods for Human Nutrition* 59(3): 113-122.

Yen G.C., Duh P.D., Chuang D.Y., (2000). Antioxidant activity of anthraquinones and anthrone. *Food chemistry* 70:437-441

Yineger H., Kelbessa E., Bekele T., Lulekal E., (2008). Plants used in traditional management of human ailments at Bale Mountains National Park, Southeastern Ethiopia. *Journal of Medicinal Plant Research.* 2 (6): 132-153.

Zhao H., Zong G., Zhang J., Wang D., Liang X., (2011). Synthesis and anti-fungal activity of seven oleanolic acid glycosides. *Molecules* 2011(16):1113-1128

Zore G.B., Thakre A.D., Jadhav S., Karuppayil S.M., (2011). Terpenoids inhibit *Candida albicans* growth by affecting membrane integrity and arrest of cell cycle. *Phytomedicine* 18 (13):1181-1190

Zohra F.S., Meriem B., Samira S., Muneer A.M.S., (2012). Phytochemical Screening and identification of some compounds from Mallow. *Journal of Natural Product and Plant Resources* 2(4):512-516

APPENDICES

Appendix i: Photographs of plates of fungal cultures in presence of the control drugs and DMSO at various concentrations.

A B C D E

A: A plate culture of *C.albicans* in presence of Amphotericin, B: A plate culture of *C.albicans* in presence of Nystatin, C: A plate culture of *C.neoformans* in presence of Amphotericin B, D: A plate of *A.niger* in presence of Amphotericin B, E:A plate of *A.niger* in presence of Ketoconazole, c: DMSO.

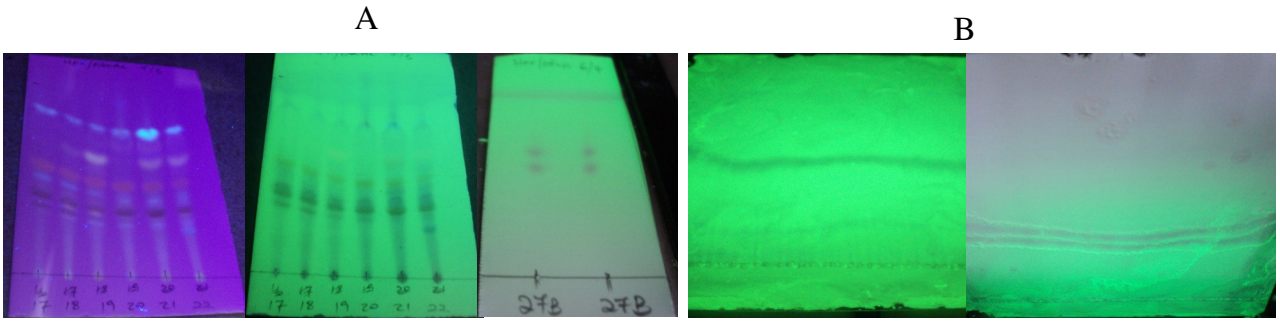
Appendix ii: Yields in (g) of crude organic extracts

Plant Name	Crude extract yield	
	Aerial	Bulb
D.cupuliflorum	9.44	15.39
G.watsonoides	3.01	10.5
G.newii	15.25	19.04
G.ukambanensis	4.43	6.94
G.goetzenii	5.92	7.64

Appendix iii: Amounts in (g) of bulb organic extracts subjected to fractionation process

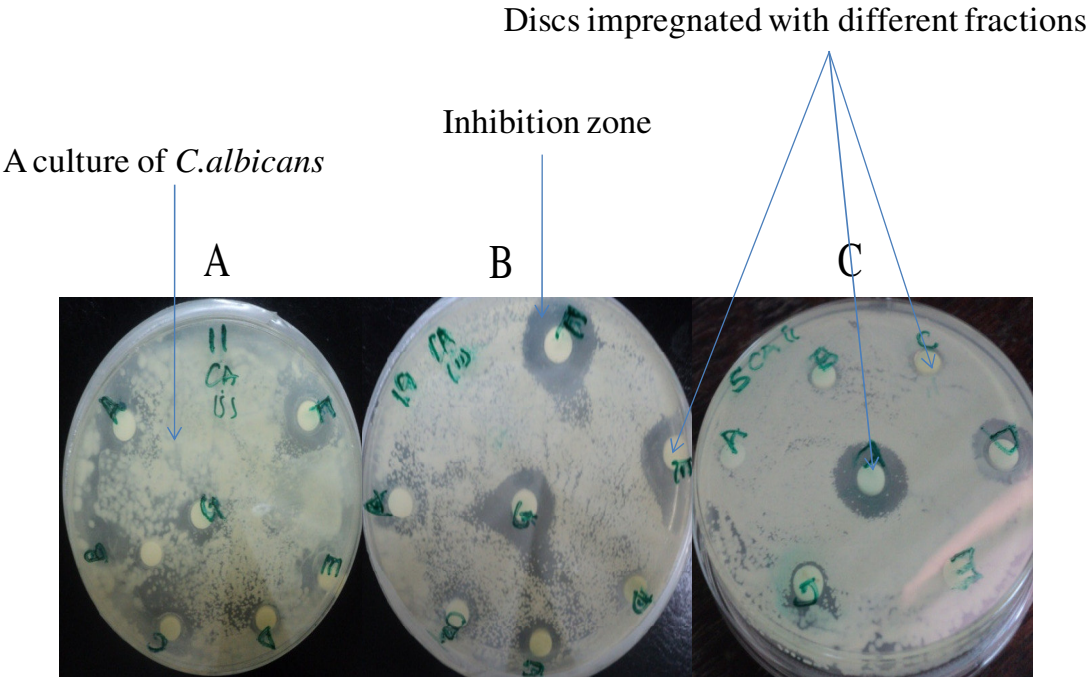
Plant Name	Bulb extract
D.cupuliflorum	100
G.watsonoides	79.58
G.newii	100
G.ukambanensis	18.68
G.goetzenii	35

Appendix iv: A photograph representation of TLC and preparative chromatography



TLC analysis of G.*ukambanensis*, B: Preparative TLC of *G.ukambanensis ;* solvent system hexane/Ethylacetate 7:3

Appendix v: Representative photographs of plates of *C.albicans* cultures in presence of various tested semi purified fractions



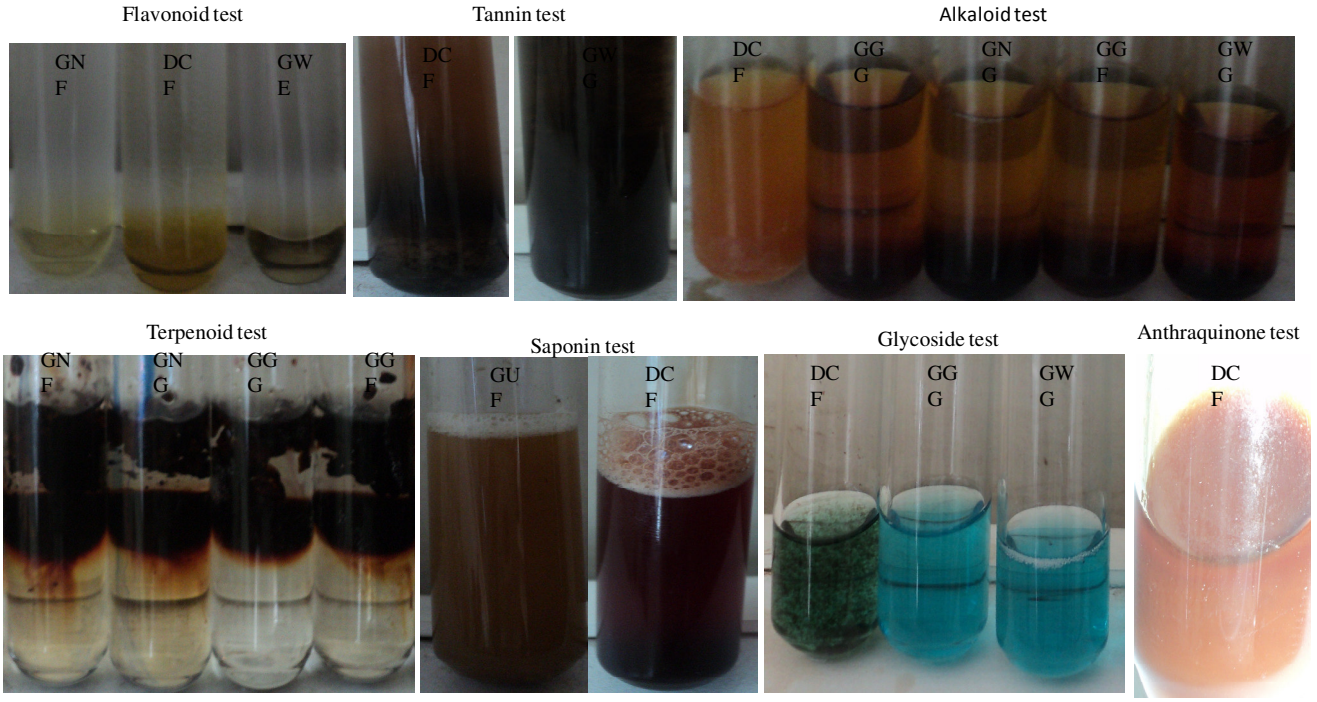
A-100% Hexane; B-DCM : Hexane (1:1); C-100% DCM; D-DCM : EtoAc (1:1) ; E-100% EtoAc ; F-EtoAc : MeoH (1:1) ; G-100% MeoH.

Appendix vi: LD_{50} values and average mortality of Brine shrimp larvae at various concentrations

Plants	Fractions	Concentration(µg/ml)			LD $_{50}$(µg/ml)
		1000	100	10	
G.newii	E	10	6.67	5.33	90.24
	F	10	10	10	5.42
	G	10	10	10	5.42
G.watsonoides	E	10	10	10	5.42
	F	10	10	10	5.42
	G	10	10	10	5.42
D.cupuliflorum	E	10	9.33	8.33	142.91
	F	10	10	10	5.42
	G	10	9.33	9	40.23
G.goetzenii	E	10	9.33	6.33	342.62
	F	10	9	7	191.37
	G	10	10	10	5.42
G. ukambanensis	E	10	9	7.67	191.37
	F	8	7	6.33	440.26
	G	7.67	6.67	5	700.12

Key: E-ethylacetate (100%) ; F-ethylacetate: methanol (1:1) ; Methanol 100%

Appendix vii: Representative photographs of phytochemical presence in the active fractions



Key: DC-*D.cupuliflorum,* GW-*G. watsonoides,* GN-*G. newii*, GU-*G.ukambanensis*, GG-*G.goetzenii* ; E –ethyl acetate fraction (100%) ; F-ethyl acetate:methanol fraction (1:1) ; G-Methanol fraction (100%)

Appendix viii: Phytochemical screening of *Dierama cupuliflorum* Klatt. (Iridaceae)

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Phytochemical screening of *Dierama cupuliflorum* Klatt. (Iridaceae)

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ABSTRACT

Background: Plants continue to play a vital role in their therapeutic value. This is because of the vast secondary metabolites that many of them produce. These natural products have been utilised as single or in combination with other compounds for utilization as source of drugs for many ailments in form of antibacterials, antifungals, antivirals, antihelminthes, and antimalarials among others. Plants evaluated phytochemically in most cases have previous reports on biological activity, ethnomedicinal or traditional medicine usage. However, many other plants with no such previous reports may be as important with variety of natural products with potential significance in pharmaceuticals for drug development. *Dierama cupuliflorum* is one such plant. The aim of the present study was to investigate the presence of phytochemicals in this plant. **Method** : The organic solvent extracts from Methanol: Dichloromethane (1:1) along with dry powder/ground portions from corms and aerial parts were screened for the presence of selected phytochemicals using standard chemical procedures. **Results**: Phytochemical screening revealed the presence of terpenoids, alkaloids, saponins, tannins, flavanoids, glycosides and anthraquinones. More phytochemicals were detected in corms than in the aerial parts. **Conclusion**: Although there is no available report on the use of this plant for medicinal purposes, the phytochemical data presented here has demonstrated that this plant has the potential to be used significantly for therapeutic purposes in many health challenges. This study has therefore laid down a good foundation for future studies on this plant whose bioactivity studies are currently underway.

Key words: Dierama, Phytochemicals, drug discovery.

1. INTRODUCTION

Plants have been used as medicine for centuries to manage various illnesses. To date, even with the development and growth in conventional medicine, a majority of our population still rely on medicinal plants as the answer to their health problems. Biological research has proved that indeed plants posses various secondary metabolites, many of which are bioactive against various pathogens while others have disease preventive properties .Still others are important in food preservation, as alternative medicine and natural therapies[1,2] . some compounds derived from plants that have been used as drugs either in their original form or semi-synthetic form to manage various ailments include ephedrine (bronchodilator), colchicines (antigout), morphine (analgesic) , and artemisinin (antimalarial) among others[3,4] .

Natural products continue to play a crucial role in drug development as they account for almost 50% new chemical entities in drug discovery and hence provide a starting point for new synthetic compounds ; in addition to this, they may also be used as templates for synthetic modification in drug discovery and developmemt. Infact natural products are source to about 90% of newly discovered pharmaceuiticals in use[4,5,6]. For many years, extensive research has been dedicated at discovering novel natural products with pharmaceuticals significance, however amongst approximately 500,000 plants species occurring world wide, only 1% have been phytochemically screened while amongst about 250,000 existing higher plants, only about 15 % have been evaluated phytochemically[2,7,8,9,10]. A lot therefore still needs to be done, more so on plants with no or scanty previous reports on ethnomedicinal usage . This is in order to unearth novel bioactive compounds for utilization as potential sources of drugs . This study was conducted to contribute towards this goal. Genus *Dierama* K. Koch belongs to the family iridaceae . it is an evergreen perennial herbs with corms which posses coarsely fibrous tunics. Leaves are several with the lower ones sheathing the stem base. Stem slender with usually branched inflorescence consisting of spikes in a lax panicle; spikes few to many , erect or pendulous. Flowers range from pink, red, purple , yellow and whiten in colour [11]. It is composed of 44 species distributed within South Africa, Tropical Africa and Ethiopia[12,13]. In parts of the world where they are found they are mainly used as ornamental flowers in gardens with other ornamental grasses[14]. The only species found in Kenya *Dierama cupuliflorum* grows as a wild flower in highland areas with no commercial utilization reported.

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Dierama cupuliflorum Klatt, is a tufted perennial herb with large panicles of delicate blue or purple flowers hanging on long thin stalks. They are common in undisturbed high altitude grassland with altitude of about 2400-3900m. Mount Elgon, Cherenganyi hills, Mau, Abardares- through Wandare route towards Satima , Karuru river and Njambini, Mount Kenya [11].

Literature search revealed no reports on the ethnomedicinal usage and biological assays. Similarly no work has been done on the phytochemical screening of this plant.

This article presents the phytochemical investigations conducted on this plant for the first time.

2. MATERIALS AND METHODS

2.1 Collection and identification of plant material

Dierama cupuliflorum was collected from Aberdares National park , through Wandare route towards Satima in the year 2011and identified by a plant taxonomist at the school of biological sciences, University of Nairobi.

2.2 Processing of the plant material to obtain crude extracts

The plants were cut into aerials and corms, which were both shredded into pieces to fasten drying under shade at room temperature. The dried plant materials were then ground into powder and kept awaiting extraction by organic solvents and phytochemical screening.

30g of powdered plant material was extracted using dichloromethane : methanol in the ratio 1:1 , the mixture was filtered using whatman filter paper No. 1. The filtrate was subjected to a rotary evaporation to obtain dry crude extracts. These were kept in the refrigerator at 4ºC awaiting phytochemical screening.

3. Qualitative phytochemical analysis

Chemical tests were conducted on organic solvent extracts and powdered plant materials of both aerial and corm parts following standard chemical procedures according to[15,16,17]. The phytochemicals analysed were alkaloids, flavanoids, saponins, tannins, glycosides, steroids, anthraquinones and terpenoids.

3.1 Screening procedure

3.1.1 Test for saponins

5ml of distilled water was added to 0.5g of powdered plant material in a vial . The mixture was shaken and heated in water bath for 2min. The presence of a stable froth indicated the presence of saponins.

3.1.2 Test for steroids

Two methods were used to determine the presence of steroids in the plant extracts. I) 0.5g of powdered plant material was mixed with 2ml acetic anhydride in a boiling tube and then cooled in ice for five minutes , 2ml concentrated sulphuric acid was added slowly along the wall of the test tube. Colour change from violet , to blue , to green was an indicative of the presence of steroids. II) concentrated sulphuric acid was slowly added to 2g of plant extract . Effervescence followed by appearance of a clear reddish brown colour at the interface was an indication of a steroidal ring.

3.1.3 Test for tannins

10 ml distilled water was added to 0.5g of powdered plant material in a test tube. The was boiled for 3min and filtered using Whatman filter paper No. 1. Ferric chloride was added and the mixture observed for dark or dirty green precipitate which indicated the presence of tannins.

3.1.4 Test for terpenoids

0.5g of powdered plant material was added in a boiling tube and 2ml chloroform carefully added, 3ml concentrated sulphuric acid was added drop wise. Presence of a reddish brown colouration at the interface showed positive results for the presence of terpenoids.

3.1.5 Test for glycosides

0.5 g of the ground plant material was added to a boiling tube. 10ml distilled water was added and stirred. This was filtered and 2ml of the filtrate hydrolyzed with few drops of concentrated hydrochloric acid. a few drops of ammonia solution was added to the mixture . Five drops of this solution was put a side in a separate test tube and 2ml of benedicts reagent added and boiled. Reddish to brown precipitate was an indicative of the presence of glycosides.

3.1.6 Test for flavonoids

0.5 g of the extract was heated with 10ml ethyl acetate over a steam bath for 3 min, the mixture was filtered and 4ml of the filtrate was shaken with 1ml dilute ammonia (50%). presence of a yellow colouration indicated the presence of flavonoids.

3.1.7 Test for alkaloids

0.5g of the powdered plants parts was added into a boiling tube . 5ml of 2% sulphuric acid was added, mixed and filtered . Few drops of Drangedorff´s reagent was added to the filtrate. Presence of an orange red precipitate indicated the presence of alkaloids.

3.1.8 Test for anthraquinones

1g of powdered plant material added into a boiling tube and boiled with 2ml of 10% hydrochloric acid for 5min. The mixture was filtered and the filtrate cooled.The filtrate was partitioned against equal volume of chloroform and the chloroform layer transferred into a clean test tube . Equal volume of volume of 10% ammonia solution was added into the chloroform layer, shaken and allowed to separate. The separated aqueuos layer was observed for a delicate rose pink colour which showed the presence of anthraquinones.

4. RESULTS AND DISCUSSION

The results of phytochemical screening of the aerials and corms of *D. cupuliflorum* showed presence of various classes of compounds in the plant. The observations were recorded depending on the colour change intensity as strongly present, moderately present, weakly

present or Not detected as in Table 1. Corms appeared to be rich in various phytochemicals as compared to the aerial portions. Steroids were not detected in this plant. All the tested phytochemicals were strongly present in the corms except anthraquionones which showed moderate presence.

The aerial portion showed strong presence of flavonoids, saponins, and tannins ; glycosides were moderately present while alkaloids, terpenoids, and anthraquinones were weakly present (Table 1).

Table 1: Phytochemical constituents from corms and aerial/ leaves parts of *Dierama cupuliflorum*

Phytochemicals	Corm	Aerial /leaves
Flavonoids	+++	+++
Saponins	+++	+++
Alkaloids	+++	+
Terpenoids	+++	+
Steroids	-	-
Tannins	+++	+++
Glycosides	+++	++
Anthraquinones	++	+

Key :
+++ = Strongly present
++= Moderately present
+ = Weakly present
- = Not detected

The presence of the phytochemicals may be of anti-infective and therapeutic significance because, the value of plants with medicinal activities lies in the natural products or chemical substances present that exert numerous biological activities in the body[7,15].

Alkaloids, a group of compounds with basic nitrogen atoms in their chemical structure are widely present in root barks, rhizomes or corms and are characterized by a bitter taste. They posses antimalarial, antibacterial, antifungal, and some have been investigated to have anticancer properties[18,19,20].

Polyphenol compounds such as flavonoids and tannins are characterized by presence of multiple phenol groups. They have avast range of pharmacological significance such as antioxidant property used for cancer management among other health complications. Their ability to scavenge for free radicals makes them have chemopreventive property[19]. Flavonoids have also been reported to have broad antimicrobial, antimalarial, anti-allergic, anti-inflammatory, hepatoprotective, anti-tumor, anti-viral, enzyme inhibition and anti-thrombotic activities while tannins are significant as anti-hemmorhoidals, hemostatic antimicrobials and also used in preparations of anti-diarhoeals[18,1,21]. Saponins, mostly found in plant skins are vital in their ability to lower cholesterol levels. They also have antioxidant, anti-inflamatory antimalarial properties.Saponins are also used in industries to to clean industrial equipments and fabrics. Terpenoids, a diverse class of organic chemicals have been reported to have antimicrobial - antibacterial activities and wound healing ability by strengthening the skin and increasing the level of antioxidants in the wounds. They also restore inflamed tissues by improving the blood supply[18,1,15,21]. In a report by [17], presence of glycosides were speculated to have anti-HIV-1 protease inhibitory activity.

5. CONCLUSION

This present study has revealed that *Dierama cupuliflorum* has a wide range of classes of compounds which have potential significance in Pharmaceuticals as source of chemotherapeutics and anti-infectives against a wide range of pathogens.

Inorder to consider therapeutic and anti-infective significance of these phytochemicals, different biological activity studies including cytotoxicity are currently underway.

Conflict of interest

All authors have none to declare.

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REFERENCES

1. Samejo QM, Sumbul A, Shah S, Memon SB, Chundrigar S. Phytochemical screening of *Tamarix dioca* Roxb. Ex Roch. *Journal of Pharmacy Reseach* 2013; 7: 181-183.
2. Pahlaviani KMRM, Issazadeh K, Massiha A, Giahi M. Chemical composition and antimicrobial activity of various extracts of O. *gratissimum* L. from Guilan, Iran. *Advanced studies in Biology* 2013; 5: (2): 51-60.
3. Salim AA, Chin Y-W, Kinghorn AD. Drug discovery from plants In: Bioactive Molecules and Medicinal plants, Ramawat KG, Merillon JM, eds. Springer 2008; 1-4.
4. Barlunas JM, and Kinghorn DA. Drug discovery from medicinal plants. *Life Sciences* 2005 ; 78 : 431-441.
5. World Health Organization. Traditional Medicine Strategy 2002-2005.
6. Moshi MJ. Current and future prospects of integrating traditional and medicine alternative medicine in the management of diseases in Tanzania. *Tanzania Health Research Bulletin* 2005; 7 :(3): 159:67.
7. Londonkar R.L, Kattegouga U.M, Shivsharanappa K, Hanchinalmath JV. Phytochemical screening and *in vitro* antimicrobial activity of *Typha angustifolia* Linn leaves extract against pathogenic gram negative micro organisms. *Journal of Pharmacy Research* 2013 ; 6: 280-283.
8. Palombo, EA. Phytochemicals from Traditional Medicinal Plants used in the Treatment of Diarrhoea: Modes of Action and Effects on Intestinal Function. *Phytotherapy Research* 2006 ; 20: 717–724.
9. Wakdikar S. Global health care challenge: Indian experiences and new prescriptions. *Journal of Biotechnology* 2004 ; 7 : 3.

10. Fabricant SD, and Farnsworth NR. The value of plants used in Traditional Medicine for Drug Discovery. Reviews in Environmental health, *Environmental Health Perspectives* 2001 ; 109 : (1): 69-75.
11. Agnew ADQ. Upland Kenya wild flowers and Ferns, 3rd Ed pg 379. East Africa Natural History Society 2013.
12. Goldblatt P, Flora of Tropical East Africa 1996 ; 1.
13. Rundall and Goldblatt. *Botanical Journal of Linean Society* 1991; 106: 329-345
14. Anonymous. *Royal Horticultural Society*, 2009.
15. Dabai TJ, Zuru AA, Hassan LG, Itodo AU, Wara SH. Phytochemical screening of crude extracts of *Amblygocarpus* and *Sclerocarya birrea. International of Traditional and Natural Medicines* 2013 ; 2: (1): 1-8
16. Dushimemaria F, Mumbengegwi RD, Preeze DI, Bock R. Qualitative phytochemical screening and in invitro antimicrobial effects of plant extracts of *Searsia tenuinervis. Journal of Research in microbes* 2012 ; 1 (2) : 088-095.
17. Mushi FN, Mbwambo ZH, Innocent E, Tewtrkul S. Antibacterial, anti-HIV-1 protease and cytotoxic activities of aqueous ethanolic extracts from *Combretum adegonium* Steud. Ex A. Rich (combretaceae). *BMC complementary & Alternative medicine* 2012 ; 12 : 163
18. Musila MF, Dossaji SF, Nguta JM, Lukhoba CW, Munyao JM. In vivo antimalarial activity , toxicity and phytochemical screening of selected antimalarial plants. *Journal of ethnopharmacology* 2013 ; 146 : 557-561.
19. Sawadogo RW, Schumacher M, Teiten HM, Dicato M, Diederich M. Traditional West African Pharmaopeia, plants and derived compounds for cancer therapy. *Biochemical pharmacology* 2012 ; 84 : 1225-1240.
20. Odhiambo JA, Siboe GM, Lukhoba CW, Dossaji S.F. Antifungal activity of crude extracts of Gladiolus dalenii Van Geel (Iridaceae). *African Journal of Traditonal, Complementary, and Alternative medicines* 2009 ; 7 : (1): 53-58.
21. Talib WH, and Mahasneh AM. Antimicrobial, cytotoxicity and phytochemical screening of Jordanian plants used in traditional medicine. *Molecules* 2010 ; 15 : 1811-1824.

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Appendix ix: Antifungal activity, brine shrimp cytotoxicity and phytochemical screening of *Gladiolus watsonoides* Baker (Iridaceae)

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Antifungal activity, brine shrimp cytotoxicity and phytochemical screening of *Gladiolus watsonoides* Baker (Iridaceae)

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ABSTRACT

Background: Plants and plant products are by far the most utilized by humans as source of medicine. They are used by various cultures in various ways. Although the beginning of the usage was by trial and error, accessibility may have also contributed to some potentially medicinal plants not being accessed and utilized, hence lack of ethnomedicinal usage in history. *Gladiolus watsonoides* Baker (iridaceae) is one such plant with high antifungal and cytotoxic activities yet no previous reports have been recorded on its usage, biological activities and phytochemicals. The aim of this study was to investigate the antifungal activity, cytotoxicity and the phytochemical components in this plant. **Method:** Organic and aqueous extracts were tested for antifungal activity against *Candida albicans, Aspergillus niger* and *Cryptococcus neoformans* by disc diffusion method. Toxicity tests were done using brine shrimp larvae – nauplii and phytochemical screening for the presence of selected classes of compounds conducted by standard chemical procedures. **Results:** Corms from Organic extracts showed moderate to high antifungal activity against the three test opportunistic fungi. The aerial extracts were not active against *Aspergillus niger* and *Cryptococcus neoformans*. Varied toxicity levels were noted against the nauplii. Amongst the classes of compounds screened, only steroids were absent in corms while glycosides, anthraquinones and steroids were absent in the aerials. **Conclusion:** Further analysis need to be undertaken before more conclusions can be made on the potential significance of this plant as lead to antifungal drug development.

KEY WORDS: *Gladiolus*, Antifungal, Cytotoxicity, Phytochemicals, Drug development

1. INTRODUCTION

Use of plants to treat a wide range of diseases by different cultures continues to date. Many of the plants used in ethnomedicine have also been followed for their biological activity, in most cases there is usually a relationship between the use of plants in ethnomedicine and the biological activity results [1]. A lot of research has been invested in such, however there are plants in the wild with no known reports of usage in ethnomedicine but may have potential natural products with significant biological activities [2]. Use of plants in traditional medicine is an ancient thing and the human race got to discover medicinal and food plants through trial, error and experience. The plants used also may have depended on their availability and place of occurrence; some other plants with various biological activities may have occurred in areas not easily accessible hence may have never been accessed for use in ethnomedicine.

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Gladiolus watsonoides is one such plant with high antifungal activity but no reports at all on ethnomedicine, biological activities and phytochemical components. It is a herb, erect with hairless leafy stems ending in a spike of up to 15 uniformly bright glossy red flowers. Mainly found in wet soils in bamboo alpine zones 2400m-4200m above the sea level [3].

Opportunistic fungal infections remain a threat to millions of lives of those individuals with compromised immune systems. Increasing incidence by infections such as Candidiasis, aspergillosis, and cryptococcosis have been reported due to growing numbers of immunocompromised cases related to HIV/AIDS, cancer, diabetes, old age, organ transplant and other surgical procedures [4 5 6]. These nfections by the three most common opportunistic human fungal pathogens have high occurences as follows: *Candida albicans* (20-40%), *Aspergillus fumigatus* (50-90%), and *Cryptococcus neoformans* (20-70%) [7] .Despite the high incidence and mortality rates caused by these fungal pathogens, there are limited numbers of antifungal drugs used to treat them. In addition to this, most of the drugs are unaffordable, some results into rapid development of resistance especially due to non compliance, and adverse side effects [4 5 8 9 10]. An

alternative source, probably from plants may therefore be necessary. Besides, plants with biological activity and no known reports for usage in ethnomedicines should be documented and investigated as in ethnomedicines are worth documented for further investigation of useful anti-infective agents. This present study investigated the antifungal activity, cytotoxicity and the nature of the major chemical classes present in *Gladiolus watsonoides* Baker (iridaceae).

2. MATERIALS AND METHOD

2.1 Collection of the plant materials

Gladiolus watsonoides was collected from Aberdares National Park , Kenya through Wandare route towards Satima in the year 2012. The plant was identified using keys in [3] ,voucher specimens in the Nairobi University herbarium and a plant taxonomist from the School of Biological Sciences, University of Nairobi. The aerial parts were separated from the corms, shredded into small pieces and dried at room temperature. Once dry, the samples were ground into powder and kept in a dry condition a waiting extraction for bioassays and phytochemical screening.

2.2 Preparation of crude extracts

30g of each of the powdered samples were extracted using water and a mixture of dichloromethane & methanol in the ratio 1:1 four times at 24 h intervals. Each of the extract was filtered and concentrated under reduced pressure at 40°C to obtain crude extracts. Dry aqueous extracts were obtained by use of a freeze drier. These were then stored in airtight containers at 4°C awaiting bioassays and phytochemical screening.

2.3 Preparation for antifungal activity

Stock solution of each sample was prepared by dissolving 0.5g of dried crude in 10ml of water in the case of aqueous extracts and Dimethylsulphoxide (DMSO) in the case of the organic solvent extracts. This was at first serially diluted such that there were five different concentration levels in which the tests were done. These were 5mg/100µl, 2.5mg/100µl, 1.25mg/100µl, 0.625mg/100µl, 0.3125mg/100µl. Samples which showed activity with the lowest concentration set were further tested at much lower concentrations at 0.15625mg/100µl, 0.07813mg/100µl and 0.03902mg/100µl. The aim of setting different concentrations was to establish the minimum inhibitory concentrations.

2.4 Determination of antifungal activity

Antifungal activity of the crude extracts was determined against three opportunistic fungal *pathogens; Candida albicans, Aspergillus niger and Cryptococcus neoformans.* All the fungi were first sub-cultured in Sabourads Dextrose Agar growth media (SDA). Broth suspensions of the organisms were prepared using distilled water and peptone media. The tests were done using disc diffusion method according to [11]. Commercially prepared sterile discs impregnated with 100µl of the solution of sample at each concentration, were aseptically transferred into petri dishes with about 25ml SDA media, freshly innocultaed with the test organism. Dimethylsulfoxide (DMSO) and water which were used to re-dissolve the crude extracts, served as the negative controls while Amphotericin B served as the positive control. The plates were prepared in replicates, sealed with parafilm to avoid contamination and incubated at 37ºc for *Candida albicans* and *Cryptococcus neoformans* while those of *Aspergillus niger* were incubated at 25ºc. The antifungal activities were evaluated by measuring the diameter of the zones of inhibition using a transparent ruler after 24 hours, 48 hours and 72hours [12 13].

2.5 Toxicity tests using brine shrimp

2.5.1 Hatching of the brine shrimp larvae

Brine shrimp *(Artemia salina)* eggs were hatched in artificial sea water prepared by dissolving 10g of sea salt (sigma chemicals, Co, UK) in 500ml of distilled water. This was added to a culture tank which had two partitions. One part was darkened, while the other was left exposed. A pinch of brine shrimp eggs was added to the darkened portion and warmed with a 60 watt lamp to provide warmth necessary for hatching. The observation was made after 24 hours and successful hatching confirmed by the presence of visible several actively moving nauplii in the culture tank. The partition wall had small holes through which the larvae (nauplii) moved to the exposed side of the tank.

2.5.2 Brine shrimp assay

This stock solutions of 1000ppm were prepared by dissolving 3mg of each extract in 3ml of DMSO in the case of organic extracts and water in the case of aqueous extracts. This stock solution 1000ppm was serially diluted to prepare other concentrations such as 500ppm, 100ppm, 10ppm and 5ppm. 10 live nauplii were transferred using a micro pipette into vials with these set concentrations. This experiment was set in triplicates (three vials per sample per concentration). Water and DMSO served as the negative controls while Rotenone served as the positive control. The observations were done after 24 hours. Cytotoxic activity was determined by recording the number of dead nauplii (immobile) in each vial per sample per concentration. This was then used to calculate the mortality mean, percentages and LD_{50} values.

2.6 Qualitative Phytochemical analysis

Chemical tests were conducted on organic solvent extracts and powdered plant materials of both aerial and corm parts following standard chemical procedures according to [14 15 16].The phytochemicals analysed were alkaloids, flavonoids, saponins, tannins, glycosides, steroids, anthraquinones and terpenoids.

2.6.1 Screening procedure

2.6.1.1 Test for saponins

5ml of distilled water was added to 0.5g of powdered plant material in a vial . The mixture was shaken and heated in water bath for 2min. The presence of a stable froth indicated the presence of saponins.

2.6.1.2 Test for steroids

Two methods were used to determine the presence of steroids in the plant extracts. I) 0.5g of powdered plant material was mixed with 2ml acetic anhydride in a boiling tube and then cooled in ice for five minutes , 2ml concentrated sulphuric acid was added slowly along the wall of the test tube. Colour change from violet , to blue , to green was an indicative of the presence of steroids. II) concentrated sulphuric acid was slowly added to 2g of plant extract . Effervescence followed by appearance of a clear reddish brown colour at the interface was an indication of a steroidal ring.

2.6.1.3 Test for tannins

10 ml distilled water was added to 0.5g of powdered plant material in a test tube. The was boiled for 3min and filtered using Whatman filter paper No. 1. Ferric chloride was added and the mixture observed for dark or dirty green precipitate which indicated the presence of tannins.

2.6.1.4 Test for terpenoids

0.5g of powdered plant material was added in a boiling tube and 2ml chloroform carefully added, 3ml concentrated sulphuric acid was added drop wise. Presence of a reddish brown colouration at the interface showed positive results for the presence of terpenoids.

2.6.1.5 Test for glycosides

0.5 g of the ground plant material was added to a boiling tube. 10ml distilled water was added and stirred. This was filtered and 2ml of the filtrate hydrolyzed with few drops of concentrated hydrochloric acid, a few drops of ammonia solution was added to the mixture . Five drops of this solution was put a side in a separate test tube and 2ml of benedicts reagent added and boiled. Reddish to brown precipitate was an indicative of the presence of glycosides.

2.6.1.6 Test for flavonoids

0.5 g of the extract was heated with 10ml ethyl acetate over a steam bath for 3 min, the mixture was filtered and 4ml of the filtrate was shaken with 1ml dilute ammonia (50%) . Presence of a yellow colouration indicated the presence of flavonoids.

2.6.1.7 Test for alkaloids

0.5g of the powdered plants parts was added into a boiling tube . 5ml of 2% sulphuric acid was added, mixed and filtered . Few drops of Drangedorff´s reagent was added to the filtrate. Presence of an orange red precipitate indicated the presence of alkaloids.

2.6.1.8 Test for anthraquinones

1g of powdered plant material added into a boiling tube and boiled with 2ml of 10% hydrochloric acid for 5min. The mixture was filtered and the filtrate cooled.The filtrate was partitioned against equal volume of chloroform and the chloroform layer transferred into a clean test tube. Equal volume of 10% ammonia solution was added into the chloroform layer, shaken and allowed to separate. The separated aqueous layer was observed for a delicate rose pink colour which showed the presence of anthraquinones.

3. RESULTS

3.1 Antifungal activity

The two different parts of the plant exhibited varied antifungal activity against one or more of the test fungi. In general, corms showed much higher antifungal activity than the aerial parts; however this activity of the corms was outweighed by the activity of the control drug Amphotericin B (Table 1).

Table 1. Minimum Inhibitory Concentrations (MIC in µg/µl) values of the crude organic extracts of *Gladiolus watsonoides* against the test fungi

	CA	AN	CN
Corms	0.3907	6.25	3.125
Aerials	0.7813	NA	NA
Amp B	<0.3907	<0.3907	<0.3907

Key; CA= Candida albicans ; AN= Aspergillus niger CN= Cryptococcus neoformans NA= No activity detected

The aerial parts were not active against *A.niger* and *C.neoformans* whereas the corms showed activity against the three fungi with highest activity revealed against *C.albicans* and the least activity revealed against the *A.niger* as shown by the Mininmum inhibitory concentrations in Table 1.

3.2 Brine shrimp assay

In general, both the plant parts showed toxicity to nauplii; artificial sea water which was a negative control was not toxic whereas rotenone, a positive control was highly toxic to the brine shrimp larvae (Table 2).

Table 2. LD_{50} values of the toxicity by the crude extracts of *G watsonoides*

	LD_{50}	+- SE
$Corms_{Aq}$	27.4503	0.1602
$Corms_{Org}$	0.5492	0.9491
$Aerials_{Aq}$	57.4512	0.2743
$Aerials_{Org}$	2.6952	0.3518
Sea water only	2771.8354	0.7657
Sea water + DMSO	147.6151	0.2816
Rotenone	0.4911	1.2426

Key ; Aq denotes aqueous extract ; Org denotes Organic extract

3.3 Phytochemical analysis

Corms of *Gladiolus watsonoides* were richer in phytochemicals than the aerial parts. All the phytochemicals tested were detected in the corms except steroids.

Table 3: Phytochemical constituents from organic extracts of corms and aerial parts of *Gladiolus watsonoides*

Phytochemicals	Corms	Aerial/leaves
Flavonoids	++	++
Saponins	+++	+++
Alkaloids	++	+
Terpenoids	+++	+
Steroids	-	-
Tannins	+++	+++
Glycosides	++	-
Anthraquinones	+	-

Key: +++: strong presence, ++: moderate presence, +: weak presence, -: not detected

The aerial parts showed presence of alkaloids saponins, flavonoids, terpenoids and tannins while steroids, glycosides and anthraquinones were not detected (Table 3).

4. DISCUSSION

Crude organic extracts of *Gladiolus watsonoides* had growth inhibiting effects on one or more of the test fungi (Table 1). The corresponding data on the aqueous extract is not shown since the activity exhibited was too little. Inactivity or less activity showed by water extracts may have been due to the presence of bioactive elements at a much lower concentration or the active elements may have been soluble only in organic solvent and therefore not present in water extracts [17 18 19].

Crude organic extracts from corms and aerials showed varied activity. Extracts from corms showed the highest activity especially against the three fungi whereas the aerial parts were active against *Candida albicans* only. Although some reports revealed *Candida albicans* to be insensitive to most extract treatments [18]. In this study it was the most sensitive to the extracts' exposure. The relatively high antifungal activity of this plant against *Candida albicans* and *Cryptoccocus neoformans* may be of interest since they are yeasts which are known to be less susceptible to the available antifungal agents mainly due to their continuously new emergence and general multidrug resistance [20].

The insensitivity of *Aspergillus niger* and *Cryptococcus neoformans* to the aerial extracts may have been due to the cell wall composition and site of action of the active ingredient being different from that of *C.albicans* [21 22], another explaination for the observation may have been that the active elements against these two fungi were either at lower undetectable levels or absent in the aerials . Still the extracts may have been active against other fungi not tested in the study.

G. watsonoides was found to be rich in phytochemicals (Table 3). Corms showed presence of all the tested classes of secondary metabolites except the steroids whereas anthraquinones, steroids and glycosides were not detected in the aerials. This may explain the varied antifungal and cytotoxic activities observed (Table 1 and Table 2). Biological activities by this plant may have been attributed to the vast presence of secondary metabolites detected. Alkaloids, mainly found in root barks, corms and rhizomes have been reported to possess antifungal, antimalarial, antibacterial and anticancer properties[1,23,24,25]. Terpenoids, flavonoids and tannins have also been reported to have among others antimicrobial activity and is used in preparations of drugs used against diarrhea [26, 27, 28].

Although not detected in this plant, cytotoxic effects of steroids have been utilized in their usage as arrow poisons They have also been investigated for their antibacterial activity [27, 29]. Saponins, glycosides and athraquinones have been reported to have anticancer, antifungal and antioxidant activities [26, 30, 31].

Toxicity tests on this plant in brine shrimp larvae revealed varied toxic levels (Table 2). This may have been attributed to presence of secondary metabolites. So far in this study, its not known which active ingredient exerted the antifungal and the cytotoxic activities. This plant therefore should be considered for further analysis.

5. CONCLUSION

Study on *Gladiolus watsonoides* revealed antifungal and cytotoxic activities along with a wide range of classes of compounds. In order to possibly consider this plant for medical relevance, further analysis on the active elements need to be undertaken before more conclusions can be made on its significance as potential lead to antifungal drug development.

Conflict of interest

All authors have none to declare

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REFERENCES

1. Musila MF, Dossaji SF, Nguta JM, Lukhoba CW, Munyao JM. In vivo antimalarial activity , toxicity and phytochemical screening of selected antimalarial plants. *Journal of ethnopharmacology* 2013 ; 146 : 557-561.
2. Phillipson, J.D., Wright, C.W., Kirby, G.C., Warhurst, D.C.Tropical Plants as sources of antiprotozoal agents. *Recent Advances in Phytochemistry*, 1993 ; 27; 1–40
3. ADQ. Upland Kenya wild flowers and Ferns, 3rd Ed pg 379. East Africa Natural History Society 2013.

4. Vandeputte P., Ferrari S., Coste T.A. Antifungal resistance and new strategies to control fungal infections . *International Journal of Microbiology* 2012
5. Pfaller A.M., Antifungal Drug Resistance: Mechanisms, Epidermiology and Consequences of treatment. *The American Journal of Medicine* 2012;125;s3-s13
6. Walsh J.T., Annaissie J.E., Denning W.D.,Herbrecht R., Kontoyiannis P.D., Burik V.J., Winghard R.J.,Patterson F.T. Treatment of Aspergillosis: Clinical practice, Guidelines of the infectious Diseases Society of America. *Clinical Infectious Diseases* 2008; 46
7. Butts A., and Krysan J. D. Antifungal Drug Discovery: Something old and Something new. *Plos pathogen* 2012; 8; 9
8. Duffalo L. Melody. Fungal opportunistic infections in HIV-disease. *Journal of Pharmacy practice* 2006;19;1:17-30
9. Dismukes E.W. Introduction to Antifungal drugs. *Clinical Infectious Diseases* 2000 30;653-657
10. Erika J.Ernst .Investigational antifungal agents. *Pharmacotherapy* 2001; 21; 165s-174s
11. Serrano, M. C. , Ramírez, M., Morilla, D.,. Valverde, A, Chávez, M., Espinel-Ingroff A., Claro, R., Fernández, A., Almeida and, C., Martín-Mazuelos, E., A comparative study of the disc diffusion method with the broth microdilution and Etest methods for voriconazole susceptibility testing of *Aspergillus* spp. *Journal of Antimicrobial chemotherapy* 2004; 53, 739-742
12. Ameh S. Obodozie O. Olorunfemi P., Okoliko I., Ochepke ., N. Potentials of corms as antimicrobial agent in food processing and traditional medicine. *Journal of Microbiology and Antimicrobials* 2011; 3;1 8-12
13. Okemo, P.O., Bais, P.H.,Vivanco, M.J., In *vitro* activities of Maesa lanceolata extracts against fungal plant pathogens. *Fitoterapia* 2003 74 : 312-316.
14. Dabai TJ, Zuru AA, Hassan LG, Itodo AU, Wara SH. Phytochemical screening of crude extracts of *Amblygocarpus* and *Sclerocarya birrea. International of Traditional and Natural Medicines* 2013 ; 2: (1): 1-8
15. Dushimemaria F, Mumbengegwi RD, Preeze DI, Bock R. Qualitative phytochemical screening and in invitro antimicrobial effects of plant extracts of *Searsia tenuinervis. Journal of Research in microbes* 2012 ; 1 (2) : 088-095.
16. Mushi FN, Mbwambo ZH, Innocent E, Tewtrkul S. Antibacterial, anti-HIV-1 protease and cytotoxic activities of aqueous ethanolic extracts from Combretum adegonium Steud. Ex A. Rich (combretaceae). *BMC complementary & Alternative medicine* 2012 ; 12 : 163
17. Fawole, O.A., Finnie, J.F., Van Staden J. Antimicrobial activity and mutagenic effects of twelve traditional medicinal plants used to treat ailments related to the gastro-intestinal tract in South Africa. *South African Journal of Botany* 2008 ; 10;1016
18. Boer, J.H., Kool, A., Mziray, W.R., Herdberg I., Levenfors J.J. Antifungal and antibacterial activity of some herbal remedies from Tanzania. *Journal of Ethnopharmacology* 2005 96 461-469
19. Clarkson , C., Maharaj, V.J., Crouch, N.R., Olwen M. Grace , O.M. Pillay, P, Matsabisa, M.G., Bhagwandin, N., Smith, P.J., Folb, P.I. In *vitro* antiplasmodial activity of medicinal plants native to or naturalised in South Africa. *Journal of Ethnopharmacology* 2004 ; 92: 177–191.
20. Espinel-Ingroff A, and Flynn T. Antifungal activity of the new triazole voriconazole against established, new and emerging yeast pathogens: a comparative study. *Antimicrobial Agents and Chemotherapy,* 1996 15-18:114
21. Edeoga, H.O., Okwu, D. E. and Mbaebie, B.O Phytochemical constituents of some Nigerian medicinal plants. *African Journal of Biotechnology*, 2005 ;4;7; 685-688
22. Gooday, G.W.Cell envelope diversity and dynamics in yeasts and filamentous fungi. *Journal of Applied Bacteriology Symposium Supplement* 1993; 74 12-20.
23. Sawadogo RW, Schumacher M, Teiten HM, Dicato M, Diederich M. Traditional West African Pharmaopeia, plants and derived compounds for cancer therapy. *Biochemical pharmacology* 2012 ; 84 : 1225-1240.
24. Shakeri A., Hazeri N.,Vlizadeh J.,Ghasemi A.,Tavallaei Z.F.Phytochemical screening, Antimicrobial and antioxidant activities of *Anabasis aphylla* L. extracts. *Kragujevac Journal of Science* 2012; 34 ;71-78
25. Odhiambo JA, Siboe GM, Lukhoba CW, Dossaji S.F. Antifungal activity of crude extracts of Gladiolus dalenii Van Geel (Iridaceae). *African Journal of Traditonal, Complementary, and Alternative medicines* 2009 ; 7 : (1): 53-58.
26. Odhiambo J., Dossaji S.,Lukhoba W., Yenesew A. Phytochemical screening of *Dierama cupuliflorum* Klatt (Iridaceae*). Journal of pharmacy Research* 2014;8;4;589-592
27. Samejo QM, Sumbul A, Shah S, Memon SB, Chundrigar S. Phytochemical screening of *Tamarix dioca* Roxb. Ex Roch. *Journal of Pharmacy Reseach* 2013; 7: 181-183
28. Talib WH, and Mahasneh AM. Antimicrobial, cytotoxicity and phytochemical screening of Jordanian plants used in traditional medicine. *Molecules* 2010 ; 15 : 1811-1824.
29. Doughari J.H.Antimicrobial activity of *Tamarindus indica* Linn.*Tropical Journal of Pharmaceutical Research*, 2006; 5 ;2: 597-603
30. Dave H.,and Ledwani L. A review on anthraquinones isolated from *Cassia* species and their applications. *Indian Journal of Natural products and Resources,* 2012; 3; 291-319
31. Thakur M., Melzig F.M., Fuchs S.,Weng A. Chemistry and pharmacology of saponins; special focus on cytotoxic properties. Botanics ; *Targets and Therapy* 2011

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