ANTI-FUNGAL ACTIVITY OF *CHLOROPHYTUM LAXUM* (*R* Br) EXTRACT ON FUNGI ISOLATES ASSOCIATED WITH TINEA CAPITIS

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ZARIA NIGERIA

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DEPARTMENT OF BIOLOGY,
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ZARIA, NIGERIA

OCTOBER, 2018

DECLARATION

I declare that the work in this thesis entitled "**Anti-fungal Activity of *Chlorophytum laxum (R Br)* Extract on Fungi isolates Associated with tinea capitis**" was carried out by me in the Department of Biology, under the supervision of Prof. I. S Ndams and Dr. I. M. K. Gadzama. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this dissertation was previously presented for another degree or diploma at this or any other institution.

MUHAMMAD, Maimuna Madaki _____ _____

Signature Date

CERTIFICATION

This dissertation entitled "**ANTI-FUNGAL ACTIVITY OF *CHLOROPHYTUM LAXUM (*R.Br*)* ON FUNGI ISOLATES ASSOCIATED WITH TINEA CAPITIS"** by Maimuna Muhammad Madaki meets the regulations governing the award of the Master of Science degree in Educational Biology of the Ahmadu Bello University, Zaria and is approved for its" contribution to knowledge and literary presentation.

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DEDICATION

This dissertation is dedicated to Almighty Allah (S.W.T) and to the memory of my beloved late parents, Alh. Muhammad Garba and Haj. Fatima Ibrahim

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To Almighty Allah (SWT) for all His mercy, bounty and blessings upon my life till this moment.

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ABSTRACT

Dermatophytosis is a common fungal infection of the skin in humans and animals. This study evaluates anti-fungal activity of *Chlorophytum laxum* (R Br) extract on fungi isolates associated with tinea capitis. The whole plant was extracted and subjected to phytochemical screening and the fractions were analyzed using gas- chromatography and mass spectrometry. The bioassay was done using food poisoning technique method. *Trichophyton spp* and *Microsporum spp* were the fungal isolated and identified. The crude extract of *Chlorophytum laxum* at 50mg/ml showed the highest zone of inhibition on the isolates: *Microsporum spp* (30.00 ± 6.1), *trichophyton spp* (37.3 ± 1.2), and *trichophyton spp* (60.3 ± 0.9) respectively significantly different ($P < 0.05$). Gas-chromatography/Mass-Spectrometry analysis identified Dimethyl sulfone, 4-Methylvaleric acid, 1-Propanol and 5H- Tetrazol-5-amine as bioactive compounds that have antifungal properties in the fractions. The data obtained were subjected to analysis of variance at $p<0.05$ using SPSS version 20 and means were separated using least significant difference (LSD) This study therefore showed that, the effectiveness of *C.laxum* inhibit *Microsporum spp* and *Trichophyton spp* which were found to causes tinea capitis infection from scalp scrapings among primary school pupils in Zaria and suggests the scientific reason for the traditional use of the plant in treatment of fungal infection.

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CHAPTER ONE

INTRODUCTION

1.1 Background of Study

Chlorophytum laxum R. Br. Family (Liliaceae) is a traditional endangered perennial herbaceous medicinal plants (Patil and Kumar, 2011; Chakraborthy *et al.*, 2014).Medicinal plants have been identified and used throughout human history. Plants have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions and defense against infections caused by insects, fungi and herbivorous mammals. Many of the herbs and spices used by humans to season food give useful medicinal compounds. Plants used in traditional medicine contain a wide range of substances that can be used to treat acute and chronic infectious disease. They contain some organic compounds also bioactive substances some of which also serve as antimicrobial in their activity, produce physiological action on the body. These bioactive substances includes: tannin, alkaloids, carbohydrate, flavonoid, saponin, (Njoku and Chidi, 2009). Antimicrobials of plants origin have great therapeutic potential on human infection particularly those involving micro-organisms such as bacteria, fungi and virus that cause serious infections in tropical and subtropical countries of world (Jumare *et al.*, 2015).

In Africa today, up to 80 % of the population use traditional medicine in primary health care (WHO, 2006). Many African plants are used in traditional medicine as antimicrobial agents but only few have been documented. However, in spite of vast improved health

and longevity in the United State and Europe, millions of their people are turning back to traditional herbal medicine in order to prevent or treat many illnesses and to circumvent resistance of many human pathogens to conventional (antibiotics), some of which have side effects like hypersensitivity and immunosuppressant (Sule *et al.*, 2010). In Nigeria, traditional medical practitioners use a variety of herbal preparation to treat different microbial diseases. For example, the Hausa tribes of Kaduna State, Nigeria use *Chlorophytum laxum* for treatment of diarrhoea and dysentery (Kunalkale and Prashant, 2013).

Chlorophytum laxum is commonly known as dwarf spider plant or wheat plant and traditionally known as' kwan makwarwa' in Hausa. The leaves and roots are edible, used in traditional medicine as general tonic for strength and vigor (Niranjan, 2011). The plants locally are used for treatment of insect bites while the tubers are used for treatment of haemorrhagic pile. Due to its diverse medicinal benefit *Chlorophytum* has been recognized as the sixth most important herb by Medicinal Plant Board of India (Praneta, 2013).

Dermatophyte is a common fungal infection of the skin in humans and animals. It present as tinea or ringworm. The name ringworm described the circular lesion produced by dermatophytes on the skin and scalp and also known as dermatophytosis. The term "Tinea" is derived from the Latin word meaning "worm" or "moth" and second part of the name identifies the part of the body infected (Fisher and Cook, 1998). Dermatophyte has a worldwide distribution but particularly common and severe in hot moist climate. The fungi that cause infection of the skin and nail which lead to variety of clinical

manifestation such as scalp (tinae capitis), feet (tinea pedis), body surface (tinea corporis), mostly affecting the epidermis. Dermatophytosis is caused by three types of dermatophytes namely: *Epidermophyton, Trichophyton* and *Microsporum*. They are named by their location and have similar treatments. Therefore, when dermatophytes infect humans they colonized the keratinized outermost layer of the skin but do not invade the living tissue (Samuel *et al.,* 2013).

1.2 Statement of Research Problem

In Nigeria, as well as in most countries of sub-Saharan Africa, fungal infection represents an increasing problem particularly with patients suffering from severe immune deficiency such as AIDs (Jumare *et al.,* 2015). Doctors estimate that at leas t 20 percent of world population will develop tinea at sometimes during life. People with higher than average risk of fungal skin infection include people who live in hot humid climate and anyone whose immune defense is weakened by illness.

In recent time, drug resistance to human pathogenic fungi has been commonly reported from all over the world in orthodox medicine (Mohammed *et al.,* 2010). The drug resistance to most of these available antifungal agents prompted herbal plants which are safer and efficient in managing infectious condition (El-mahmood and Doughari, 2008).

Furthermore, the problem of the side effects of the synthetic drugs and their unavailability has become overwhelming. However, traditional medicinal practitioners in Nigeria use herbal preparations to treat microbial infections including those associated with skin with no scientific basis (Jumare *et al*., 2015).

1.3 Justification of the Study

There is need for scientific basis to support the curative claims of *Chlorophytum laxum* made by traditional healers.Therefore; there is interest to explore the potency of *Chlorophytum laxum* usage on fungal isolates which causes tinea capitis. Plant materials can serve as available, safe and cheap alternative agents for treatment of fungal infections (Erdogrill, 2002).

Ringworm and scalp related infection has been a common infecton among primary school pupils in Zaria resulting been absence in class rooms and not associating with peer groups. This make most parents spent a lot of resources on orthodox medicine for curation with little or no change

It is necessary to explore a new antifungal agent of plant origin that could be useful for the treatment of fungal infections.

1.4 Aim of the Study

The aim of this study was to evaluate the anti-fungal activity of *Chlorophytum laxum* (*R.Br*) extract on fungal isolates associated with tinea capitis.

1.5 Objectives of the Study

The objectives of this study were to:

1. Determine the phytochemical constituents of *Chlorophytum laxum* crude the extract.

2. Isolate and identify dermatophytes from scalp scrapings among primary school pupils.
3. Determine the antifungal activity of *C. laxum* crude extract on the isolates.
4. Identify the chemical compound(s) present in the fractions of *C. laxum.*

1.6 Research Hypotheses

1. The phytochemical constituents present in *Chlorophytum laxum* crude extract cannot be determined.
2. There are nodermatophytes isolates present in scalp scrapings among primary school pupils.
3. There is no antifugal activity of *C. laxum* crude extracton the isolates.
4. There are no chemical compounds present in the active fractions of *C. laxum* crude extract.

CHAPTER TWO

LITERATURE REVIEW

2.1 Nomenclature

2.1.1 Botanical classification of *Chlorophytum laxum*

Below is the taxonomic classification of *Chlorophytum laxum*R. Br.

Botanical Name: *Chlorophytum laxum the* synonyms are *Chlorophytum Bichetti* and *Anthericum parviflorum (wight)*

KINGDOM: Plantae

SUBKINGDOM: Tracheobionta

SUPER DIVISION: Spermatophyta

DIVISION: Tracheophyta

CLASS: Liliopsida

SUBCLASS: Hamamelidae

ORDER: Asparagales

FAMILY: Liliaceae

GENUS: *Chlorophytum*

SPECIES: *laxum*

2.1.2 Description of the plant *Chlorophytum laxum*R.Br

Chlorophytum laxum it is a clump-forming variegated species that is moderately fast growing and petite in stature, about 30 cm tall with a spread at 60 cm or more on ground but less if contained in pots. Measures height of 1 ft with shealthing leaf base acute

acuminate with entire margin,tufts of lovely lime green grass-like recurving leave with thin ivory-white margin. The leaves are slender and elongated with tapering tips, paper-thin, smooth and shiny, distichous and sessile, ranging between 10 - 40 cm in length and about 1.5 cm broad. It bears tiny inconspicuous star shaped 6-petated flower in white on a 10 -20 cm non-arching scape that is almost erect, tuberous root is cylindrical and measuring 10 - 14 cm^3 long and 1-1.4 cm^3 in diameter(Niranja, 2011).

2.2 Distribution of *Chlrophytum laxum*

Chlorophytum laxum is native to South Africa but wide spread elsewhere in India, Sri Lanka, China, Myanmar, Thailand, Malaysia, Indonesia, North Australia and tropics of Africa like Nigeria, Morocco, Tunisia, Libya and Egypt. It is found in rain forest areas, as erect growing plant along the forest margins, grassy slopes and rocky places along valley (Patil and Deokule, 2010).

2.3 Ethnomedicinal uses of *Chlrophytum laxum*

Chlorophytum are noted for their medicinal benefits in Ayurvedic, and Unani system of medicine (Devendra *et al.*, 2012).Traditionally, roots of these species are reputed to possess various pharmacological effecs like immune-modulation, adaptogenic, aphrodisiac, neurodegenerative, rejuvenators, hepatoprotective, antioxidant, lactation, gynecological disorders, arthritis, diabetes mellitus and nutritional value (Purohit and Prajapati, 2003; Govindarajan *et al.*, 2005; Thakur and Dixit, 2006; Joshi and Parle, 2006; Patil and Kumar, 2011).

2.4 Biological activities of *Chlrophytum laxum*

Chlorophytum species are rich in both monodesmosidic saponins (oligosaccharide chain attached at C3 position) and bidesmosidic saponins (an additional sugar moiety at the C26 or C28 positions) (Marais and Reilly 1978; Kothari, 2004). *Chlorophtyum laxum* contains proteins (8-9%), carbohydrates (41%), roots fibres (4%) and saponins (2-17%). Saponin is the chief medicinal compound present in the roots. Saponins and alkaloids present in the plant are the primary phytochemical constituentof medicinal properties (Pullaiah, 2002). There are almost 215 species that have been reported in the genus *Chlorophytum* (Manish*et al*., 2010; Kunal and Prashant, 2013). All are perennial rhizomatous herbs. Rhizomes are often short and inconspicuous while roots are usually thicker or slightly fleshy. The important species which have so far been explored include *C. adescendens, C. borivilianum, C. laxum, C. tuberosum and C. comosum* (Kaushik, 2005).

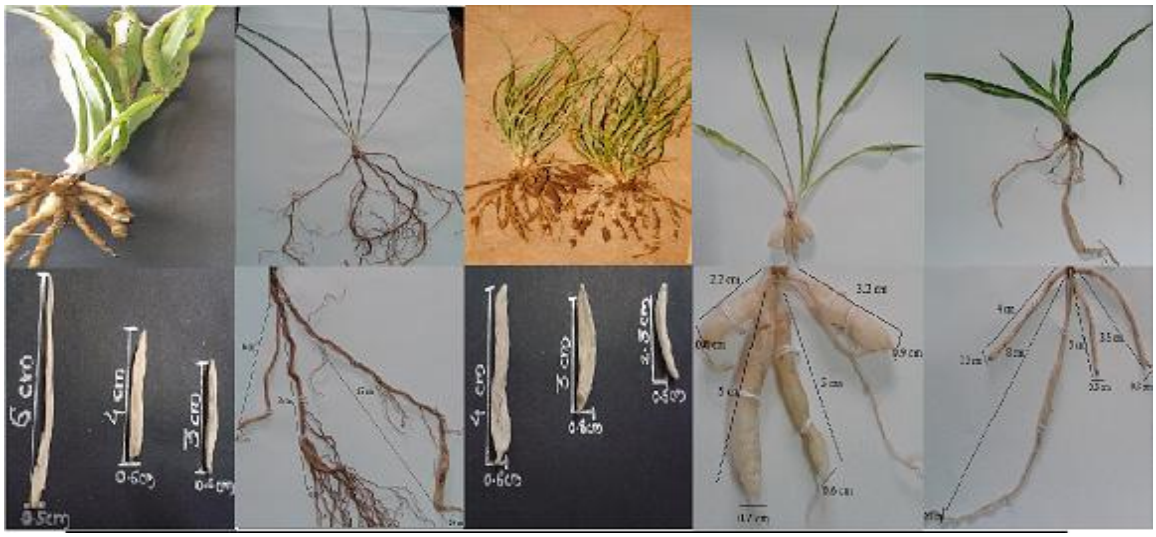

Figure 2.1: Different species of *Chlorophytum* leaves and tubers
(Adapted From Sharada *et al*, 2015).

2.5 Dermtophytes

Dermatophytes are a monophyletic group of fungi known for affecting the skin of animals and humans (Martinez *et al.,* 2012). These fungi cause a variety of skin diseases, including athlete's foot (clinically termed tinea pedis), jock itch (tinea cruris), tinea barbae (beard area), tinea manuum (hands), tinea unguuim (nails), and scalp (tinea capitis or tinea corporis, depending on area of the body infected. The estimated lifetime risk of acquiring a dermatophyte infection is between 10 and 20 percent. Dermatophytes are fungi that can cause infections of the skin, hair, and nails due to their ability to utilize keratin. The organisms colonize the keratin tissues and inflammation is caused by host response to their metabolic bye-products. Occasionally, the organisms do invade the subcutaneous tissues, resulting in kerion development.

The dermatophytes are transmitted by either direct contact with infected host (human or animal) or indirect contact with infected exfoliated skin or hair in combs, hairbrushes, clothing, furniture, theatre seats, caps, bed linens, towels, hotel rugs, and locker room floors(Caputo *et al.,* 2001)

Depending on the species, the organisms may be viable in the environment for up to 15 months. There is an increased susceptibility to infection when there is a preexisting injury to the skin such as scars, burns, excessive temperature and humidity.

Dermatophytes cause a variety of clinical conditions. They are among the most common infectious agents of humans. Collectively, the group of diseases is termed dermatophytosis. From the site of infection the fungal hyphae grow centrifugally in the stratum corneum. The fungus continues downward growth into the hair invading keratin as it is formed. The zone of involvement extends upward at the rate at which the hair grows and it is visible above the skin surface by days 12-14. Infected hairs are brittle and by the third week broken hair are evident. The infection continues (for 8-10 weeks) to spread in the stratum corneum to involve other hairs at which point, the infected area extends to approximately 3.5-7.0 cm in diameter. The spontaneous cure of naturally occurring infection at puberty is a familiar clinical observation. These fungi have worldwide distribution, and at present, there are 40 recognized species in the dermatophyte Genera. Of these, about 25 species belonging to the genera *Epidermophyton*, *Microsporum* and *Trichophyton* are presently known to infect man. Dermatophytes cause some of the most common fungal infections in the world and are endemic to all continents, excluding Antarctica. Infection by *M. audouinii* is of historical interest in many parts of the world because it was responsible for epidemics in Europe in the 19th century before spreading to the Americas and then finally almost disappearing 50 years ago (Arenas, 2008).

While these infections, are difficult to treat and contribute to high morbidity, pain, and suffering, especially in aging populations. The impact to our economy is striking, as over 500 million dollars is spent on the treatment of dermatophytes worldwide every year (Achterman and White, 2012a, b).

Dermatophytes are in the family Arthrodermataceae, a group of filamentous fungi closely related to dimorphic fungi in the Order Onygenales, most closely to the genus *Coccidioides*, a human pulmonary pathogen. While three dermatophyte genera (*Trichophyton*, *Microsporum*, and *Epidermophyton*) have been described, the phylogenetic relationships of the dermatophyte species do not support three distinct divisions, suggesting that these genera are not monophyletic (Gräser *et al.,* 2008). For example, some *Trichophyton* species are more distantly related to *T. rubrum*, the major cause of athlete's foot, than some *Microsporum* species (Gräser *et al.,* 2008; White *et al.,* 2008).

2.5.1 Classification of dermatophytes

Dermatophytes are hyaline and well septate moulds which include more than 100 species. Of these, only 42 species have been considered of importance and about less than half of these species are pathogenic. (Chowdhry et al., 2013), classified dermatophytes into three anamorphic (asexual or imperfect) genera, namely, *Microsporum*, *Trichophyton* and *Epidermophyton* of the class Hyphomycetes of the deuteromycota (Fungi imperfecti). The classification of dermatophytes is based on the formation of conidia and their morphology and is updated with the discovery of 8 new species.

2.5.1.1 *Microsporum*

There are about 16 known species belonging to the genus *Microsporum* which are associated with the skin and hair infections. However, they are not associated with nail

infections. *Microsporum audouinii* is the prototype of this genus. The shape of macroconidia varies from spindle or fusiform to obovate (egg shaped) in *M. nanum* and cylindrofusiform in *M. vanbruseghemii* (Ali et al., 2009). Macroconida may be septate having 1-15 septa, the size of which may vary from 6µm-160µm by 6µm-25µm. Some of the commonly observed species of *Microsporum* are; *M. audouinii M. canis, M. gypseum, M. nanum, M. ferrugineum, M. cookie, M. vanbreuseghemii, M. persicolor* (Figure 2.2)

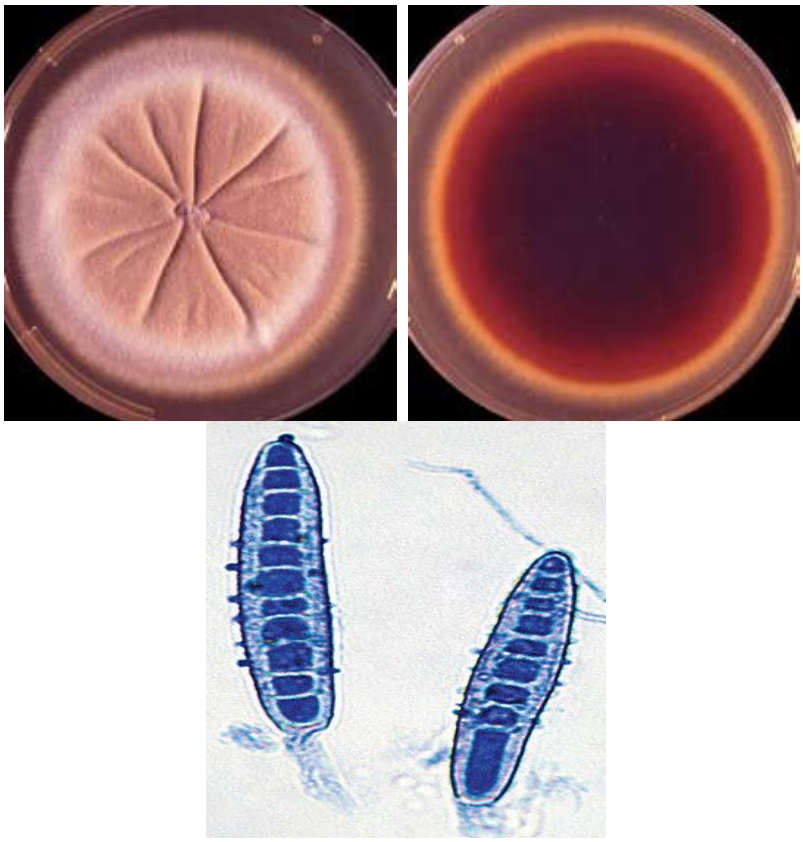

Figure 2.2: Culture and macroconidia of *Microsporum cookei.*
(Adapted from Elis *et al.* 2007).

2.5.1.2*Trichophyton*

Twenty four known species of the genus *Trichophyton* have been identified. *T. tonsurans* is the type species of genus *Trichophyton. Trichophyton* spp. usually infects skin, hair and nails Mucoma, 2000). They posses well septate, pencil-fusiform or cylindrical macroconida having 1-12 septa with smooth and thin wall that may be observed on microscopic examination. The macroconidia may be present singly or in clusters, each macroconidium ranging from 8µm-86µm x 4µm-14µm in size. Micoconidia are numerous and their shape may vary from globose, pyriform to spherical. Most common species of *Trichophyton* are; *T. tonsurans***,** *T. mentagrophyte, T. rubrum, T. schoenleinii, T. verrucosum, T. violaceum, T. concentricum* (Figure 2.3)

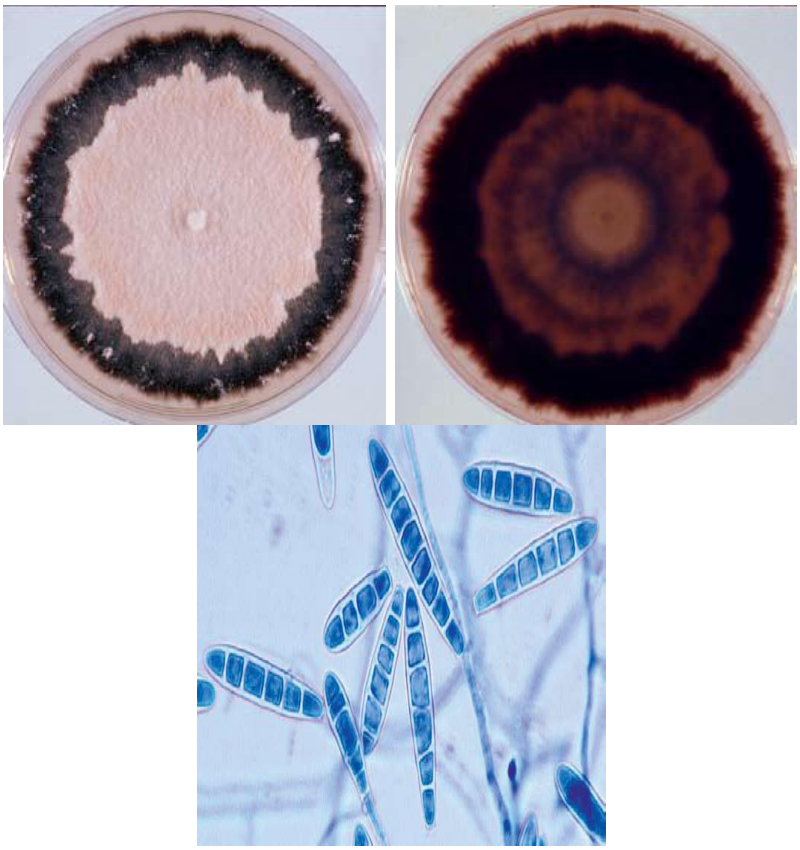

Figure 2.3: Culture and macroconidia of *Trichophyton ajelloi*
(Adapted from Elis *et al.* 2007).

2.5.1.3 *Epidermophyton*

This genus *Epidermophyon* has only two known species, *E. floccosum* and *E. stockdaleae.* The former is the type species of this genus which is pathogenic (Sabouraud, 1910). These fungi produce thin to thick, smooth walled and septate macroconida having 1-9 septa in number which may be observed on microscopic examinations. The size of each macroconidia ranges from 20µm-60µm x 4µm-13µm. The microconidia are absent (Hussain *et al.*, 2012) (Figure 2.4).

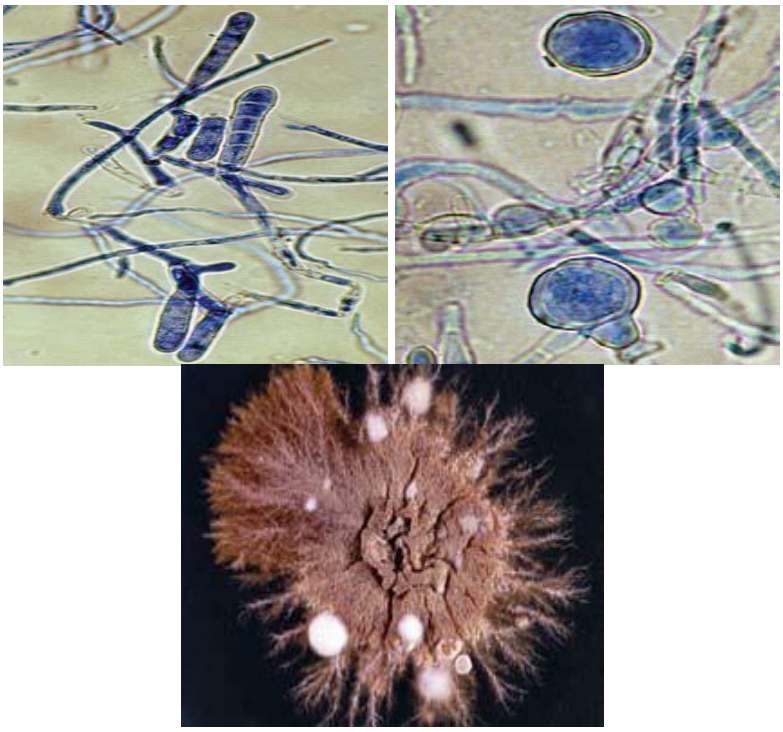

Figure 2.4: Macroconidia, chlamydospores and Culture of *E. floccosum.* (Adapted from Elis *et al.* 2007).

2.6 Epidemiology of Dermatophytosis

The distribution, frequency, and etiological agents of dermatophytosis vary according to the geographic region studied, the climatic variations, the socioeconomic level of the population, the time of study, the presence of domestic animals and age of the individual (Al-Rubiay and Al-Rubiay, 2006). Epidemiology is important in the control of infection and public health issues related to different types of dermatophytosis. Various epidemiological factors such as (Age, sex, occupation of patient, cultural, environmental and geographical conditions) would facilitate better management of the diseases conditions. Although age, sex and the occupation have a little impact on the frequency of dermatophytosis but, these factors have correlation with the incidence of the dermatophytosis.

2.6.1 Age

Prepubertal children are particularly susceptible to dermatophytic infections because of their poor personal hygiene habits and poor environmental sanitation. Higher incidence of dermatophytosis have been reported in the age group of 3-8 years (Sarma and Borthakur, 2007; Patel *et al*., 2010).

2.6.2 Sex

In countries like India males are more affected by dermatophytosis as compared to females. This may be due to the occupational exposure of male because short hairs help implantation of the spores (Singh and Beena, 2003).

2.6.3 Occupational factors

Studies have shown that occupation of the people also affect the incidence of dermatophytosis. Laborers, farmers and industrial workers who carryout physical works involving dust attraction are more prone to the dermatophytic infections (Singh and Beena, 2003).

2.6.4 Cultural factors

Large incidence of tinea corporis has been reported in Indian women as they wear sarees. The relatively high frequency of tinea capitis due to poor scalp hygiene has been reported from South India. Tinea pedis is more common among the people who wear occlusive footwear and use community washing facilities such as in army camp, boarding schools etc (Singh and Beena, 2003).

2.6.5 Environmental conditions

These conditions play an important role in the incidence and prevalence of dermatophytes in a particular region. The hot and humid climatic conditions of the tropical and sub-tropical regions are best suited for the growth of the dermatophytes (Deshmukh *et al.*, 2010). The prevalence and endemicity of a dermatophyte species in a particular region is the combined effect of all the aforesaid factors as a specific pattern of the disease cannot be predicted on the basis of a single factor. Also, it is difficult to establish overall incidence and prevalence of various dermatophyte infections in different parts of the world (Ameen, 2010).

2.7 Dermatophytosis in Africa

Different types of clinical manifestations of dermatophytosis were reported in African subcontinent in which different dermatophyte species were implicated as compared to Europe. *T. audouinii*, *T. violaceum* and *T. soudanense* were the common dermatophytes in some African countries (Havlickova *et al*., 2008). *T. gourvilii* is endemic in Africa and is mainly associated with tinea capitis. The black dot infection caused by *T. tonsurans* and *T. violaceum* is also endemic in Africa. *T. schoenleinii* is found in closely dense population of Africa. *T. soudanense* is commonly associated with tinea capitis especially in north-western Africa. *T. rubrum* and *T. mentagrophyte* are less commonly isolated and are particularly associated with tinea corporis and tinea cruris. In some parts of Africa, tinea faciei and tinea barbae are caused by *M. gypseum* and *M. ferrugineum* (Havlickova *et al*., 2008).

Nigeria:*M. audouinii* (46.8%), *T. mentagrophyte* (25.5%), *T. rubrum* (21.3%), *E. floccosum* (4.3%) and *T. tounsurans* (2.1%) were isolated from the different tinea conditions in school children in Nigeria (Uneke *et al.,* 2006)

2.8 Pathogenesis of Dermatophytosis

Dermatophytes grow within dead, keratinized tissue only. The fungal cells produce keratinolytic proteases *in vivo* and *in vitro* which provide means of entry into living cells. The metabolic products diffuse through the Malphigian layer of epidermis to cause scabs and scales. The fungal hyphae grow rapidly, become old, break into arthrospores and are shed off from the epidermis. Dermatophytes colonize the horny layers of the skin, hair and nails initially. The intensity of the disease depends on several other factors such as nature of the host, strain of the dermatophyte, species variation and the site of infection.

The dermatophytic infection spreads centrifugally in all directions on the glabrous skin showing the classical ringworm pattern. However, it is still not clear how dermatophytic fungi regulate the utilization of different proteases against various cornified layers of the host in addition to the other roles played by these proteins especially in adherence or immunomodulation etc. When compared to the other fungal diseases, dermatophytes infect the immunocompetent patients irrespective of the other fungal diseases where the incidence is more in immunocopromised patients. The dermatophytic infection can be acute and may rapidly be eliminated through efficient host immune responses (Vermount *et al*., 2007).

2.9 Etiology of Tinea capitis

The predominance of specific pathogens causing tinea capitis varies with significant geographic patterns, environments, climates, occupations, ethnic groups and life styles to infection. For example, tinea pedis is more common in developed countries, while tinea capitis is more common in developing countries (Martinez *et al.*, 2012). The dermatophytes that cause tinea capitiscan invade other parts of the body such as the nails and the body, but rarely the feet or groins. Children or adults who have neither signs nor symptoms of infection, but from whose scalps causative fungi can be grown are described as "carriers" (H P A, 2007).

The disease is caused by species of genera *Microsporum* and *Trichophyton* (Emele and Oyeka, 2008). The most important causative agents are species, which cause an endothrix infection, such as *Microsporum audouinii*, *Microsporum canis* and *Microsporum gypseum* (Ngwogu and Otokunefor, 2007). *Trichophyton gourvilli*, *Trichophyton soudanense*, *Trichophyton tonsurans*, *Trichophyton violaceum* and *Trichophyton*

Yaounde (Ngwogu and Otokunefor, 2007). Ecto-endothrix invasion of the hair is often associated with *M. audouinii, M. canis, M. distortum, M. ferrugineum, M. gypseum, M. nanum*, and *T. verrucosum*. Where some fluorescence under Wood light (Rebollo *et al*., 2008).

The organism that commonly causes *Tinea capitis* in the Western world is *Trichophyton tonsurans.* In Africa countries the causative agent are *Trichophyton soudanense* and *Microsporum audouinii* (Havlickova *et al*., 2008).

2.10 Clinical Manifestations of Tinea capitis

There are three clinical types of tinea: tinea capitis superficial (non-inflammatory), tinea capitis profunda (inflammatory) and tinea capitis favosa (favus). Inflammatory tinea capitis presents with painful, inflammatory, indurated, and postulated mass that can be accompanied by regional lymphadenopathy (Aktas *et al*., 2009). Massive follicular destruction and big nodules presenting with pustule and sinus tractions can rarely occur. This acute inflammatory nodule is called kerion celci which occur as a result of intense hypersensitivity reaction to dermatophyte infections (Aktas *et al*., 2009). If the zoophilic dermatophytes are the causative agent, pustules and deep indurations can occur (Corting, 2009). Genetic immunological predispositions and also genetic differences of keratins affect the ability of a fungus to attach to the stratum corneum (Joshi *et al*., 2011). Dermatophytes have the ability to form molecular attachments to keratin and use it as a source of nutrients allowing them to colonize keratinized tissues, including the stratum corneum of the epidermis, hair, nails (Kemal *et al*., 2013). Resistance factors to the colonization of fungi are composed of UV light, variation in temperature and moisture,

and fungistatic fatty acids and sphingosines produced by keratinocytes (Kemal *et al*., 2013).

2.11 Transmission of Tinea capitis

The etiological agents originate from different sources based on host preference and habitat. The natural reservoir of dermatophytes can be humans (anthropophilic dermatophytes), animals (zoophilic dermatophytes), or soil (geophilic dermatophytes) (Adamski and Batura-Gabryel, 2007).

- Anthropophilic dermatophytes are restricted to human hosts and produce a mild, chronic inflammation.

- Zoophilic organisms are found in animals and cause marked inflammatory reactions in humans who have contact with infected cats, dogs, cattle, horses, birds, or other animals. This is followed by a rapid termination of the infection.
- Geophilic species are usually recovered from the soil but occasionally infect humans and animals. They cause a marked inflammatory reaction, which limits the spread of the infection and may lead to a spontaneous cure but may also leave scars.

Transmission requires contact with intact or detached hair. Human-to–human transmission usually requires close contact with infected subject or person because dermatophytes are of low infectivity and virulence. In most cases transmission takes place within families or in situations involving direct contact with detached hair; for example in barber shops and sharing the same head rest.

The source of infection of zoophilic dermatophytes in children and adults are mostly domestic animals – cats, dogs, hamsters, guinea pigs, rabbits or even some birds. Farmers

also often suffer from dermatomycoses transmitted from breeding cattle, pigs, sheep, horses and goats (Adamski and Batura-Gabryel 2007). Infection with geophilic dermatophytes usually happens as a result of contact with soil and it is common among people who cultivate the soil (gardeners, farmers) (Kalinowska, 2012). The disease more often affects males than females working without protective gloves and unsuitable hygiene is conductive for transmission of pathogen. Infection through direct contact with ill people occurs rather rarely (Kalinowska, 2012).

Occasionally, dermatophytes infection may become chronic and wide spread. This progression has been related to both host and organism factors (Fathi and Al-Samarai, 2000). Approximately half of these patients have underlying diseases affecting their immune response or are receiving treatments which compromise T-lymphocyte function. On the basis of the type of hair invasion, dermatophytes are also classified as endothrix, ectothrix or favus. In endothrix infection the fungus grows completely within the hair shaft, the hyphae are converted to arthroconidia (spores) within the hair while the cuticle surface of the hair remains intact (Fuller *et al.*, 2003).

In ectothrix infection hair invasion develops in a manner similar to endothrix except that the hyphae destroy the hair cuticle and grow around the exterior of the hair shaft. Arthroconidia may develop both within and outside the hair shaft. Elongated hyphae, parallel to the long axis of the hair, persist within the hair. Favus is a rare type of tinea capitis characterized by typical honey-colored, cup-shaped, follicular crusts called scutula (Brajesh and Mahadeva, 2013). Ectothrix anthropophilic infections potentially spread rapidly whereas endothrix and favic infections are less contagious (Rebollo, *et al.*, 2008).

Fungal conidia are shed in the air, and may remain viable for long periods on combs, brushes, blankets and telephones (Habif *et al*., 2005).

These dermatophytes can be transmitted from person to person and through fomites (Panasitti *et al*., 2006). The clinical presentation of the disease varies depending on the etiological agent and type of hair invasion, the level of host resistance and the degree of inflammatory host response (Liu *et al*., 2000). Asymptomatic carriage (AC) seems to be organism specific. Anthropophilic dermatophytes such as *T. tonsurans, T. violaceum,* and *M. audouinii* have been associated with high rates of AC (Ginter-Hanselmeyer *et al*., 2007). These organisms generally produce mild signs of infection. Asymptomatic carriers at home or school are potentially important sources of disease transmission (Ginter-Hanslmeyer *et al*., 2007). Other studies have determined that carrier rate may increase to as high as 44 % for the siblings of index cases (Chen and Friedlander, 2001).

Dermatophytes are keratinophilic fungi, which parasitize on corneous structures, such as Stratum corneum, hair or nails (Kalinowska *et al*., 2012). Of great importance may also be some specific anatomic regions of the skin, greatly facilitating the colonization by fungi. Scalp hair can therefore arrest arthrospores spread by air. Similarly, spores are arrested in the hyponychium under or in the interdigital spaces, or in the folds of the skin where additionally occlusion helps them to develop (Dworacka-Kaszak, 2004). The spores are particularly resistant to environmental conditions, such as variable temperature and drying (Hryncewicz-Gwozdz *et al*., 2005). In addition to the progressive migration and climate change, social and economic conditions that affect skin exposure to fungal pathogens, and therapeutic methods are also important (Szepietowski and Baran, 2005). Transmission is increased with decreased personal hygiene and low socioeconomic

status. Asymptomatic carriers are common, making TC difficult to eradicate (Kawachi *et al*., 2010).

Table 2.1 Characteristic of Some Commonly Isolated Dermatophytes

DERMATOPHYTES	COLONIAL MORPHOLOGY	GROWTH RATE	MICROSCOPIC IDENTIFICATION
Microsporum Audouinli	Downy white to Salmon Pink colony.	2 week	Sterile hypae: terminal chlamydosporesm,favic chandeliers, and pectinate bodies; macroconidia rarely seen – bizarre shaped if seen; micronodia rare or absent
M. canis	Colony is usually membranous with feathery periphery; centre of colony is white to butt over orange –yellow or lemon yellow or yellow orange apron and reverse.	1 week	Thick walled, spindle shaped, multiseptate, rough walled, macroconidia some with macroconidia rarely seen
Microsporum Gypscum	Cinnamon coloured, powdery, colony reverse light tan	1 week	Thick-walled, rough, elliptical, multisapate, macroconidia, microconidia few or absent.

Table 2.1 Characteristic of Some Commonly Isolated Dermatophytes Continuation……..

Epidermophyton Floccosum	Center of colony tends to be folded and is khaki green, periphery is yellow; reverse yellowish brown with observable folds	1 week	Macroconidia large, smooth-walled multisepate, clavate and borne singly or in cluster of two or three microconidia not formed by this species.
Trichophyton Mentagrophytes	Different colonial types; white or pinkish, granular and fluffly varieties; occasional light yellow periphery in younger cultures, reverse buff to reddish brown.	7-10 days	Many round to globose microconidia most commonly borne in grapelike cluster or laterally along the hyphea; spiral hyphae in 30% of isolates, macroconidia are thin walled, smooth, club shaped, and multisepate, numerous or are depending upon strain.
Trichophyton Rubrum	Colonial types vary from white dowry to pink granular, rugal folds are common, reverse yellow when colony is young however, wine red colour commonly develop with age.	2 weeks	Microconidia usually Tear drop, most commonly borne along sides of the hyphae, macroconidia usually absent, but when present are smooth thin walled and pencil shaped.
Trichophyton Tonsurans	White, tan to yellow or rust, suedelike to powdery; wrinkled with heaped or sunken center; reverse yellow to tan	7-14 days	Microconidia are teardrop or club shaped with flat bottoms; vary in size but usually larger than other dermatophytes; macroconidia rare and

Table 2.1 Characteristic of Some Commonly Isolated Dermatophytes Continuation

	to rust red.		balloon forms found when present
Trichophyton Schoenleinii	Irregularly heaped, smooth white to cream colony with radiating grooves; reverse white.	2-3 weeks	Hyphae usually sterile; many antler-type hyphae seen (favic chandeliers)
Trichophyton Violaceum	Port wine to deep violet colony, may be heaped or flat with waxy glabroussurface; pigment may be lost on subculture	2-3 weeks	Branched, tortuous hyphae that are sterile; chlamydospores commonly aligned in chains
Trichophyton Verrucosum	Glabrous to velvety white colonies; rare stains produce yellow-brown colour; rugal folds with tendency to sink into agar surface	2-3 weeks	Microconidia rare; large and tear-drop when seen; macroconidia extremely rare, but forms characteristic 'rattail' types when seen; many chlamydospores seen in chains, particularly when colony is incubated at 37° C

(Koneman and Roberts1985).

2.12 Epidemiology of Tinea capitis

The epidemiology of TC has changed with the advent of griseofulvin and the sensitivity of *M. audouinii* to this antifungal medication (Elewski, 2000). Since the 1970's, there has been a progressive spread of infections caused by *Trichophyton tonsurans* through inner city areas of much of the USA and more recently in the UK and other European cities. By contrast *Trichophyton schoenleinii*, which causes a characteristic scalp infection, favus, is becoming less common, partly because it's striking clinical appearances and the tendency to scar are recognized even in remote communities. Patients with favus, or their parents, are more likely to present for treatment (H P A, 2007).

Trichophyton tonsurans arrived in the Americas with the Spanish conquistadores and currently, in Mexico, this organism accounts for between 15 % and 28 % of cases (Arenas, 2002). In the United States of America, it is the predominant causative organism of TC (98 %), whereas the dermatophyte *Microsporum canis* is more common in some parts of Europe, Arab countries, Iran, Brazil, Mexico, and the Dominican Republic (Arenas, 2002). The Countries with the highest incidence are Italy and other Mediterranean countries, although other nearby countries such as Austria, Hungary, Germany, and Poland also has high incidences (Ginter-Hanselmayer *et al*., 2007). The increase in anthropophilic dermatophytes is due to *Trichophyton tonsurans*, mainly in the United Kingdom and to *Trichophyton soudanense* and *Microsporum audouinii* in France (Panasiti *et al*., 2007).

The commonest cause of this infection in the UK is *Microsporum canis*. Its geographic range is, however, worldwide as it is spread from cats or dogs. In many parts of the UK,

Microsporum canis infections are infrequent but still the commonest forms of TC in those locations. *Microsporum canis* infections are also seen in children who do not have a history of exposure to cats or dogs (H P A, 2007). The likely explanation is that they have acquired the infection from a contaminated environment. In addition, other anthropophilic fungi such as *T. violaceum, T. soudanense* and *M. audouinii* are seen in cities. A dramatic increase in *T. tonsurans* infections has been reported in the USA (Nelson *et al*., 2003). Additionally, *T. tonsurans* had become the most common cause and today more than 95 % of *Tinea capitis* cases are caused by *T. tonsurans* (Foster *et al*., 2004).

In the USA *T. tonsurans* is also the most frequent isolate; it appears to be common in urban populations, particularly black American children, than in other cultural or ethnic groups. Little is known about the risk factors for anthropophilic infection (HPA, 2007). Previous studies have indicated; race, socio economic conditions, cultural patterns and public health measures as some of the predisposing factors to the infections (Ayanbimpe *et al*., 2003, Anosike *et al*., 2005, Bassiri and Khaksar, 2006). The emergence of *T. tonsurans* infection in developed countries has been attributed to low socioeconomic status, crowded living conditions, and the sharing of combs (Fuller *et al*., 2003).

In a recent US survey, TC was found in 6.6 % of the population (Fungal Research Trust, 2011). However an infection range from 0 % to 19.4 % is more common in deprived areas and black children, suggesting a global prevalence of 200 million cases (Fungal Research Trust, 2011). In Germany, before World War II, *Microsporum audouinii* and *Epidermophyton floccosum* occupied the top of the list of causative organisms by

frequency, but from the 1950s onwards *T. rubrum* (80 % to 90 %) has been the predominant dermatophyte at all sites apart from the head (Seebacher *et al*., 2008).

In the Netherlands, Sweden, and Belgium, there have been increases in *M. canis* TC, but there are also increases of anthropophilic TC caused by *T. violaceum*, *T. soudanense*, and *T. tonsurans*, which is a reflection of immigration patterns, particularly from East Africa (Kolivras *et al*., 2003; Hallgren *et al*., 2004). The most common species responsible for TC in Australia and New Zealand are *M. canis* and *T. Tonsurans* (Ameen, 2010). However, since the early 1990s, *T. soudanense*, *T. violaceum*, and *M. audouinii* TC have been increasingly reported in children who have migrated from East Africa, in particular, with evidence for transmission of these agents to local populations (Mc Pherson *et al*., 2008).

In Africa, however, tinea capitis continues to be an important public health problem, where it has been reported to affect 10 % to 30 % of school-aged children (Sidat *et al*., 2007). Hair infection (tinea capitis) is most common among children, often resulting in bald patches with psychological consequences. Although TC, like other dermatophytoses, is of public health importance, it is not a notifiable disease and as a result, the actual prevalence figures are unknown in many endemic areas (Ayaya *et al*., 2001; Ameh and Okolo, 2004; Anosike *et al*., 2005).

In Nigeria, the head is affected in 13.7 % of cases and the most common causative organisms are *Trichophyton soudanense* (30.6 %), *Microsporum ferrugineum* (7.7 %), and *Microsporum audouinii* (7.7 %), with cases involving *Trichophyton tonsurans* occurring less frequently (Ayanbimpe *et al*., 2008). In Mozambique, the prevalence of TC is 9.6 %, due mainly to *Microsporum audouinii*, *Trichophyton violaceum*, and

Trichophyton mentagrophytes (Sidat *et al.*, 2007). There is limited data on TC infections in Kenya. However, for those that have evaluated the prevalence of TC among school going children, have shown 33.3 % infection rates with the prevalence of *T. tonsurans* being 77.8 % and 4 % for *T. rubrum* (Ayaya *et al.*, 2001*).*

The distribution of dermatophyte infections and their causative agents varies with geographical region. It is influenced by a wide range of factors, such as type of population, climatic factors, lifestyle, migration of people, cultural practices and socioeconomic conditions (Havlickova *et al*., 2008; Ameen, 2010). Some dermatophyte species appear to be homogeneously distributed worldwide whereas others show a geographic restriction (Havlickova *et al*., 2008). In recent decades, an ever-growing etiological role of some anthropophilic dermatophytes has become evident all over the world (Jankowska-Konsur *et al*., 2011).

2.13 Diagnosis of Tinea capitis

Apart from cultural characteristics for identification of dermatophytes, clinically TC agents such as *M. audouinii* and *M. canis*, can mimic impetigo and pediculosis or psoriasis and seborrhea, respectively. However, for impetigo, the pain is generally more severe and individual hairs do not appear to be broken. In psoriasis, the scales on the scalp are thicker, but the hair is not broken off (Johnson and Nunley, 2000). *Alopecia areata* also causes hair loss and may mimic *T. tonsurans* infections, but does not cause scaling of the scalp (Sarabi, 2008). Tinea capitis is diagnosed by several methods.

A Wood's lamp examination may show hairs that turn blue-green. A potassium hydroxide test on the hair or scalp may show fungi under the microscope. A fungal culture of the hair or scalp may show what type of fungus is causing the infection

(Mounsey and Reed, 2009). Clinical diagnosis can also be applied. However, dependence on the clinical diagnosis of tinea capitis is unreliable and has a low specificity even though certain signs such as lymphadenopathy are useful predictors of the infection. For this reason, wherever possible, the diagnosis should be confirmed by appropriate laboratory tests.

2.14 Wood's Lamp Direct Examination

The Wood's lamp is used for the detection of fungal infection of hairs, it transmit invisible long-wave ultraviolet light (340-450 nm wave length). The Wood's glass consists of barium silicate with 9% nickel oxide. Filtered ultraviolet (Wood's) light elicits a green fluorescence from some dermatophyte fungi, mainly *Microsporum* species, in hair infections. Exposure to Wood's light is a useful screening procedure for taking specimens from *Microsporum* infections (H P A, 2007). The first use of Wood's lamp for the detection of tinea capitis was based on the fact that some dermatophyte species produce characteristic fluorescence under UV light. The chemical responsible for the fluorescence is pteridine. Wood's lamp is helpful in the diagnosis and treatment of an individual patient as well as for mass screening and control of epidemics in schools. It can also be helpful in assessing the length and response to treatment; the end point being emergence of non-fluorescent hair. Different fungi produce different types of fluorescence **a.** Bright green: *M. audouinii, M. canis and M. ferrugineum* **b.** Dull green: *T. schoenleinii* **c.** Golden yellow: Pityriasis versicolor (*Malessezia furfur*) **d.** No florescence: Dermatophytes that cause fluorescence are generally members of the *Microsporum* genus, non-fluorescing fungi such as *T. tonsurans*, *T. verrucosum* and other

dermatophyte species implicated in tinea capitis. However, the absence of fluorescence does not necessarily rule out tinea capitis as most *Trichophyton* species, with the exception of *T. schoenleinii*, are non-fluorescent (Gupta and Singhi, 2004).

Wood's light fluorescence is helpful but not diagnostic as it is only positive if the responsible organism fluoresces and fluorescence is sometimes seen for other reasons. The diagnosis of tinea capitis should be confirmed by microscopy and culture of skin scrapings and hair pulled out by the roots (Higgins *et al.,* 2000). However, with tinea capitis infections that are caused by the *Trichophyton* species, the fungal spores form on the inside of the hair shaft (endothrix), and there is no fluorescence (Fuller *et al.*, 2003). Therefore, the Wood's light examination is not a reliable method for diagnosing tinea capitis caused by the *Trichophyton* species because this species does not fluoresce.

2.15 Mycological culture of Tinea capitis

Culture documentation of the infection is a crucial component to treatment of TC (Roberts and Friedlander, 2005). Plucked hair fragments and skin scrapings are placed directly in culture media. The most commonly used media is Sabouraud agar. Chlorampenicol and Cycloheximide are used to inhibit bacteria and saprophytic fungi. Cultures are incubated at 25ºC for 3-4 weeks and if *T. verrucosum*, *T. violaceum* or *T. soudanense* are suspected, they are incubated for 6 weeks. Fungal identification is based on macroscopic (pigmentation formation) and microscopic morphology (macroconidia or microconidia formation).

2.16 Identification of Dermatological Agents Causing Tinea capitis

Like a number of fungi, dermatophytes may exhibit two phases in their life cycle: the anamorph state (imperfect or asexual phase), which is isolated in the laboratory; and the teleomorph state (perfect or sexual phase) (Enany *et al*., 2013). Not all of the teleomorph dermatophyte species have been identified (Mukherjee *et al*, 2011). Anamorphic states of dermatophytes include genera *Epidermophyton*, *Microsporum* and *Trichophyton* and belong to the class Hyphomycetes and phylum Deuteromycota (Enany *et al*., 2013). Teleomorphic states include majority of geophilic and zoophilic species of *Microsporum* and *Trichophyton.* They are classified in the teleomorphic genus Arthroderma, order Onygenales, phylum Ascomycota, and are usually found in their anamorphic state (Molina, 2011).

There are 3 major genera of Dermatophytes. These are *Epidermophyton*, *Microsporum* and *Trichophyton. Epidermophyton* are characterised by large thin-walled, multicellular, club-shaped and clustered bunches of macroconidia (Ayorinde *et al*., 2013). No Microconidia are produced. However, *Microsporum* produces both microconidia and macroconidia (Ayorinde *et al*., 2013). Macroconidia are multiseptate, with echinulations on the cell wall. The thickness of the cell wall and shape varies depending on the species (Simpanya, 2000). *Trichophyton* produces smooth walled macroconidia. Macroconidia are thin walled and cigar-shaped (Centre for Food Security and Public Health, 2005).

Microsporum canis grow on culture media to form white cotton radiated colony, golden yellow on reverse (Marques *et al.*, 2005). Macroconidia are fusoid, thick and rough-walled with curved apex with greater than 6 cells (Mcdonald, 2000). *Epidermophyton flocossum* grow with khaki pigmentation on front and yellow brown reverse (Ellis *et al*.,

2007). The macroconidia are similar to those of *Microsporum* except that they are smooth thin walled, club shaped and they occur in clusters and appear to be directly growing from the hyphae (Mcdonald, 2000) but with no microconidia.

The cultural morphhology and microscopic chararacteristics for *Trichophyton* species include; numerous smooth walled and clavate to pyriform microconidia. Macroconidia are less distinctive and often absent in this genus (Mcdolnald, 2000). *Trichophyton tonsurans* show dark-brown pigmentation with reddish brown to mahogany reverse after 21 days. In microscopy it reveals numerous microconidia of varying sizes and shape which appear to be formed at right angle to the hypha (Ellis *et al.,* 2007).

Trichophyton mentagrophytes produce a flat, white to cream and a powdery appearance in potato dextrose agar with a pinkish brown reverse. The microscopy show single-celled spherical microconidia and spiral hyphae. *T. rubrum* produce white to cream flat colony with yellow-brown reverse after 21 days. On microscopy, numerous pyriform microconidia are observed (Ellis *et al.,* 2007). *T. mentagrophytes* is urease positive after 7 days while *T. rubrum* is urease negative after 7 days (Mcdolnald, 2000). The Colony of *T. verrucosum* grows slowly in potato dextrose agar. It is small, button or disc-shaped, white to cream-coloured, with a suede-like to velvety surface, a raised centre, and flat periphery with a yellow reverse. On microscopy, it is observed to produce clavate to pyriform microconidia borne singly along the hyphae (Ellis *et al.,* 2007).

Traditionally, most commercially available identification systems of dermatophytes are based on physiological (growth temperature), nutritional (sugar assimilation and/or fermentation, enzyme production profiles) and morphological characteristics (Elsayed *et al.*, 2010). Tinea capitis is generally identified by the presence of branching hyphae and

spores on KOH microscopy (Ayorinde *et al*., 2013). If hyphae and spores are not visualized, Wood's lamp examination can be performed. If KOH microscopy and Wood's lamp examinations are negative, fungal culture may be considered when *Tinea capitis* is strongly suspected (Barry and Hainer, 2003).

Laboratory diagnosis is routinely performed by direct microscopic examination of a clinical specimen followed by sample culture in specific agars (Côbo *et al*., 2010). This combination of techniques is time-consuming and notoriously low in sensitivity (Uchida *et al*., 2009). Furthermore, sample cultures on agar have a high risk of contamination by non-dermatophytic moulds and yeasts (Arabtzis *et al*., 2007). The final diagnosis must be made based on isolation of the organism from affected tissues and visualization of tissue invasion by organisms with compatible morphology (Arabtzis *et al*., 2007).

PCR-based techniques used in dermatophytes diagnosis, are highly specific and sensitive methodologies but demand well-equipped laboratories, expensive reagents, laboratory personnel expertise and protocol standardization, so, actually, molecular techniques for dermatophytes diagnosis are far from being routinely used and need more studies before implementation (Garg *et al*., 2009). Some associations with other techniques, such as histology, are proposed to increase diagnosis sensitivity (Karimzadegan-Nia *et al*., 2007) but this would bring more difficulties in the routine, demanding professionals, equipments and more time (Côbo *et al*., 2010).

2.17 Direct Microscopy

Microscopic examination and/or fungal culture should be used to confirm the clinical diagnosis of TC because of the extended nature of most treatment regimens (Ali *et al*., 2007). Microscopic examination consists of scraping the scales of the lesions onto a slide

and viewing the sample, which is prepared with a 20 % potassium hydroxide (KOH) solution, under the microscope to look for the presence of hyphae (Chen and Friedlander, 2001). This test may be difficult to interpret or may be falsely negative with early or inflammatory lesions. Therefore, the final diagnosis of TC should be made by culture.

2.18 Molecular Diagnosis

The diagnosis of dermatophytosis is based primarily on cultural characteristics and microscopic examinations. However, these techniques are less sensitive and more time consuming. Moreover, these methods require a skilled and experienced person especially for species level identification of dermatophytes (Malinovschi *et al*., 2009). The molecular diagnostic methods give faster and more precise identification and overcome the limitations of the conventional methods (Kim *et al*., 2011). These methods include: polymerase chain reaction (PCR), multiplex PCR, nested PCR, arbitrarily primed PCR, random amplified polymorphic DNA analysis (RAPD) (Kim *et al*., 2011).

Ribosomal DNA (rDNA) has long been used as a potential marker for phylogenetic studies (Avise, 2004). rRNA genes are organized in clusters of tandem repeats, each of which consists of coding regions (18S, 5.8S, and 28S) and two ITS regions (ITS-1 and ITS-2) and one non-transcribed spacer (NTS) region. Coding regions are evolutionarily conserved and are utilized for phylogenetic inferences for major phyla. (Mohini and Deshpande 2010) reported the phylogenetic classification and species identification of dermatophytes strains based on DNA sequences of nuclear riobosomal internal transcribed spacer-1 region.

2.19 Treatments

Griseofulvin was first approved by the U.S. Food and Drug Administration (FDA) for systemic treatment of TC in 1958. Before then, the only available treatments were shaving the head, applying mercury/sulfur to the scalp, or resorting to high-fat diets (Möhrenschlager *et al.,* 2005). However, griseofulvin quickly became the mainstay of treatment and the use of terbinafine and itraconazole in patients allergic to griseofulvin were also successful (Trivino-Duran *et al.,* 2005). In a meta-analysis study, Fleece *et al* (2004) showed terbinafine treatment for up to four weeks to be as effective in treating *Trichophyton* spp. as 8 weeks of griseofulvin treatment. Griseofulvin is a mitotic inhibitor and interferes with nucleic acid, protein, and cell wall synthesis of replicating dermatophyte cells (Brendan, 2012). There is also evidence that griseofulvin has an anti-inflammatory effect, which is unique among the systemic antifungal agents (Brendan, 2012). A recent extensive review quoted mycologic cure rates of 80 % to 95 % and effective therapy rates of 88 % to 100 % for griseofulvin (Gupta and Cooper, 2008).

In 2007, terbinafine became the second FDA-approved drug to treat TC in children. The drug is an allyl-amine whose antifungal effect is due to inhibition of squa-lene epoxidase (Brendan, 2012). However the responses of *Microsporum* species to terbinafine are generally slower than those of *Trichophyton* and in some patients there is treatment failure (Caceres-Rios *et al*., 2000). However higher doses of terbinafine, more than 6 mg/kg per day, appear to produce good responses (Devliotou-Panagiotidou and Koussidou-Eremondi, 2004).

Itraconazole has both fungistatic and fungicidal activity, depending on its tissue concentration, unlike other azoles, its principal mechanism of action is fungistatic,

through depletion of ergosterol in the cell membrane, leading to alteration of membrane permeability (Rebollo *et al*., 2008). It is highly lipophilic and keratinophilic, and it persists in the stratum corneum for 3 to 4 weeks after suspension of treatment, allowing it to be used in pulses of 1 week separated by periods of 2 weeks without treatment (Higgins *et al*., 2000).

Currently, the recommended therapy for TC is a 6 to 8 week course of oral griseofulvin. Griseofulvin has a long-standing history of safety and efficacy when used to treat fungal scalp infections in children (Bennet *et al*., 2000). Griseofulvin is effective when treating *Microsporum, Epidermophyton,* and *Trichophyton* (Chan and Friedlander, 2004). When compared to other treatments in *Trichophyton* infections, griseofulvin and terbinafine are equally effective, but griseofulvin is most effective against *Microsporum* infections (Fuller *et al.,* 2001). Despite the existence of antifungal agents effective on dermatophytes, there is need to search for alternatives (Tra Bi *et al*., 2005). The relatively high cost and constraints due to the length of the modern treatment curb the control of the dermatomycoses in developing countries (Tra Bi *et al*., 2005). Resource-poor people from remote areas still use traditional medicine for the treatment of various diseases of microbial and non microbial origin (Kone *et al*., 2002).

2.20 Antifungal Susceptibility Testing

Large number of antifungal agents has been introduced during past two decades for treating dermatophytosis (Chadeganipor *et al*., 2004). Different susceptibility patterns of different dermatophyte species to various antifungal agents have been reported. Different methods have been employed by different groups of researchers for determining the *in*

vitro antifungal susceptibility to new and existing antifungal agents. These methods include; broth macro and microdilution methods, agar dilution, E test, colorimetric microdilution, disk diffusion method etc. (Perera *et al*., 2001; Fernandez-Torres *et al.,* 2003, Karaca and Koc, 2004; Santos and Hamdan, 2005). Clinical and Laboratory Standards Institute (CLSI) published a reference method M38-A2 document in 2008, in which the protocol for determining the MICs of several antifungal agents against filamentous fungi including dermatophytes was mentioned. Araujo *et al.,* (2009) tested antifungal activities of fluconazole, itraconazole, ketoconazole, terbinafine and griseofulvin by broth microdilution technique, against dermatophyte species recovered from nails and skin specimens from Goiania city in Brazil. The low MIC values 0.03 µg/ml were 39 found for 33.3, 31.6 and 15% of isolates for itraconazole, ketoconazole and terbinafne, respectively. Adimi *et al.,* (2013) and co workers evaluated the efficacy of ten antifungal agents (fluconazole, itraconazole, ketoconazole, terbinafine and griseofulvin, voriconazole, clotrimazole, ciclopirox olamine, amorolfine and naftifine) against large number of dermatophyte strains using CLSI broth microdilution method (M38-A). Itraconazole and terbinafine were found highly effective as compared to other antifungal agents while fluconazole was found least effective in their study. Yadav *et al*., (2013) determined the susceptibility of the clinical isolates of dermatophytes using commercially available antifungal disks (Himedia 10ug/disk) of griseofulvin, miconazole, terbinafine, clotrimazole, fluconazole and ketoconazole in the disk diffusion method. Clotrimazole was found the best antimycotic agent against dermatophytes followed by miconazole and ketoconazole. Similarly, Nweze *et al.,* (2010) also used disk diffusion method for determining the susceptibility of dermatophyte isolates against eight

antifungal agents. These researchers conducted that disk diffusion method was reproducible, simple and could be used to determine the antifungal susceptibility of dermatophytes.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

The study was carried out in Zaria, a major city in Kaduna State in North West Nigeria. There are two Local Government Areas in Zaria metropolis namely, Sabon Gari and Zaria Local Government Area. Zaria is known as a centre for education. The population of Zaria is estimated as 547,000 from the 2006 Nigerian census. It is situated on latitude 11°7′, 11°12′N and longitude 7°41′E (Mamman *et al*., 2000). Relative humidity in Zaria is between (63.2- 68.8 %) with an average rainfall of (155.9-182.1mm), temperature range of (25-30.2°C), and with a low evaporation rate (154.2-163.91mm). The vegetation of Zaria is within the guinea savannah, the community cultivates tomatoes, maize, sweet potatoes, beans, rice, sugar cane, millet and pepper. Livestocks such as cow, sheeps, goats, cats, dgs and poultry are kept in medium to scale ffarms where they are attached to people

3.2 Source and Preparation of Plant Materials

The plant material was collected from Botanical garden of Biological Sciences, ABU main campus. This was brought and identified with voucher number 1098 at the Herbarium Unit of the Department of Botany, Ahmadu Bello University Zaria. The whole plant was air-dried and made into powder using a clean mortar and pestle and subsequently referred to as powdered material and stored in dessicator for further analysis.

3.3 Preparation of Crude Methanolic Extract of *Chlorophytum laxum*

The ground plant was subjected to extraction at the Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Ahmadu Bello University Zaria. A total of 500 g of the dried plant of *C. laxum* was subjected to extraction with 5 liters of 70% (v/v) methanol by cold maceration at room temperature for 3 days. The total mixture was filtered with cotton wool. The filtrate was concentrated to dryness on a water bath at 50°C so as to obtain the dry extract whichwas then held in desiccators for further analysis (Evans, 2002).

3.4 Differential Fractionation of the Methanolic Extract of *C. laxum* in Different Solvents

The dried methanolic extract obtained from the plant of *C.laxum*(30g) was suspended in distilled water and partitioned in sequence with hexane, ethyl acetate and n-butanol each for several times. The different solvent fractions were concentrated on a water bath at 100^{o} C so as to obtain the dry extract.

3.5 Preliminary Phytochemical Screening

The extract was subjected to various phytochemical tests to identify the chemical constituents present using standard methods as described by Evans (2002), Musa (2005), Sofowora (2008). One gram(1 g) of the extract was weighed and dissolved in 10ml of sterile distilled water and filtered using Whatman No. 1 filter paper, 1ml of the dissolved filtrate extracts, was dispensed into various test tubes and used for the following tests;

3.5.1 Test for carbohydrates.

3.5.1.1 Molisch's test

To a portion of each extract in a test tube, few drops of molisch reagent was 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.

3.5.2 Test for unsaturated steroid and triterpenes

3.5.2.1 Liebermann-Bucchard test

To a portion of each extract, equal volume of acetic acid anhydride was added and mixed gently. One (1ml) of concentrated sulphuric acid was added down the side of the test tube to form a lower layer. Changes in color were observed immediately and over a period of one hour. Blue to blue-green colour in the upper layer and a reddish, pink or purple colour in lower layer indicate the presence of triterpenes.

3.5.2.2 Test for unsaturated sterols

To a portion of each extract, 2-3 drops of concentrated sulphuric acid was added at the side of the test tube. Immediate colour change at the interphase of the extract and sulphuric acid was noted as well as colour change over one hour period (cherry red colour usually indicates the presence of unsaturated sterols (salwoski test).

3.5.3 Test for cardiac glycosides

3.5.3.1 Keller- Kiliani test

A portion of each 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 that indicates the presence of deoxy sugars and a pale green colour in the upper acetic acid layer indicating the presence of cardiac glycosides.

3.5.4 Test for saponin glycoside

3.5.4.1Frothing test

About 10ml of distilled water was added to a portion of each extract and was shaken vigorously for 30seconds. The tube was allowed to stand in a vertical position and was observed for 30mins. A honeycomb froth that persists for 10-15mins indicates presence of saponins.

3.5.5 Test for tannins

3.5.5.1 Lead Sub-acetate test

To a portion of each extract, 3-5 drops of lead acetate solution was added. A coloured precipitate indicates the presence of tannins.

3.5.6 Test for flavonoids

3.5.6.1 Shinoda's Test

A portion of the extract was dissolved 1-2ml of 50% methanol in heat Metallic magnesium chips and few drops of concentrated hydrochloric acid were added. Appearance of red coloured indicates presence of flavonoids.

3.5.7 Test for alkaloids

3.5.7.1 Mayer's test

To test tubes containing 1ml of each extract, few drops of Meyer's reagent were added. The development of a cream precipitate indicates presence of alkaloids.

3.5.7.2 Dragendoff's test

To test tubes containing 1ml of each extract, few drops of Dragendorff's reagent were added. A reddish brown precipitate indicates presence of alkaloids.

3.5.7.3 Wagner's test

Few drops of wagner's reagent were added to a portion of each extract, whitish precipitate indicates presence of alkaloids.

3.5.8 Test for free anthracene derivatives (Bontrager's test)

To a portion of the extract in a dry test tube, 5ml of chloroform was added and was shaken for at least 5 minutes. This was filtered and the filtrate shaken with equal volume

of 10% ammonia solution.The appearance of a bright pink colour in the aqueous (upper) layer indicates the presence of free anthraquinones (Sofowora, 2008).

3.6 Ethical Clearance and Informed Consent

Ethical clearance was obtained from Kaduna State Ministry Of Health for this research (appendix xii).Permissions were obtained from head teachers while verbal consent was obtained from the pupils that was took in the study.informed verbal and written consents were obtained from the parents/ guardians for participation of their chidren or ward in the study.

3.7 Specimen Collection and Isolation of Test Fungi Organisms

Parental consent was sought in order to collect samples from the pupil in Salmanduna and Anguwan Bishar LEA, Primary School in Zaria city. Children within the age of 4-13years were examined in a well-lit room and the scalp was examined for scaly grey patches, lusterless hair strands and purulent lesions (Higgins *et al.*, 2000). Those that presented with lesions and sign of pathogenic infections were selected for the study. The site of infection was cleaned and disinfected with70% alcohol as well as to remove traces of any ointment or local applications present and followed by the collection of scalp scrapings of the affectedpart of the scalp with sterile surgical blades. Hair specimens were collected by removing dull broken hair from the margin of the lesion using sterile surgical blade. The scrapings were handled separately and no individual scrapings were allowed to mix up with the other and transported in folded labelled square clean paper. Sabouraud dextrose agar (SDA) was prepared as described by manufacture's instruction,

supplemented with cycloheximide and chlorophenicol. Fifteen (15) ml of SDA was aseptically dispensed into Petri-dishes and allowed to set. (Ayanbimpe *et al.,* 2003).

3.8. Isolation of Test Organisms on Sabouraud's Dextrose Agar (SDA).

A portion of each sample collected from the school children was placed onto the centre of the agar plate media.The media were incubated at room temperature (28 °C) and observed daily for seven (7) days. After the incubation period, suspected isolates were sub- cultured onto SDA repeatedly to get pure cultures and stored in SDA slants. From these slants, subsequent sub-culturing at regular intervals was undertaken.

3.8.1 Morphological identification of the isolated dermatophytes

Identification of fungal isolates was carried out by both macroscopic and microscopic examinations using the method described by Refai *et al* (2013).

3.8.2 Macroscopic examination of the cultures

Examination involved rate of growth, colour on the surface, texture of the colony or consistency (cottony, velvety, folded, fluffy, suede-like and wiry), surface topography (flat, folded, plicate, rugose), colonial morphology and reverse side of colony (pigmentation of the media), margins, elevation and detachability from the agar surface.

3.8.3 Microscopic examination of the cultures

A drop of lactophenol cotton blue was placed on a clean glass slide and a pin-size of the culture was placed on the slide. It was gently teased with flamed inoculation needles. When it was satisfactorily spread, cover slip was placed on it, pressed gently to avoid air

bubbles. The preparation was examined with x 10 and x 40 objectives lens of light microscope Refai *et al* (2013).

3.9 Systematic Description of Fungi Isolates

Isolated fungi were identified using both macroscopic and microscopic characteristics and compared with already described species using the identification key by Samson *et al*. (2004) and Refai *et al* (2013).

3.10 Effect of Different Concentrations of Crude Methanolic Extracts of *Chlorophytum laxum* on the Growth of Fungi species

A total of 5 mL of varying concentrations viz: 50, 25, 12.5, and 6.25 mg/mL of crude methanolic extract were dispensed onto the 15ml molten medium in petri dishes with the help of a syringe. These was thoroughly mixed and allowed to solidify at room temperature (28 °C). A portion of fungal growth from the periphery was cut with the and of a sterilized cork borer of 5mm in diameter and placed, onto the middle of each petri dish and incubated at room temperature in triplicate. Seven days (7) after incubation, measurement of the colony was taken directly with the help of a ruler in millimeter (mm). The difference in the measurement between the mycelial growth of the fungi and that of the cork borer calculated to determine the effect of different concentrations of *Chlorophytum laxum* on the growth of the isolates. Negative control was set up without adding any plant extract. Antifungal activity of the extract was recorded in terms of percentage mycelial growth inhibition by comparing colony in control with colony in treatment using food poisoning technique formular as described by (Vinesh and Davendra, 2013).

$$I = \frac{DC - DT}{DC} \times 100$$

Where, I = Percentage inhibition

DC = Average diameter of control (cm)

DT = Average diameter of growth with treatment (cm)

3.11 Antifungal Activity of Extracts Fractions

The antifungal activity of each fraction of *C. laxum* obtained from the crude methanolic extract against the test organisms was evaluated by using poisoned-food technique described by (Vinesh and Davendra, 2013)). Varying concentrations in mg/ ml of 50, 25, 12.5 and 6.25of 5 ml of the various fractions: hexane, ethyl acetate, n-butanol and aqueous were dispensed onto the 15ml molten medium in petri dishes aseptically. They were thoroughly mixed and allowed to solidify. A portion of fungal growth from the periphery was cut with the help of a sterilized cork borer of 5mm in diameter and placed onto the middle on each petri dish and incubated at room temperature. A week after incubation, measurement of the colony was taken directly with the aide of a scale. Differences in the measurement between the mycelial growth of the fungi and that of the cork borer gave the growth of colony and the effect of different concentration of various fractionson the growth of the isolates. Control experiment was set up without adding plant extract.

3.12 Thin Layer Chromatography (TLC) of the Fractions

Thin Layer Chromatography was used to separate the chemical components of*C.laxum* extracts using silica gel TLC plates in accordance with the procedure adopted by Patra *et al*. (2012). The silica gel plates (Merck F_{254} Darmstadt Germany) of 5×10 cm each were prepared for the extract. The plate was spotted with the *C. laxum* extracts at 1.5cm origin line using a micro capillary tube and then subjected to separation in the developing chamber containing Ethylacetate: Methanol (10:2 v/v). After development, the plate was removed, air dried, and sprayed with p-anisaldehyde sulphuric acid. This was placed for 5 seconds in an oven to reveal the separated components. The following formula was used to measure the retention factor (Rf) which is the distance the compound travels to the distance the solvent travels (Patra *et al*., 2012).

3.13 Gas-Chromatography/Mass-Spectrometry (GC-MS) Principle of the Assay:

The gas-chromatography/mass-spectometry (GC/MS) is composed of two major building blocks; the gas chromatograph and the mass spectrometer. GC analysis separates all of the components in a sample and provides a representative spectral output. The technician injects the sample into the injection port of the GC device. The GC instrument vaporizes the sample and then separates and analyzes the various components. Each component produces a specific spectral peak that may be recorded on a paper chart or electronically. The time elapsed between injection and elution is called the "retention time." The retention time can help to differentiate between some compounds. The size of the peaks is proportional to the quantity of the corresponding substances in the specimen analyzed. The peak is measured from the baseline to the tip of the peak. MS

identifies substances by electrically charging the specimen molecules, accelerating them through a magnetic field, breaking the molecules into charged fragments and detecting the different charges. A spectral plot displays the mass of each fragment (Amirav *et al*., 2008).

To identify the actual chemical compounds in the active fractions of the extracts, the fraction was further analysed by GC/MS, following the procedure as described by the manufacturer, the samples were inserted into the GC/MS inlet port in a GC/MS vial. The results were printed out from the computer system connected to the GC/MS machine (Amirav *et al*., 2008).

3.14 Data Analysis

The data generated were presented in tables and charts and were analysed statistically using the SPSS version 20.0, the details are attached in the appendices. Means were compared using student's *t*-test analysis of means to determine the level of significance. Analysis of variance (ANOVA) was used to compare means of the plant extracts at different concentrations against growth of the isolated fungi species the means were separated using Duncan Multiple Range Test (DMRT).

CHAPTER FOUR

RESULTS

4.1 Yield of Extracts of *Chlorophytum laxum*

The results of phytochemical properties are carbohydrate, unsaturated sterols and triterpenes, cardiac glycoside, tannins, saponin glycoside, flavonoid and alkaloids in methanolic extract of *C.laxum* while free Anthracene derivatives was absent, presented in Table 4.1.

The yield was 35 g (7%) of the whole *C.laxum* crude methanolic extract. The hexane fraction yielded 0.13 g (0.03%), ethyl acetate fraction yielded 1.47g (0.29%), n-butanol fraction yielded 1.77 g (0.35), and from distilled water fraction 26.63 g (5.3%) was obtained, presented in Table 4.2.

4.2 Morphologic and Microscopic Identification of Dermatophyte

Dermatophyte species identification was done based on pigmentation on the front and backof Sabouraud dextrose agar and microscopic features.

Microsporum spp Colonies were smooth, woody and heaped velvety. Obverse colony colour werecream to rusty orange while the reverse colour was cream to brownish. Mycelium is septate branched, long hyphae, with profusely branched hyaline (Plate I)

Trichophyton spp 1 colonies were powdery and suede-like with concentric folds. Obverse morphology was white and grayish while reverse was yellow. Mycelium is septated with variable shape microconidia along the hyphae (Plate II)

Trichophyton spp 2 colonies are suede-like and flat. Both obverse and reverse colony colour was yellow to orange (Plate III).

Table 4.1: Phytochemical Constituents of *C.laxum* using methanolic Extraction

Phytochemicals	Type of test	*Chlorophytum laxum*
Carbohydrate	Molish	+
Unsaturated Sterols	Salkowski	+
Triterpenes	Liebermann-Bucchard	+
Cardiac Glycoside	Keller-Kiliani	+
Saponin Glycoside	Frothing	+
Tannins	Lead sub-acetate	+
Flavonoids	Sodium hydroxide	+
Alkaloids	Mayer's	+
	Dragendorff's	+
	Wagner's	+
Free Anthracene Derivatives	Bontra ger's	-

+ = Presence, - = Absence,

Table: 4.2 Thin Layer Chromatography profile (TLC) of the Fractions of *Chlorophylum laxum*

Metabolites	Detecting reagent	Haxane	Ethyl acetate	Butanol	Aqueous
Phenolic compound	Ferric chloride	+	+	+	+
Terpenoid/ Steroids	Liebermann-Bucchard	+	+	+	+
Anthraquinones	Borntragers	+	+	+	+
Flavonoid	Aluminium chloride	+	+	+	+
Alkaloids	Dragendorff	+	+	+	+

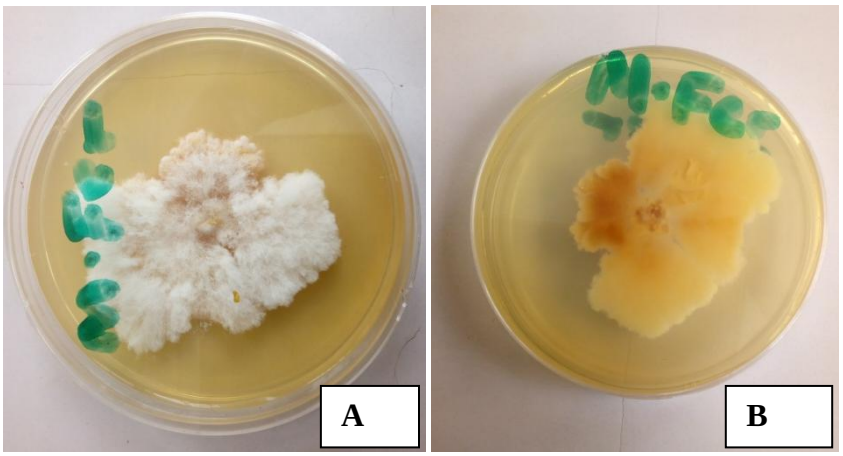

Plate I: Pure culture of *Microsporum spp:* A - upper surface, B - reverse side

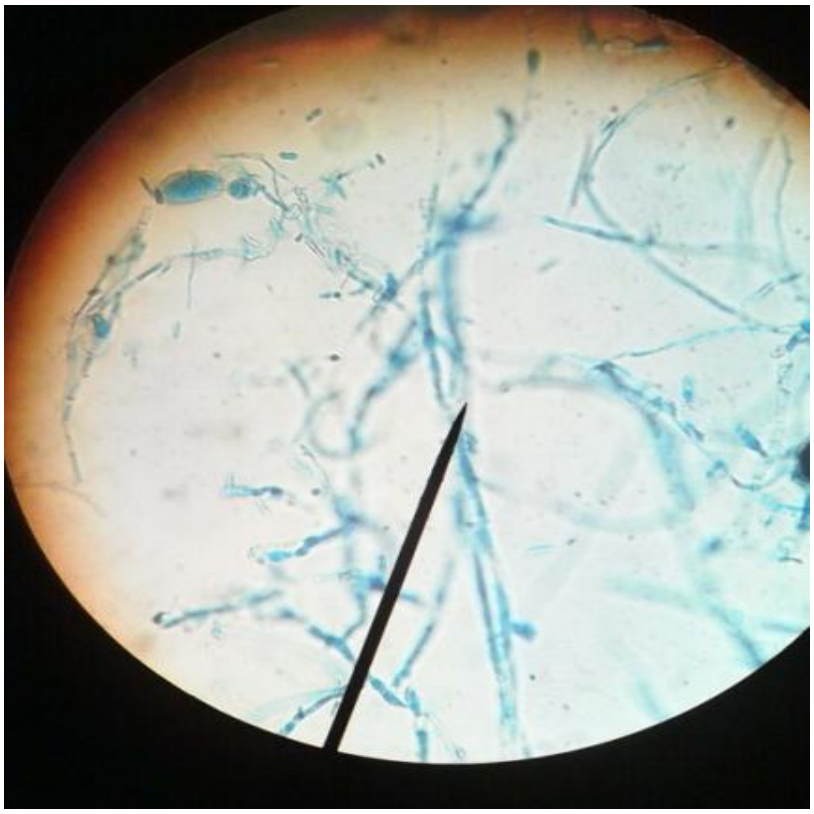

Plate II: Microscopic features of *Microsporum spp*
Objective len ×*10*

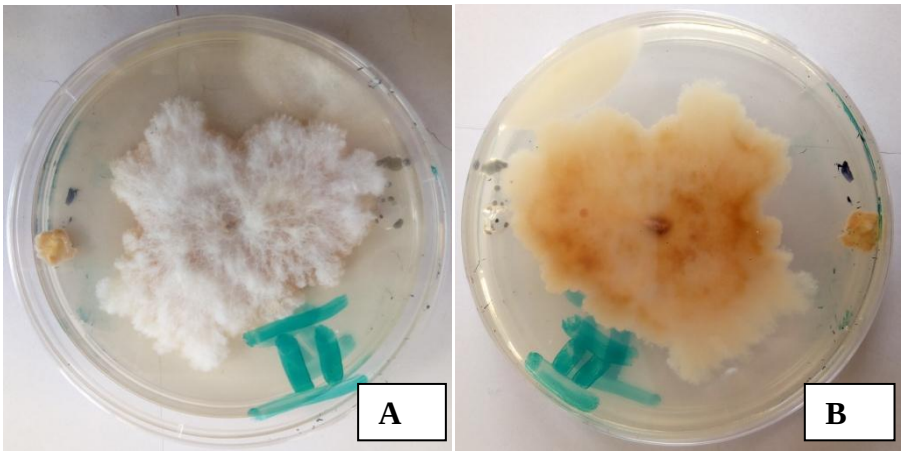

Plate III: Pure culture of *Trichophyton spp* 1*:* A - upper surface, B - reverse side

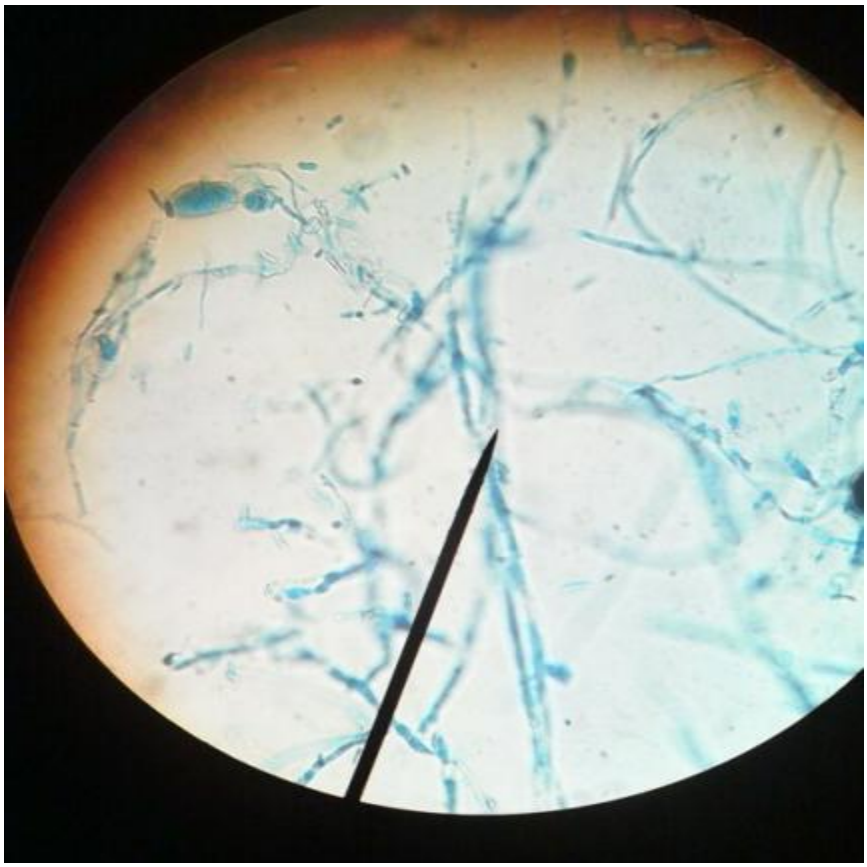

Plate IV: Microscopic features of *Trichophyton spp1*
Objective len ×*10*

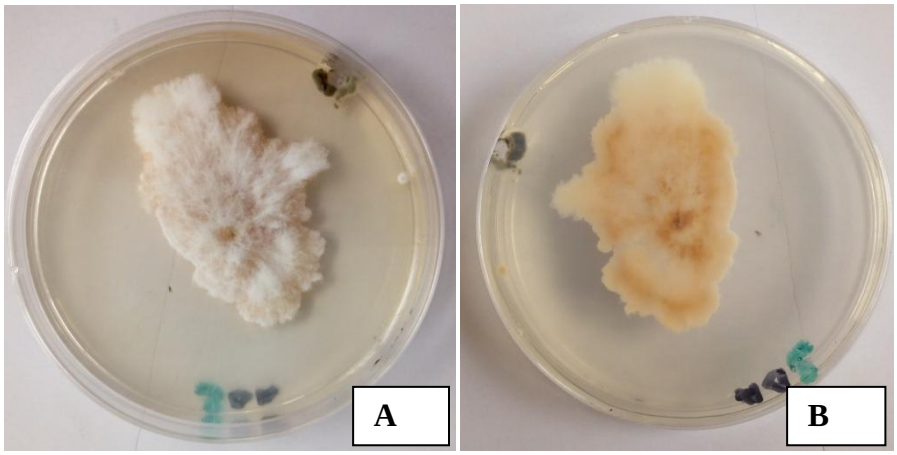

Plate V: Pure culture of *Trichophyton spp* 2: A - upper surface, B - reverse side

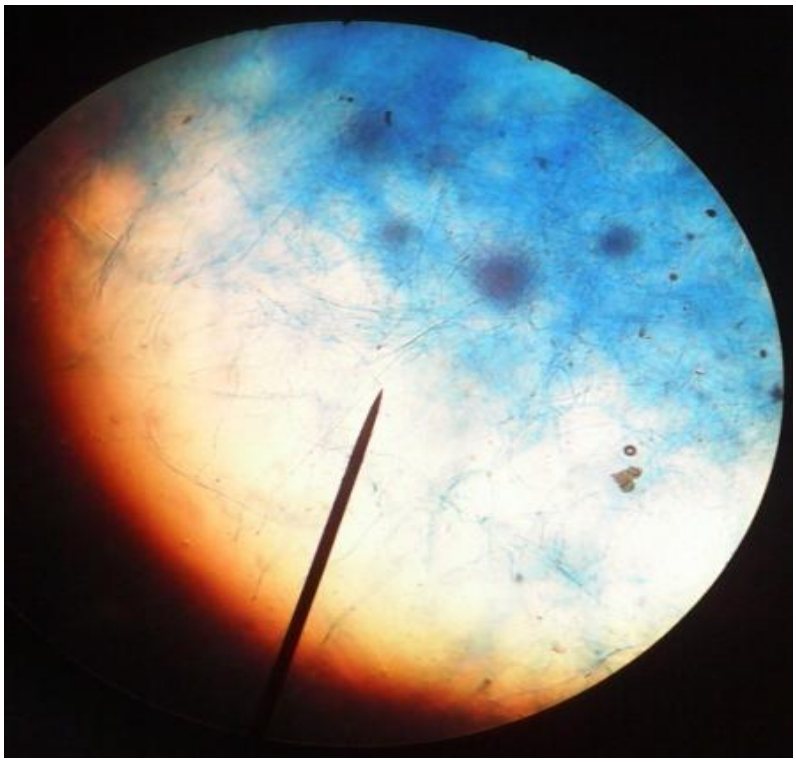

Plate VI: Microscopic features of *Trichophyton spp 2*
Objective len ×*10*

4.3 Effect of Different Concentrations Crude Methanolic Extract of *Chlorophytum laxum* on Mycelia Growth zone of Inhibition on Fungi Isolated from primary school pupils.

The crude methanolic extract possesses toxic activities on the isolated pathogens. The table further revealed that the inhibitory activity vary with concentration and the test organisms. The higher the concentration of the extract, the higher the zone of inhibition and vice-versa. Thus the highest inhibitory activity of the concentration was seen at 50 Mg/mL for each of the test organisms: *Microsporum species* 30.00 ± 6.11mm, *Trichophyton species (1)* 37.33 ± 1.20mm, and *Trichophyton species (2)* 6 0.33 ± 0.88mm respectively. Similarly, the lowest inhibitory effect of the concentration occurred at 6.25 Mg/mL for each of the isolated fungal species: *Microsporum species* 20.67 ± 4.63mm, *Trichophyton species (1)* 29.33 ± 1.45mm, *Trichophyton species (2)* 50.67 ± 1.76mm. However, there was no significant difference among the concentrations of the crude methanolic extract of *Chlorophytum laxum* on the mycelia growth inhibition of the isolated fungi species but varied from the control ($P \leq 0.05$)It is evident from Table 4.2.

Table 4.3 Effect of Crude Methanolic Extract of *Chlorophytum laxum* on Isolated Fungi from primary school pupils

	Test organisms		
Conc. (%)	***M. species***	***T. species 1***	***T. spcies 2 (mm)***
0.0	0.0± 0.0	0.0 ± 0.0	0.0 ±0.0
6.25	20.7± 4.6 [a]	29.3± 1.5 [a]	50.7± 1.8 [a]
12.5	27.0± 4.5 [a]	31.7± 1.5 [a]	53.0± 8.5 [a]
25.0	28.3± 6.6 [a]	35.7± 1.7 [a]	55.7± 4.3 [a]
50.0	30.0±6.1 [a]	37.3± 1.2 [a]	60.3± 0.9 [a]

Values are expressed as means ± SEM (Standard error of means). Means having different superscripted alphabets along columns are significantly different at P ≤ 0.05

4.4 Effect of Chlorophytum laxum Fractions on mycelial growth zone of inhibition on the Test Organisms

Antifungal activities of fractions concentration on percentage mycelia growth inhibition of the test organisms expressed as mean inhibitory activity with different superscripted alphabets along columns and are significantly different at P< 0.05. The Highest antifungal activities in *Microsporum species, Trichophyton species 1,* and *Trichophyton species 2* were observed in 50mg/ml concentration regardless of the fraction method. The highest mean values of 78.00±5.51mm, 80.00±3.51mm and 85.00±2.65mm were observed at 50mg/ml concentration of aqueous fractions, with the least mean values of 23.00±1.15mm, 30.33±3.18mm and 48.33±1.33mm effect in 6.25Mg/mL concentaction of Hexane. The effects of the *chlorophyton laxum* fractions concentrations on the test organisms were significantly different in figures below.

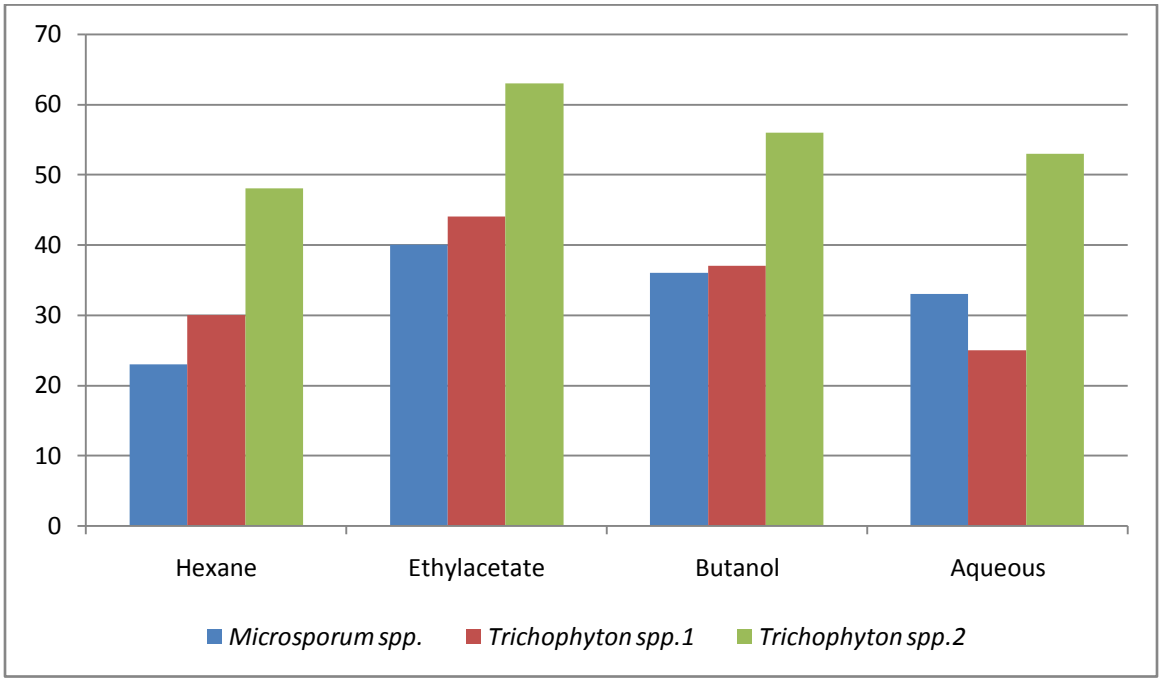

Figure 4.1: Effect of *Chlorophytum laxum* Fractions concentrations at 6.25mg/ml on the Test Organisms

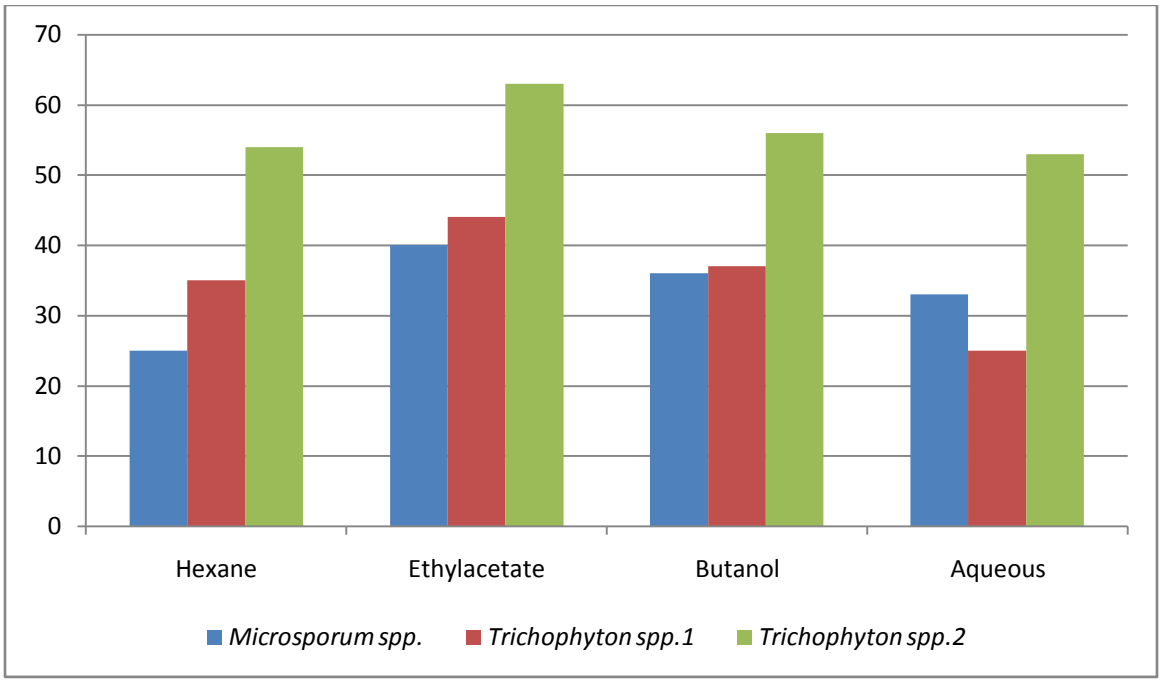

Figure 4.2: Effect of *Chlorophytum laxum* Fractions concentrations at 12.5 mg/ml on the Test Organisms

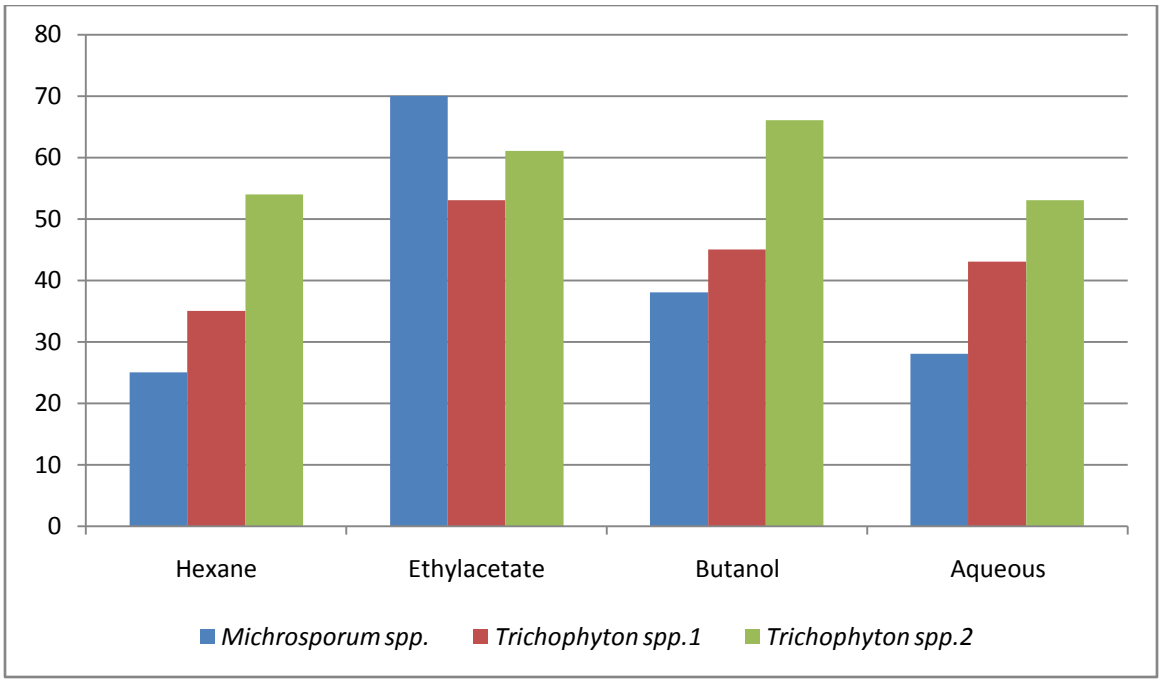

Figure 4.3: Effect of *Chlorophytum laxum* Fractions concentrations at 25 mg/ml on the Test Organisms

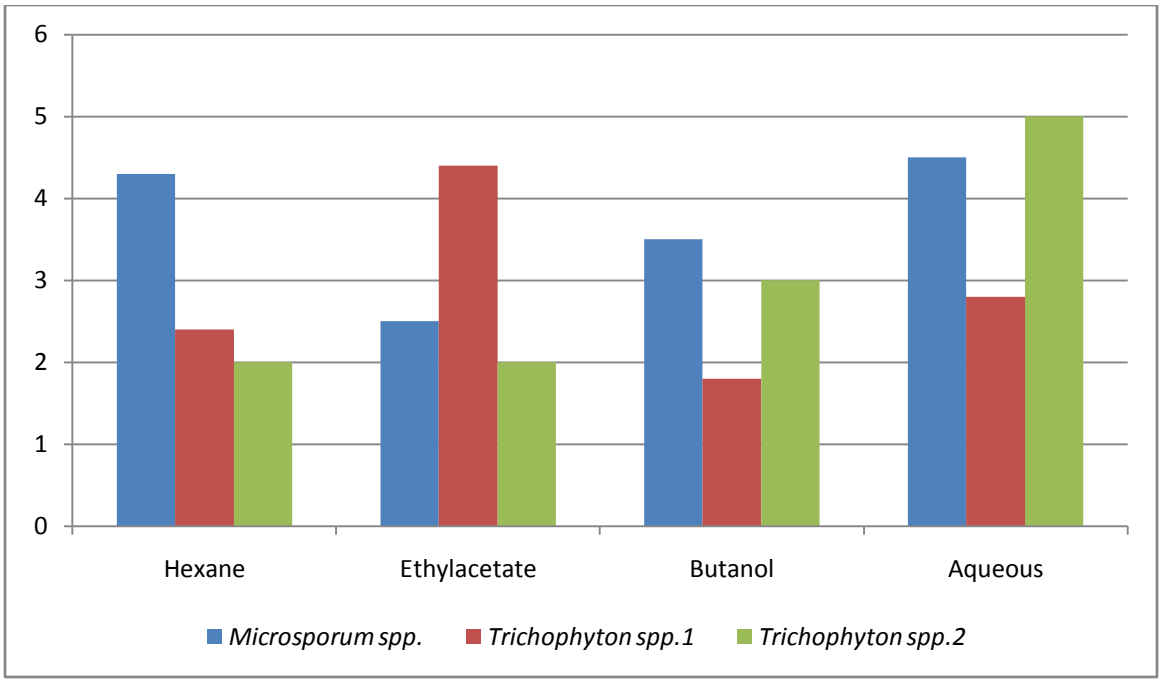

Figure 4.4: Effect of *Chlorophytum laxum* Fractions concentrations on the Test Organisms

4.5 Thin Layer Chromatography (TLC) of the Fractions

The thin layer chromatography (TLC) profile of hexane, ethyl acetate, butane and aqueous fractions which shows the presence of phenolic compounds, alkaloids, cardiac glycosides chloride and terpenoid visualized with specific detecting reagents Constituents of crude *C.laxum* methanolic extract using TLC technique is presented in Table 4.4.

4.6 Gas-Chromatography/Mass-Spectrometry (GC/MS) Analysis of Fractions of Crude methanolic extract of *Chlorophytum laxum*

In the present study leaves and bark components of the *Chlorophytum laxum* were identified by Gas-Chromatography/Mass-Spectrometry (GC/MS) analysis.Thirty (30) different active principles with their retention time (RT), molecular formula, molecular weight (mw) and concentration (%) in crude methanolic extract of *Chlorophytum laxum* in different fractions were detected.Tables 4.4, 4.5, 4.6 and 4.7alongside figures 4.1, 4.2,4.3 and 4.4 of ten(10) compounds respectively.Table 4.5 shows the interpretation based on peak spectra of Hexane fraction, Dimethyl sulfone had the highest peak area (81.06) and 1-Propanol was least (0.78).Figure 4.1shows the GC/MS chromatogram obtained in Hexane fraction of methanolic extract of crude *C.laxum*. Dimethyl sulfone had the highest retention time (6.643) 1-Propanol had the lowest (33.910).Table 4.6 shows the interpretation based on peak spectra of Ethyl acetate fraction of the extract. The highest peak area was observed in 4-Methylvaleric acid (39.34) and the lowest in 2-Pentene (0.93). The GC/MS chromatogram (Fig. 4.2) obtained in Ethyl acetate fraction of methanolic extract of crude *C.laxum* shows the highest retention time in 4-Methylvaleric acid (46.497) and Dimethyl Sulfoxide (10.866) had the least.The interpretation based on

peak spectra of Butanol fraction of the extract (Table 4.7) shows the highest peak area obtained in 1-Propanol (9.68) and Chlorine dioxidehad the lowest (1.72). Figure 4.3shows the GC/MS chromatogram obtained in Butanol fraction of methanolic extract of crude *C.laxum* indicating Chlorine dioxide with the highest retention time (36.901) and Pyrrole had the least (5.871).Table 4.8 shows the interpretation based on peak spectra of aqueous fraction with 5H-Tetrazol-5-amine having the highest peak area(4.77) and lowest both N-Ethylformamide and Pyridine (2.02) respectively. The chromatogram (Fig. 4.2) obtained in aqueous fraction of methanolic extract of crude *C.laxum* shows the highest retention time in Pyrrole (38.103) and Pyridine (5.745) had the least with a molecular weight of 67.091 and 79.1 g/mol respectively.

Table 4.4 Phytocomponents Identified in the Hexane Fraction of *C. laxum* by GC/MS Analysis

S/No	Rate Time	Name of compound	MF	MW g/mol	Peak Area (%)
1	6.643	Dimethyl sulfone	$C_2H_6O_2S$	94.128	81.06
2	46.491	1H-Indene	C_2H_8	116.163	3.35
3	36.529	1-Azabicyclo[3.1.0]hexane	C_3H_9N	83.132	1.70
4	36.998	Nitric acid	HNO_3	63.01	1.05
5	30.206	1-Butanamine	$C_7H_{17}N$	115.22	0.89
6	29.308	Azetidine	C_3H_7N	57.09	0.84
7	29.411	Phenethylisocyanate	C_9H_9NO	147.177	0.82
8	31.494	Azetidine	C_3H_7N	57.09	0.80
9	34.910	(S)-(+)-5-Methyl-1-heptanol	$C_8H_{18}O$	130.228	0.79
10	33.582	1-Propanol	C_3H_8O	60.095	0.78

Key: MF= Molecular Formula, MW= Molecular Weight

Table 4.5 Phytocomponents Identified in the Ethylacetate Fraction of *C. laxum* by GC/MS Analysis

s/No	Rate Time	Name of compound	MF	MW (g/mol)	Peak Area (%)
1	46.497	4-Methylvaleric acid	$C_2H_{12}O_2$	116.158	39.34
2	33.617	Propanoic acid	$C_3H_6O_2$	74.079	4.58
3	44.969	Arsenous acid	AsH_5NO	125.943	3.27
4	12.056	2-Propenamide	C_3H_5NO	71.079	2.76
5	37.250	1-Azabicyclo[3.1.0]hexane	C_3H_9N	83.132	1.19
6	37.582	3-Heptanol	$C_7H_{16}O$	116.204	2.50
7	41.112	Propanamide	C_3H_7NO	73.095	1.08
8	36.998	1,6-Heptadiene	C_7H_{12}	96.170	1.00
9	10.866	Dimethyl Sulfoxide	C_2H_6OS	78.13	0.99
10	36.529	2-Pentene	C_5H_{10}	70.135	0.93

Key: MF= Molecular Formula, MW= Molecular Weight

Table 4.6 Phytocomponents Identified in the Butanol Fraction of *C. laxum* by GC/ MS Analysis

s/No	Rate Time	Name of compound	MF	MW (g/mol)	Peak Area (%)
1	33.623	1-Propanol	C_3H_8O	60.095	9.68
2	12.039	5H- Tetrazol-5-amine	CH_3N_5	85.07	4.59
3	36.901	Chlorine dioxide	ClO_2	67.448	2.08
4	7.050	Methylacrylonitrile	C_4H_5N	67.091	2.01
5	7.571	Pyrrole	C_4H_5N	67.091	1.96
6	5.871	Pyrrole	C_4H_5N	67.091	1.95
7	6.913	Chlorine dioxide	ClO_2	67.448	1.86
8	36.055	N- Ethylformamide	C_3H_7NO	73.095	1.85
9	11.067	N- Ethylformamide	C_3H_7NO	73.095	1.74
10	7.021	Chlorine dioxide	ClO_2	67.448	1.72

Key: MF= Molecular Formula, MW= Molecular Weight

Table 4.7 Phytocomponents Identified in the Aqueous Fraction of *C. laxum* by GC/MS Analysis

s/No	Rate Time	Name of compound	MF	MW (g/mol)	Peak Area (%)
1	12.045	5H- Tetrazol-5-amine	CH_3N_5	85.07	4.77
2	38.103	Pyrrole	C_4H_5N	67.091	3.62
3	36.512	2-Butanamine	$C_4H_{11}N$	73.139	3.35
4	33.611	1-Propanol	C_3H_8O	60.095	3.13
5	37.565	Cyclopentaneundecanoic acid	$C_{16}H_{30}O_2$	254.414	2.69
6	37.233	2-Butanamine	$C_4H_{11}N$	73.139	2.52
7	9.664	N- Ethylformamide	C_3H_7NO	73.095	2.28
8	8.823	Methylacrylonitrile	C_4H_5N	67.091	2.05
9	35.642	N- Ethylformamide	C_3H_7NO	73.095	2.02
10	5.745	Pyridine	C_5H_5N	79.1	2.02

Key: MF= Molecular Formula, MW= Molecular Weight

CHAPTER FIVE

5.0 DISCUSSION

The phytochemical screening of the crude methanolic extract of *Chlorophytum laxum* whole plant revealed the presence of phytochemicals such as carbohydrate, unsaturated sterols and triterpenes, anthraquinone, tannins, saponins, glycoside,flavonoid and alkaloids while anthracene derivatives was absent, this agrees with Sani and Aliyu (2011), that the plant contains a wide range of substances which are known to treat diseases. Kauskik (2005) and Niranja (2011) reported that saponins of *Chlorophytum* spp are bidesmosidic in nature which may be responsible for its better steroidal bioavailability. Aboh (2014), agreed that saponins are naturally occurring surface-active glycosides and possessed strong antifungal activities. Similarly Adekunle and Ikumapayi, (2006) Gugulothu *et al*., (2009) and Chakraborthy *et al*., (2014), reported that antifugal activities of some species of *Chlorophytum* contain tannins which act by coagulating the protoplasma of the microorganism. The possible mechanism of action of tannins has been linked to interference with energy generation by uncoupling oxidative phosphorylation or interference with glycoprotein of cell surface. The terpenoids have also been shown to possess hypocholesterolemic and antidiabetic properties. The findings in this study is in line with Sharada *et al*. (2015) who evaluatedcomparative pharmacognostic, phytochemical and biological activities of *Chlorophytum* species and reported that *C. laxum* phytochemical screening of the methanol (hexane, ethyl acetate, butanol and aqueous) fractions using the TLC technique revealed the presence of terpenoids, steroids, anthraquinones, phenolic compounds, flavonoids and alkaloids. Sharada *et al*.2015 reported the presence of carbohydrate,flavonoids, saponinand

alkaloidsin phytochemical evaluation of *Chlorophytum*.Similarly, Aboh (2014) in a study on the evaluation of antifungal activities of crude extracts of the arial parts of *Mitracarpus villosus* reported that plants with antimicrobial activity contain bioactive constituents such as tannins, alkaloids and saponins which are responsible for the biological properties of such plants. Also, Deore *et al.*, (2015) in a study on the comparative pharmacognostic, phytochemical and biological evaluation between *Chlorophytum* species reported the presence of similar phytochemicals.

This study identified two genera of dermatophyte agents causing tinea capitis. These species included; two species *Trichophyton spp.(1&2)* and one *Microsporum spp*. Similar organisms were isolated in previous studies carried out in Western Kenya, Libya, Nigeria and Korea on tinea capitisinfection (Ayaya *et al.*, 2001; Ellabib *et al.*, 2002; Enemour and Amendu, 2009; Adefemi *et al*., 2016; Kim *et al*., 2016).

Similarly, this was in agreement with Sajjan and Mangalgi (2012), that isolation of different species of dermatophytes varies from one ecological niche to another. This observation was also made in the previous studies carried out in Madagascar, Jamaica, Iraq, Kenya and Nigeria (Ayaya *et al*., 2001; Audonneau *et al*., 2006; East-Innis *et al*., 2006; Al Samarai, 2007; Garg *et al*., 2009; Adefemi *et al*., 2016). Adverse environmental conditions could be responsible for dermatophyte resistance; therefore it can stay for a longer time on surfaces making it easily transferred by asymptomatic carriers. This results do not match other studies carried out in Western regions of the world, Pakistan, Nepal, Ethiopia, Egypt, India, Kenya and Nigeria (Elewski, 2000; Ahmed *et al*., 2006; Jha *et al*., 2006; Woldeamanuel *et al*., 2005; Chepchirchir *et al*., 2009; Azab *et al*., 2012; Grover *et al*., 2012) in which *T. violaceum*was the most frequent species isolated.*T. soudanense* was predominant in studies carried out in Nigeria and Gabon (Ayanbimpe *et al*., 2008 and Hogewoning *et al*., 2011). While *T. mentagrophytes* and *T. verroccusum* were common in studies conducted in India, Iran and Nigeria (Yazdanfar, 2010; Bose *et al*., 2011; Adefemi *et al*., 2016).

This study is in constrat with reports from India and Nigeria Avasn *et al*. (2008) and Emele and Oyeka, (2008), whoreported *M. audonii* as the most prevalent species isolated. In Egypt, Amer *et al*. (2007), reported *M. canis*, as the most frequently isolated.

These distribution patterns of dermatophytes infection in different parts of the world has been attributed to factors such as geographical location, climate, life-style, and prevalence of immunodeficiency diseases in the community and also the reluctance of patients to seek treatment because of embarrassment or minor nature of disease unless the condition becomes sufficiently serious to affect the quality of life (Hashem al sheikh, 2009).

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The etiological agents of tinea capitis vary from one geographical location to another. In Sub-Saharan West Africa, *Microsporum audouinii* and *Trichophyton soudanense* have been isolated (Ayanbimpe *et al*., 2003; Anosikke *et al*., 2005) In the Western regions of the world, *Trichophyton tonsurans* has emerged the predominant cause of tinea (Ghannoum *et al*., 2003). In a previous study conducted in Kisumu in Western part of Kenya, the etiological agents isolated were *T. violeceum, M. audonii* and *M. canis* which are zoophilic organisms.

The study revealed that tinea capitis infection is caused by fungal species of *Trichophyton* and *Microsporum*. It is the most common children dermatophyte infection worldwide. According to world health report of infectious diseases (2000), overcoming antimicrobial resistance is the major issue of WHO for the next millennium. Hence, the last decade witnessed an increase in the investigation of plants as analternative for human disease management.

The highest zone of inhibition of aqueous fraction (50mg/ml) of *C. laxum* observed in this study might be due to the fungal isolatessensitivity to the aqueous fraction as compared to the other fractions. Kure *et al*. (2016) reported that methanolic leafextract of *Chlorophytum borivilianum* showed more antifungal activity than ethanolic and aqueous leaf extracts on two species of *Aspergillus*. Even though the plants used were \the same genus, the difference in the activity observed might be attributed to the different fungal species in this study.

Gas- Chromatography-Mass Spectrometry (GC/MS) is an important tool for reliable identification of bioactive compounds (Johnson*et al*., 2011).Gas-Chromatography/Mass-Spectrometry (GC/MS) analysis showed the presence of Dimethyl sulfone, 4-Methylvaleric acid, 1-Propanol and 5H- Tetrazol-5-amine as main compounds with other 9 compounds in trace quantity in the hexane, ethyl acetate, butanol and aqueous fractions of *C. laxum*. These identified compounds might be responsible for the antifungal activity of *C. laxum*on the fungal isolates. Lakshmi *et al*. (2014) isolated 21 bioactive compounds using GC/MS analysis in methanolic extract of *Lactuca runcinata* DC. Gas-Chromatography/Mass-Spectrometry (GC/MS) was used to confirm the presence of

thirteen bioactive compounds in ethanolic bark extract of *Ficus religiosa linn* (Saravanan *et al*., 2014).

In the present study both the leaves, stem, roots and bark components of *Chlorophytum laxum*, were identified by gas chromatography-mass spectrometry (GC/MS) analysis containing compounds which show wide a range of pharmacological activity including fungicide, antimicrobial, anti-inflammatory,analgesic, anti-oxidant, anticholinesterase and amyloid beta inhibitory activities, which is in accordance with (Eller *et al.,* 2005).

Dimethyl sulfone readily penetrates cellular membrane. The membrane penetrating ability of Dimethyl sulfone may enhance diffusion of other substance through the skin for this reasoncanbe used for topical treatment of skin disease. Phenethylisocyanate possess appropriate absorption, distribution, metabolism and excretion properties necessary to make it suitable for use a a drug, 1-Propanol are used as solvent in pharmaceutical industry mainly for resin and cellulose esters and 1-butamine possess fungicide and antimicrobial bioactive compounds (Gobalakrishan *et al.,* 2014).

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

It was observed that carbohydrate, unsaturated sterols and triterpenes, anthraquinone, tannins, saponins, glycoside, flavonoid and alkaloids are the phytochemical constitients of crude methanolic extract of *C. laxum*.

The major etiological agents isolated from the primary school pupils infected with Tinea capitis are *Trichophy spp 1*, *Microsporum spp* and *Trichophyton spp 2*.

The highest antifungal activities with highest zone of inhibition are *Microsporum spp,Trichophyton spp 1,* and*Trichophyton spp 2* are observed in 50mg/ml concentration with 30.00 ± 6.1mm, 37.30± 1.2mm and 60.30± 0.90mm respectively as the highest mean values observed at 50 mg/ml concentration of crude methanolic extract of *Chlorophytum laxum*.

The probable active compounds identified by GC/MS analysis such as Dimethyl sulfone, 4-Methylvaleric acid, 1-Propanol and 5H- Tetrazol-5-amine may be responsible for the antifungal activity of the fractions in Hexane, ethyl acetate, Butanol and aqueuous respectively of the crude methanolic extract of *C. laxum*.

6.2 Recommendations

1. Crude methanolic extracts should be use as the most efficient method of extracting *Chlorophytum laxum* solvents.

2. It is recommended that molecular work should be done on fungal isolates and other etiological agent responsible for causing tinea capitis.

3. It is recommended that future work in line with this study should be carried out to determine toxicity, side effects properties of the compounds identified since the results stipulates significant capacity and possess antifungal properties in pharmaceutical industries.

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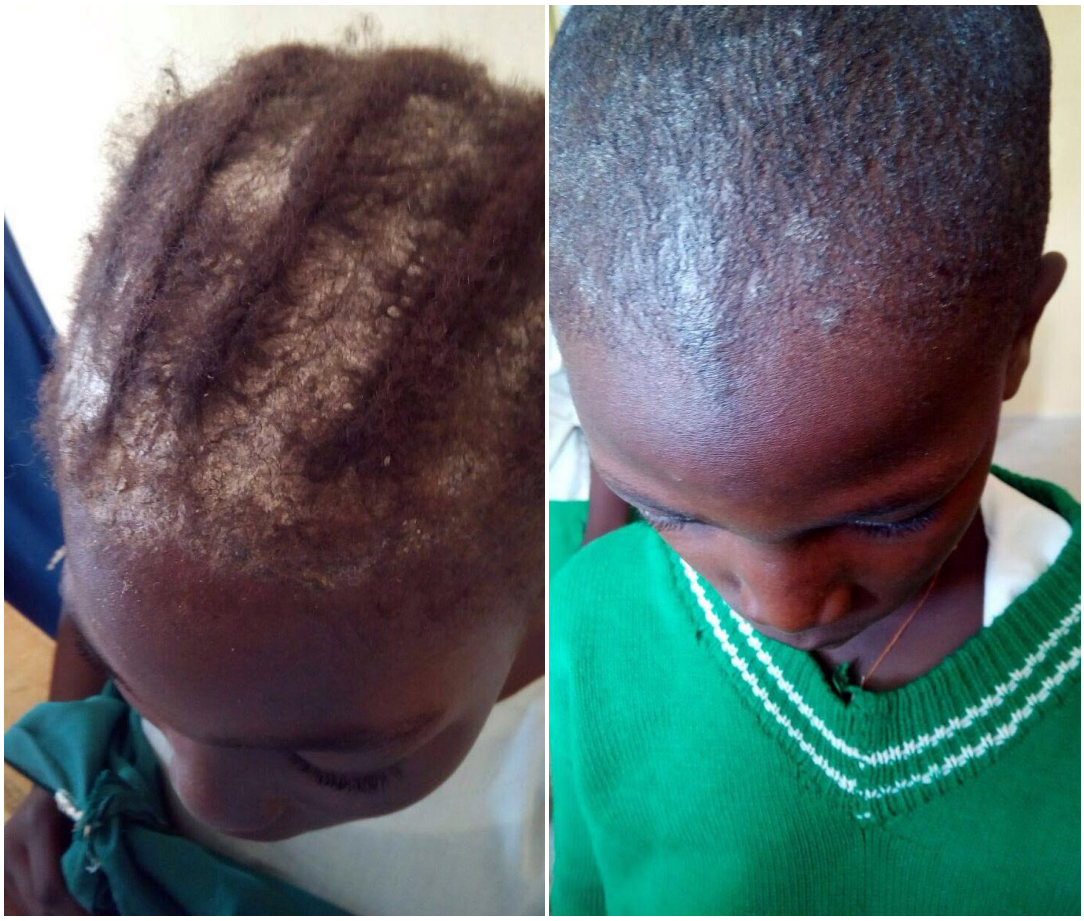
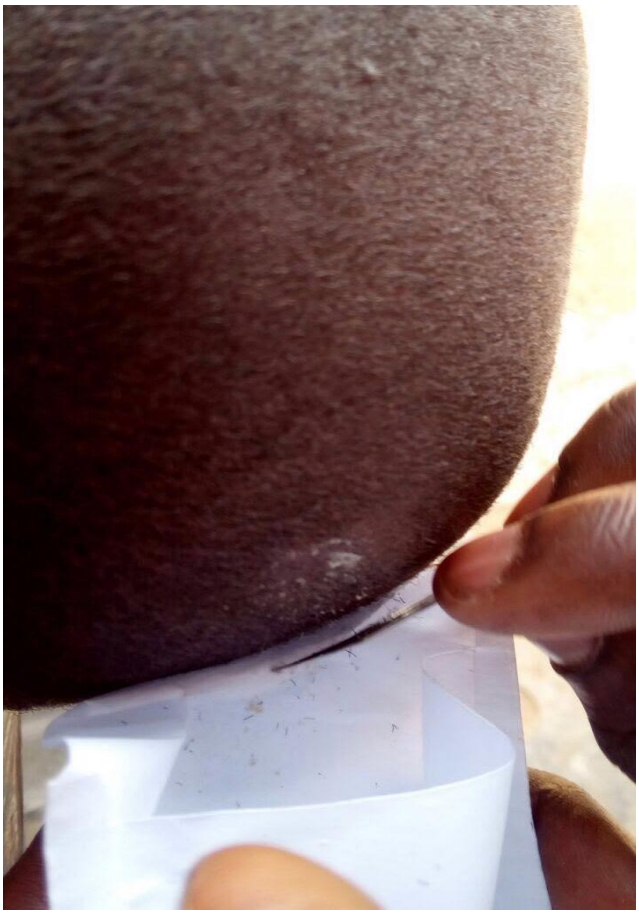
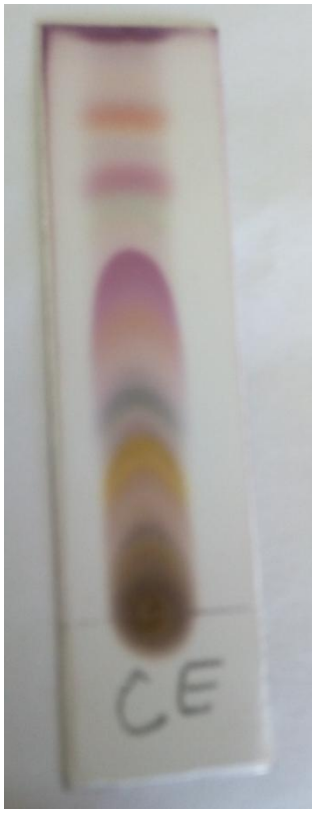


Appendix I: Pupils infected with Tinea capitis

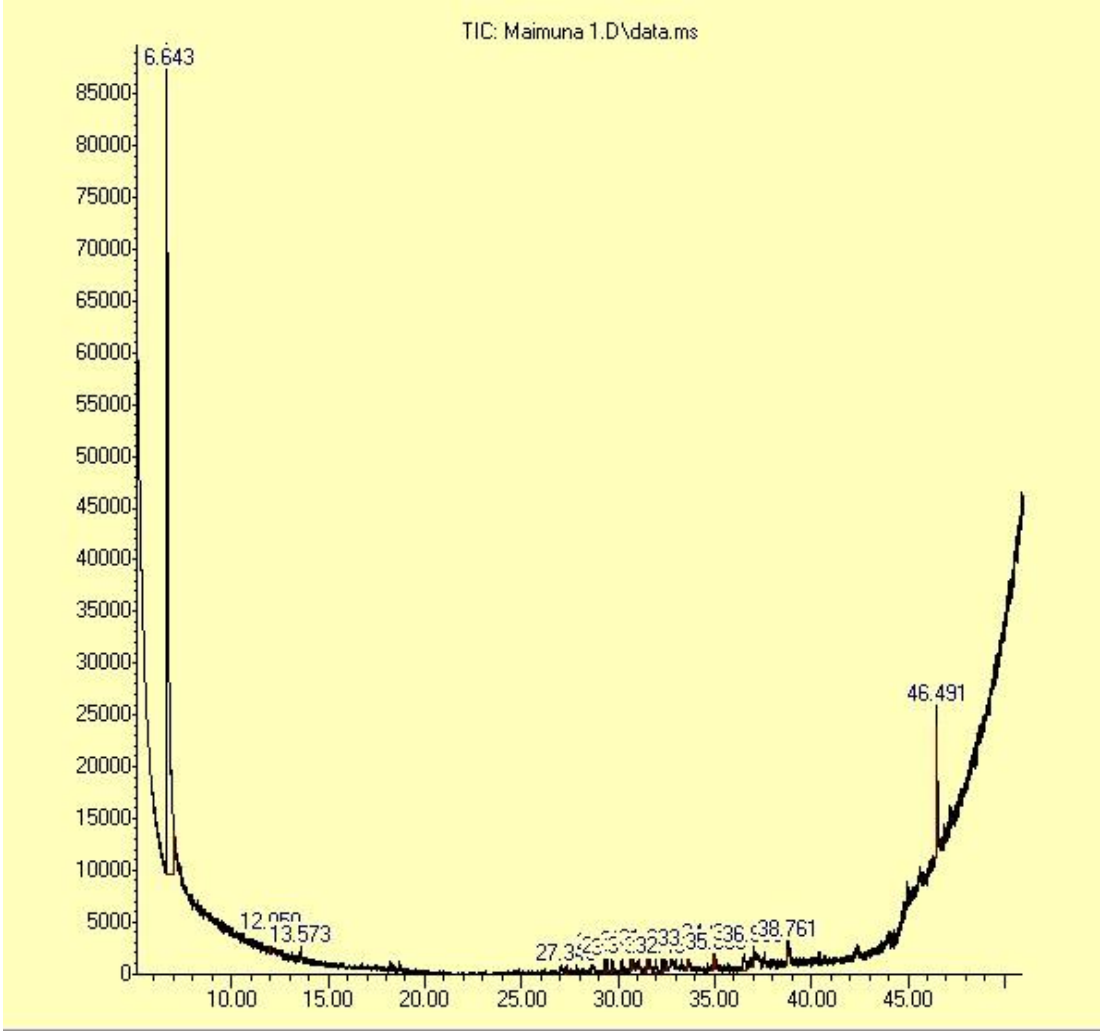


Appendix II: Skin scrapings collected from scalp of infected Pupil

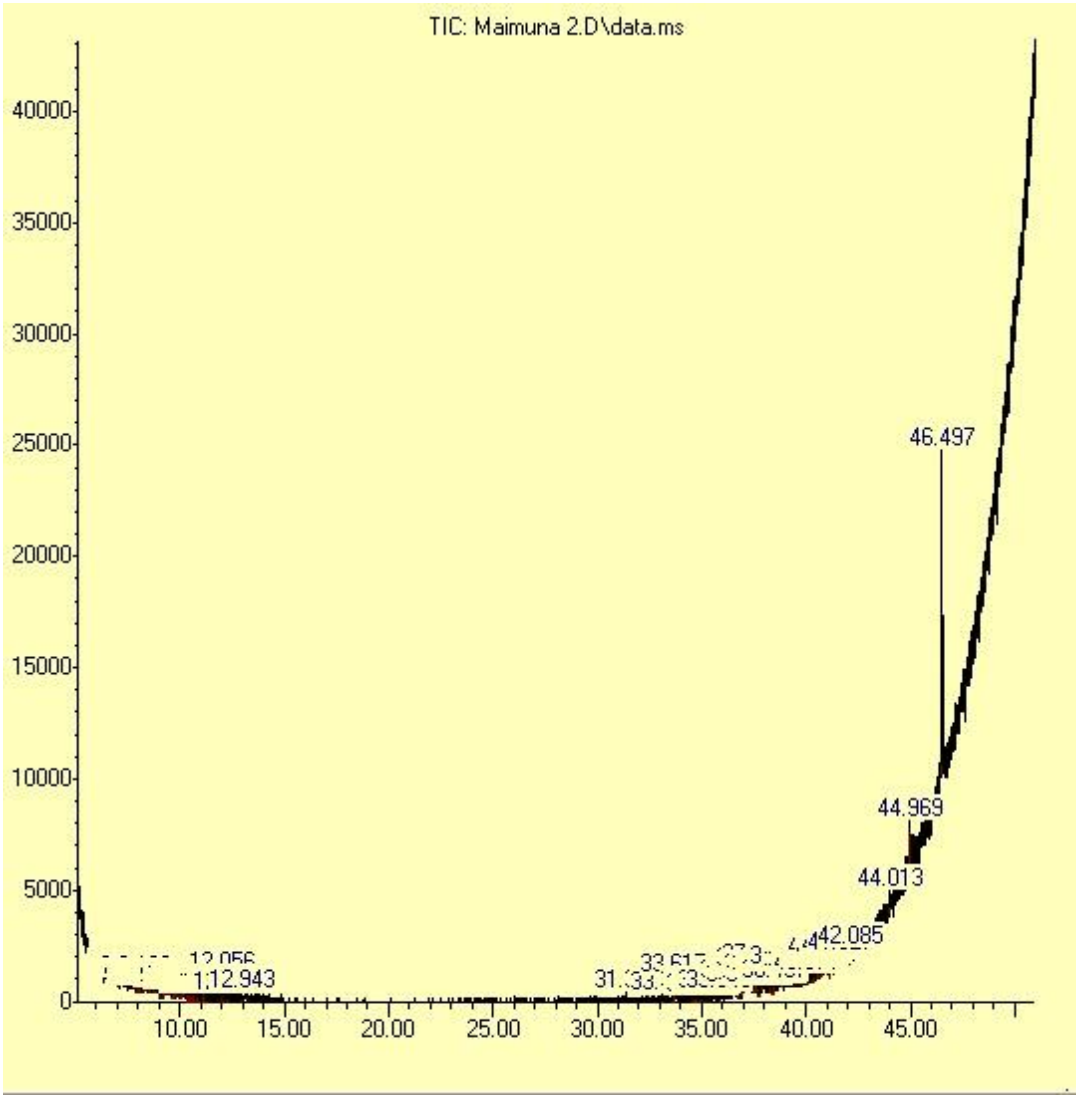
Appendix III: Butanol and Aqueous fractions TLC profile



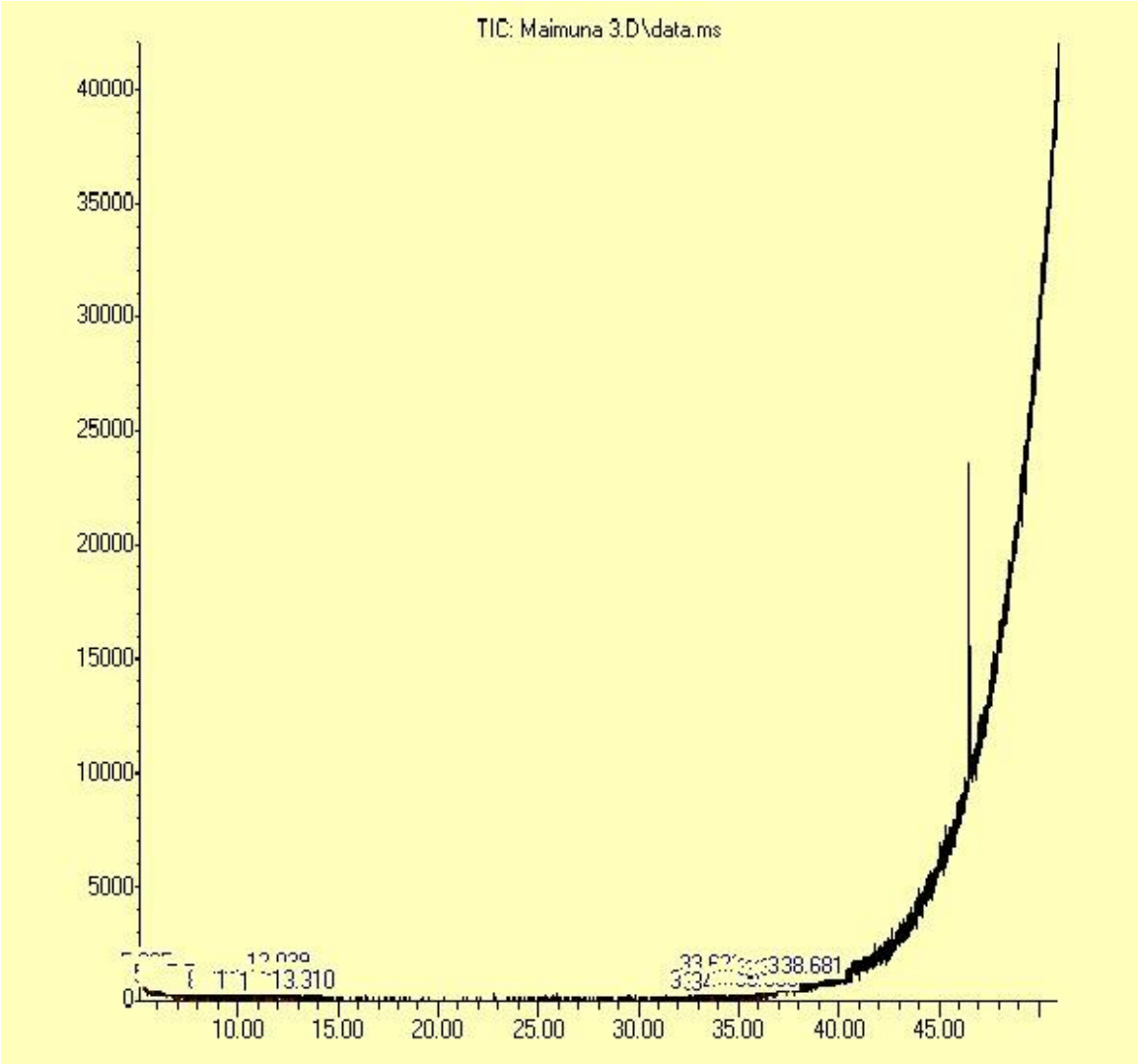
Appendix IV: Ethyl acetate fraction TLC profile



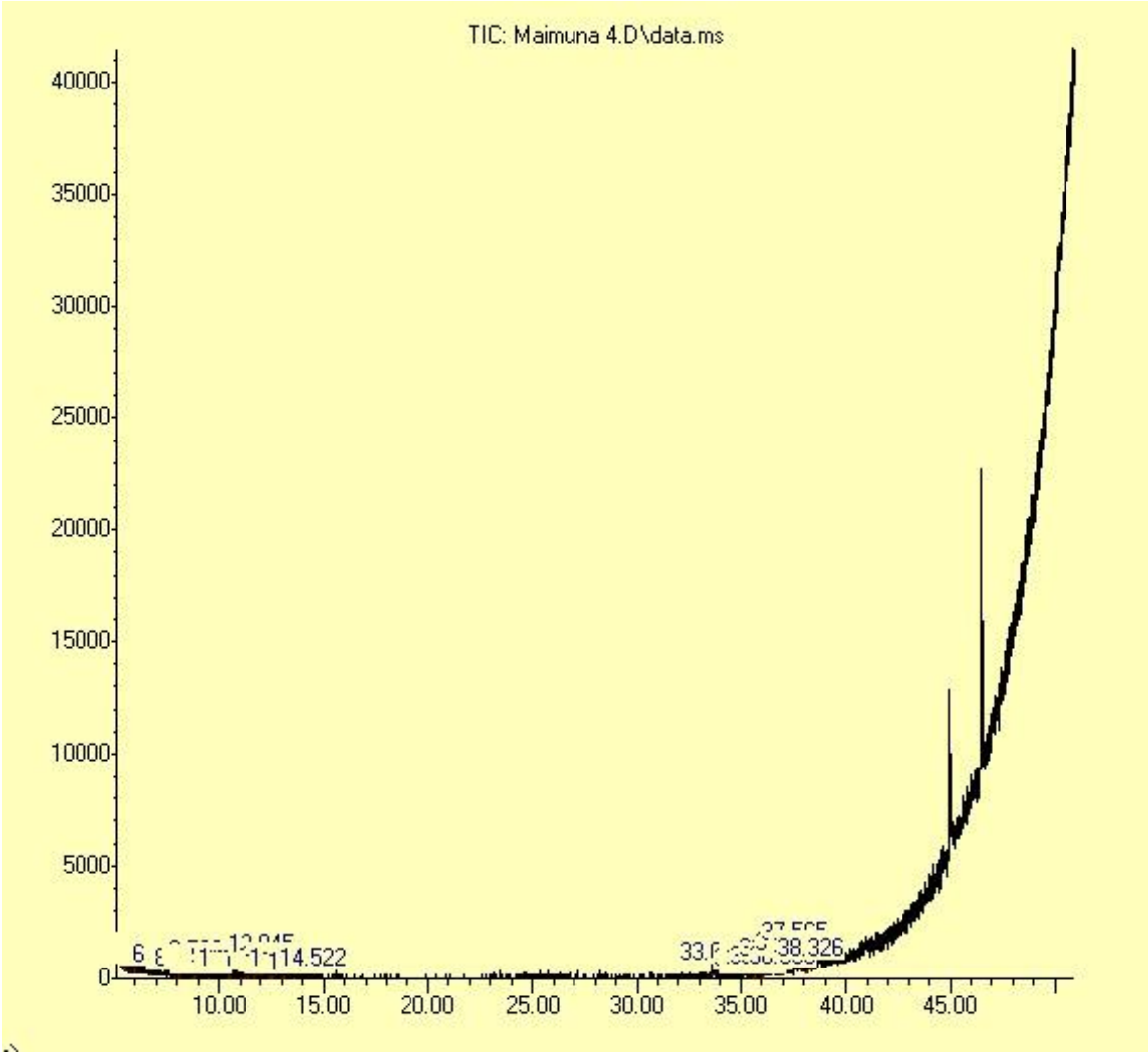
Appendix V: GC/MS Chromatogram of Hexane Fraction of the Crude Methanolic Fraction of *C. laxum*



Appendix VI: GC/MS Chromatogram of Ethylacetate Fraction of the Crude Methanolic Fraction of *C. laxum*



Appendix VII: GC/MS Chromatogram of Butanol Fraction of the Crude Methanolic Fraction of *C. laxum*



Appendix VIII: GC/MS Chromatogram of Aqueous Fraction of the Crude Methanolic Fraction of *C. laxum*

Appendix IX: *CHLOROPHYTUM LAXUM*

Appendix X: *CHLOROPHYTUM LAXUM* ON ITS HABITAT

Appendix XI: GAS-CHROMATOGRAPHY/ MASS-SPECTROMETRY MACHINE

Appendix XII: Consent form

INFORMED CONSENT FORM FOR TINEA CAPITIS (scalp infection) INFECTION PUPILS ATTENDING

LOCAL GOVERNMENT EDUCATIONAL AUTHORITY, ZARIA

Name of principal investigator	MUHAMMAD MAIMUNA MADAKI
Name of Organisation	Department of Biological sciences, Ahmadu Bello University, Zaria
Name of proposal	Anti-fungal activity of methanolic extract of *Chlorophytum laxum* on Tinea capitis agent.
Phone	O8036627732

Introduction: I am Maimuna Muhammad madaki of Biological sciences Department, Ahmadu Bello University Zaria. Carryingout a research on ringworm, a disease which is very common in our country. I am going to give you information and invite you to be part of the research. Before you decide, you can talk to any one you feel comfortable with about the research. If there is any thing you do not understand, please feel free to ask questions at any time.

- **Purpose of research:** Tinea capitis (fungal infection of scalp) is a contagious disease which is common in Nigeria. Some of the drugs used in managing people with this infection are expensive and unsafe after long period of usage or high dosage. This research is to find less expensive and safer way of managing the disease.

Type of research intervention: The research would involve the collection of scalp scrapings from the head. You may feel a slight pain in the process, but it will not cause you any harm. The hair will be collected only once throughout the research period which will last for about six months.

RISK AND BENEFIT FACTORS

There is no risk attached to this research and the benefit is that all participants that are found positive during the research will be refer to the rightful channel where they would receive proper treatment.

Participant selection: Pupils between the ages of 4- 14 attending primary schools at Zaria city are to participate in this research.

Voluntary participation: Your participation in this study is totally voluntary and you may refuse to participate or you may withdraw from participation at any time without losing the educational privilege that you are entitled to receive.

Confidentiality: We would not share the identity of those participating in the research. However, the data collected from this research may be published in order to allow other people to benefit from it.

SECTION B: Certificate of consent

I hereby consent to the collection of the hair sample of myself/my child or ward, and have the understanding that this sample is being collected solely for the purpose of research and no harm shall come to me (my child/ward) by providing the samples. The results of the study may or may not be of immediate benefit to the patient.

The above statement has been read out or explained to me, and having understood the same, I put my signature or thumb impression

Print Name of Participant_____

Signature of Participant _____

Date _____

Day/month/year

If illiterate

I have witnessed the accurate reading of the consent form to the participant, and the individual has had the opportunity to ask questions. I confirm that the individual has given consent freely.

Print name of witness_____ or Thumb print of participant

Signature of witness _____

Date _____

Day/month/year

Statement by the researcher

1. Scalp scrapings will be collected from the participant.
2. I confirm that the participant was given an opportunity to ask questions about the study, and all the questions asked were answered to the best of my ability
3. I confirm that the individual is not forced into giving consent, and the consent was given freely and voluntarily.
4. A copy of this informed consent form was provided to the participant.

Name of researcher ----------

Signature ----------

Date ----------

Appendix XII: Ethical clearance

MINISTRY OF HEALTH AND HUMAN SERVICES
KADUNA STATE, NIGERIA

MOH/ADM/744/VOL.1/454 8TH SEPTEMBER, 2016

NOTICE OF APPROVAL AFTER FULL COMMITTEE REVIEW

INVITRO INHIBITORY ACTIVITY OF CHLOROPHYTUM LAXUM EXTRACTS ON THE TINEA CAPITIS ISOLATED AMONG PRIMARY SCHOOL PUPILS IN ZARIA LOCAL GOVERNMENT, NIGERIA

Name of Principal Investigator :	MUHAMMAD MAIMUNA MADAKI
Address of Ethical Approval :	DEPARTMENT OF BIOLOGICAL SCIENCES, FACULTY OF SCIENCE, AHMADU BELLO UNIVERSITY, ZARIA, KADUNA STATE
Date of receipt of Application :	26TH JULY, 2016
Date of Ethical Approval :	17TH AUGUST , 2016

This is to inform you that the Research described in the submitted Protocol, the Consent Forms, advertisements and other participant information materials have been reviewed and given full approval by the the Health Research Ethics committee (HREC).

If there is delay in starting the research or any change, inform the HREC so that the dates of approval can be adjusted accordingly.

However, Researcher is kindly requested to submit a copy of his/her findings to the State Ministry of Health, please.

Dr. BUTAWA NN
Chairman

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