PHYTOCHEMICAL SCREENING AND ANTIFUNGAL EFFECTS OF *EUPHORBIA HETEROPHYLLA* LINN. AND *MITRACARPUS SCABER* ZUCC. EXTRACTS

BY

Michael Adeniyi SHITTU,
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A DISSERTATION SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES AHMADU BELLO UNIVERSITY ZARIA IN PARTIAL FULFILMENT OF REQUIREMENTS FOR THE AWARD OF A MASTERS DEGREE IN EDUCATIONAL BIOLOGY

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FACULTY OF SCIENCE.
AHMADU BELLO UNIVERSITY, ZARIA,
NIGERIA

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DECLARATION

I hereby declare that the work in this dissertation titled "PHYTOCHEMICAL SCREENING AND ANTIFUNGAL EFFECTS OF *EUPHORBIA HETEROPHYLLA* LINN. AND *MITRACARPUS SCABER* ZUCC." EXTRACTS was performed by me in the Department of Biological Sciences. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this work has been presented for another degree at any institution.

Michael Adeniyi SHITTU _____ _____

Signature Date

CERTIFICATION

This dissertation titled "PHYTOCHEMICAL SCREENING AND ANTIFUNGAL EFFECTS OF *EUPHORBIA HETEROPHYLLA* LINN. AND *MITRACARPUS SCABER* ZUCC. EXTRACTS*"* by Michael Adeniyi SHITTU meets the regulations governing the award of the degree of master of Educational Biology of Ahmadu Bello University and is approved for its contribution to knowledge and literary presentation.

Dr. M.A Adelanwa Chairman, Supervisory Committee	_____ Signature	_____ Date
Prof. S.P. Bako Member, Supervisory Committee	_____ Signature	_____ Date
Prof. A. K. Adamu Head, Department of Biological Sciences	_____ Signature	_____ Date
Prof. K. BALA, Dean, School of Postgraduate Studies	_____ Signature	_____ Date

DEDICATION

This work is dedicated to my wife and family who stood by me from the beginning to the end of this study.

ACKNOWLEDGEMENTS

I am first and foremost grateful to God Almighty, the father of all mercies for the successful completion of this work. I am particularly indebted to Dr. M.A. Adelanwa, the chairman supervisory committee of this research work who despite his tight schedule of duties never relented in his efforts in seeing that I am successful at any stage of the research. His wise, useful and constructive suggestions made the production of this work possible.

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ABSTRACT

The whole plant extracts of *Euphorbia heterophylla* and *Mitracarpus scaber* were screened for their phytochemical properties and antifungal effects. Aqueous, methanol, ethyl acetate and n-hexane were used as solvents for extraction of the plant samples. The plant samples were also screened qualitatively and quantitatively for their bioactive constituents. The aqueous, methanol, ethyl acetate and n- hexane extracts of the whole plant of *Euphorbia heterophylla* and *M. scaber* were concentrated at 100mg/ml, 50mg/ml, 25mg/ml and 12.5mg/ml respectively. The antifungi activities of the plant samples were tested against *Candida albicans, Trichophyton mentagrophytes, Microsporum auduounii* and *Aspergillus flavus*. The MIC and MFC of the extracts was also determined for all the fungi species. The result revealed that there were no significant differences in bioactive constituents of both plants qualitatively and quantitatively. Ethyl acetate extract of *Euphorbia heterophylla* and *Mitracarpus scaber* had highest zone of inhibition of 24.50±0.71mm and 26.00±0.00mm on *Candida albicans,* 24.00±0.00mm and 24.00±0.00mm on *Trichophyton mentagrophytes* while aqueous extract inhibition was 20.00±.00mm and 22.00±0.00mm on *Candida albicans,* 18.00±0.00mm and 18.00±0.00mm on *Trichophyton mentagrophytes* respectively. No zone of inhibition was produced in *Microsporum auduouinii* and *A. flavus* in all the solvent extracts used. The combined effects of *Euphorbia heterophylla* and *Mitracarpus scaber* plant extracts using the same solvents (aqueous, methanol, ethyl acetate and n–hexane) showed significant differences in all the solvent extracts at 100mg/ml of aqueous (23.00±0.00mm and 20.00±0.00mm), methanol (19.00±0.00mm and 18.00±0.00mm), ethyl acetate (28.00±0.00mm and 26.00±0.00mm) and n-hexane (20.00±0.00mm and 17.00±0.00mm) on *Candida albicans* and *Trichophyton mentagrophytes* respectively. No zone of inhibition was shown in *Aspergillus flavus* and *Microsporum auduouinii*. The MIC and MFC ranged from 50 – 6.25mg (MIC) and 100 – 6.25mg (MFC). Thus the traditional claims of the uses of the plants as antifungal agents were therefore justified.

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of the Study

Medicinal plants have been used for centuries as remedies for human diseases because they contain components of therapeutic value (Nostro *et al.*, 2000; Tanaka *et al.*, 2002). According to the World Health Organization (WHO) in 2008, more than 80% of the World's population relies on traditional medicine for their primary health care needs. Traditional medicine is an important part of African cultures and local medicinal systems vary among cultural groups and regions (Makhubu, 2006). Herbs are now very popular in developing countries on account of improved knowledge about the safety, efficiency and quality assurance of ethno- medicine (Makhubu, 2006). In recent years, secondary plant metabolites (phytochemicals) have been extensively investigated as a source of medicinal agents. Thus it is anticipated that phytochemicals with good anti-fungal activity will be used for the treatment of fungal infections. This is because according to Arora and Keur (1999), the success story of chemotherapy lies in the continuous search for new drugs to counter the challenges posed by resistant strains of micro-organisms. Studies indicate that in some plants, there are many substances such as peptides, tannins, alkaloids, essential oils, phenols and flavonoids among others which could serve as sources of antimicrobial production. These substances or compounds have potentially significant therapeutic applications against human pathogens including bacteria, fungi and viruses (Arora and Keur, 1999, Okigbo and Omodamiro, 2006). The development of microbial resistance to the available antibiotics has led researchers to investigate the antimicrobial activity of medicinal plants (Bisignano *et al.*, 1996, Hammer *et al.*,1999). Antibiotic resistance has become a global concern (Westh *et al.*, 2004) as the clinical

efficacy of many existing antibiotics is being threatened by the emergence of multi-drug resistant pathogens (Bandow *et al.*, 2003). Natural products either as pure compounds or as standardized plant extracts provide unlimited opportunities for the development of novel drugs because of the great diversity in their chemical structure. There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structure and novel mechanisms of action for new and re-emerging infectious diseases (Rogas *et al.*, 2004). Therefore, researchers are increasingly turning their attention to ethno-medicine, looking for new leads to develop more effective drugs against microbial infections (Benkeblia, 2004) and this has led to the screening of several medicinal plants for potential antimicrobial activity (Colombo and Bosisio, 1996; Iwu *et al*., 1999). Phytochemical composition, bioactivity and wound healing potential of *Euphorbia heterophylla* leaf extract by Omole and Emmanuel (2000) showed that the phytochemical analysis revealed the presence of medicinally active constituents of the plant which is rich in Alkaloids, Flavonoids, Saponins and Tannins. The order of decreasing concentration of phytochemical from aqueous and ethanol extracts of fresh and dried sample are Alkaloids >Cyanide> Tannins> Flavonoids > Saponins.

The research into biologically active compounds from natural sources has always been of great interest for scientists looking for new sources of useful drugs against infectious diseases. *Mitracarpus scaber* is widely employed in traditional medicine in West Africa for headaches, toothache, amenorrhoea, dyspepsia, hepatic diseases, venereal diseases and leprosy (Bisignano *et al*., 2000). Among the folkloric uses, the juice of the plants is applied topically for the treatment of skin diseases (infectious dermatitis, eczema and scabies). Daiziel, (1937); Kerharo and Adam, (1974) observed that a lotion and a skin ointment made with the aerial part of *M. scaber* are used for skin infections or skin diseases and other infectious.

Previous studies by Moulis *et al.* (1992) reported the isolation of pentalogin from fresh, aerial parts of *Mitracapus scaber* which demonstrates a potent antifungal activity against *Candiba albicans* and *Trichophyton soudanense.* Other investigations (Sanogo *et al.,* 1996) showed that different extracts of *M. scaber* exhibited broad antibacterial and antifungal activity against standard strains and clinical isolates of *Staphylococcus aureus* and *C. albicans* responsible for common skin infections. More recently, Germano *et al.* (1999) reported the hepato protective effects of *Mitracarpus scaber* decoction on tetrachloromethane (CCl_4) induced hepatotoxicity *in vivo* as well as *in vitro* using isolated hepatocytes.

1.2 Statement of Research Problem

The use of *Euphorbia heterophylla* and *Mitracarpus scaber* have been on the basis of trial and error in different communities in Africa without any scientific basis. Plant parts have been used in different locations for the treatment of different ailments which sometimes bring about conflicting results.

Often traditional healers use plants according to their analogy and morphological similarities to the ailment being treated. For example, plants containing red juice are used to treat ailments connected to menstruation problems and bleedings (Neuwinger, 2000). There is therefore the need to ascertain the basis for the claims of the efficacy of the plants used locally in ethno-medicine.

1.3 Justification

The increasing resistance to antibiotics has resulted in the research to form new organic molecules from plants with antimicrobial properties for treating diseases since some micro-organisms have developed resistance to many orthodox drugs (Sofowora, 2006). There is the need to find an alternative approach in the treatment of infectious diseases. Using local plants will be a welcome development as the cost will be minimal.

The leaves and stem bark extracts of *Euphorbia hererophylla* and *Mitracarpus scaber* have been reported in the treatment of various ailments such as ulcer, cancer, skin diseases e.t.c. It is therefore important to scientifically investigate these plant parts to ascertain their therapeutic potentials.

Determination of their chemical composition as well as antimicrobial efficacy against specific pathogens is important in the recognition of this plant as a potent commercial medicinal plant. Tests can determine its efficacy against a pathogen and thus, establish the minimal dosage required for the treatment of ailments.

1.4 Aim of Study

To evaluate the phytochemical constituents and antifungal effect of n-hexane, ethyl acetate, methanol and aqueous extracts of the whole plant of *Euphorbia heterophylla* and *Mitracarpus scaber*.

1.5 Objectives

1. To obtain the methanol, ethyl acetate, n- hexane and aqueous extracts of whole plants of *Euphorbia heterophylla* and *Mitracarpus scaber*.
2. To determine qualitatively and quantitatively secondary metabolites present in the methanol, ethyl acetate, n- hexane and aqueous extracts of whole plants of *Euphorbia heterophylla* and *Mitracarpus scabers*.
3. To assess the antifungal activities of the aqueous, methanol, ethyl acetate and n-hexane extracts of single and combined whole plants of *E. heterophylla* and *M. scaber* on some selected microorganisms.
4. To determine the Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) of aqueous, methanol, ethyl acetate and n-hexane extracts of single and combined plants of *E. heterophylla* and *M. scaber* against different species of isolated fungal.

1.6 Hypotheses

i. There are no significant differences qualitatively and quantitatively between secondary metabolites present in the aqueous, methanol, ethyl acetate and n-hexane extracts plant of *E .heterophylla* and *M. scaber*.

ii. There are no significant differences in the antifungal activities of the aqueous, methanol, ethyl acetate and n- hexane extracts of single and combined whole plant *E. heterophylla* and *M. scaber* on some selected microorganisms.

iii. There are no significant differences in the MIC and MFC of aqueous, methanol, ethyl acetate and n-hexane extracts of single and combined whole plant *E .heterophylla* and *M. scaber* against different species of isolated fungal microorganisms

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Mycoses

2.1.1 Concept of Mycoses

Collective nouns for all forms of impaired health which may be caused by fungi themselves, their toxins that invade tissues and their allergens are myelophthisis, causing superficial, subcutaneous or systemic fungal diseases. (Male, 1991).

2.1.2 Types of mycoses

Myelophthisis are subdivided into five groups:

i. Mycoses**:** Pathogen fungi attack and destroy living cell, tissues and organs.

ii. Mycotisation is noso-parasitism by fungi. It is defined as saprobic/nosoparasitic colonization of dead or damaged tissue such as burns or ulcer, cavities or diverticulas by fungi which may be non-pathogenic or potentially pathogenic (opportunistic), such as *Aspergillus* or mucor species (Male, 1991).

iii. Mycoallergies are allergic reactions caused by the allergens of non-pathogenic or pathogenic fungi, which come into contact with a usually predisposed person or animal by inhalation, ingestion or immediate contact. The latter may take place directly on the skin or the adjacent mucous membrane (mainly the genitals) or with a mycotic infection or a mycotisation (Male, 1991).

iv. Mycotoxicoses is alimentary poisonings by the toxin of certain fungi. For example, when moulds which have contaminated the foods or feeds; poisoning mostly occurs as a result of consuming the contaminated food and less often by eating products of

contaminated grains. The mycotoxins are highly thermostable and tolerate temperature up to 300°C (Noble and Somervialle, 1981).

v. Mycetism is alimentary poisoning by the toxins (alkaloids) of macromycetes resulting from a confusion of edible with poisonous mushrooms (example *Agaricus spp.* with *Amanita spp.*) (Male, 1991).

2.1.3 Morphology and identification of dermatophyte

Dermatophyte can be classified into three groups; namely *Epidermophyton*, *Microsporum* and *Trichophyton*. In keratinized tissue, dermatophytes form only hyphae and arthrospores. Dermatophyte are worldwide in distribution, with some species displaying a higher incidence in certain regions than in others. For example, *Trichophyton schenleinii* is found mainly in the mediterranian and *Trichophyton rubrum* in tropical climate (Male, 1991).

Representative colonies form on the sabouraud's dextrose agar at room temperature. Conidia formation may be observed by means of slide cultures, Sabouraud's dextrose medium is the most suitable medium for the isolation of dermatophyte with the addition of cycloheximide, which inhibits non-pathogenic environment fungi contaminant (Germain and Summerbell, 1999).

2.2 Trichophyton

Trichophyton is a dermatophyte which thrives in the soil, humans or animals. Based on its natural habitats, the genus includes anthropophilic, zoophilic and geophilic species. Some species are cosmopolitan; others have a restricted geographical distribution. *Trichophyton cencentricum,* for example, is endemic to the Pacific Islands, Southern Asia, and Central

America. *Trichophyton* is one of the leading causes of hair, skin and nail infection in humans (Germain and Summerbell, 1999).

The genus *Trichophyton* is divided into several species, the most common being *Trichophyton, mentagrophytes, Trichophyton rubrum, Trichophyton schoenlenii. T. Rubrum* is the commonest causative agent of dermatophytis worldwide. Species may cause invasive infection in immuno-compromised hosts. The growth rate of *Trichophyton* colonies is slow to moderately rapid. The texture is waxy, glabrous to cottony (resembling cotton). From the front, the colour is white to bright yellowish or red violet. The reverse or below is pale, yellowish brown or reddish brown (De hoog *et al.*, 2000). *Trichophyton* has separate hyaline hyphae, conidiophores, microconidia and athroconidia. Chlamydospores may also be produced. Conidiophores are poorly differentiated from the hyphae. Microconidia (also known as the microaleuriconidia) are one-celled and round or pyriform in shape. They are numerous and can be solitary or arranged in clusters. Microconidia are often the predominant type of conidia produced by *Trichophyton* (Germain and Summerbell, 1999).

Macroconidia (also known as the Macroaleuriconidia) are multicellular (2 or more celled), smooth, thin, or thick-walled and cylindrical, clavate or cigar shaped. They are usually not formed or produced in very few numbers. Some species may be sterile and the use of specific media is required to induce sporulation (De hoog *et al*., 2000). *Trichophyton* differs from *Microsporum* and *Epidermophyton* by having cylindrical shape, thin walled or thick walled smooth Macroconidia (Germain and Summerbell, 1999).

2.3 Microsporum

Microsporum is a filamentous keratiophilic fungus included in the group of dermatophytes. The natural habitat of some of the *Microsporum* species is the soil (the geophilic species).

Others primarily affect various animals (the zoophilic species) or human (the anthropophilic species) some species are isolated from both soil and animals (geophilic and zoophilic). Most of the *Microsporum* species are widely distributed in the world while some have restricted geographic distribution. *Microsporum* has the asexual state that is the telemorph phase which is usually referred to as arthroderma (De hoog *et al*., 2000). The genus *Microsporum* includes 17 conventional species. Among these, the most significant ones are: *M. audouinii, M. nanum, M. gypseum, M. distortum, M. ferrugineum, M. gallinae. Microsporum* colonies are glabrous, downy, woolly or powdery. The growth on sabourand's dextrose agar at 25°C may be slow or rapid and the diameter of the colony varies from 1 to 9 cm after 7 days of incubation. The colour of the colony varies depending on the species. It may be white or yellow to cinnamon. From the reverse, it may be yellow to red-brown (Germain and Summerbell, 1999*). Microsorum* acroaleurioconidia are hypae-like. Microaleurioconidia are unicellular, solitary, oval to elevate in shape, smooth, hyaline and thin-walled (Germain and Summerbell,1999*).* Macroaleurioconidia hyaline, echinulate to roughed, thin to thick-walled, typically fusiform (spindle in shape) and multicellular (2-15 cells). They often have an annular trill. Inoculations on specific media, such as potato dextrose agar or sabouraud dextrose agar supplemented with 3 to 5% sodium chloride may be required to stimulate macroconidia production of some strains. *Microsporum* differs from *Trichophyton* and *Epidermophyton* by having spindle-shaped macroconidia with echinulate to rough walls (Germain and Summerbell, 1999).

2.4 Epidermophyton

Epidermophyton is a filamentous fungus. It is distributed worldwide. Man is the primary host of *Epidermophyton floccosum*, the only species which is pathogenic. The natural habitat of the related but nonpathogenic species *Epidermophyton stockdales* is the soil (De hoog *et al.*,

2000). The genus *Epidermophyton* contains two species: *Epidermophyton floccosum* and *Epidermophyton stockdales.* The E. *stockdales* is known to be non-pathogenic, leaving *Epidermophyton floccosum* as the only species causing infection to humans. The colonies of *Epidermophyton floccosum* grow moderately rapidly and mature within 10 days. Following incubation at 25°C on potato dextrose agar, the colonies are brownish yellow to olive-green or khaki or green-yellow from the front. From the reverse, they are orange to brown with an occasional yellow border. The texture is flat and grainy initially and become radially grooved and velvet by aging. The colonies quickly become downy and sterile (Germain and Summerbell, 1998; De hoog *et al.,* 2000). Septate hyaline, macronidia and occasionally, chlamydoconidium-like cells are seen. Micronidia are typically absent. Macronidia (10-40 x 6-12um) are thin-walled, 3 to 5-celled, smooth and clavate-shaped with rounded end. They are found singly or in clusters. Chlamydoconidium-like cells, as well as arthroconidia are common in older cultures (Germain and Summerbell, 1998, De hoog *et al.,* 2000). *Epidermophyton floccosum* is differentiated from *Microsporum and Trichophyton* by the absence of microconidia. (Germain and Summerbell, 1998, De hoog *et al*., 2000*).*

2.5 Natural habitat of dermatophyte (mycotic agents)

The most important habitat of all fungi is soil and decaying organic materials. Those of yeast are the mucous membranes or secretions, excretions and excrements of man and animals. The yeast and mould can also be found in contaminated water (Male, 1991).

The major biological function of the dermatophyte is the destruction of keratinized materials such as hide, fur, claws, nails and horns of dead animals. The major biological function of many moulds is the organic destruction of foliage and dead plant materials. Biphasic fungi also exist in soil, plant, moss and moulding wood (Male, 1991)

2.6 Pathogenesis of Mycoses

The pathogenesis of mycoses can either be formal, representing the way and mode of infection or can be casual referring to the mechanism of the disease.

2.6.1 Formal pathogenesis

The formal pathogenesis can be contacted by Contact between fungus and the host skin and or the adjacent mucous membranes. This originates all dermatophyte of epidermis, hair, and nail. They cause also all candidose of skin and genitals as well as *Pityriasis versicolor* and *Tinea nigra*. Infection by trauma, wounds and burns are mostly infected by opportunistic moulds, for example, *Aspergillus, Rhizopus* and *Mucor* species. Deep injuries by wooden objects in which the object paricle remains in the tissue can lead to infection by a group of moulds called the dermatiaceous, sporotichosis or mycetoma in temperate climate. Candida is found to infect open surgery especially of the heart. Transmission can also be through catheters, following long-term urethral catheterization. Heamatogenic propagation of the pathogens can occur by inhalation of the organism, for example, Conidia of moulds and biphasic fungi. Some organisms can be ingested in small amounts with contaminated foods. They may multiply rapidly in the intestine where they are favoured by some pathogens and some antibiotics such as amino glycosides and some newer cephlalosporins. For the establishment of mycoses, the pathogen must be able to infect the host tissue by tolerating the physical, chemical and immunological condition of the host. They should also be able to enzymatically break down and utilise the host tissues on the other hand. In a situation where infections occur, when the pathogenic potential of the pathogen overcomes the resistance of the host, such fungi are called primary or obligatory pathogenic and the patients infection is called primary mycoses. In case where the resistance of the host is relatively smaller than the pathogenic potential of such

pathogens is called secondary, potentially pathogenic or opportunistic and the respective infections is called secondary mycoses (Male, 1991)

2.7 Mycoses Caused by Dermatophytes

Dermatophytes are filamentous fungi which cause ring worm in man and animal (Noble *et al.*, 1998).The dermatophyte as described earlier may be divided into three (3) group geophilic, commonly inhibiting soil, zoophilic primary invading animal and man (Male, 1991).

2.7.1 Microbiology

Dermatophyte grows well on sabourauds dextrose agar (SDA) and is distinguished by the nature of their spores, especially the microconidia (Noble *et al* 1998). A key to their identification can be found in Rebell and Taplin (1970).

2.8 Epidermal Dermatophyte

2.8.1 *Tinea manuum* (Tinea pedis)

This is tinea of the feet (toes webs), the hand (palm). Symptom is interdigital scaling involving the third and fourth interdigital space, which may extend to the planter surface often with fissures and secondary bacterial infection, hyper keratosis inflammation, erosion and itching. The pathogens are anthrophilic *Trichophyton* species *(T. rubrum, T. interdigitale, E. floccosum)*. (Male, 1991).

2.8.2 Onychomycosis (*Tinea unguis*)

Tinea of the nails, about 85% of the cases of this disease are found on toes, 12% on both the toes and finger (Male, 1991). Symptoms are a small area of white, yellow or brownish

discoloration on side of the nail, close to lateral nail fold, accumulation of subungal keratinisation and crumbling of nail plate. The Pathogen is *T. rubrum, T. mentagrophytes, and E. floccosum*.

2.8.3 *Tinea capitis*

Tinea of the hair skin: it is caused by anthrophilic geophilic and zoophilic dermatophyte. Example *T. tonsurans, T. mentogrophytes, T. verrucossum,* and *T. Schoenleinii* (Male, 1991).

The symptoms including lesion of *Tinea capitis* are associated with loss of hair with partial alopecia, hair breakage with short stubble or mating of hair in crust and scale. The lesion may consist of highly inflammatory deep reaching nodular scales any peripilar pustles and crusts (Male, 1991)

2.8.4 *Tinea corporis*

This is a tinea that affects anypart of the skin except the scalp, beard, groins, hands and feet and caused by *Trichophyton mentagrophytes*.

Symptoms: It may have a variety of appearances; most easily identifiable are the enlarging, raised red rings with a central area of clearing (ringworm). Sometimes the skin surrounding the rash may be dry and flaky. Almost invariably, there will be hair loss in area of the infection (Male,1991).

2.8.5 *Tinea cruris*

This is Tinea of the groin. It rarely occurs in women and unusual in men before puberty. It is most common during summer on persons who use tight occlusive clothing.

Symptom: the lesion occurs in the inner thigh. It appears as annular plaque with well-defined borders. The disease could extensively spread over the buttock and waistline without involving the scrotum.

Tinea cruris caused by *E. floccosum* is associated with little central clearing scaling and a characteristics satellite lesions. With *T. rubrum* it may spread beyond the groins and more severe inflammation is caused by *T. mentogrophyte.* The border of the affected area is vesicular with probably follicular papules or pustles (Male, 1991).

2.8.6 Candidose

About 85% - 95% of mycoses are caused by *Candida albicans* and the remainder are due to other Candida species particularly C.*tropicalis, C. guilliermandis, C. parapslosis, C. pseudotropicalis*.

The *Candida* genus is yeast-like fungi. They are frequently symbiotic inhabitant of gastrointestinal tract and vagina of normal individual. Under suitable conditions they may cause infection either on skin or on mucous membrane. *Candida* is not a virulent organism and is probably never pathogenic in a normal healthy tissue. When host resistance is impaired by systemic or local factors, the fungus may proliferate and become invasive. Systematic predisposing factors are: diabetis mellitus, pregnancy, debilitation and therapy with broad-spectrum antibiotics, corticosteroids, cytotoxic agents and birth control pills. Candidoses may also occur in individuals with certain peculiar specific cellular or general immune response, e.g Acquired Immune Deficiency Syndrome (AIDS) or Severe Combined Immune-Defiency (SCIDS). Symptoms which are mucous membrane infections (thrush) in infants and adults appear as white plaques on the tongue and buccal surfaces as well as mucous membrane of the vagina. Perimucosal skin infection may occur around the rectum and genitalia. In the groin the

lesion is inflamed, bright red pique with poorly defined margins while a small amount of weeping and crushing lesion may occur in other intertriginous area and webs of fingers (Ryan and Ray, 2004).

2.8.7 Aspergillosis

Aspergillus spp. is the most commonly isolated invasion moulds (Denning *et al.,* 1998). Only a few of the 200 species are pathogenic to man, primarily *Aspergillus fumigatus.Aspergillus flavus* and *Aspergillus niger. Aspergillus fumigatus* remains the mould most frequently isolated, but the epidemiology appears to be changing .*Aspergillus fumigatus* accounted for 82% of cases of invasive *Aspergillosis* in 1985, compared to 66% in 1999 in stem cell transplant patients (Marr *et al.,* 2000) .*Aspergillus terreus* is increasingly recognized as a pathogen accounting for 15% of isolates in 2001, compared to < 2% in 1996 in one study (Baddley *et al*.,2003). *Aspergillus* spp can lead to invasive *Aspergillosis*, trachea bronchitis, *Aspergilloma* and chronic necrotizins *aspergillosis*, but colonization without infection can occur. Infections caused by *Aspergillus spp*. have significantly increased in recent years. This is probably as a result of increasing numbers of patients at risk including the HIV immuno-compromised individuals. Despite a better understanding of the epidemiology of *Aspergillus* infections, important diagnostic limitations persist. *Aspergillus spp.* can cause disease in humans by colonization and subsequent allergic reactions, colonization of pre-existing cavities or tissue invasion. *Aspergillus flavus* is the second leading cause of invasive and non-invasive aspergillosis (Hedayati *et al.,* 2007) and is more virulent than *Aspergillus fumigatus. Aspergillus flavus* is a common cause of fungal sinusitis and cutaneous infections. Chronic conditions such as chronic cavitary pulmonary aspergillosis and sinuses fungal balls have rarely been associated with *Aspergilus flavus.* However, infection by *Aspergillus fumigates* is

the most prevalent species in the genus (Guinea *et al.,* 2005). Amongst the several secondary metabolites produced by *Aspergillus flavus* are aflabolites, the most toxic and potent carcinogenic natural compounds ever characterized (Hedayati *et al.,* 2007).

2.8.8 Treatment of Mycoses

Superficial mycoses mainly nail and hair infections are currently treated with griseofulvin, ketoconazole, fluconizole and itraconazole.Topical application of tonaftate is recommended for the treatment of most zoophilic and geophilic tinea. Oral griseofulvin is usually required to eliminate more chronic anthrophilic infection at a dosage of 500mg daily for adults; while in children the dosage may be half or three fourth of the adult dose preferably after fatty meal. However, these antifungal are associated with some side effects and other problems of resistance. For example, amphotericin a highly nephrotoxic, while 5-flourocytoxin is associated with severe problems of resistance and amphotericin B, micoazole, ketoconazole and itraconnazole are unsatisfactory from the point of view of tissue penetration (Male, 1991).

Since the few antifungal agents available are not active in a sufficient measure against all the heterogenous fungal species responsible for systemic mycoses, there is an urgent need for further development of systematically applicable preparations (Male, 1991).

2.9 Concept of Mycoses and Its Management in Traditional Medicine

Traditional medicine practitioner believed that mycotic agent is an inherent part of every individual and can pass from generation to generation. The diseases only manifest themselves when the concentration of the pathogen is extremely high (Principle, 1991).

2.9.1 The medicinal plants

The World Health Organization (WHO) considers medicinal herb as any vegetable containing in one or more of its organs; any substance that can be used for therapeutic purpose or as raw materials from chemical-pharmaceutical synthesis (Principle, 1991).

2.9.2 Historical review of medicinal plants

One of the earliest recorded plants in medicine dates back to about 3000BC and this was oil obtained from the plant *Hydrocarpus gaertn,* used for the treatment of leprosy. Even before then other plants like opium poppy *(Papaver somniferum)* and Castor oil seed (*Ricinus communis* L.) were being used in Egypt. The earliest use of herb plants in traditional medicine can be found in Ebers Papyrus written in 1500BC, while for Chinese, it can be found in Chinese pharmacopoeia written around 1122BC. The father of medicine, Hippocrates, included about 400 recipes of herbal origin in his material. The list includes Opium, mint, sage and Rosemary (Principle, 1991).

Mexico and Peru had provided some useful medicinal plants such as Coco and Tobacco, as far back as 1531BC. It was also discovered in the 18^{th} century that Australian aborigines used medicinal herbs along with ritual rites. The ancient Ayurveda, Indian system of medicine, was described in about 1200BC with a list of over 14000 dispensaries in India. The later civilization of Greece, Rome, Arabia, Europe and America all recorded their use of plants as medicine. Most of the plants recognized by these societies as having medicinal properties are still in use or have provided the chemical model for the derivatives of their natural product (Principle, 1991)

Nigerian ancients have used medicinal plants for hundreds of years for the treatment of many common ailments. Many traditional practitioners have claimed that their ancestors in various ways transferred their knowledge and use of herbs to them. Herbs or some parts of plants or combination of plant and some herbs are used right from conception to the time the child is born and raised to adulthood (Owonibi, 1989).

The accomplishment of native people in understanding plant properties so thoroughly must be simply as a result of long and intimate association with floras and their dependence on them. Consequently, and especially since so much aboriginal knowledge is based on experiment, these achievements warrant careful and critical attention on the part of modern scientific research. This medical practitioners start to take advantage of the plants that still exist in many parts of the world, lest it be lost with inexorable spread of civilization and the extinction of one primitive culture after another (Owonibi, 1989).

2.9.3 The state of medicinal plants in the world today

Hussain (1991) reported that plants continue to provide basic raw materials for some of the most important drugs, despite the increase in development of new drugs from synthetic sources.

According to WHO (Farnsworth *et al*.,1985) about 30% of the inhabitants of the world rely on traditional medicine for their primary health care needs and the major parts of traditional therapy involve the use of plant extracts or their active principle. All the regions of the world have contributed to herbal medicine as discussed below.

2.9.3.1 American region

The World Wild Life Fund in USA at Botanical Museum of Harvard University not only finances modest programme of field investigation in parts of tropical South America, whose botanical knowledge is threatened by Western civilization, but has also set up an ethno botanical specialist group consisting of botanists, anthropologists and experts in related fields of discipline around the world. This helps in bringing together investigations dedicated to ethnobotany in many countries (Plotlain, 1983). Furthermore, Sofowora (1993) reported that about 25% of all the prescriptions dispensed in community pharmacies in the USA contain drugs extracted from higher plants.

2.9.3.2 European region

The proportion of prescription containing plant extracts is more than 60% in Eastern Europe, while it is between 35-40% in West Germany (Hussain, 1991). In Europe, researches on medicinal plants were carried out in University departments, leading to an increasing trend in the use of herbs and homeopatheic medicine (Hussain, 1991).

2.9.3.3 Asian region

Medicinal products compound from herbs are common sight in the drugs stores in Asia. This is because the Chinese system of medicine widely practised in China, Japan, Korea, Hong Kong and Malaysia gained more than 80% of its medicaments from plants. Similarly, the traditional system of Ayurveda, Siddha and Unani practised in India, Pakistan, Bangladesh, Sri -lanka and Nepal are mainly based on drugs of plant origin (Hag and Hussain, 1993).

2.9.3.4 African region

The Organization of African Unity now African Union (AU) through its Scientific Technical and Research Commission (OAU/STRC) has financed research on African medicinal plants in many countries. These countries include Angola, Egypt, Senegal, Nigeria, etc. (Sofowora, 1993).

2.9.3.5 Nigeria

Awosika (1993) reported that about 80% of Nigeria depends on traditional medicine as a major approach to curing diseases. This may be due to the fact that local medicinal plants are readily available, effective, economical and a less complicated method of curing diseases.

2.10 Economic Impact of Medicinal Plants

The pharmaceutical industry in the United States has attained in the prescription market alone, annual sales in excess of $3,000,000,000 from medicine isolated first from plants. Many of them were discovered in use amongst unlettered people in aboriginal society around the world (Schulte and Farnsworth, 1980). Recent report by Principle (1991) assumed that the present value through the year 2000 of current plant-based pharmaceuticals in Western European Countries, United States, Japan and Turkey is about US$1500.00 billion which is approximately equivalent to the annual GDP of France or the United Kingdom. In China, the World Health Organization reported that the products of traditional medicine were valued at US$571 million and the country-wide sales of crude plant drugs is up to US$1.4 billion annually (Akerele, 1991). In Singapore alone the trade is worth more than US$49 million annually (Ooi, 1993).

Ekwunife and Aguwa (2010). Reported that the drugs imported to Nigeria were about £120 million (US$ 200 million). Such import bills suggest the need for screening of our vast flora for alternative drugs. Another problem is that synthetic drugs are often far more expensive. For example reserpine can be commercially extracted from natural sources for about US$1.25 per gram (67%). This shows that the difference is much larger with the synthesis drugs because of increased cost of production which is higher than natural products (Principle, 1991).

2.10.1 Origin and distribution of *Euphorbia heterophylla*

Euphorbia is one of the most diverse genera in the family *Euphorbiaceae*. Members of the family and genus are sometimes referred to as spurges. The leaves of *Euphorbia heterophylla* are commonly used as a lactogentic agent by taking a decoction of it or massaging the breast with the poultice to induce milk flow (Dokosi, 1998). They are also used in traditional medical practice as laxative and to treat gonorrhoea, migraine and viral warts while the plant latex is used as fish poison, insecticide and ordeal poisons (Rodriguez *et al.,* 1976; Falodun *et al*., 2003). Recently their anti-tum or anticancer properties and their activities against the Human Immuno deficiency Virus (HIV) have also been reported in *E. hetrophylla* leaf (Williams *et al*., 1995).

Euphorbia heterophylla Linn is native to Central and South America, but now widely distributed throughout the tropics and subtropics. It occurs throughout most tropical Africa and the Indian Ocean islands, as well as in the Mediterranean region and South Africa (Aerestrup *et al.,* 2008). *Euphorbia heterophylla* Linn is widely used in traditional African medicine and elsewhere in Tropical countries. In Africa a decoction or infusion of the stem and fresh or dried leaves is taken as a purgative and laxative to treat stomach–ache and

constipation and to expel intestinal worms. A leaf infusion is used to treat skin problems, including fungal diseases and abscesses. In Nigeria, the latex and preparations of the leaves and roots are applied to treat skin tumours. In East Africa, the roots are used in the treatment of gonorrhea or to increase milk production in breast–feeding women. The latex is irritant to the skin and eyes and may be employed as ruberfacient and to remove warts. However, the latex is also used as an antidote against the irritation caused by the latex of other *Euphorbia* species. In peninsular Malaysia, a leaf extract is taken to treat body pain. The latex is used in the preparation of arrow and fish poison (Falodun *et al*., 2003).

In Benin the leaves are eaten as vegetable or famine food, despite their laxative action. The plant is grazed by livestock, and can be fed to guinea pigs as an addition to fresh forage. Honey bees collect nectar from the flower. All parts of *Euphorbia heterophylla* contain latex: leaves 0.42% stem 0.11%, root 0.06% and whole plant up to 0.77% (Falodun *et al* 2003).

A water extract from the leaves exhibited strong purgative effect when given orally to rats. The hexane, chloroform and ethyl acetate extracts from the roots showed significant antinociceptive activity in rats at doses of 150–300mg/kg. *Euphorbia heterophylla* is propagated by seed. Fresh seed germinates readily under tropical conditions, but remain dormant under temperate circumstance. Both light and temperature influence the breaking of dormancy. The turning of soil favour germination and seeds germinate even when at a depth of 10cm in the soil (Burkill, 1994).

2.10.2 Habitat*:* *E. heterophylla* grow in moist tropical and subtropical regions on a wide range of soil, principally in shaded waste places and in cultivated areas (Parsons and Cuthbertson, 1992).

2.10.3 Classification of *E. heterophylla* .Linn.

Euphorbia heterophylla belongs to Kingdom–plantae (plant), Division-Magnoliophyta (flowering plants), Class-Magnoliopsida (dicotyledon), Order-Euphorbiales, Family-Euphorbiaceae, (spurge family) Genus-Euphorbia (spurge) and Species-*E. heterophylla* Common name is Mexican fire plant (Linnaeus, C. 1753).



Plate I: *Euphorbia heterophylla* Linn.

2.11 Origin and Distribution of *Mitracarpus scaber* Zucc.

Mitracarpus scaber Zucc (*Rubiaceae*) which is also known as *Mitracarpus villosus* (SW) DC, is a very common weed of cultivated or fallowed land. Recent studies have shown that alcohol extracts of the aerial parts of *M.villosus* had *in-vitro* antimicrobial activities against *Dermatophilus congolensis*. Experiments have proved that ointment containing alcohol extracts of *M. villosus* used topically had a high efficiency against bovine *Dermatophilosis* and cured tested animals without recurrence (Ali-Emmanuel *et al*., 2003).

Mitracarpus scaber is native to sub-saharan Asia (Thailand) but has been planted worldwide. *Rubiaceae* family is a large family of 630 genera and about 1300 species found worldwide especially in tropical and warm regions. These plants are not only ornamental but are also used in African folk medicine to treat several diseases (Ali-Emmanuel *et al*., 2003). In sub-Saharan Africa, *M. scaber* chemical composition and the screened pharmacological activities are used to treat skin infection (Ali- Emmanuel *et al*., 2003).

It is a common weed in upland areas of savanna. The plant is widely used traditionally among most communities as poultice in the treatment of skin disease such as scabies, eczema, itching and dandruff (Bisignano *et al*., 2000). *M. scaber* is widely employed in traditional medicine in West Africa for headache, venerial diseases, amenorrhoea, dyspepsia, hepatic disease and leprosy (Bisignano *et al*., 2000).

The inflorescence is dense and clustered together with small white flowers at the leaf axils that are usually thick. *M. scaber* vary in its habitat and reproduces by seed.

2.12 Classification of *Mitracarpus scaber* Zucc.

Mitracarpus scaber (ZUCC) belongs to the Kingdom -Plantae, Division -Magnoliophyta, Class-Magnoliopsida, Order-Gentianales, Family-Rubiaceae, Genus-Mitracarpus and Species *M. scaber*. Common name is Botton grasses, Gogamasu (Hausa) (Zuccarini, J.G. 1848).



Plate II: *Mitracarpus scaber* Zucc.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Collection and Identification of Plant Materials

The fresh whole plants of *Euphorbia heterophylla* and *Mitracarpus scaber* were collected from a site opposite Sun-seed Company New Jos Road, Dakache, Zaria, Kaduna State. Samples from the plant were taken to the Herbarium Section of the Department of Biological Sciences, Ahmadu Bello University, Zaria for authentication. The voucher specimen is deposited in Herbarium Section for future reference as follows *Euphorbia heterophylla* Linn, (588) and *Mitracarpus scaber* Zucc. (900007).

The collected whole plants of *E. heterophylla* and *M. scaber* were washed and dried at room temperature in the laboratory for two weeks. Samples were ground to powder form using a mortar and pestle. The powdered plant samples were kept in polythene bags until extraction.

3.2 Preparation of Extracts

The 500g powdered sample was extracted with Hexane (2 L) using maceration method. The residue (marc) were dried and then extracted with ethyl acetate (2 L). The residue (marc) was extracted with methanol (2 L) and finally with water (2 L). Phytochemical test (screening) according to the method of Wagner and Bladt (1996).

3.3 Phytochemical Screening

Phytochemical screening (qualitatively) was carried out on the extracts using the standard methods described by Trease and Evans (1996) to determine the following bioactive ingredients: carbohydrates, cardiac glycosides, saponins, alkaloids, tannins, steroids and triterpenes, flavonoids, and anthraquinones.

3.4 Test for Carbohydrates

3.4.1 Molisch's test

To a small portion of 1g extract in a test tube, some drops of Molisch's reagent were added and concentrated sulphuric acid was added down the side of the test tube to form a lower layer. A reddish coloured ring at the interphase indicates presence of carbohydrates (Trease and Evans, 1996).

3.4.2 Fehling's test

To a small portion of 1g extract in a test tube, 5ml of an equal mixture of Fehling's solution A and B was added and boiled in a water bath. Brick red precipitate indicates presence of reducing sugar (Trease and Evans, 1996).

3.5 Test for Cardiac Glycosides

3.5.1 Keller-Kiliani Test

A portion of 1g extract was dissolved in 1ml of glacial acetic acid containing traces of Ferric Chloride solution. This was then transferred into a dry test tube and 1ml of concentrated Sulphuric acid was added down the side of the test tube to form a lower layer at the bottom. This was observed carefully at the interphase for purple-brown ring which indicated the presence of cardiac glycosides (Trease and Evans, 1996).

3.5.2 Kedde's test

To a portion of 1g extract, 1ml of 2% solution of 3, 5-Dinitrobenzoic acid in 95% alcohol was added. The solution was made alkaline with 5% sodium hydroxide. Appearance of purple-blue colour indicates the presence of cardenolides (Trease and Evans, 1996).

3.6 Test for Saponins

3.6.1 Frothing test

About 10ml of distilled water was added to a portion of 1g extract and was shaken vigorously for 30 seconds. The tube was allowed to stand in a vertical position and was observed for 30 mins. A honeycomb froth that persisted for 10-15mins indicates the presence of saponins (Trease and Evans, 1996).

3.7 Test for Steroids and Triterpenes

3.7.1 Liebermann-Bucchard test

To a portion of 1g extract, equal volume of acetic acid anhydride was added and mixed gently. 1ml of concentrated sulphuric acid was added down the side of the test tube to form a layer. Colour changes were observed immediately and over a period of one hour. Blue to blue-green colour in the upper layer indicates the presence of steroids and a reddish, pink or purple colour indicated the presence of triterpenes (Trease and Evans, 1996).

3.8 Test for Flavonoids

3.8.1 Shinoda test

A portion of 1g extract was dissolved in l-2ml of 50% methanol, warmed and filtered, three pieces of metallic magnesium chips was added, followed by a few drops of concentrated sulphuric acid. An orange colour indicates presence of flavonones, flavonols and flavonoids (Silva et al 1998).

3.8.2 Sodium Hydroxide test

A few drops of 10% sodium hydroxide were added to 1g extract. Yellow coloration indicates the presence of flavonoid (Trease and Evans, 1996).

3.8.3 Ferric Chloride test

A few drops of ferric chloride solution were added to a portion of 1g extract. A green precipitate indicated presence of phenols (Trease and Evans, 1996).

3.9 Test for Tannins

3.9.1 Ferric Chloride test

To a portion of 1g extract, 3-5 drops of ferric chloride solution was added. A greenish-black precipitate indicates the presence of condensed tannins while hydrolysable tannins gave a blue or brownish-blue precipitate (Trease and Evans, 1996).

3.9.2 Bromine water test

Few drops of bromine water were added to 1g extracts in a test tube. A buff (pale yellow-brown) coloured precipitate indicates condensed tannins, while hydrolysable tannins gave none at all (Trease and Evans, 1996).

3.9.3 Lead sub-acetate test

To a small portion of 1g extract, 3-5 drops of lead sub-acetate solution were added. A yellow coloured precipitate indicates the presence of tannins (Trease and Evans, 1996).

3.10 Test for Alkaloids

3.10.1 Mayer's test

To a portion of 1g extract, a few drops of Mayer's reagent were added. A cream precipitate indicates the presence of alkaloids (Trease and Evans, 1996).

3.10.2 Dragendoff's test

To a portion of 1g extract, a few drops of Dragendoff's reagent were added. A reddish brown precipitate indicates the presence of alkaloids (Trease and Evans, 1996).

3.10.3 Wagner's test

A few drops of Wagner's reagent were added to a portion of 1g extract. A whitish precipitate indicates the presence of alkaloids (Trease and Evans, 1996).

3.10.4 Picric acid test

A few drops of 1% Picric acid solution were added to a portion of 1g extract in a test tube. Yellow colouration indicates the presence of alkaloids (Trease and Evans, 1996).

3.11 Test for free Anthraquinones

3.11.1 Bontrager's test

To a portion of 1g extract in a dry test tube, 5ml of chloroform was added and shaken for at least 5 minutes. This was filtered and the filtrate shaken with equal volume of 10% ammonia solution. A bright pink colour in the aqueous (upper) layer indicates the presence of free anthraquinones (Trease and Evans, 1996).

3.12 Test for Combined Anthracene

3.12.1 Modified Bontrager's test

Small portion of 1g extract in a test tube was boiled with 5ml of 10% hydrochloric acid for 2-3mins. This would hydrolyse the glycosides to yield aglycones which are soluble in hot water. This was then filtered and the filtrate was cooled and extracted with 5ml of benzene. The benzene layer was pipetted off and shaken gently in a test tube with half of its volume 10% ammonium hydroxide. If the lower ammonia layer becomes rose pink to cherry red, the extract contains anthraquinones derivatives free or in combined state (Trease and Evans, 1996).

3.13 Quantitative Analysis of the Phytochemical Constituents

3.13.1 Alkaloid determination using Harborne (1973) method

About 5g of the sample is weighed into a 250ml beaker and 200ml of 10% acetic acid in ethanol will be added, covered and allowed to stand for 4 hours. This was filtered and the extract was concentrated on a water bath to one quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue was the alkaloid, which was dried and weighed.

3.13.2 Flavonoid determination by the method of Boham and Kocipai–Abyazan (1994)

About l0g of the plant sample will be extracted repeatedly with 100ml of 80% aqueous methanol at room temperature. The whole solution was filtered through whatman filter paper No. 42(125mm). The filtrate was later transferred into a crucible and evaporated into dryness over a water bath and weighed to a constant weight.

3.13.3 Saponin determination

The method of Obadoni and Ochuko (2001) was used. Out of the ground samples 20g was weighed for each and placed in a conical flask and 100ml of 20% aqueous ethanol was added. The sample was heated over a hot water bath for 4 hours with continuous stirring at about 55°C. The mixture was filtered and the residue re-extracted with another 200ml 20% ethanol. The combined extracts were reduced to 40ml over water bath at 90°C. The concentrated extracts were transferred into a 250ml separatory funnel and 20ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated and 60ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation, the samples were dried in the oven to a constant weight; the saponin content was calculated as a percentage.

3.13.4 Tannin determination by Van-Burden and Robinson (1981) Method

About 500mg of the sample was weighed into a 50ml plastic bottle and 50ml of distilled water was added and shaken for 1 hour on a mechanical shaker. This was filtered into a 50ml volumetric flask and made up to the mark. Then 5ml of the filtered sample was pipetted out into a test tube and mixed with 2ml of 0.1M $FeC1_3$ in 0.1M HC1 and 0.008M potassium ferrocyanide. The absorbance was measured at 120 mm within 10 minutes.

3.13.5 Determination of total Phenols by Spectrophotometric Method

The fat free sample was boiled with 50ml of ether for the extraction of the Phenolic component for 15 minutes. About 5ml of the extract was pipetted into a 50ml flask; then10ml of distilled water was added. About 2ml of ammonium hydroxide solution and 5ml of concentrated amylacohol was added also. The sample was made up to mark and left to react for about 30

minutes for colour development. This was measured at 550nm with an UV-visible spectrophotometer.

3.14 Collection of the Fungi

The fungi used were obtained from the cultured microorganisms collected from Department of Medical Microbiology ABUTH Shika, Zaria. This sample was subcultured and maintained in Sabouraud's Dextrose Agar (slants) at 4^0C.

3.15 Identification of Isolated Fungi

The fungi species were identified by their cultural macroscopic and microscopic characters. The cultural macroscopic properties were studied. (ie the hyphae, presence of conidiophores, colour, shape of the conidia head e.t.c. James and Natalie, 2001).

The microscopic identification was aided by appropriate taxonomic keys and atlas of Dermatophyte (James and Natalie, 2001). Pure cultures were inoculated into slant bottles containing sterile SDA to serve as reservoir and store under low temperature inside the refrigerator (8-15^0C) to arrest their growth.

Lactophenol cotton blue stain was dropped on a clean slide using dropping pipette, while a small portion of mycelium was teased on the slide, using a needle. The slide was covered with pressure to eliminate air bubbles. The slide was then mounted and observed under the microscope at x10 and x40 objective lenses respectively. The species encountered were identified in accordance with Cheesbrough (2000).

3.16 Preparation of Fungal Media

3.16.1 Sabouraud's Dextrose Agar (SDA)

The medium was prepared according to manufacturer's instruction (Oxoids Limited Basingstoke, Hampshire England).

This was prepared by dissolving 42g of its powder in distilled water to produce a litre and then heated gently to dissolve the agar completely. The SDA was distributed into l0mls and 20mls bottles, capped and autoclaved at 121^0C for 15 minutes. After sterilization, 10ml SDA bottles were slanted and allowed to set firmly to give agar slope (slants).

3.16.2 Sabouraud's Dextrose Liquid Medium (SDLM)

The medium was prepared by dissolving 30 grammes of SDLM-powder (Oxiods) in one litre of distilled water and distributed into the final containers and sterilized at 121°C for 15minutes.The media were allowed to cool to 45°C and 20ml of the sterilized medium was poured into sterile petri dishes and allowed to cool and solidify. (Olowosulu *et al*., 2005).

3.16.3 Preparation of test organisms

The fungal culture was prepared on sabouraud's dextrose agar incorporated with 0.5% cycloheximide and 0.45% chloramphenicol and incubated at 30°C for 7days, thereafter, kept in triplicates as stock fungal spores cultures at 4°C (Aberkane *et al*., 2002).

3.16.4 Maintenance of Test Fungal Stock Culture

To do this, six freshly prepared sabouraud's dextrose agar slants were inoculated each and harvested, washed isolated and incubated at 30°C for 5-7 days. (Aberkane *et al*., 2002).

3.16.5 Preparation of spore suspension

This was prepared by harvesting SDA slants containing stock fungal culture. The harvested fungal spores were washed using harvesting medium. The supernatants were discarded and

spore-pellets resuspended in harvesting medium, standardized and stored at 4°C until required for use within two weeks (Olowosulu *et al*., 2005).

3.17 Determination of Zone of Inhibition

This was done by flooding prepared SDA plates with standardized spores suspension of the test fungus and draining the excess. Cups were bored in the set agar with the aid of flamed cork-borer. Using micropipette, 0.lml volume of graded concentration of the crude extract were transferred into each cup on the SDA plates and the plates allowed to stand for one hour for diffusion of the antifungal agents into the agar. The plates were incubated at 30°C for 3-4 days. The zones of inhibition of fungal growth on SDA were measured using caliper to measure the diameter in millimeter (Otimenyin *et al.,* 2008)

3.18 Determination of Minimum Inhibitory Concentration (MIC)

Agar dilution technique was employed for this. Here, a 10ml volume of double-strength SDA was melted and 10 ml volume of graded concentration of crude extract were mixed, poured aseptically in plates and allowed to set. Sterile paper disc in duplicates were aseptically placed on the set agar. The standardized fungal spores were inoculated with sterile paper disc aseptically. The minimum concentration that showed no growth was the MIC of the crude extract. The same procedure was repeated for other extracts and the standard antifungal agents such as Terbinafine (Shanmugapriya *et al.,* 2012).

3.19 Determination of Minimum Fungicidal Concentration (MFC)

The discs from the plates in MIC determination that showed no growth were removed and the fungal spores were harvested after 7 days. SDA Slant culture was washed with 10ml normal saline inoculated into SDLM with 0.05% Tween 80 with glass beads aseptically. These broths

were incubated for 48hrs at 37°C and then examined for the presence or absence of visible growth. Positive and negative controls were set up to confirm the viability of the organism (positive control), the tube contained the media inoculated with sterile water and test organism while in the negative control, and the tubes contained only media inoculated with sterile distilled water. Re-inoculation was done into recovery SDLM and incubation was done for 5days at 30°C. Visual observation of visible growth was made and the fungicidal concentration of the crude extract for that organism was examined (Hafidh *et al.,* 2011)

3.20 Statistical Analysis

SPSS version 20 statistical software package was used. One-way analysis of variance (ANOVA) was used to assess the efficiency of the plant parts in terms of the activity as was shown by the zone of inhibition. Duncan's Multiple Range Test (DMRT) was used to separate the means where the differences were significant based on ranking.

CHAPTER FOUR

4.0 RESULTS

4.1 Phytochemical Composition (qualitative) Extracts of Whole Plants of *Euphorbia heterophylla* and *Mitracarpus scaber*

The qualitative phytochemical screening carried out in the aqueous, methanol, ethyl acetate and hexane extracts of the whole plant of *Euphorbia heterophylla* and *Mitracarpus scaber* are presented in Table 4.1 and Table 4.2.

Among the eleven bioactive compounds tested, the free anthracene, combined anthracene and free anthraquinones were absent in all the plant extracts. Both extracts of *Euphorbia heterophylla* and *Mitracarpus scaber* obtained with the entire solvent used for extraction contained similar bioactive ingredients. Carbohydrate (Fehling's test), Saponins (Frothing S.H. test) was absent in the ethyl acetate and hexane extract of the two plants.

Also, Cardiac glycoside (Kedde's test), Tannins (Ferric chloride test), Flavonoids (Shinoda, Sodium hydroxide), Alkaloids (Dragendoff's and Wagners test), were absent in hexane, methanol, ethyl acetate extracts of both plants while they were present in the remaining solvent extracts.

Table 4.1: The Phytochemical Composition (qualitative) of extracts of whole plant of *Euphorbia heterophylla.*

Phytochemical Screening		**Methanol**	**Aqueous**	**Ethyl Acetate**	**n- hexane**
Carbohydrate	Molisch Test	+	+	+	+
	Fehling's Test	+	+	-	-
Cardiac Glycoside	Keller Killiani	+	+	+	+
	Kedde's	+	+	+	-
Saponins	Fronthing				
Tannins	Ferric Chloride	+	+	+	-
	Lead Sub Acetate	+	+	+	+
Flavonoids	Shinoda	+	+	+	-
	Sodium Hydroxide	-	+	-	+
Alkaloids	Dragendoff's	-	+	-	+
	Wagner	-	+	-	+
Free Anthraquinone	Combined Anthracene	-	-	-	-
	Free Anthracene	-	-	-	-
Anthraquinone	Bentrager	-	-	-	-
Sterolds	Buchard	+	+	+	+
	Triterpene	+	+	+	+

Key: + = Present, - =Absent

Table 4.2: The phytochemical Composition (qualitative) of extracts of whole plant *Mitracarpus scaber*

Phytochemical Screening		**Methanol**	**Aqueous**	**Ethyl Acetate**	**n – hexane**
Carbohydrate	Molisch's Test	+	+	+	+
	Fehling's Test	+	+	-	-
Cardiac Glycoside	Keller Killiani	+	+	+	+
	Kedde's	+	+	+	-
Saponins	Fronthing				
Tannins	Ferric Chloride	+	+	+	-
	Lead Sub Acetate	+	+	+	+
Flavonoids	Shinoda	+	+	+	-
	Sodium Hydroxide	-	+	-	+
Alkaloids	Dragendoff's	-	+	-	+
	Wagner	-	+	-	+
Free Anthraquinone	Combined Anthracene	-	-	-	-
	Free Anthracene	-	-	-	-
Anthraquinone	Bentrager	-	-	-	-
Sterolds	Buchard	+	+	+	+
	Triterpene	+	+	+	+

Key: + = Present, - =Absent

4.2 Percentage Composition of Phytochemical in *Euphorbia heterophylla* Extracts for four Different Solvents.

The phytochemical constituents of *Euphorbia heterophylla* extracts using several solvents (methanol, aqueous, ethyl acetate and n-hexane) (Table 4.3 and figure 4.1) showed that aqueous extract (6.70%) yielded the highest quantity of the phytochemical constituents followed by methanolic extract (5.44%) while n-hexane extract (3.43%) had the least yield of the constituents. In all the extracts, phenols had the highest yield of 11.20%, 9.65% and 12.30% in methanolic, n-hexane and aqueous extracts respectively while tannins (7.00%) yielded highest with ethyl acetate.

Saponins had the least yields of 0.80%, 0.50% and 3.20% with ethyl acetate, n-hexane and aqueous extracts respectively and alkaloid (1.00%) yielded least with methanolic extract. Significant difference was observed among the various phytochemical constituents using methanolic, ethyl acetate and aqueous extracts while no significant difference was observed in n-hexane extract and in phenols yielded by the various extracts

Table 4.3: Percentage Composition of Phytochemicals in *Euphorbia heterophylla* extracts from four Different Solvents

Extract	**Alkaloids**	**Flavonoids**	**Tannins**	**Saponins**	**Phenols**
Methanol	1.00±0.00^{b}	3.00±0.20^{ab}	9.00±0.50^{a}	3.00±0.40^{a}	11.20±0.20^{a}
Ethyl Acetate	1.00±0.10^{b}	2.50±0.50^{b}	7.00±0.00^{b}	0.80±0.00^{b}	6.00±0.00^{a}
n. hexane	3.00±0.90^{a}	1.00±0.00^{c}	3.00±0.00^{c}	0.50±0.05^{b}	9.65±7.35^{a}
Aqueous	4.00±0.00^{a}	4.00±0.50^{a}	10.00±0.20^{a}	3.20±0.20^{a}	12.30±0.70^{a}
Total	**2.25±0.52**	**2.63±0.43**	**7.25±1.02**	**1.88±0.47**	**9.79±1.66**
P value	**0.021***	**0.019***	**0.000****	**0.002****	**0.672ns**

Note: Means along column with the different alphabets are significantly different at P<0.05.

4.3 Percentage composition of Phytochemicals in *Mitracarpus scaber* extracts from four Different Solvents.

The phytochemical constituents of *Mitracarpus scaber* extracts using several solvents (methanol, aqueous, ethyl acetate and n-hexane) (Table 4.4 and figure 4.2) showed that Significant difference was observed in all the extracts, meanwhile, no significant difference was observed in alkaloid and flavonoids yielded by the various extracts.

Aqueous extract (5.72%) also yielded the highest quantity of the phytochemical constituents followed by methanolic extract (5.12%), ethyl acetate extract (3.46%) while n-hexane extract (1.48%) had the least yield of the constituents. In all the extracts, phenols had the highest yield of 9.70%, 5.20% and 10.00% using methanolic, ethyl acetate and aqueous extracts respectively while alkaloid and tannins yielded highest with n-hexane with 2.00% each. Alkaloid had the least yield with methanolic (0.90%) and aqueous (2.00%) extracts while saponins had the least yield with ethyl acetate (1.00%) and n-hexane (0.30%). Significant differences were observed in the percentage composition of extract in phytochemicals (alkaloids, flavonoids, tannins and saponins) but no significant difference was observed between the different solvent extract of Phenols.

Table 4.4: Percentage Composition of Phytochemical in *Mitracarpus scaber* Extracts from four Different Solvents.

Extract	**Alkaloids**	**Flavonoids**	**Tannins**	**Saponins**	**Phenols**
Methanol	0.90±0.00[b]	2.00±0.50[a]	8.00±0.70[a]	5.00±0.10[a]	9.70±0.70[a]
Ethyl Acetate	2.00±0.40[a]	2.00±0.40[a]	4.70±0.30[b]	1.00±0.00[b]	5.20±0.10[b]
n. hexane	2.00±0.30[a]	1.80±0.20[a]	2.00±0.10[c]	.30±0.00[c]	1.30±0.10[c]
Aqueous	2.00±0.20[a]	2.80±0.20[a]	8.50±0.50[a]	5.30±0.30[a]	10.00±0.70[a]
Total	**1.73±0.21**	**2.15±0.20**	**5.80±1.01**	**2.90±0.86**	**6.55±1.37**
P value	**0.101ns**	**0.321ns**	**0.002****	**0.000****	**0.001****

Note: Means along column with the different alphabets are significantly different at P<0.05.

4.4: Effect of different extracts of *Euphorbia heterophylla* on the Growth of Fungal Species (mg/ml).

The inhibitory effect of four extracts of *E. heterophylla* is presented in Table 4.5 and figure 4.3. Inhibitory effect was only observed in ethyl acetate and aqueous extracts. No inhibition was observed using methanolic and n-hexane extract.

Ethyl acetate and aqueous extract only showed inhibition on *Candida albicans* and *Trichophyton mentagrophytes* with no inhibition on *Microsporum audouinii* and *Aspergillus flavus*. Ethyl acetate and Aqueous extract on *C. albicans* and *T. mentagrophytes*, Terbinafin (control) had the highest zone of inhibition.

In ethyl acetate extract, 100 mg/ml had the highest inhibition on both *C. albicans* (24.50 mm) and *T. Mentagrophytes* (24.00 mm), followed by 50 mg/ml with inhibition of 22.50 mm and 22.00 mm on *C. albicans* and *T. mentagrophytes* respectively while 12.5 mg/ml had the least inhibition of 17.00mm and 13.50mm on *C. albicans* and *T. mentagrophytes* respectively.

In Aqueous extract, 100 mg/ml also had the highest inhibition on both organisms with *C. albicans* having 20.00 mm and *T. Mentagrophytes* having 18.00 mm inhibition, followed by 50 mg/ml with inhibition of 18.00 mm and 16.00 mm on *C. albicans* and *T. Mentagrophytes* respectively. The least inhibition of 5.00 mm was observed at 12 mg/ml on *C. albicans*. No inhibition at 25 mg/ml on *C. albicans* and *T. mentagrophytes* and at 12.5 mg/ml on *T. mentagrophytes*. Significant difference was observed among the concentrations on inhibition of *Candida albicans* using ethyl acetate and aqueous extract and on *T. mentagrophytes* using aqueous extract.

Table 4.5: Effect of Different Extracts of *Euphorbia heterophylla* on the Growth of four Fungal Species (mg/ml**)**

Extract	**Concentration (mg/mL)**	***Candida albicans***	***Trichophyton mentagrophytes***	***Microsporum audouinii***	***Aspergillus flavus***
Methanolic	100	0.00±0.00^{b}	0.00±0.00	0.00±0.00^{b}	0.00±0.00^{b}
	50	0.00±0.00^{b}	0.00±0.00^{b}	0.00±0.00^{b}	0.00±0.00^{b}
	25	0.00±0.00^{b}	0.00±0.00^{b}	0.00±0.00^{b}	0.00±0.00^{b}
	12.5	0.00±0.00^{b}	0.00±0.00^{b}	0.00±0.00^{b}	0.00±0.00^{b}
	Terbin (100mg/ml)	29.00±0.00^{a}	30.00±0.00^{a}	29.00±0.00^{a}	30.00±0.00^{a}
Ethyl Acetate	100	24.50±0.71^{b}	24.00±0.00^{b}	0.00±0.00^{b}	0.00±0.00^{b}
	50	22.50±0.71^{c}	22.00±0.00^{c}	0.00±0.00^{b}	0.00±0.00^{b}
	25	20.00±0.00^{d}	19.50±0.00^{d}	0.00±0.00^{b}	0.00±0.00^{b}
	12.5	17.00±1.41^{e}	13.50±0.50^{e}	0.00±0.00^{b}	0.00±0.00^{b}
	Terbin (100mg/ml)	29.00±0.00^{a}	30.00±0.00^{a}	29.00±0.00^{a}	30.00±0.00^{a}
n- hexane	100	0.00±0.00^{b}	0.00±0.00^{b}	0.00±0.00^{b}	0.00±0.00^{b}
	50	0.00±0.00^{b}	0.00±0.00^{b}	0.00±0.00^{b}	0.00±0.00^{b}
	25	0.00±0.00^{b}	0.00±0.00^{b}	0.00±0.00^{b}	0.00±0.00^{b}
	12.5	0.00±0.00^{b}	0.00±0.00^{b}	0.00±0.00^{b}	0.00±0.00^{b}
	Terbin (100mg/ml)	29.00±0.00^{a}	30.00±0.00^{a}	29.00±0.00^{a}	30.00±0.00^{a}
Aqueous	100	20.00±0.00^{b}	18.00±0.00^{b}	0.00±0.00^{b}	0.00±0.00^{b}
	50	18.00±0.00^{b}	16.00±0.00^{b}	0.00±0.00^{b}	0.00±0.00^{b}
	25	0.00±0.00^{c}	0.00±0.00^{c}	0.00±0.00^{b}	0.00±0.00^{b}
	12.5	5.00±7.07^{c}	0.00±0.00^{c}	0.00±0.00^{b}	0.00±0.00^{b}
	Terbin (100mg/ml)	30.00±0.00^{a}	30.00±0.00^{a}	29.00±0.00^{a}	30.00±0.00^{a}

Note: Means along column with the different alphabets are significantly different at $P<0.05$.

4.5 Effect of Different Extracts of *Mitracarpus scaber* on the Growth of four Fungal Species (mg/ml).

The inhibitory effect of four extracts of *Mitracarpus scaber* (Table 4.6 and figure4.4) showed that Inhibitory effect was observed in ethyl acetate and aqueous extracts. No inhibition was observed using methanolic and n-hexane extract.

Ethyl acetate and aqueous extract only showed inhibition on *Candida albicans* and *Trichophyton mentagrophytes* with no inhibition on *Microsporum audouinii* and *Aspergillus flavus*. Ethyl acetate and Aqueous extract on *C. Albicans* and *T. Mentagrophytes*, Terbinafin (control) had the highest zone of inhibition.

In ethyl acetate extract of *M. scaber*, 100 mg/ml had the highest inhibition on both *C. albicans* (26.00 mm) and *T. Mentagrophytes* (24.00 mm), followed by 50 mg/ml of *M. Scaber* with inhibition of 24.00 mm and 21.00 mm on *C. albicans* and *T. Mentagrophytes* respectively. While 12.5 mg/ml of *M. scaber* had the least inhibition of 18.00 on *C. albicans* and no inhibition on *T. mentagrophytes.* In Aqueous extract of *M. scaber*, 100 mg/ml also had the highest inhibition on both organisms with inhibition of 22.00 mm on *C. albicans* and 18.00 mm on *T. mentagrophytes*, followed by 50 mg/ml with inhibition of 18.00 mm and 15.00 mm on *C. albicans* and *T. Mentagrophytes* respectively. No inhibition was observed at 12.5 mg/ml and 25 mg/ml on both organisms.

Table 4.6 Effect of different Extracts of *Mitracarpus scaber* on the Growth of four Fungal Species (mg/ml)

Extract	**Concentration (mg/mL)**	***Candida albicans***	***Trichophyton mentagrophytes***	***Microsporum audouinii***	***Aspergillus flavus***
Methanolic	100	0.00±0.00^{b}	0.00±0.00^{b}	0.00±0.00^{b}	0.00±0.00^{b}
	50	0.00±0.00^{b}	0.00±0.00^{b}	0.00±0.00^{b}	0.00±0.00^{b}
	25	0.00±0.00^{b}	0.00±0.00^{b}	0.00±0.00^{b}	0.00±0.00^{b}
	12.5	0.00±0.00^{b}	0.00±0.00^{b}	0.00±0.00^{b}	0.00±0.00^{b}
	Terbin (100mg/ml)	29.00±0.00^{a}	28.00±0.00^{a}	29.50±0.71^{a}	30.00±0.00^{a}
Ethyl Acetate	100	26.00±0.00^{b}	24.00±0.00^{b}	0.00±0.00^{b}	0.00±0.00^{b}
	50	24.00±0.00^{b}	21.00±0.00^{b}	0.00±0.00^{b}	0.00±0.00^{b}
	25	20.00±0.00^{c}	18.00±0.00^{c}	0.00±0.00^{b}	0.00±0.00^{b}
	12.5	18.00±0.00^{c}	0.00±0.00^{d}	0.00±0.00^{b}	0.00±0.00^{b}
	Terbin (100mg/ml)	29.00±0.00^{a}	30.00±0.00^{a}	29.00±0.00^{a}	30.00±0.00^{a}
n- hexane	100	0.00±0.00^{b}	0.00±0.00^{b}	0.00±0.00^{b}	0.00±0.00^{b}
	50	0.00±0.00^{b}	0.00±0.00^{b}	0.00±0.00^{b}	0.00±0.00^{b}
	25	0.00±0.00^{b}	0.00±0.00^{b}	0.00±0.00^{b}	0.00±0.00^{b}
	12.5	0.00±0.00^{b}	0.00±0.00^{b}	0.00±0.00^{b}	0.00±0.00^{b}
	Terbin (100mg/ml)	29.00±0.00^{a}	30.00±0.00^{a}	30.00±0.00^{a}	30.00±0.00^{a}
Aqueous	100	22.00±0.00^{b}	18.00±0.00^{b}	0.00±0.00^{b}	0.00±0.00^{b}
	50	18.00±0.00^{c}	15.00±0.00^{b}	0.00±0.00^{b}	0.00±0.00^{b}
	25	0.00±0.00	0.00±0.00^{c}	0.00±0.00^{b}	0.00±0.00^{b}
	12.5	0.00±0.00	0.00±0.00^{c}	0.00±0.00^{b}	0.00±0.00^{b}
	Terbin (100mg/ml)	30.00±0.00^{a}	30.00±0.00^{a}	30.00±0.00^{a}	30.00±0.00^{a}

Note: Means along column with different alphabets are significantly different at P<0.05.

4.6 Effect of Combined Extracts of *Mitracarpus scaber* and *Euphorbia heterophylla* on the Growth of four Fungal Species (mg/ml)

All the extracts of combined *M. scaber* and *E.heterophylla* were observed to show inhibition on *C. albicans* and *T. Mentagrophytes* with no inhibition on *M. audouinii* and *A. flavus* (Table 4.7 and figure 4.5). Methanolic extract showed the highest inhibition of 19.00 mm and 18.00 mm on *C. albicans* and *T. mentagrophytes* respectively at 100 mg/ml with the least inhibition at 50 mg/ml on *C. albicans* (17.00 mm) and *T. mentagrophytes* (16.00 mm). No inhibition at 25 mg/ml and 12.5 mg/ml was observed. Ethyl acetate extract of the combined plants showed thehighest inhibition at 100 mg/ml on *C. albicans* (28.00 mm) and *T. mentagrophytes* (26.00 mm), followed by 50 mg/ml with inhibition of 26.00 mm and 24.00 mm on *C. albicans* and *T. mentagrophytes* respectively while 12.5 mg/ml had the least inhibition on *C. albicans* (20.00 mm) and *T. mentagrophytes* (18.00 mm).

In n-hexane extract of both plants combined, inhibition was only at 100 mg/ml and 50 mg/ml with 100 mg/ml having the highest inhibition of 20.00 mm on *C. albicans* and 17.00 mm on *T. mentagrophytes*. Combined aqueous extract of *M. scaber* and *E. heterophylla* was observed to have the highest inhibition of 23.00 mm on *C. albicans* and 20.00 mm on *T. mentagrophytes* with least inhibition of 16.00 mm at 12.5 mg/ml on *C. albicans* and 18.00 mm at 50 mg/ml on *T. mentagrophytes*. No inhibition at 25 mg/ml and 12.5 mg/ml on *T. mentagrophytes* was observed.

Table 4.7: Effect of Combined Extracts of *Mitracarpus scaber* and *Euphorbia heterophylla* on the Growth of four Fungal Species (mg/ml)

Extract	**Concentration (mg/mL)**	***Candida albicans***	***Trichophyton mentagrophytes***	***Microsporum audouinii***	***Aspergillus flavus***
Combined Methanolic	100	19.00±0.00^{b}	18.00±0.00^{b}	0.00±0.00^{b}	0.00±0.00^{b}
	50	17.00±0.00^{b}	16.00±0.00^{b}	0.00±0.00^{b}	0.00±0.00^{b}
	25	0.00±0.00^{c}	0.00±0.00^{c}	0.00±0.00^{b}	0.00±0.00^{b}
	12.5	0.00±0.00^{c}	0.00±0.00^{c}	0.00±0.00^{b}	0.00±0.00^{b}
	Terbin (100mg/ml)	29.00±0.00^{a}	28.00±0.00^{a}	29.50±0.71^{a}	30.00±0.00^{a}
Combined Ethyl Acetate	100	28.00±0.00^{a}	26.00±0.00^{b}	0.00±0.00^{b}	0.00±0.00^{b}
	50	26.00±0.00^{a}	24.00±0.00^{b}	0.00±0.00^{b}	0.00±0.00^{b}
	25	24.00±0.00^{b}	22.00±0.00^{c}	0.00±0.00^{b}	0.00±0.00^{b}
	12.5	20.00±0.00^{c}	18.00±0.00^{c}	0.00±0.00^{b}	0.00±0.00^{b}
	Terbin (100mg/ml)	29.00±0.00^{a}	30.00±0.00^{a}	29.00±0.00^{a}	30.00±0.00^{a}
Combined n- hexane	100	20.00±0.00^{b}	17.00±0.00^{b}	0.00±0.00^{b}	0.00±0.00^{b}
	50	18.00±0.00^{b}	16.00±0.00^{b}	0.00±0.00^{b}	0.00±0.00^{b}
	25	0.00±0.00^{c}	0.00±0.00^{c}	0.00±0.00^{b}	0.00±0.00^{b}
	12.5	0.00±0.00^{c}	0.00±0.00^{c}	0.00±0.00^{b}	0.00±0.00^{b}
	Terbin (100mg/ml)	29.00±0.00^{a}	30.00±0.00^{a}	30.00±0.00^{a}	30.00±0.00^{a}
Combined Aqueous	100	23.00±0.00^{b}	20.00±0.00^{b}	0.00±0.00^{b}	0.00±0.00^{b}
	50	20.00±0.00^{b}	18.00±0.00^{b}	0.00±0.00^{b}	0.00±0.00^{b}
	25	18.00±0.00^{c}	0.00±0.00^{c}	0.00±0.00^{b}	0.00±0.00^{b}
	12.5	16.00±0.00^{c}	0.00±0.00^{c}	0.00±0.00^{b}	0.00±0.00^{b}
	Terbin (100mg/ml)	29.00±0.00^{a}	30.00±0.00^{a}	29.00±0.00^{a}	30.00±0.00^{a}

Note: Means along column with different alphabets are significantly different at P<0.05.

4.7 Minimum Inhibitory Concentrations (MIC) of different extracts of *E. heterophylla* and *M. scaber* (mg/ml) on four species of fungi

The minimum inhibitory concentration of the various extracts on *E. heterophylla* and *M. scaber* single and combined is presented in Table 4.8 and figure 4.6.

Minimum inhibitory concentration of 12.5 mg/ml on *C. albicans* and *T. mentagrophytes* using ethyl acetate extract of *E. heterophylla*. 25 mg/ml on *C. albicans* and 50 mg/ml on *T. mentagrophytes* using aqueous extract of *E. heterophylla*.

In *M. scaber,* ethyl acetate had an MIC of 6.25 mg/ml on *C. albicans* and 12.5 mg/ml on *T. mentagrophytes* while aqueous extract had an MIC of 12.5 mg/ml on both *C. albicans* and *T. mentagrophytes*. In the combined extracts, ethyl acetate had an MIC of 6.25 mg/ml and 12.5 mg/ml on *C. albicans* and *T. mentagrophytes* respectively. Aqueous extract of the combined had an MIC of 25 mg/ml and 50 mg/ml on *C. albicans* and *T. mentagrophytes*. Hexane and Methanol had an MIC of 50 mg/ml on both *C. albicans* and *T. mentagrophytes* respectively. No inhibition by the extracts on *M. audouinii* and *A. flavus* was observed. Terbinafine (control) had MIC of 0.78mg/ml on C. albicans, T. mentagrophytes, M. audouinii and A. flavus. When compared the solvent extracts with the standard drug Terbinafine had the highest resistant of the microorganisms.

4.8 Minimum Fungicidal Concentrations (MFC) of different extracts of *E. heterophylla* and *M. scaber* (mg/ml) on four species of fungi

The Minimum Fungicidal Concentration of the various extracts on *E. heterophylla* and *M. scaber* single and combined is presented in Table 4.9 and figure 4.7.

Ethyl acetate extract of *Euphorbia heterophylla* had Minimum Fungicidal Concentration at 50mg/ml on *Candida albicans* and *Trichophyton mentagrophytes.* Aqueous extract of *Euphorbia heterophylla* had Minimum Fungicidal Concentration at 100mg/ml on *Candida*

albicans. There was no inhibitory effect on *Trichophyton mentagrophytes* using aqueous extract.

In *M. scaber*, ethyl acetate had the MFC of 6.25 mg/ml on *C. albicans* and 12.5 mg/ml on *T. mentagrophytes* while aqueous extract had an MFC of 25 mg/ml and 50 mg/ml on *C. albicans* and *T. mentagrophytes* respectively.

In the combined extracts, ethyl acetate had an MFC of 12.5 mg/ml and 50 mg/ml on *C. albicans* and *T. mentagrophytes* respectively. Aqueous extract of the combined extracts had an MFC of 100 mg/ml on *C. albicans* with no inhibition at all on *T. mentagrophytes*. Hexane and methanol extracts had no inhibition on both *C. albicans* and *T. mentagrophytes*. There was no inhibition by the extracts of both plants on *M. audouinii* and *A. flavus.* Terbinafine (control) had MFC of 1.56mg/ml on C. albicans, T. mentagrophytes, M. audouinii and A. flavus. When compared the solvent extracts with the standard drug Terbinafine had the highest resistant of the microorganisms. Thus, this solvent extracts may be used for treatment of dermatophytes.

Table 4.8: Minimum Inhibitory Concentration (MIC) of different extracts of *E. heterophylla* and *M. scaber* (mg/ml) On four species of fungi

Plant	**Extract**	***C. albicans***	***T. mentagrophytes***	***M. audouinii***	***A. flavus***
E. heterophylla	Ethyl Acetate	12.50	12.50	-	-
	Aqueous	25.00	50.00	-	-
M. scaber	Ethyl Acetate	6.25	12.50	-	-
	Aqueous	12.50	12.50	-	-
Combined	Ethyl Acetate	6.25	12.50	-	-
	Aqueous	25.00	50.00	-	-
	Hexane	50.00	50.00	-	-
	Methanol	50.00	50.00	-	-
Control	Terbinafin	0.78	0.78	0.78	0.78

Table 4.9: Minimum Fungicidal Concentration (MFC) of Different extracts of *E. heterophylla* and *M. scaber* (mg/ml) on four species of fungi

Plant	**Extract**	***C. albicans***	***T. mentagrophytes***	***M. audouinii***	***A. flavus***
E. heterophylla	Ethyl acetate	50.00	50.00	-	-
	Aqueous	100.00	-	-	-
M. scaber	Ethyl acetate	6.25	12.5	-	-
	Aqueous	25.00	50.00	-	-
Combined	Ethyl acetate	12.50	50.00	-	-
	Aqueous	100.00	-	-	-
	Hexane	-	-	-	-
	Methanol	-	-	-	-
Control	Terbinafin	1.56	1.56	1.56	1.56

CHAPTER FIVE

5.0 DISCUSSION

All plant parts synthesize some chemicals which metabolize their physiological activities. These phytochemicals are used to cure diseases in herbal and homeopathic medicine (Kesavan *et al.*, 2007). In this present day, many people like to use traditional methods to cure general diseases (Tyagi and Bohra, 2002).

5.1 Phytochemical Profile (*Euphorbia heterophylla* and *Mitracarpus scaber*)

The qualitative phytochemical profile of the whole plant extracts (aqueous, methanol, ethyl acetate and hexane) of the two plant samples used in this research showed the presence of the following bioactive constituents: carbohydrates, steroids, glycosides, alkaloids, saponins, tannins, triterpenes and flavonoids. Some of the observed phytochemical constituents present in this plant have been reported to possess antifungal properties (George *et al*., 2002; Ubani *et al*., 2012). Tannins have been described to contain a large number of complex substances that are widely distributed in almost all parts of the plant. They are usually localized in the various parts of the plant such as the leaves, stem, roots and barks (Oyi, 2001). Phytochemicals are the natural bioactive compounds found in plants. The phytochemicals work nutrients and fibres to form an integrated part of defence system against various diseases and stress condition (Koche *et al*., 2010). The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids, steroids terpenoids, carbohydrates and phenolic compound (Pascaline *et al*., 2011).

5.2 Secondary Metabolites

5.2.1 Secondary Metabolites in *Euphorbia heterophylla*

The quantitative phytochemical constituents of the whole plant of *Euphorbia heterophylla* extracts using several solvents (aqueous, methanol, ethyl acetate and n-hexane) yielded

different proportions of extracts of plant being investigated. This showed that phenol had the highest yield of 11.20% 9.65% and 12.30% in methanolic, n-hexane and aqueous extract respectively. The reason for the high yield of phenol obtained with aqueous solvent (12.30%) is because aqueous solvent is the most polar among these three solvents used for extraction, followed by methanol and then n-hexane which is the least polar solvent. The extraction potential of solvents increase with increasing polarity i.e from non-polar to polar solvent. Tannins had the highest yield in ethyl acetate extract (7.00%). Saponins had the highest yield in aqueous extract (3.20%) while its lowest yield was obtained in the n-hexane extract (1.00%). The methanol extract gave the lowest yield of alkaloids (1.00%). n-hexane gave the lowest yield because it is the least polar or non-polar among the solvent used for extraction of the plant. An increase in the concentration of the extracts leads to an increase in the quantity of phytochemical constituents ($p<0.05$). This is similar to the findings of Edeoga *et al* (2005) in their studies on phytochemical constituents of some Nigeria medicinal plants in which they revealed the presence of alkaloids (0.86%), Phenol (0.10%), Tannins (12.46%), Flavonoids (0.74% and Saponins (0.00%), Steroids (0.00%).

Omole and Emmanuel (2010), in their studies on phytochemical composition, bioactivity and wound healing potential of *E. heterophylla* leaf extract, revealed that the plant crude extract estimated in percentage is rich in some extent in alkaloids, flavonoids, saponins and tannins, they concluded that *E. heterophylla* contains high amount of alkaloids and low saponins content. The development is likely as a result of the method of extraction used and the location where plant samples were collected. The order of decreasing concentration of the phytochemicals from aqueous and ethanol extracts of fresh and dried samples is alkalolds>cyanide>tannins>flavonoids>saponins.

Natural phenolic and flavonoids compounds are widespread in the plant kingdom. They are found in leaves, fruits, barks and wood and can accumulate in large amounts in particular organs or tissues of the plant (Nieminen *et al*., 2002).The mode of antifungal action of Phenolic and Flavonoids extract might be related to their ability to inactive adhesions, enzymes, cell envelope transport protein or related to the sites and number of OH group on the phenolic rings (Chabot *et al*., 1992 and Cowan1999). It has been reported that tannins act as an antifungal agent at higher concentrations by coagulating the protoplasm of the micro-organism (Adekunle and Ikumapayi, 2006). Similarly, the possible mechanism of action of tannins has been linked to interference with energy generation by uncoupling oxidation phosphorylation or interference with glycoprotein of cell surface (Harekrishna *et al.,* 2010). Saponins are naturally occurring surface-active glycosides and they have been reported to possess strong antifungal activities in their interaction with membrane sterols (George *et al.,* 2002)

5.2.2 Secondary metabolites of *Mitracarpus scaber*

The phytochemical constituents of *Mitracarpus scaber* extracts using several solvents (aqueous, methanol, ethyl acetate and n-hexane) showed that phenols had the highest yield of 9.70%, 5.20% and 10.00% in methanol, ethyl acetate and aqueous extracts respectively. The reason for the high yield of phenol obtained with aqueous solvent (12.30%) is because aqueous solvent is the most polar among these three solvents used for extraction, followed by methanol and then n-hexane which is the least polar solvent. The extraction potential of solvents increase with increasing polarity i.e. from non-polar to polar solvent. Alkaloids and tannins yielded the highest with 2.00% each, alkaloids had the least with methanolic (0.90%) and aqueous (2.00%) extract while saponins had the least with ethyl acetate (1.00%) and n-hexane (0.30%). The higher yield of the aqueous extract over the methanol extract and other less polar solvent could be due to their difference in polarity. During

extraction solvent diffused into the solid plant material and solubilised compounds with similar polarity (Prashant *et al.* 2011). In a study, Cimanga *et al.* (2004) reported that the percentage yield of the n-hexane extract of *Mitracarpus villosus* was 14.80%. This was higher than the percentage yield of n-hexane extract recorded in this study (0.30%). This could be due to the difference in the method of extraction used. Successive cold maceration method with various solvents of increasing polarity was employed in this study, while batch cold maceration only was employed in the study by Cimanga *et al.* (2004). This shows that *Mitracarpus scaber* and *Euphorbia heterophylla* can be used as herbal mixture for the treatment of skin infections caused by some fungi.

5.3 Antifungal Activities of Crude Extracts

5.3.1 Antifungal activities of *Euphorbia heterophylla* extracts

The inhibitory effect of four extracts (aqueous, methanol, ethyl acetate and n–hexane) of *Euphorbia heterophylla* showed that ethyl acetate and aqueous extracts had inhibition zones on *Candida albicans* and *Trichophyton mentagrophytes*. There was no zone of inhibition on *Microsporum auduonii* and *Aspergillus flavus.* An increase in the concentration of the extracts used for this analysis leads to an increase in the zone of inhibition ($p<0.05$). This result is in contrast with the result of Fred-Jaiyesimi and Abo (2010) who investigated the phytochemical and antimicrobial analysis of methanol extract, petroleum ether and chloroform fraction of the whole plant of *E. heterophylla*. They observed that the bioactive constituents present in the whole plant of *E. heterophylla* have varying polarities against the test organisms with the methanolic extract showing significant activity against *Staphylococcus albus, Proteus mirabilis, E. coli, Salmonella typhi* and *Klebsiella pneumoniae* at the dose tested and no effect on *Staphylococcus aureus, Fasarium oxysporum, Aspergillus flavus* and *Candida albicans*.

5.3.2 Antifungal activities of *Mitracarpus scaber* extracts

The inhibitory effect of four extracts (aqueous, methanol, ethyl acetate and n-hexane) of *Mitracarpus scaber* showed that ethyl acetate and aqueous extract had inhibition of zone on *Candida albicans* and *Trichophyton mentagrophytes*. There was no zone of inhibition using methanol and n-hexane extracts on *Microsporum auduounii* and *Aspergillus flavus*. The biological activities of the extracts have been shown to be highly dependent on solvent polarity (Zohra and Fawzia, 2011). Polarity is the relative ability of a molecule to engage in strong interactions with other polar molecules (Barwick, 1997). Polarity therefore represents the ability of a molecule to enter into interactions of all kinds. Polar solvents have properly of dipole interaction forces, particularly hydrogen-bond formation for which solvating molecules become soluble and leads to the solubility of the compound (Barwick, 1997). From the results of this study, the degree of antifungal activities of the test plant varied from one test microorganism to another. It was observed that there was an increase in antifungal activity with increase in the concentration of extract used. In the susceptibility test of the fungi to the different extracts, it was observed that the ethyl acetate extract of *Mitracarpus scaber* produced the highest antifungal activity against two (2) of the test fungi (Yeast and Dermatophytes). This was indicated by the diameter of zone of inhibition which was shown to increase with the increase in concentration of the extracts. The increased antifungal activity of the ethyl acetate extract over the other solvent extracts (aqueous, methanol and n-hexane) of *Mitracarpus villosus* is an indication that ethyl acetate was able to extract out most of the active components of the plant. The zone of inhibition of the ethyl acetate extracts against all the fungal species tested at 50mg/ml was comparable to the standard drugs (Terbinafine) used. The mode of action of ethyl acetate could be related to their ability to alter membrane properties leading to cell death (Aqil *et al*., 2012).

5.4 Antifungal Activities of Combined Extracts of *Euphorbia heterophylla* and *Mitracarpus scaber* against some Dermatophytes

The zone of inhibition of combined aqueous, methanol, ethyl acetate, n-hexane extracts of *Mitracarpus scaber* and *Euphorbia heterophylla* showed that ethyl acetate extract had highest zone of inhibition at 100mg/ml (28mm and 26mm) against *Candida albicans* and *Trichophyton mentagrophytes.* This was followed by aqueous extract at 100mg/ml (23mm and 20mm), n- hexane extract at 100mg/ml (20mm and 17mm) in both organisms of *Candida albicans* and *Trichophyton mentagrophytes* while methanol extract at 100mg/ml (19mm and 18mm) was the least zone of inhibition against *Candida albicans* and *Trichophyton mentagrophytes.* All the extracts had no activity on *Microsporum aduounnii* and *Aspergillus flavus*. This is in line with the finding of Abalaka *et al*. (2011) who carried out a study on determination of *in-vitro* susceptibility of typhoid pathogen to synergistic action of *Euphorbia hirta*, *Euphorbia heterophylla* and *Phyllanthus niruri* for possible development of effective Anti- Typhoid drugs. They observed that the test organisms showed very degree of susceptibility to various concentrations of the extracts from the three medicinal plants with diameter of zone of inhibition as wide as 26 ± 0.20mm, 22 ± 0.10mm and 18± 0.00mm respectively. MIC was 0.04 – 12mg/ml of the extract against the fungi compared to those of standard antibiotics used. The results of synergistic actions of the extract on the organisms revealed far wider diameter zones of clearing compared with those created by each of the extracts. This justifies the reason herbalists usually prescribe the mixture of different herbs to cure various ailments. The Herbalists seldom prescribe one herb for the cure of any disease.

Similarly, Doughari and Obidah (2008) investigated the anti-fungal activity of stem back extracts of *Leptadenia lancifolia* and they reported that when various concentrations of the methanol extracts (5 – 50mg/ml were combined with fluconozole 0.5mg/ml) were used,

there was an increase in activity in each of the combinations. MlC and MFC values of the extract against the test fungi (*Aspergillus flavus*, *Aspergillus fumigatus*, *Crytococcus neoformans* and *Candida albicans*) ranged between 0.5 – 6.0mg/ml

5.5 Minimum Inhibitory Concentration (MIC) of Crude Extracts against some Dermatophytes

The minimum inhibitory concentration (MIC) of various extracts on *E. heterophylla* and *M. scaber*, single and combined revealed an MIC of 12.5mg/ml ethyl acetate extract of *E. heterophylla* and 25mg/ml on *C.albicans* and 50mg/ml on *T. mentagrophytes* using aqueous extract of *E. heterophylla.* In *M. scaber*, ethyl acetate had an MIC of 6.25mg/ml on *C. albicans* and 12.5mg/ml on *T. mentagrophytes* while aqueous extract had an MIC of 12.5mg/ml on both *C. albicans* and *T. mentagrophytes*. In the combined, ethyl acetate had an MIC of 6.25mg/ml and 12.5mg/ml on *C. albicans* and *T. mentagrophytes* respectively. The combined aqueous extracts had an MIC of 25mg/ml and 50mg/ml on *C. albicans* and *T. mentagrophytes*. n – hexane and methanol had an MIC of 50mg/ml on both *C. albicans* and *T. mentagrophytes*. There was no inhibition of the extracts on *M. auduounii* and *A. flavus.* Terbinafine (control) had MIC of 0.78mg/ml on C. albicans, T. mentagrophytes, M. audouinii and A. flavus. When compared the solvent extracts with the standard drug Terbinafine had the highest resistant on the four species of fungi. The result of minimum inhibitory concentration of extracts suggests that the extracts may act as fungicidal agents to these micro-organisms. This finding is similar to the work of Uduak *et al.,* (2010), who carried out a study on Antifungal activities of some *Euphorbiaceae* plants used in traditional medicine in Nigeria. They observed that the inhibitory effect validated the use of the plants to treat infections caused by these microorganisms.

5.6 Minimum Fungicidal Concentration (MFC) of Crude Extracts on some Dermatophytes

The minimum fungicidal concentration (MFC) of the various extract on *E. heterophylla* and *M. scaber*, single and combined revealed that MFC of 50mg/ml on both *C. albicans* and *T. mentagrophytes* using ethyl acetate extract of *E. heterophylla* and 100mg/ml using aqueous extract on *C. albicans.* No inhibition on *T. mentagrophytes* using aqueous extract was observed. In *M. scaber*, ethyl acetate extracts had an MFC of 6.25mg/ml on *C. albicans* and 12.5mg/ml on *T. mentagrophytes* while aqueous extract had an MFC of 25mg/ml and 50mg/ml on *Candida albicans* respectively.

In combined effects, ethyl acetate extract had MFC of 12.5mg/ml and 50mg/ml on *C. albicans* and *T. mentagrophytes* had an MFC of 100mg/ml on *C. albicans* with no inhibition at all on *T. mentagrophytes*. There was no inhibition by the extracts of both plants on *M. auduouni* and *A. flavus*. Terbinafine (control) had MFC of 1.56mg/ml on C. albicans, T. mentagrophytes, M. audouinii and A. flavus. When compared the solvent extracts with the standard drug Terbinafine had the highest resistant on the four species of fungi. Thus, this solvent extracts may be used for treatment of dermatophytes. Doughari and Obidah (2008) investigated the anti-fungal activity of stem back extracts of *Leptadenia lancifolia* and they reported that when various concentrations of the methanol extracts (5–50mg/ml were combined with fluconozole (0.5 mg/ml) and used, there was increase in activity in each of the combinations. MFC values of the extract against the test fungi (*Asperigillus flavus*, *Aspergillus fumigatus*, *Crytococcus neoformans* and *Candida albicans*) ranged between 0.5–6.0mg/ml.

The bioactive compounds responsible for the inhibitory effects of these plants were detected in their phytochemical screening, some of which were reported in literature as antimicrobial constituents. Flavonoids are known to be antimicrobial in nature (Joseph *et*

al., 2002). Flavonoids isolated from the leaves of *Euclea crispa* sub sp. *crispa* were reported to give antimicrobial activity (Pretorius *et al*., 2003). Tannins identified from *Vaccinium vitis-idaea*, terpenes from *Vernoniaa mygdalina* and saponins from *Allium minutifolium* were all established as antimicrobial constituents of the plants (Ho *et al*., 2001; Barile *et al*., 2007). Therefore, the whole plant of *E. heterophylla* and *M. scaber* either singly or combined could be used in the treatment of fungi.

CHAPTER SIX

6.0 SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

6.1 Summary

The qualilative and quantitative phytochemcial screening of the whole plant extracts (aqueous, methanol, ethyl acetate and n–hexane) of the two plant samples used in this research showed the presence of bioactive constituents such as carbohydrate, steroids, glycosides, alkaloids, saponins, tannins, triterpens and flavonoids. Some of the observed phytochemcial constituents present in these plants may be responsible for curing the dermatophytes diseases. The zones of inhibition in ethyl acetate and aqueous extracts of both *E. heterophylla* and *M. scaber* on *Candida albicans* and *T. mentagrophytes* showed inhibition, with no zone of inhibition on *M. auduounii* and *A. flavus*. For example, at 100mg/ml ethyl acetate and aqueous extracts of *Euphorbia heterophylla* had zone of inhibition of 24.50±0.71mm and 20.00 ± 0.00mm on *Candida albicans* and 24.00±0.00mm and 18.00±0.00mm on *T. mentagrophytes.* In *M. scaber* extracts ethyl acetate and aqueous extracts showed a significant zone of inhibition on *C. albicans* and *T. mentagrophytes.* For example, at 100mg/ml ethyl acetate and aqueous extracts of E. heterophylla had zone of inhibition of 26.00± 0.00mm and 22.00± 0.00mm respectively on *T. mentagrophytes*. The standard drugs, Terbinafine (control) had zone of inhibition between 18.00± 0.00mm to 30.00 ±0.00mm for all extracts (aqueous and ethyl acetate) on all the organisms of fungi used.

In combined effects of all the extracts, there is a significant inhibition of growth in all extracts used (aqueous, methanol, ethyl acetate and n-hexane).The zone of inhibition (mm) of combined extracts (aqueous, methanol, ethyl acetate and n–hexane) of both whole plants of *E. heterophylla* and *M. scaber* revealed that at all concentration of 100mg/ml, 50mg/ml,

25mg/ml and 12.5mg/ml, the inhibition zone that was observed were 28.00±0.00mm, 26.00±0.00mm,24.00±0.00mm and 20.00±0.00mm on (*Candida albicans* and 26.00±0.00mm, 24.00±0.00mm, 22.00± 0.00mm and 18.00±0.00mm on *T. Mentagrophytes* followed by aqueous, n- hexane and methanol extract of both plants with no inhibition of growth on *M. auduounii* and *A. flavus* in all plant extracts. The standard drugs (positive control) Terbinafine had zone of inhibition in all extracts and all microorganisms.

The MIC and MFC of both plants extract can help in antifungal activities. The MIC of 12.5mg/ml ethyl acetate of *E. heterophylla* and 25mg/ml on *C. albicans* and 50mg/ml on *T. mentagrophytes*. In *M. scaber*, ethyl acetate had an MIC of 6.25mg/ml on *C. albicans* and 12.5mg/ml on *T. Mentagrophytes*. In combined, ethyl acetate had an MIC of 6.25mg/ml and 12.5mg/ml on *C. albicans* and *T. mentagrophytes* respectively. The combined aqueous extracts had an MIC of 25mg/ml and 50mg/ml on *C. albicans* and *T. mentagrophytes*, n- hexane and methanol had an MIC of 50mg/ml on both *C. albicans* and *T. mentagrophytes*. There was no inhibition of the extracts on *M. auduounii* and *A. Flavus*. Terbinafine (control) had MIC of 0.78mg/ml on C. albicans, T. mentagrophytes, M. audouinii and A. flavus. When compared the solvent extracts with the standard drug Terbinafine had the highest resistant on the four species of fungi.

MFC of 50mg/ml on both *C. albicans* and *T. mentagrophytes* using ethyl acetate extract of *E. heterophylla* and 100mg/ml using aqueous extract on *C. albicans.* No inhibition on *T. mentagrophytes* using aqueous extract was observed. In *M. scaber*, ethyl acetate extracts had an MFC of 6.25mg/ml on *C. albicans* and 12.5mg/ml on *T. mentagrophytes* while aqueous extract had an MFC of 25mg/ml and 50mg/ml on *Candida albicans* respectively.

In combined effects, ethyl acetate extract had MFC of 12.5mg/ml and 50mg/ml on *C. albicans* and *T. mentagrophytes* had an MFC of 100mg/ml on *C. albicans* with no inhibition at all on *T. mentagrophytes*. There was no inhibition by the extracts of both plants on *M. auduouni* and *A. flavus.* Terbinafine (control) had MFC of 1.56mg/ml on C. albicans, T. mentagrophytes, M. audouinii and A. flavus. When compared the solvent extracts with the standard drug Terbinafine had the highest resistant on the four species of fungi. Thus, this solvent extracts may be used for treatment of dermatophytes.

6.2 Conclusion

- The phytochemical constituents did not differ qualitatively and quantitatively between the two plants. However, the aqueous extracts of both plants had the highest constituent concentration (phenol with highest value 12.30±0.70mm and 10.00±0.70mm for *E. heterophylla* and *M. scaber* respectively).
- There is a significant difference ($p<0.5$) in the antifungal activities for single and combined plants. Ethyl acetate extracts of *E. heterophylla* and *M. scaber* had the highest effects of zone of inhibition at 100mg/ml of both plants on *C. albicans* and *T. mentagrophytes* (24.50±0.71mm and 24.00±0.00mm for *E. heterophylla* and 26.00±0.00mm and 24.00±0.00mm for *M. scaber*). In combined effects, ethyl acetate extracts of *E. heterophylla* and *M. scaber* also had the highest zone of inhibition at 100mg/ml on *C. albicans* (25.40±1.07mm) and *T. mentagrophytes* (24.00±1.33mm). *M. audouinii* and *A. Flavus* showed differently significant resistance to both plants.
- The MIC and MFC of both plants indicated that *M. scaber* had the highest effects, compared to *E. heterophylla* against the test organisms.

6.3 Recommendations

Based on the findings above, it is recommended that:

1. The ethyl acetate extracts of *Mitracarpus scaber* with Minimum Fungicidal Concentrations (MFC) of 6.25mg/ml and 12.5mg/ml against the growth of *C. albicans* and *T. Mentagrophytes* are recommended for drug formation in the treatment of diseases caused by these microorganisms.
2. Further studies should be carried out on the active compounds obtained from bioactive plant extracts in this studies that are responsible for the antifungal activities.
3. Further studies should be carried out using *Mitracarpus scaber* extract on other enteric microorganisms

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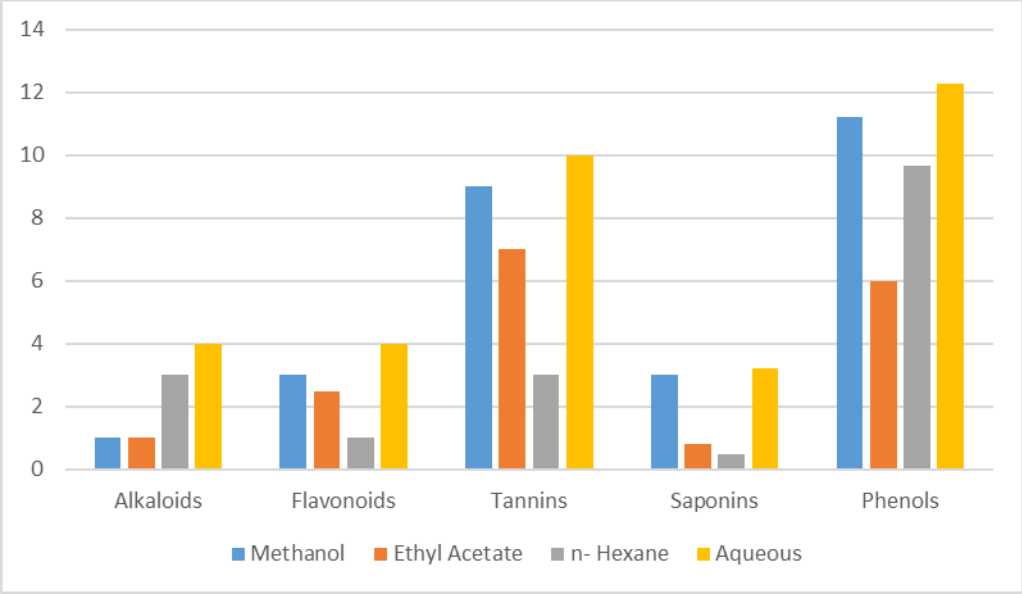
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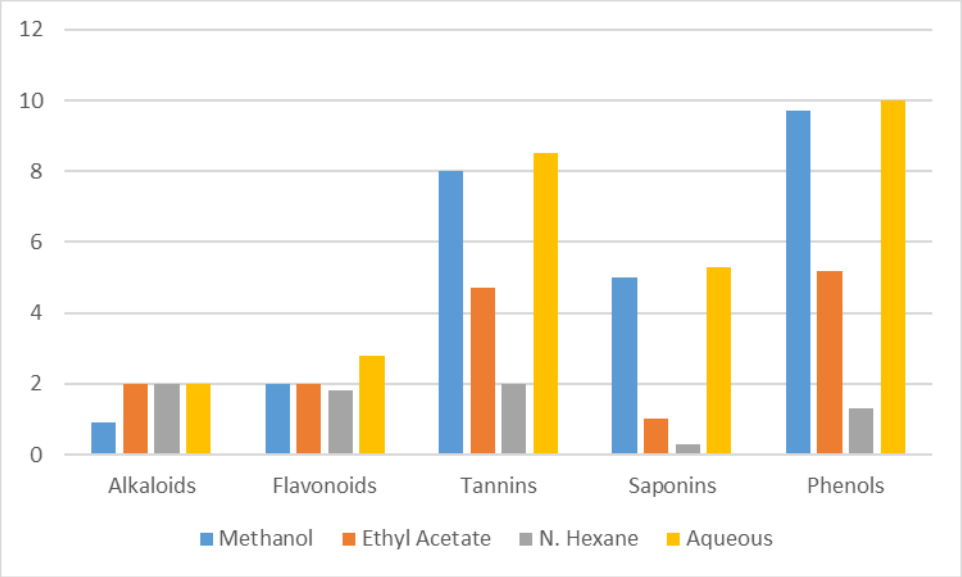
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APPENDICES

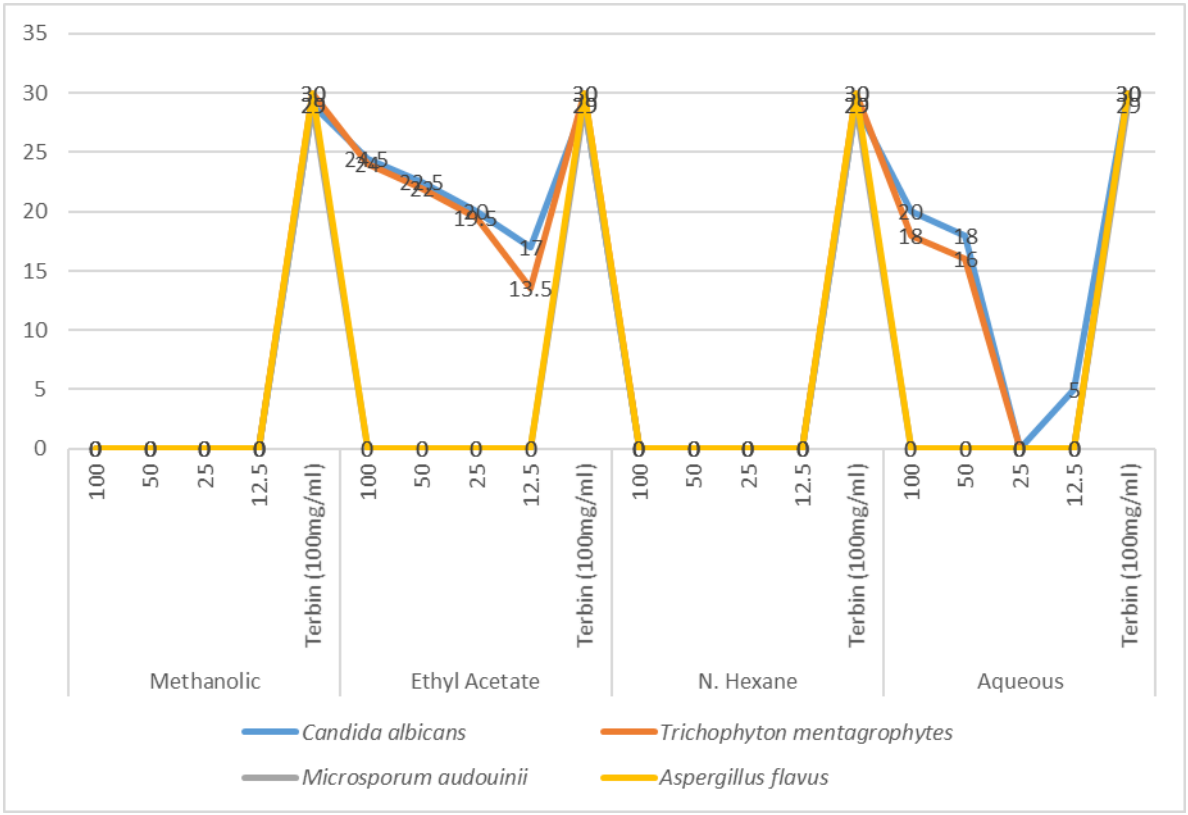
Appendix I: percentage composition of Phytochemicals in *Euphorbia heterophylla* extract from four different solvents.



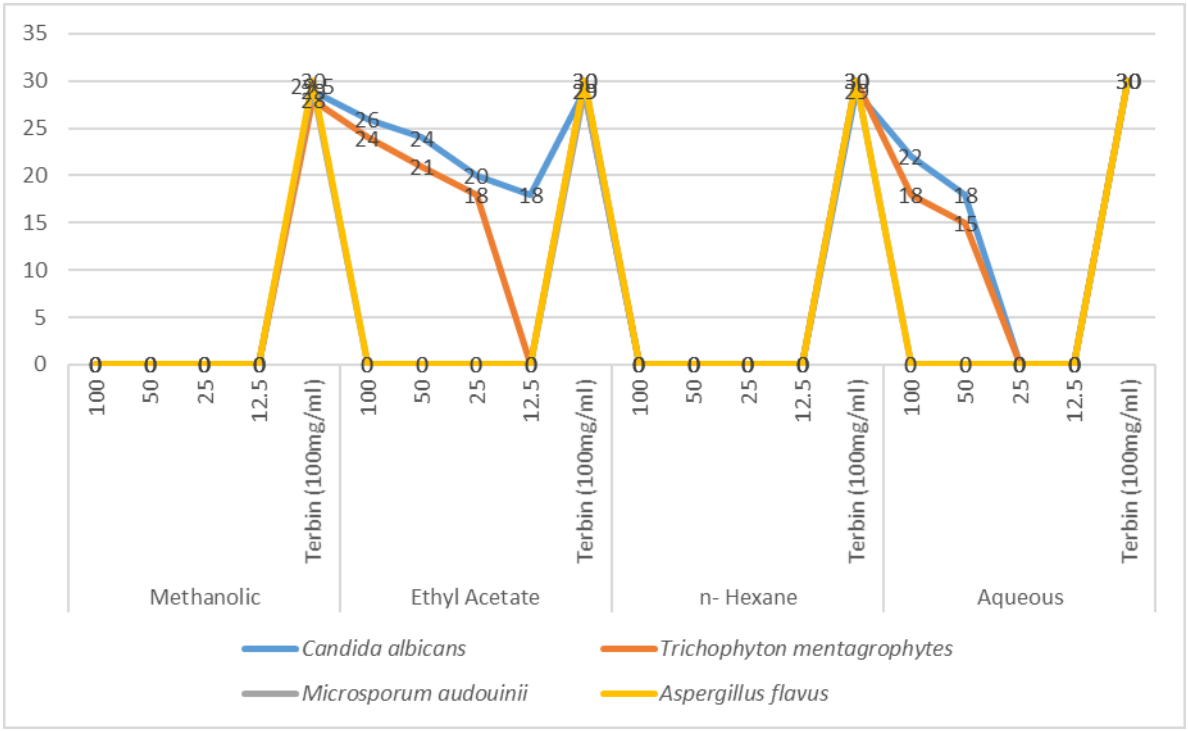
Appendix 2: percentage composition of phytochemical in *Mitracarpus scaber* extract from four different solvents.



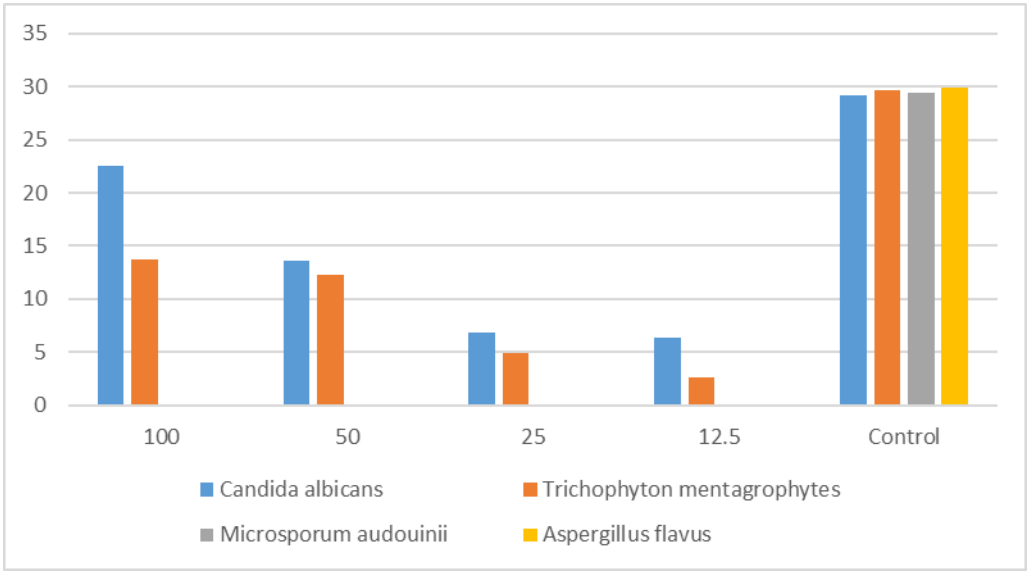
Appendix 3**:** Effect of different extracts of *Euphorbia heterophylla* on the growth of four fungal species.



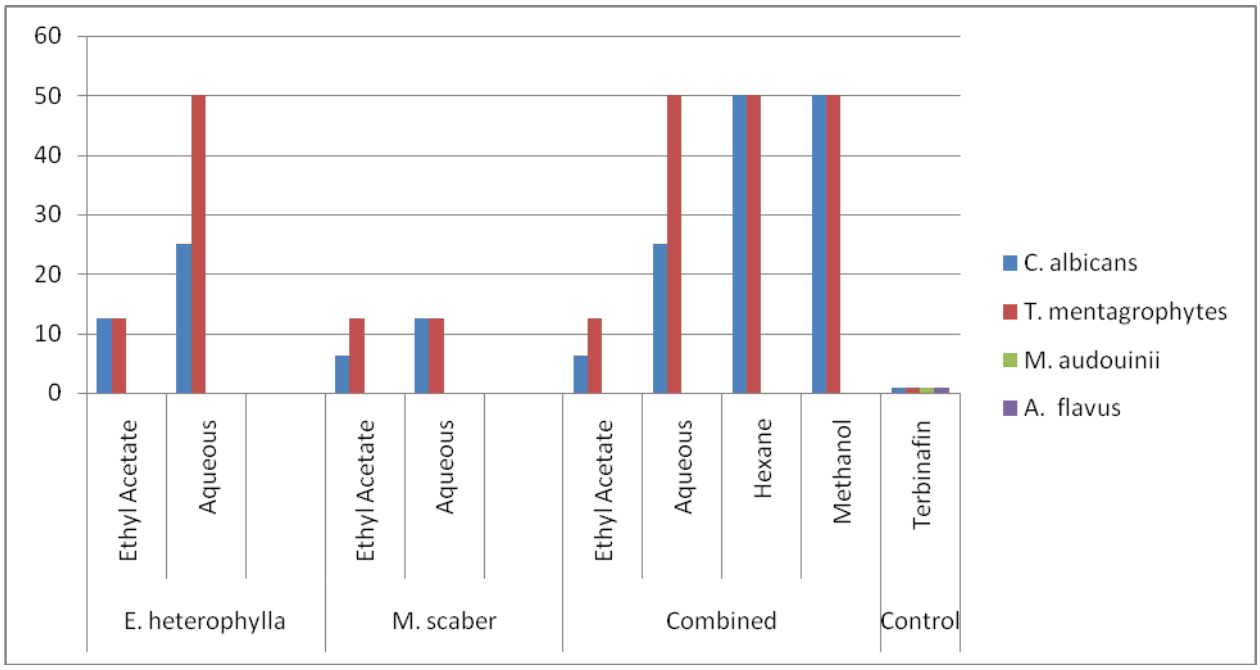
Appendix 4: Effect of different extracts of *Mitracarpus scaber* on the growth of four fungal species.



Appendix 5: **Effect of Combined Different Extract of *Mitracarpus scaber* and *Euphorbia heterophylla* on the Growth of four Fungal Species**



Appendix 6: **Minimum Inhibitory Concentration (MIC) of Different extracts of *E. heterophylla* and *M. scaber* on four species of fungi**



Appendix 7: **Minimum Fugicidal Concentration (MFC) of Different extracts of *E. heterophylla* and *M. scaber* (mg/ml) on four species of fungi**

