

**DETECTION OF DERMATOPHYTES AND THE ANTIFUNGAL ACTIVITY OF
Buchholzia coriacea Engler (WONDERFUL KOLA) ON THE ISOLATES FROM GOATS
IN GWAGWALADA, FEDERAL CAPITAL TERRITORY, NIGERIA**

BY

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DECLARATION

I hereby declare that the work in this dissertation titled “**Detection of Dermatophytes and the Antifungal Activity of *Buchholzia coriacea* Engler (Wonderful Kola) on the Isolates from Goats in Gwagwalada, Federal Capital Territory, Nigeria,**” was performed by me in the Department of Veterinary Microbiology Ahmadu Bello University, Zaria, under the supervision of Prof. H.M. Kazeem and Dr. A. Ahmed. 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.

Name of student

Signature

Date

CERTIFICATION

This Dissertation entitled “**DETECTION OF DERMATOPHYTES AND THE ANTIFUNGAL ACTIVITY OF *Buchholzia coriacea* Engler (WONDERFUL KOLA) ON THE ISOLATES FROM GOATS IN GWAGWALADA, FEDERAL CAPITAL TERRITORY, NIGERIA**” meets the regulation governing the award of the degree of Master of Science of Ahmadu Bello University, Zaria and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

To my family

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ABSTRACT

Dermatophytes are a group of closely related pathogenic fungi with the capacity to invade the keratinized tissues of humans and animals. This study was aimed at detecting dermatophytes from goats in Gwagwalada, Federal Capital Territory (F.C.T), and evaluating the antifungal activity of different extracts of *Buchholzia coriacea* (wonderful kola) on the isolates. One hundred and twenty four samples were aseptically collected from goats showing clinically suggestive lesions from August, 2015 to January, 2016. Each sample was observed by microscopy before primary culture on Sabouraud dextrose agar (SDA) and secondary culture on Potato dextrose agar (PDA). Dermatophyte isolates were identified grossly using surface and reverse pigmentation, topography, texture and growth rate and microscopically using shape, size and arrangement of macro and micro conidia. The seeds of *Buchholzia coriacea* were extracted serially by cold maceration using n-hexane, ethyl acetate, distilled water and methanol. The extracts were tested on each isolate using the broth macro dilution technique. Commercially available antifungal discs (Himedia©) containing Amphotericin B (100 units), Clotrimazole (10µg), Fluconazole (25µg), Itraconazole (10µg) Ketoconazole (10µg) and Nystatin (100 units) were used to assess the susceptibility profile of the isolates. Out of the 124 samples collected, 20 (16.13%) were positive for dermatophytes. Accordingly, *Trichophyton spp* had 13 (10.48%) isolates, *Microsporum spp*, 6 (4.84%) isolates and *Epidermophyton spp*, 1 (0.81%) isolate. Seven species of dermatophytes were identified with *Trichophyton verrucosum* having the highest number of isolates, 6 (4.84%). This was closely followed by *Trichophyton tonsurans*, 5 (4.03%). *Microsporum audouinii* accounted for 4(3.23%) while *Microsporum gypseum* had 2 (1.61%). *Epidermophyton floccosum*, *Trichophyton mentagrophytes*, and *Trichophyton ajelloi* had 1 (0.81%) isolate each, respectively. The methanol extract of *B.coriaceae* showed *in vitro* antifungal activity on all the isolates with MIC and MFC values ranging from 62.5 mg/ml to 500 mg/ml while the hexane and ethyl acetate extract showed *in vitro* antifungal activity on three and two isolates respectively. The aqueous extract did not exhibit any inhibitory activity on the isolates. The antifungal activity of the isolates were determined for the six antifungal drugs revealing that the isolates were most susceptible to Amphotericin B (70%), followed by nystatin (60%), ketoconazole(60%), itraconazole(55%), clotrimazole(30%), and finally fluconazole (15%). *Trichophyton mentagrophytes* was resistant to all the antifungal agents used. All *Microsporum* species were resistant to fluconazole and all but one was resistant to clotrimazole. *Epidermophyton floccosum* was susceptible to all the agents except clotrimazole. Dermatophytosis is a health issue in goats in Gwagwalada and the methanol extract of *B.coriaceae* has an antifungal effect on the dermatophytes isolated. There is need to practise good hygiene and carry out proper diagnosis and treatment to prevent and control the spread of dermatophytes to humans and other animals.

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LIST OF ABBREVIATIONS

AIDS	Acquired Immune Deficiency Syndrome
AP	Amphotericin B
BFBC	Butanol Faction of <i>Buchholzia coriacea</i>
CC	Clotrimazole
CLSI	Clinical and Laboratory Standards Institute
EUCAST	European Committee for Antimicrobial Susceptibility Testing
FCT	Federal Capital Territory
FLC	Fluconazole
HIV	Human Immune Deficiency Virus
IT	Itraconazole
KT	Ketaconazole
LCB	Lactophenol Cotton Blue
M	Microsporum
MFC	Minimum Fungicidal Concentration
MIC	Minimum Inhibitory Concentration
NS	Nystatin
PCV	Packed Cell Volume
PDA	Potato Dextrose Agar
R	Resistant
S	Susceptible
SDA	Sabouraud's Dextrose Agar
SPSS	Statistical Package for Social Sciences
STZ	Streptozocin
T	Trichophyton

CHAPTER ONE

1. INTRODUCTION

1.1 Background

Dermatophytes are a group of closely related pathogenic fungi that have the capacity to invade keratinized tissue (skin, hair and nails) of humans and animals (Maraki *et al.*, 2007). They hold two imperative properties: they are keratinophilic and keratinolytic agents (Kushwaha *et al.*, 2000). The superficial mycoses caused by dermatophytes are called dermatophytosis but more commonly referred to as “tinea” or “ring-worm” infections (Lakshmipathy *et al.*, 2010). Dermatophytes consists of three genera namely: *Epidermophyton*, *Microsporum* and *Trichophyton*, with only a few species belonging to the genera *Microsporum* and *Trichophyton* being the usual cause of dermatophytosis in domestic animals (Cabanés, 2000).

Based on their host specificity dermatophytes are classified into three ecological groups namely geophiles (soil), anthropophiles (man) and zoophiles (animals) (Rippon, 1982). The geophilic dermatophytes are generally saprophytic and derive nutrients from keratinous substrates (Swai and Sanka, 2012). Rarely these pathogens cause infection in animals and man. Geophilic dermatophytes include *Trichophyton ajelloi*, *Trichophyton terrestre*, *Microsporum fulvum*, *Microsporum gypseum*, *Microsporum cookie* and *Epidermophyton stockdaleae* (De Vroey, 1984; Baxter and Pearson, 1969; Connole, 1990). Zoophiles are pathogens with animal hosts and grow as saprophytes on animal materials. Zoophiles are also reported to infect human beings who acquire the infection through contact (Swai and Sanka, 2012). *Trichophyton simii* (monkeys), *Trichophyton verrucosum* (ruminants), *Trichophyton mentagrophytes* (rodents), *Trichophyton equinum* (horses), *Microsporum canis* (cats) and *Microsporum nannum* (pigs) (English, 1972; Marples, 1956). The primary hosts of anthropophilic dermatophytes are human beings but they

may also cause infection in animals. Examples include *Trichophyton rubrum*, *Trichophyton kanei*, *Trichophyton schoenleini*, *Trichophyton concentricum*, *Trichophyton tonsurans*, *Microsporum gypseum*, *Microsporum audouinii*, *Microsporum ferrugineum* and *Epidermophyton floccosum* (Georg, 1960; Kaplan and Gump, 1958).

The incidence of dermatophytosis varies according to climate and natural reservoirs. The infections are mostly common in developing countries due to poor hygienic conditions, close proximity to animals, poor socio-economy and the climate which supports the growth of dermatophytes (Weitzman and Summerbell, 1995; Peerapur *et al.*, 2004). Hot, humid environments predispose to infection and young animals tend to be more commonly affected. Dermatophytosis is more common in housed animals, rather than animals turned out to pasture and the highest incidence of the disease occurs during the winter months (Papini *et al.*, 2009) although it may resolve spontaneously in the spring and summer. However, the pattern of the species of dermatophytes involved in dermatophytosis may be different in similar geographical conditions, both in humans and animals. It has been related, among other factors, to the decline in the incidence of animal ringworm in the areas or the degree and closeness of animal to human contact (Pier *et al.*, 1994). However, the disease appears to be more common in tropical than temperate climates, and particularly in countries or areas having hot and humid climatic conditions (Radostits *et al.*, 1997).

Infections caused by dermatophytes have increased dramatically (Nweze, 2001; Mendez *et al.*, 2008). They have gained prominence due to their rising incidence in patients with immunocompromised states such as cancer, diabetes mellitus, HIV/AIDS and organ

transplantation (Shehata *et al.*, 2008). Prior to this development, dermatophytosis have been recognized as a public health problem in many parts of the world and have even reached endemic proportions in some countries especially in Africa (Nweze, 2001; Nweze *et al.*, 2005; Nweze, 2010). Human beings are usually infected from animals, mostly through direct contact or via fungus-bearing hair and scales from infected animals. Dermatophytes have been cited among the most frequent cause of dermatological problems in domestic animals (Cabanes, 2000; Ranganathan *et al.*, 1998).

Goats are domestic animals which are of great importance in Nigeria's economy (Ugwu, 2007). Ugwu stated that goats do not only serve as a source of food, but also as sources of income, hides and skin, manure for agriculture and for social or recreational purposes. The National Bureau of Statistics of the Federal Ministry of Agriculture and Rural Development carried out in 2010 a National Agricultural Sample Survey which indicated that Nigeria was endowed with a population of 72.5 million goats, which was more than the population of cattle and sheep at 19.5 million and 41.3 million respectively (www.nigerianstat.gov.ng/pages/downloads/66). Goats are constantly in contact with man, the soil and other animals which makes them susceptible to infection from these sources. *T. verrucosum* has been cited as the major agent encountered in cases of ruminant ringworm. Other species such as *M. canis*, *T. mentagrophytes* and *T. equinum* have been isolated from some of these ruminants (Pier *et al.*, 1994; Pepin and Austwick, 1968; Stenwig, 1985).

It is a well-known fact that treating fungal infections can be challenging. Only a few classes of antifungal drugs, such as polyenes, azoles, echinocandins, allylamines, and flucytosine, are

available to treat the myriad of fungal infections (Sanglard *et al.*, 2009). Some topical antifungal agents that have been used with accorded success in various fungal conditions include iodine preparations (tincture of iodine, potassium iodide, iodophors), Copper preparations (Copper sulphate, Copper naphthenate, cuprimyxin), sulfur preparations (monosulfiram, benzoyl disulfide), phenols (phenol, thymol), fatty acids and salts (propionates, undecylenates), organic acids (benzoic acid, salicylic acids), dyes (crystal [gentian] violet, carbolfuchsin), hydroxyquinolines (iodochlorhydroxyquin), nitrofurans (nitrofuraxine, nitrofurfurylmethyl ether), imidazoles (miconazole, ticonazole, clotrimazole, econazole, thiabendazole), polyene antibiotics (amphotericin B, nystatin, pimaricin, candicidin, hachimycin), allylamines (naftifene, terbinafine), thiocarbamates (tolnaftate), and miscellaneous agents (acrisorcin, haloprogin, ciclopirox, olamine, dichlorophen, hexetidine, chlorphenesin, tiacetin, polynoxylin, amorolfine (Mercks Manual, 2008).

More than 80% of the population in developing countries depend on plants for their medical needs against various ailments (Farnsworth, 1988). Although, dermatophytes respond well to conventional antifungal agents (Weitzmann and Summerbell, 1995; Nweze *et al.*, 2007), many patients usually cannot afford the cost and instead use local medicinal plants to treat infections. Some laboratory tests were carried out using some of these plant extracts found in Nigeria against dermatophytes recovered from patients and some were found to have good *in vitro* antifungal activities against dermatophytes (Okafor *et al.*, 2001; Nweze *et al.*, 2004).

Buchholzia coriacea also known as ‘‘Wonderful kola’’ belongs to the *Capparaceae* family (Keay, 1989). The kola is recommended for treatment of migraines and malaria in humans and

its leaves and seeds have been reported to have anthelmintic activity (Nweze and Asuzu, 2006). The ethanolic extract of *Buchholzia coriacea* seeds have been shown to have antitrypanosomal activity on mice experimentally infected with *Trypanosoma brucei brucei* (Nweze *et al.*, 2009). The seeds have also been shown to have antimicrobial properties in studies carried out by Ezekiel and Onyeziri, (2009). The authors further stated that the fresh seed as well as hexane and methanolic extracts showed antimicrobial activities against some food borne bacteria like *Escherichia coli*, *Enterococcus faecalis*, *Staphylococcus aureus*, *Trichoderma viridae* and *Aspergillus niger*.

1.1 Statement of Research Problem

Dermatophytes cause dermatological problems in domestic animals which in turn can spread to humans. In sheep and goats, dermatophytes cause damages to their skin and hide and also affect their productivity. Sheep and goats may be involved in the spreading of pathogenic fungi in the environment, representing a source of infection for humans and other animals. In Nigeria, many small ruminants are housed in the same compound or very close to their human handlers. Animal handlers and nomads are at higher risk of infection by zoophilic dermatophytes because they are in regular and direct contact with the animals (George, 1956). In a study carried out in Borno State by Nweze (2001), *T. verrucossum* ranked second among seven different species of dermatophytes recovered from school children. Incidentally, the major occupation of the inhabitants of Borno State is rearing of animals. It thus follows that animal dermatophytosis has an important implication for human dermatophytic infection (Ogunbiyi *et al.*, 2005). Fungal diseases are assuming new importance because of the inappropriate use of antibacterials that eliminate the natural beneficial microflora which otherwise suppress the growth of fungi (De

Lucca, 2007). An increasing number of antifungal agents have been used for treating dermatophytosis (Barchiesi *et al.*, 2001; Chadeganipor *et al.*, 2004). However, not all species have the same susceptibility patterns, and relative or absolute microbial resistance may occur in relation to some dermatophytes (Fernández-Torres *et al.*, 2002).

1.2 Justification for the Study

Animals serve as reservoirs of zoophilic dermatophytes, and their infections have considerable zoonotic importance. Most of the dermatophytes causing lesions in animals are also capable of producing ringworm in humans (Quinn *et al.*, 1994). Both in humans and animals, infection occurs through mutual contact or contact with fomites (such as water troughs and brushes) that contain the spores. The fungus spreads easily in damp, warm environment of a barn. Ringworm also occurs in animals at pasture. Environmental factors, seasonal influences and the animal's age play only a minor role in the occurrence and development of the disease. Ringworm has an adverse effect on animal's performance like poor development or fattening performance and reduced milk yield. Also important is the lasting damage to the skin, which becomes visible on tanning and significantly reduces the quality of the leather. Economic importance of animal ringworm relies on its contagiousness among animal communities, high cost of treatment, difficulty of control measures, and its public health consequences because the majority of dermatophytes isolated from animals are zoonotic (Chermette *et al.*, 2008). These zoophilic dermatophytes produce clinical lesions in human that are more inflammatory than those caused by the typical anthropophilic fungi normally transmitted from person to person (Radentz, 1991). Nowadays, animal dermatophytosis is an important issue not only for veterinary doctors but also for dermatologists (Takahashi, 2003). On the other hand, many domestic animals could carry

dermatophytes spores on their coats without showing any signs of infection. (El-Bahay and Refai, 1973). Information on dermatophytosis in goats will help in decreasing the incidence of the condition in goats and susceptible hosts and will also pave the way for improvement in the quality of hides and skins.

To the best of our knowledge, there is a dearth of information on dermatophytes isolated from animals in Gwagwalada.

There has been a growing interest in developing new antimicrobial agents from various sources to combat microbial resistance in fungi. In the past, dermatophytosis were treated with synthetic antimicrobial drugs but now due to inadequate drug exposure and incomplete therapy, the fungus has developed resistance against them (Martinez-Rossi *et al.*, 2008). The drugs used against dermatophytosis also exhibit several side effects, have limited efficacy and are very expensive (Kyle and Dahl, 2004; Gupta and Cooper, 2008). Such considerations have led to the search for alternative treatment methods which have included plant extracts or plant derived compounds based on the knowledge that plants have their own defense against fungal pathogens (Gurgel *et al.*, 2005). The advent of acquired immunodeficiency syndrome (AIDS) in human patients has been important to the development of new strains of resistant organisms, and there remains a continuing need for development of new antifungal agents (Boothe, 2001). Plant based antimicrobials have enormous therapeutic potentials as they can serve the purpose with lesser side effects that are often associated with synthetic antimicrobials (Roja and Rao, 2000). Plant remedies have a strong efficacy against several assorted diseases such as skin disease caused by fungi and moulds. Their essential oils are best candidature in presence of their cytotoxic aptitude

against fungus (Sharma *et al.*, 2014). Wonderful kola is widely distributed in most African countries and its parts (e.g. bark, leaf, seed and stem) have been used to treat a variety of health conditions in most rural communities in Nigeria (Burkill, 1985). As a result of its supported broad-spectrum affinity, there is need to further study the potential utilization of wonderful kola. In a study carried out by Ibrahim and Fagbohun (2013), the extracts of the seed of *B.coriacea* showed significant antifungal activity on different fungi used including *Trichoderma viridae* and *Aspergillus niger*. The antifungal activity of the seed extracts was observed using methanol and ethanol. Many scientists, (Anjum and Khan, 2003; Adedokun *et al.*, 2002; Bajwa *et al.*, 2006; Thebo and Abro, 2000; Pirzada *et al.*, 2007; Sanjay and Ashok, 2006; Farsos, 2009), have worked on the antifungal activities of medicinal plants from different regions of the world. Investigations have been carried out to discover plant products that inhibit fungi like *Aspergillus spp*, *Trichophyton rubrum* and *Rhizopus spp*.

These fungal species cause infections in humans which are difficult to control effectively and the pharmaceutical arsenal currently available are rather limited (Gupta and Garg, 1991). Thus the use of plant extracts that inhibit fungal growth without harming the host represents potential therapeutic agents. *In vitro* antifungal susceptibility testing will be useful in discovering and selecting effective antifungal agents (Carrillo-Munoz *et al.*, 2006).

1.3 Aim of the Study

This study was designed to determine the prevalence of dermatophytosis in goats in Gwagwalada, F.C.T and to evaluate the antifungal activity of the different extracts of *Buchholzia coriacea* (wonderful kola) on the isolates.

1.4 Research Questions

1. What is the level of dermatophytosis from goats in Gwagwalada, F.C.T?
2. What are the effects of the different extracts of *B.coriaceae* (wonderful kola) on the dermatophytes isolated?
3. Are the dermatophytes isolated susceptible to common antifungal agents?

1.5 Objectives of the Study

The objectives of the study are:

1. To determine the level of infection due to dermatophytes in goats in Gwagwalada, F.C.T by isolation.
2. To evaluate the effect of n-hexane, ethyl acetate, methanol and aqueous extracts of *B. coriaceae* (wonderful kola) on the recovered isolates.
3. To determine the antifungal activities of commercially available antifungal agents on identified isolates.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Review of Literature on Dermatophytes

2.1.1 History of dermatophytes

History credits three biologists for the creation of medical mycology: Robert Remak, Johann L. Schönlein, and David Gruby, each of whom studied dermatophytes at one point or another (Weitzman and Summerbell,1995).In 1837 Robert Remak, a Polish physician of the Medical Faculty of Berlin University, noted hyphae in the crusts of the disease known as favus. This was an epochal discovery since for the first time a microorganism was incriminated as being the cause of a human disease. Schönlein then identified the specimen having fungal origin. However the real founder of dermatomycology was David Gruby based on his discoveries from 1841 to 1844, his communications to the French Academy of Science, and his publications during this period (Gruby 1841, 1843, 1844).Independently, and unaware of previous work that had been done in this field, he described the causative agent of favus, both clinically and in microscopic details of the crusts, and established the contagious nature of the disease (Gruby, 1841). He also described ectothrix invasion of the beard and scalp, naming the aetiologic agent of the latter *Microsporum* (referring to the small spores around the hair shaft) *audouinii* (Gruby, 1843), and described endothrix hair invasion by *Herpes (Trichophyton) tonsurans* (Gruby, 1844). In addition to his observations on dermatophytes, he also described the clinical and microscopic appearance of thrush in children.

Raimond Sabouraud, one of the best known and most influential of the early medical mycologists, began his scientific studies of the dermatophytes around 1890, culminating in the

publication of his classic volume, *Les Teignes*, in 1910 (Sabouraud,1910).Sabouraud's contributions included his studies on the taxonomy, morphology, and methods of culturing the dermatophytes and the therapy of the dermatophytoses. He classified the dermatophytes into four genera, *Achorion*, *Epidermophyton*, *Microsporum*, and *Trichophyton*, primarily on the basis of the clinical aspects of the disease, combined with cultural and microscopic observations.In 1934, Chester Emmons (Emmons, 1934) modernized the taxonomic scheme of Sabouraud and others and established the current classification of the dermatophytes on the bases of spore morphology and accessory organs. He eliminated the genus *Achorion* and recognized only the three genera *Microsporum*, *Trichophyton*, and *Epidermophyton* on the basis of mycological principles.

Nutritional and physiological studies of the dermatophytes pioneered at Columbia University (Benham, 1953, Silva and Benham, 1952) and at the Center for Disease Control (C.D.C), in Georgia, (Ajello and Georg, 1957, Georg, 1952, Georg and Camp, 1957, Swartz and Georg, 1955) simplified the identification of dermatophytes that led to reduction of the number of species and varieties.

Dermatophytosis has been prevalent as far back as 1906 (Sequeira, 1906), at which time ringworm was treated with compounds of mercury or sometimes sulphur or iodine. Hairy areas of skin were considered too difficult to treat because remedies applied could not penetrate the hair shaft sufficiently, so the scalp was treated with X-rays and followed up with antifungal medication. Another treatment from around the same time was application of Araroba

powder. The Araroba or goa powder is an extract of *Andira araroba*, a South American tree, recognized for its effective treatment of dermatophytosis ((Sequeira, 1906) .

2.1.2 Aetiological agents of dermatophytosis

The aetiologic agents of the dermatophytoses are classified in three anamorphic (asexual or imperfect) genera, *Epidermophyton*, *Microsporum*, and *Trichophyton*, of anamorphic class *Hyphomycetes* of the *Deuteromycota* (Fungi Imperfecti). The descriptions of the genera essentially follow the classification scheme of Emmons (Emmons, 1934) on the bases of conidial morphology and formation of conidia and are updated following the discovery of new species (Ajello, 1968, Ajello, 1977, Matsumoto and Ajello. 1987). The genera and their descriptions are as follows:

- ***Epidermophyton spp.*** The type species is *Epidermophyton floccosum*. The macroconidia are broadly clavate with typically smooth, thin to moderately thick walls and one to nine septa, 20 to 60 by 4 to 13 mm in size. They are usually abundant and borne singly or in clusters. Microconidia are absent. This genus has only two known species to date, and only *E. floccosum* is pathogenic.
- ***Microsporum spp.*** The type species is *Microsporum audouinii*. Macroconidia are characterized by the presence of rough walls which may be asperulate, echinulate, or verrucose. Originally, the macroconidia were described by Emmons as spindle shaped or fusiform, but the discovery of new species extended the range from obovate (egg shaped) as in *Microsporum nanum* (Fuentes, 1956) to cylindrofusiform as in *Microsporum vanbreuseghemii* (Georg *et al.*, 1962). The

macroconidia may have thin, moderately thick to thick walls and 1 to 15 septa and range in size from 6 to 160 by 6 to 25 μm . Microconidia are sessile or stalked and clavate and usually arranged singly along the hyphae or in racemes as in *Microsporum racemosum*, a rare pathogen (Borelli, 1965).

- ***Trichophyton spp.*** The type species is *Trichophyton tonsurans*. Macroconidia, when present, have smooth, usually thin walls and one to 12 septa, are borne singly or in clusters, and may be elongate and pencil shaped, clavate, fusiform, or cylindrical. They range in size from 8 to 86 by 4 to 14 μm . Microconidia, are usually more abundant than macroconidia and may be globose, pyriform or clavate, or sessile or stalked, and are borne singly along the sides of the hyphae or in grape-like clusters.

2.1.3 Epidemiology of dermatophytes

The epidemiology of dermatophytoses in developed countries has exhibited notable changes over the past decades as a consequence of variation in some environmental conditions, and the distribution of the etiological agents usually reflects the changing clinical patterns of dermatophytoses (Ameen *et al.*, 2010). Dermatophytes are among the few fungi causing communicable disease. All but one of the species known to cause disease primarily affects mammals. The exception, *Microsporum gallinae*, is primarily established in gallinaceous fowl. Their etiological agents and predominating anatomical infection patterns vary with geographical location and environmental and cultural factors (Havlickova *et al.*, 2008). Such fungi grow at surface temperatures of 25°C - 28°C with warm and humid conditions which is supported for infection on human skin. Infections by fungi are relatively common in tropical countries due to

wearing of dirty and pungent clothing, low socioeconomic status, crowded living conditions, superficial skin infections, a low tendency to self-limitation and poor medical care help to increase the epidemic spread of skin mycoses (Weitzman and Summerbell, 1995; Peerapur *et al.*, 2004). Also, booming tourism, international sports activities and increasing migration are responsible for disseminating an imported fungal group of mycoses (Chowdhry *et al.*, 2013). Apart from those species usually associated with disease, transitional species exist which appear to be primarily saprobic organisms occasionally or rarely causing infection. Dermatophytic species significantly vary in their ability to invade hair, nail, and skin on their unique enzyme production and nutritional demands (Simpanya, 2000). Dermatophytes and their congeners have long been divided into anthropophilic, zoophilic, and geophilic species on the basis of their primary habitat associations (Ajello, 1962, Georg, 1960). Anthropophilic dermatophytes are primarily associated with humans and rarely infect other animals. Zoophilic dermatophytes usually infect animals or are associated with animals but occasionally infect humans (Achterman and White, 2012). Geophilic dermatophytes are primarily associated with keratinous materials such as hair, feathers, hooves, and horns after these materials have been dissociated from living animals and are in the process of decomposition. These species may cause human and animal infection. Geophilic species are thought to have been ancestral to the pathogenic dermatophytes, preadapted to cutaneous pathogenesis by their ability to decompose keratin and their consequent close association with animals living in hair and feather-lined nests in contact with soil (De Vroey, 1984). Dermatophytes are known to grow best in warm and humid environments and are, therefore, more common in tropical and subtropical regions and this probably explains why they are very common in Africa (Nweze, 2010). For instance, some species of dermatophytes such as *Trichophyton mentagrophytes* var. *interdigitale*, *Microsporum canis*, *Epidermophyton floccosum*

and *Trichophyton rubrum* are distributed all over the world. However, other species probably have partial geographic restriction. For example, *Trichophyton schoenleinii* is found in Africa and Eurasia while *Trichophyton soudanense* is also restricted within Africa (Weitzmann and Summerbell, 1995). Others are *Trichophyton violaceum* which are associated to Asia, Africa and Europe and *Trichophyton concentricum* which is known to be common in the Far East, India and the Pacifics (Ameen, 2010).

2.1.4 Dermatophytosis in West Africa

Dermatophytosis is one of the most common cutaneous infections all over the world (Ameen, 2010; Nweze and Okafor, 2005). It causes superficial fungal infections that pose public health problems to man and animals (Havlickova *et al.*, 2008). Dermatophyte infections can be *al* disfiguring and recurrent and generally need long-term treatment with antifungal agents (Nweze *et al.*, 2007) In Africa and several other countries in Latin America and the Middle East, there is a kind of variability and geographical/regional associations in the pattern of dermatophytic infections. For instance, tinea capitis is known to be very common in Western Africa especially among children and several species of dermatophytes are known to be responsible (Moriarty *et al.*, 2012). Tinea cruris, tinea pedis, tinea corporis and tinea unguium are caused by *T. rubrum* in many urban areas of developing countries (Hernandez-Salazar *et al.*, 2007) and even in developed countries (Borman *et al.*, 2007; Foster *et al.*, 2004). *Microsporum audouinii* is the predominant dermatophyte species in many parts of Africa. *T. violaceum* is reportedly endemic in several parts of South and Northern Africa and *T. soudanense* in central Northwestern parts of Africa (Ellabib *et al.*, 2002; Morar *et al.*, 2004; Woldeamanuel *et al.*, 2005). Conversely, *M. canis* predominates other dermatophytes in Southern and Central European countries as the most

common cause of tinea capitis while *T. mentagrophytes* and *T. rubrum* are the cause of increasing cases of tinea unguium and pedis, respectively (Tao-Xiang *et al.*, 2005; Tan, 2005).

In Togo, a study involving 374 children from primary schools in North and Southern part of the country revealed that 11% of the children in the North (dry and urban area) and 20% in the South (wet and rural area) had obvious clinical lesions. Two species of dermatophytes were isolated: *Microsporum langeronii* and *T.soudanense*; this second dermatophyte being uncommon in the South (Dupouy-Camet *et al.*, 1988). The authors further established that 15% of the children in the North and 42% of the children in the South were asymptomatic carriers. This suggests that the locality predispose to dermatophytoses and concurs with the findings in Nigeria (Nweze, 2001; Nweze and Okafor, 2005). In another study in Germany involving children arriving from Togo including an 8 week-old male baby, *T. soudanense* was recovered from discrete lesions on the hairy scalp and neck of these patients, thus confirming that this agent was indeed common in Togo (Faulhaber and Korting, 1999). Similarly, a ten year retrospective study in Togo had indicated that dermatophytosis was a significant public health problem in the country (Napo-Koura *et al.*, 1997).

The prevalence and pattern of distribution of dermatophytosis seems to vary across various regions of Senegal. Develoux *et al.*, (2002) found a frequency of 26.4% in Dakar, whereas Silverberg *et al.*, (2002) and Cremer *et al.*, (1997) reported 11.4 and 11%, respectively. In a later two year study involving three dermatology Centers in Senegal, 16% of HIV patients with dermatosis had dermatophytic infections suggesting that dermatophytoses could pose a future

problem in the country with the increasing incidence of HIV/AIDS among the inhabitants of that country (Monsel *et al.*, 2008).

In the Northern flanks of the West African countries of Guinea Conakry and Burkina Faso , *T.violaceum*, *T. rubrum* and *T. soudanense* were identified as the the predominant aetiological agents of dermatophytoses (Menan *et al.*, 2002; Guiguemde *et al.*, 1992).

A study conducted at the Dermatology Center of Treichville Hospital in Abidjan, Cote d'Ivoire by Adou-Bryn *et al.*, (2004), found a threefold higher incidence of tinea capitis in boys than in girls and a peak during childhood especially those aged between 5 and 9 years. In their reports, the authors concluded that the most frequent aetiologic agents were *T. soudanense* and *M. audouinii var langeronii* in 63.6 and 31.3% of cases respectively.

In Ghana, studies conducted among 463 children in the Greater Accra region showed that *T. violaceum* (26%) was the most prominent species, followed by *T. tonsurans* (22%). The percentage occurrence of *M. audouinii* (15%) was relatively low compared to other studies performed in Africa (Nweze, 2010). The prevalence of *T. rubrum* was 11% and no *T. soudanense* was recovered in the study (Hogewoning *et al.*, 2006). Tietz *et al.*, (2002) recovered a rare species of the *T.rubrum* complex (*Trichophyton raubitschekii*) in Germany from a set of four African immigrant patients who were presented with typical lesions of tinea corporis. One of the patients was from Ghana and the other three were from Cameroon. In a similar incident in the USA, two African children adopted from Liberia in West Africa, residing in Cincinnati,

Ohio, presented a case of tinea capitis associated with *T. soudanense*, a dermatophyte that is not common in the whole of North America (Markey *et al.*, 2003).

In a review carried out by Nweze (2010), he summarized that the most common aetiological agents of dermatophytoses in the West African sub-region are *T. soudanense* and *M. audouinii*, with *T. rubrum*, *T. mentagrophytes*, *T. tonsurans* and *T. violaceum* recently playing dominant roles in some locations in the region.

2.1.5 Dermatophytosis in Nigeria

In Nigeria, there are varying reports of dermatophytosis in different cities and communities (Nweze and Okafor, 2005). As early as 1977, studies have been carried out to determine the prevalence of dermatophytosis especially among school children. In Lagos, Adetosoye (1977) screened 3860 school children. The prevalence at the time was just 2.1%. Seven species of dermatophytes were recovered from specimens collected from the hair, skin and scalp scrapings of 81 school children. *T. soudanense* was the most aetiological agent followed by *M. canis*. More recently in a study involving several states in Central Nigeria, a total of 28505 primary school children aged between 3 and 16 years were sampled from 12 primary schools. Tinea capitis was found to be the most prevalent superficial mycoses. The most common aetiological agent was *T. soudanense*, (30.6%), followed by *M. ferrugineum*, (7.7%) and *M. audouinii*, (7.7%) (Ayanbimpe *et al.*, 2008). In Ogun state, South Western Nigeria, a total of 2772 randomly selected junior secondary school pupils between the ages of 8-14 years from 60 schools were examined. The prevalence of dermatophytosis was 23.21%. Aetiological agents identified were *M. canis*

(30.19%), *M. audouinii* (32.92%), *T. interdigitale* (14.37%), *T. soudanense* (9.73%) and *T. tonsurans* (12.05%). Most of the dermatophytes encountered were anthropophilic species. *M. canis* was the only zoophilic dermatophyte (Popoola *et al.*, 2006).

Emele and Oyeka (2008) in another larger study which involved a total of 47723 primary school children residing in different regions of Anambra State, found that 4498 (9.4%) had tinea capitis. The highest prevalence of the disease occurred in the Southern region of the state (12.6%). Schools in urban areas recorded lower prevalence of the disease. Moreso, tinea capitis occurred significantly more in children below 10 years of age than in those above this age. *M. audouinii* was the most prevalent (42%), followed by *M. ferrugineum* (17%) and *T. mentagrophytes* (16%). In a similar study carried out in Kano State Nigeria, 2150 itinerant quranic scholars were screened. Only 9.5% were found to be infected and the age group 10-14 years was most affected. *T. rubum* (50.2%) was the most prevalent followed by *M. audouinii* (26.5%). *T. rubrum* was the only dermatophyte that was recovered from all sites apart from the buttocks (Adeleke *et al.*, 2008).

2.1.6 Dermatophytosis in humans

Superficial mycoses are among the most frequent forms of human infections, being estimated to affect more than 20-25% of the world's population, and their incidence is constantly increasing (Havlickova *et al.*, 2008). The infection initially presents itself with red patches on affected areas of the skin and later spreads to other parts of the body. The infection may affect the skin of the scalp, feet, groin, beard, or other areas. Traditionally, infections caused by dermatophytes are named according to the anatomic locations involved and by adding the Latin term describing the

body part after the word tinea, e.g., tinea capitis for ringworm of the scalp. The clinical manifestations are as follows:

- (i) tinea barbae (ringworm of the beard and mustache);
- (ii) tinea capitis (scalp, eyebrows, and eyelashes);
- (iii) tinea corporis (glabrous skin);
- (iv) tinea cruris (groin);
- (v) tinea favosa (favus);
- (vi) tinea imbricata (ringworm caused by *T. concentricum*);
- (vii) tinea manuum (hand);
- (viii) tinea pedis (feet);
- (ix) tinea unguium (nails).

Several anatomic sites may be infected by a single dermatophyte species, and different species may produce clinically identical lesions. The incubation period in humans is usually 1 to 2 weeks. Dermatophytosis is common especially among people who play sports involving skin-to-skin contact, wrestling in particular. Wrestlers with ringworm may be withheld from competition until their skin condition is deemed noninfectious by the proper authorities (Decorby, 2009).

2.1.7 Dermatophytosis in animals

Different species of dermatophytes have been isolated from different species of animals worldwide. The most important animal pathogens worldwide are *M. canis*, *M. gypseum*, *T. mentagrophytes*, *T. equinum*, *T. verrucosum*, and *M. nanum*. These species are zoonotic,

especially *M. canis* infections of domestic cats and *T. verrucosum* of cattle and lambs (Merck veterinary Manual, 2008).

Typical dermatophytic lesions are circular or irregular lesions depending on infecting organism. The symptoms of dermatophytosis vary, depending on the infecting organism, affected tissues (e.g., skin, hair or and these may nails) and area of the body. In unhaired (glabrous) skin, the lesions are usually characterized by inflammation that is most severe at the edges, with erythema, scaling and occasionally blister formation. The central area may be clear, resulting in the formation of classic “ringworm” lesion. In hairy parts of the body, the hairs become brittle and areas of alopecia may appear. Scaly and crusty patches and alopecia will be seen in these areas due to broken hair shafts and hairs lost from inflamed regions of the skin. Follicular papules and pustules will also be present. There is usually a varying degree of pruritus.

There were few studies specifically carried out in the world among a large species spectrum of animals aimed at identifying the fungal species associated with the carrier state of dermatophytes and their prevalence. From four older studies carried out in Nigeria that screened animals for dermatophytes, three were conducted in Nigeria’s western State of Oyo which comprised about 3.5% of Nigeria’s population (Adeyefa, 1986; Efuntoye and Fashanu, 2002), while the fourth one investigated dermatophytes amongst rodents in eastern Nigeria (Okafor and Gugnani, 1981). Nweze (2011) sampled ten different species of domestic animals numbering 538, across seven states (Enugu, Anambra, Ebonyi, Abia, Imo, Kogi and Delta) of Nigeria between the months of August 2006 and January 2009. Ten species of dermatophytes were recovered in the

study. They were mostly zoophilic species and included *M. canis*, *T. mentagrophytes*, *T. verrucosum*, *M. gypseum*, *M. gallinae*, *T. equinum*, *M. nanum*, *M. equinum*, *M. persicolor* and *T. gallinae*. Out of these, *M. canis* was the most predominant species consisting of 37.4% of all positive samples. This was followed by *T. mentagrophytes* (22.9%), *T. verrucosum* (15.9%), *M. gypseum* (7.0%), *M. gallinae* (6.1%), *T. equinum* (5.6%), *M. nanum* (3.3%) and *M. equinum* (1%), *M. persicolor* and *T. gallinae* jointly recorded the least prevalence (0.5%) in the study.

2.1.8 Pathogenesis of dermatophytosis

Primary infection starts through a small skin break. (Laham *et al.*, 2011; Achterman and White, 2012; Mikaili *et al.*, 2012). Infection is caused by arthrospores or conidia. The pathogen invades the uppermost, non-living, keratinized layer of the skin namely the stratum corneum, produces exo-enzyme keratinase and induces inflammatory reaction at the site of infection (Wawrzkievicz *et al.*, 1991; Lopez-Martinez *et al.*, 1994; Siesenop and Bohm, 1995; Muhsin *et al.*, 1997). The customary signs of inflammatory reactions such as redness (ruber), swelling (induration), heat and alopecia (loss of hair) are seen at the infection site. Inflammation causes the pathogen to move away from the site of infection and take residence at a new site. This movement of the organism away from the infection site produces the classical ringed lesion (Dahl, 1994).

2.1.9 Diagnosis

Diagnosis is based on the history, physical examination, and microscopic examination of scrapings and hairs from the lesions, in conjunction with fungal culture and other techniques such as Wood's lamp examination and histology of the tissues. According to the Centre for Food

Security and Public Health of the Iowa State University (2013), dermatophytes can often be detected by microscopic examination of infected hairs and skin or nail scrapings. Hyphae rounding up into arthroconidia are diagnostic, but hyphae alone could be caused by other fungi, including contaminants. In hairs, arthroconidia may be found outside (ectothrix) or inside (endothrix) the hair shaft. Skin scrapings should be taken from the edge of the lesion, and hairs should be plucked (not cut) from this area. The best hairs to select are those that fluoresce under a Wood's lamp, or are broken or scaly. Nail scrapings are generally taken from the nail bed, or from deeper portions of the nail after removing the outer layers (except in cases where the infection is entirely superficial). Samples are usually cleared with potassium hydroxide (KOH) or other agents to help visualize the organism. Various stains such as chlorazol black E, Parker blue-black ink, Swartz-Lamkin stain or Congo red stain may be added. Fluorescence microscopy, using calcofluor white or other stains, can also be used to visualize dermatophyte structures. Fungal cultures, which identify the species of dermatophyte, can be useful in understanding the source of the infection and targeting preventive measures appropriately. Culture may also be necessary if the diagnosis is uncertain, or the infection is resistant to standard treatment. However, recommendations vary in the literature, and uncomplicated cases are not always cultured in practice. Samples for culture include hair, skin and nail samples, as for microscopic examination. In some situations (e.g., infections in sensitive sites, or the identification of asymptomatic carriers), other techniques such as brushing the hair, using adhesive tape to collect samples, or rubbing the area with a sterile toothbrush or moistened, sterile cotton swab may also be effective. Colonies appear in 5 days to 4 weeks, depending on the organism. Colony morphology can differ with the medium. Descriptions are usually based on Sabouraud agar, but dermatophyte medium or other fungal culture media can also be used for isolation.

Dermatophyte species can be identified by the colony morphology; the appearance of microconidia, macroconidia and other microscopic structures; biochemical characteristics such as urease production; and nutritional requirements. Specialized tests such as the ability to penetrate hairs *in vitro*, or mating tests (which are usually available only at reference laboratories) may be used occasionally. Differential media (e.g., bromocresol purple - milk solids glucose) can be helpful during differentiation. Some fungal cultures from infected people are negative.

2.1.10 Treatment

Treatment is recommended to alleviate symptoms (pruritus), reduce risk for secondary bacterial infection, and limit spread of the infection to other body sites or other individuals. Topical antifungal therapy is the treatment of choice for most patients. Systemic antifungal agents are primarily reserved for patients who fail topical therapy.

Terbinafine emerged as a new therapeutic option for dermatophytoses in both humans and animals (Mancianti *et al.*, 1999). Terbinafine inhibits the growth of dermatophytes of all genera and is the main drug of choice for the treatment of dermatophytoses, especially with chronic conditions. Griseofulvin is used exclusively to control the development of keratinized tissue infection by presenting only fungistatic and not fungicidal action. This drug is orally administered and treatment varies according to the clinical form of mycosis (Gupta *et al.*, 2001).

Other antifungal treatments include topical agents such as miconazole, clotrimazole, ketoconazole, or tolnaftate applied twice daily until symptoms resolve — usually within one or two weeks. Topical treatments should then be continued for a further 7 days after resolution of visible symptoms to prevent recurrence (Kyle and Dahl, 2004; McClellan *et al.*, 1999). The total duration of treatment is therefore generally two weeks, but may be as long as three (Canadian Pediatric Society, 2008).

In immunocompetent patients, topical agents are usually effective in cases that are limited to glabrous skin (e.g., tinea corporis, tinea cruris, and tinea pedis). Dermatophyte infections of the nails (tinea unguium) are usually treated with oral antifungal drugs. Concurrent therapies may include debridement of the nail or nail avulsion. Treatment should consider sources of reinfection, such as pets, family members or other close contacts. Some authors suggest treating all family members when the case is caused by certain anthropophilic organisms.

2.1.11 Mechanism of action of antifungal drugs

Antifungals work by exploiting differences between mammalian and fungal cells to kill the fungal organism with fewer adverse effects to the host. Unlike bacteria, both fungi and humans are eukaryotes. Thus, fungal and human cells are similar at the biological level. This makes it more difficult to discover drugs that target fungi without affecting human cells. As a consequence, many antifungal drugs cause side-effects. Some of these side-effects can be life-threatening if the drugs are not used properly. Antifungals are classified based on structure and mechanism of action not based on site of action.

The four major classes of antifungal agents in clinical use include the azoles, polyenes, echinocandins and allylamine/thiocarbamates. They all owe their antifungal activities to inhibition of synthesis of or direct interaction with ergosterol. Ergosterol is the predominant component of the fungal cell membrane (Parks and Casey, 1996).

Polyene antifungals are amphiphilic. The polyene antimycotics bind with sterols in the fungal cell membrane, principally ergosterol. This changes the transition temperature (T_g) of the cell membrane, thereby placing the membrane in a less fluid, more crystalline state. (In ordinary circumstances membrane sterols increase the packing of the phospholipid bilayer making the plasma membrane denser.) As a result, the cell's content including monovalent ions (K^+ , Na^+ , H^+ , and Cl^-), small organic molecules leak and this is regarded one of the primary ways cell dies (Baginski and Czub, 2009). Animal cells contain cholesterol instead of ergosterol and so they are much less susceptible. However, at therapeutic doses, amphotericin B may bind to animal membrane cholesterol, increasing the risk of human toxicity (Groll *et al.*, 1998).

Azole antifungal drugs (except for abafungin) inhibit the enzyme lanosterol 14 α -demethylase; the enzyme necessary to convert lanosterol to ergosterol. Depletion of ergosterol in fungal membrane disrupts the structure and many functions of fungal membrane leading to inhibition of fungal growth (Groll *et al.*, 1998). Examples include ketoconazole followed by fluconazole, itraconazole, and voriconazole.

Allylamines inhibit squalene epoxidase, another enzyme required for ergosterol synthesis. Examples of the allylamines include amorolfin, butenafine, naftifine, and terbinafine (Ameen, 2010).

Echinocandins may be used for systemic fungal infections in immunocompromised patients; they inhibit the synthesis of glucan in the cell wall via the enzyme Beta (1-3) glucan synthase. Echinocandins are poorly absorbed when administered orally. When administered by injection they will reach most tissues and organs with concentrations sufficient to treat localized and systemic fungal infections (*Chang et al., 2017*).

2.1.12 Control and prevention of dermatophytosis

Up till now, there is no approved human vaccine against dermatophytosis. For horses, dogs and cats an approved inactivated vaccine called Insol Dermatophyton, is available (Boehringer Ingelheim) which provides time-limited protection against several *Trichophyton* and *Microsporum* fungal strains (Grovet, 2016).

2.1.13 Resistance to antifungal agents

The study of resistance to antifungal agents has lagged behind that of antibacterial resistance for several reasons. Perhaps most importantly, fungal diseases were not recognized as important pathogens until relatively recently (*Weyet al., 1988; Anaissie and Bodey, 1989*). For example, the annual death rate due to candidiasis was steady between 1950 and about 1970. Since 1970, this

rate increased significantly in association with several changes in medical practice, including more widespread use of therapies that depress the immune system, the frequent and often indiscriminate use of broad-spectrum antibacterial agents, the common use of indwelling intravenous devices, and the advent of chronic immunosuppressive viral infections such as AIDS. These developments and the associated increase in fungal infections (Beck-Sagué *et al.*, 1993) intensified the search for new, safer, and more efficacious agents to combat serious fungal infections.

Antifungal resistance occurrence has to be considered independently for each antifungal class and for each fungal genus. However antifungal drug resistance appears to essentially be due to point mutations in either drug targets or transcription factors regulating actors of the resistance.

2.1.14 Plants used as treatment for dermatophytosis

Quite a number of plants are being used to treat dermatophytosis. They include tea tree oil, garlic pawpaw, coconut oil, lemon grass, aloe vera, turmeric, grapefruit seed extract, olive leaf extract, oregano and lavender oil, neem, amongst a host of others. Ameh and Okolo (2014) reported that in Sokoto, the white juice of a common weed, the Dead sea apple (*Calotropis procera*) is usually used to treat ringworm infections in children. Healing is usually observed 1-2 weeks after topical application. Some have been used together with conventional antifungal agents to potentiate their positive effects and diminish their negative effects. The *Otacanthus azureus* (Linden) Ronse essential oil alone or in combination with azoles is a promising antifungal agent in the treatment for human dermatomycoses caused by filamentous fungi (Houel *et al.*, 2013).

The combination of ketoconazole and *P. graveolens*'s essential oil for treatment of infections caused by *Trichophyton* species reduce the minimum effective dose of ketoconazole, and thus minimize the side-effects of ketoconazole (Shin and Lim, 2003).

Several studies in Nigeria and outside Nigeria have reported on the inhibitory effect and antifungal activities of *Ocimum gratissimum* against dermatophytes and other pathogenic fungi. Nwosu and Okafor (1995) reported the antifungal activities of extracts of *O. gratissimum* collected from southeastern Nigeria against seven pathogenic fungi including *Trichophyton rubrum* and *T. mentagrophytes*.

Similarly in a study carried out by Mbakwem – Aniebo *et al.*, (2012), the extracts of the leaves of *Ocimum gratissimum* had effect against *Trichophyton*, *Microsporum*, *Epidermophyton* and *Malassezia furfur*

The antifungal effect of *Hypercom perforatum*, *Eucalyptus globules* (88%), *Catharanthus roseus* (88%), *Ocimum sanctum* (85.50%), *Azadirachta indica* (84.66%), *Ricinus communis* (75%), *Lawsonia inermis* (74.33%) *jatropha curcas* (10%) *Eucalyptus intertexta* and *Eucalyptus largiflorens* have been shown against *Epidermophyton*, *Microsporum* and *Trichophyton* (Ghasemi *et al.*, 2014; Venugopal and Venugopal, 1994; Suklampoo *et al.*, 2012; Scott *et al.*, 2006).

2.2 Review of Literature on *Buchholzia coriacea*

2.2.1 Description and taxonomic classification of *Buchholzia coriacea*

Buchholzia coriacea was named after R.W. Buchholz who collected the plants in Cameroon in the late 19th century (Keay *et al.*, 1989). It belongs to the family *Capparidaceae*. Below is the taxonomic classification:

Family: *capparaceae* Juss,

Super order: *Rosanae Takht*,

Order: *brassicales* Bromhead

Genus: *Buchholzia* engl

Class: *Eqissetopsida* c. Agardh

Sub class: *magnoliidae* nov'ak ex takht

Specie: *Coriaceae*

In Nigeria, *Buchholzia coriacea* is known as “uworo,” “owi,” and “uke” among the Yoruba, Edo and Igbo tribes, respectively (Sofowora, 2008). Among the people of Central African, the fruit is known as—esson bossi (Cruickshank *et al.*, 1980). *B. coriacea* is a forest tree with large, glossy leaves and conspicuous cream white flowers in racemes at the end of the branches (Mbata *et al.*, 2009).

The plant *Buchholzia coriacea* is a shrub or medium-sized tree, evergreen, with a dense crown, large glossy leathery leaves arranged spirally and clustered at the ends of the branches, and conspicuous cream-white flowers in racemes at the end of the branches. The bark of the plant *Buchholzia coriacea* is smooth, blackish-brown or dark-green. Slashes are deep red turning dark brown (Akpayung *et al.*, 1995; Awouters *et al.*, 1995). The leaves are large, obovate, oblanceolate to elliptic, shortly acuminate or acute at the apex, cuneate at the base, 15-30×5-11 cm, thinly coriaceous, glabrous, midrib very prominent below, about 10 lateral nerves, each running directly into the one above and forming distinct loops close to the margin, prominent below, stalk 10-15 cm long, swollen for about 1 cm at both ends, pale green. The flowers can be described as simple or lightly-branched lax racemes among the leaves at the ends of the shoots, up to 24 cm long, individual flowers with a stalk less than 1.3 cm. Four small rounded sepals bent right back exposing the thick saucer-shaped purplish receptacle, without petals, 40 to 45 stamens with cream-yellow filaments and small purplish-black anthers and a narrow elongated ovary projecting beyond the stamens at the end of a thin stalk. The fruits of the plant *Buchholzia coriacea* are large, long stalked, ellipsoid, resembling avocado pears, 12×5-8 cm, endocarp up to 1.3 cm thick and woody, yellowish when ripe, flesh yellow, edible, containing a few large blackish seeds, about 2.5 cm long (Culpeper, 1995; Grieve Maud, 1984). The plant *Buchholzia coriacea* is a tree of the lowland rain forest in the region of Guinea, Cameroon, and in Gabon. In Gabon the plant *Buchholzia coriacea* is sometimes cultivated as a medicinal and fetish plant (Gbile *et al.*, 1993). Vernacular names of the plant are Cola pimento, elephant cola and oignon de Gorille (Palombo, 2006; Andrews, 1982; Arber, 1986).



Plate I: *Buchholzia coriacea* seed



Plate II: *Buchholzia coriacea* plant: leaves and flowers

2.2.2 Traditional uses

The name 'wonderful kola' was coined from the seeds of *Buchholzia coriacea* because of its usage in traditional medicine. The seeds are covered in purple aril which are chewed in Ivory Coast and has a pungent taste. The bark can be made into a pulp for inhalation or into a snuff to relieve headache, sinusitis, and nasal congestion in Ivory Coast; smallpox or skin itching in Gabon. The pulped bark is applied to the chest to treat chest pains and also boils. In Liberia, the seeds are used on skin eruption and internally for worms. In Ivory Coast, the crushed up seeds, are pasted over the stomach for difficult childbirth. It is also considered anthelmintic (worm expeller). It is used as cough medicine, and in the treatment of ulcer. It is also used in the treatment of hypertension by drinking the fluid squeezed out of the leaves with pea leaves and small salt. In the Ivory coast the twig bark decoction of the plant *Buchholzia coriacea* is used for the treatment of rheumatism and kidney pain, it is also used for the treatment of infections of the eye (bark gruel poured into the flat of the hand and inhaled) and for the treatment of pain in the back (fruit pulp massaged in). For the treatment of earache, seeds are pounded in a little bit of water and the resulting liquid is dropped into the ear (Nwachukwuet *al.*, 2014). The Ebri tribes bathe smallpox victims with the bark decoction of the plant *Buchholzia coriacea*. Young leaves of the plant *Buchholzia coriacea* are used in a gruel poultice for ulcers and boils (Anowi *et al.*, 2012). In Gabon pounded bark of the plant *Buchholzia coriacea* is used as a lotion against scabies, the fruit of the plant *Buchholzia coriacea* as an anthelmintic. In former times young warriors were given fresh roots of the plant *Buchholzia coriacea* to stimulate them before battle. The seeds of the plant *Buchholzia coriacea* are edible and that they have a spicy taste and that they can be used as a condiment (spice). Okoli *et al.*, (2010) reported the anti-plasmodial

properties of the plant, the ground seeds were therefore routinely mixed with palm oil and taken orally as treatment for malaria (Adjanohoun *et al.*, 1996). The Cameroonians use the seed as remedy to relieve chest pain (Thomas *et al.*, 1989). It was also reported to have analgesic effects (Ezeja *et al.*, 2011) and anthelmintic potentials (Nweze and Asuzu, 2006).

2.2.3 Phytochemistry

The medicinal value of plants lies in some chemical substances that produce a definite physiologic action on the human body (Himal *et al.*, 2008). The most important of these bioactive compounds of plants are alkaloids, flavonoids, tannins, and phenolic compounds. The phytochemical research based on ethnopharmacological information is generally considered an effective approach in the discovery of new infective agents from higher plants (Duraipandiyar *et al.*, 2006). Knowledge of the chemical constituents of plants is desirable, not only for the discovery of therapeutic agents, but also because such information may be of value in disclosing new sources of such economic materials as tannins oils, gums, precursors for the synthesis of complex chemical substances. In addition, the knowledge of the chemical constituents of plants would further be valuable in discovering the actual value of folkloric remedies (Mojab *et al.*, 2003).

Several studies have been carried out on various parts of the plant *Buchholzia coriacea*. Obiudu *et al.*, (2015) carried out phytochemical screening of the aqueous and methanol extracts of *Buchholzia coriacea* seeds and found the presence of alkaloids, flavonoids, tannins, saponins, anthraquinones and cardiac glycosides. The presence of the various identified phytochemicals

may be responsible for the therapeutic usage of *Buchholzia coriacea* seeds in the treatment of various illnesses such as diabetes mellitus. Furthermore phytochemical analysis has shown the presence of alkaloids, saponins, cardiac glycosides and flavones glycosides (Adisa *et al.*, 2011). Saponins, anthraquinones, alkaloids, cyanogenic glycosides have been reported by Fred-Jaiyesimi *et al.*, (2011) from the seeds of the plant. Tannins and cardiac glycosides were also reported by Mbata *et al.*, (2009) and Ayo *et al.*, (2012) in the seeds of *Buchholzia coriacea*. Ibrahim and Fagbohun (2012) showed that the methanol and ethanol extracts of dried seeds of *Buchholzia coriacea* contained alkaloids, glycosides, saponin, tannin, flavonoids, terpenes, reducing compounds, and phenols. In their research the From their work the methanolic extract had a higher percentage yield than the ethanolic extract. Nwachukwu *et al.*, (2014) screened fresh, oven dried uncooked and cooked seed samples of *Buchholzia coriacea* in order to compare their phytoconstituents and determine their effects on hepatocellular integrity. They found flavonoids, saponins, oxalates, tannins, phytates, cyanogenic glycosides and alkaloids to be present in the seed of the plant. The concentrations of all the phytoconstituents investigated in their study were highest in fresh seed samples of *Buchholzia coriacea*. Processing of the oven dried uncooked and cooked samples could be the cause of the difference in concentrations.

Anowi *et al.*, (2012) revealed the presence of tannins, flavonoids, alkaloids, glycosides, and saponins in the leaves of the methanol extract of *B. coriacea*. Similarly, alkaloids, tannins, reducing sugars, terpenoids, flavonoids, saponins, and cardiac glycosides were shown to be present in the leaves of *B. coriacea* by Onyekeba *et al.*, (2011).

2.2.4 Pharmacological actions

Studies on the antidiabetic properties of *Buchholzia coriacea* showed that the methanol seed extract of *B. coriacea* exhibited hypoglycaemic effects, and the effect was synergistic when used with metformin (a standard oral hypoglycaemic agent). Oral administration of *B. coriacea* at 100, 200, 400 mg/kg reduced blood glucose by 37.73, 12.30 and 11.30% respectively after 4 hours of administration. The combination of the extract (100 mg/kg) and metformin (100 mg/kg) reduced blood glucose by 73.4 and 72.2% respectively (Theophine *et al.*, 2012)

Adisa, *et al.*, (2011) evaluated the hypoglycemic activity and ameliorative effects of oral administration of ethanol extracts (EEBC) and butanol fraction (BFBC) of *Buchholzia coriacea* seeds in streptozotocin (STZ)-induced diabetic mice and rats. The extracts significantly ($P < 0.05$) decreased fasting blood glucose (FBG) in hyperglycemic mice and normoglycemic rats within 4 and 12 hours of administration.

Oral administration of the extract with glibenclamide (a standard antidiabetic agent) caused a significant ($p < 0.05$) reduction in FBG , serum alanine aminotransferase and aspartate aminotransferase levels as well as serum creatinine, urea, total cholesterol, triglyceride and thiobarbituric acid reactive species (TBARS) products in STZ induced diabetic rats. Adisa *et al.*, (2011) proposed that *B. coriacea* seeds contain a potent hypoglycemic and antioxidant agent suggested to be a flavone glycoside concentrated in BFBC which may find clinical usefulness in ameliorating diabetes-induced secondary complications.

Chinaka *et al.*, (2012) showed that oral administration of the methanol extract of the seeds of *B. coriacea* at 150, 300 and 600 mg/kg of methanol fruit extract of *Buchholzia coriacea* caused significant dose-dependent decrease in fasting blood glucose in rats. The serum concentration of catalase and reduced glutathione were significantly higher in rats treated with glibenclamide (2 mg/kg) and various doses (150, 300 and 600 mg/kg) of the extract. In contrast to the values in negative control rats, the extract also decreased serum triglyceride and total serum cholesterol levels. The fruit extract dose dependently reduced lipid peroxidation in diabetic rats.

Ezekiel and Onyeoziri, (2009) carried out a study on the effect of the fresh kola, hexane and methanol extracts of *B. coriacea* on some food borne pathogens (*Escherichia coli*, *Enterococcus faecalis*, *Staphylococcus aureus*, *Trichoderma viride* and *Aspergillus niger*). The fresh kola showed inhibitory zones with the test bacteria: *E. coli* (62 mm), *E. faecalis* (40 mm) and *S. aureus* (50 mm). The growth of the two test fungi *T. viride* and *A. niger* was completely inhibited. The hexane extract showed inhibitory zones ranging from 20 to 40 mm with the test bacteria: *E. coli* (21 mm), *E. faecalis* (20 mm) and *S. aureus* (40 mm). It however showed no inhibitory effect on *T. viride* and *A. niger*. The extract also elicited inhibitory zones ranging between 20 to 30 mm with some of the test pathogens: *E. coli* (30 mm) *E. faecalis* (25 mm) and *S. aureus* (20 mm), *T. viride* (15 mm). It did not show inhibitory effect on *A. niger*.

The study also carried out by Ajaiyeoba *et al.*, (2003) on fractions of the methanol extract of the stem bark *Buchholzia coriacea* showed a high concentration-dependent antibacterial and antifungal activity of the fractions when compared to the standard antibiotic, ampicillin and tioconazole. The methanol extract was found to be non-toxic with an LC₅₀ of 1031 µ/ml in the

brine shrimp lethality (BSL) assay. Lupeol and B-sitosterol were the two main compounds present in the most active fraction.

The antibacterial effect of the leaves of *Buchholzia coriacea* were evaluated against Gram positive and Gram negative clinical isolates including ESBL positive *E. coli* isolates by Chika *et al.*, (2012). The n-hexane, methanol and chloroform extracts of the leaves of *B. coriacea* elicited antibacterial activities against the test isolates with activity against *E. coli*, *Staphylococcus aureus*, *Shigella* species, *Klebsiella pneumoniae* and *Bacillus subtilis*. The n-hexane and methanol extracts showed moderate inhibitory effects, however, chloroform extract did not exhibit activity against the ESBL. The minimum inhibitory concentration (MIC) values ranged between 6.25 mg/ml and 12.5 mg/ml for all the test isolates. MIC values for all the ESBL positive *E. coli* isolates were 50 mg/ml. The study showed that the extracts of the leaves of *B. coriacea* possess promising antibacterial effects which can be considered for pharmaceutical and medicinal purposes.

Nweze *et al.*, (2011) investigated the activity of the methanol seed extract of *Buchholzia coriacea* against a field strain of *Trypanosoma congolense* in experimentally infected mice. Results of the study showed no significant difference ($P < 0.05$) in body weights of the mice. The packed cell volume (PCV) of infected mice was significantly ($p < 0.05$) reduced than those uninfected. The methanol extract of *Buchholzia coriacea* seeds did not show antitrypanosomal activity against mice infected with *Trypanosoma congolense*. Similarly the anthelmintic properties of *Buchholzia coriacea* and *Gynandropsis gynandra* methanol leaves and stem

extracts were investigated against *Fasciola gigantica*, *Taenia solium* and *Pheritima pasthuma*, respectively. The plants showed *in vitro* effect against all the tested parasites at concentrations between 10 and 100 mg/ml (Ajaiyeoba *et al.*, 2003).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

The study was carried out in Gwagwalada area council, Abuja. Gwagwalada is the second largest Area Council in the Federal Capital Territory (FCT), Abuja. Rainfall is seasonal, characterized by heavy thunder storm and torrential down pour. Vegetation in the area is the guinea savanna type with patches of riparian vegetation. The major occupation of the local indigenes is subsistence farming (Mabogunje, 1977). It is one of the semi-urban settlements in the F.C.T and is located between latitude and longitude N 8° 56' 29" and E 7° 5' 30" respectively. The city has a tropical climate with two major seasons; rainy season between April to October and dry season between November to March. It is known for its high temperatures throughout the year averaging annually at 27.2 °C. Annual rainfall is about 1271 mm with a relative humidity of between 20-30%. It is made up of ten wards namely Zuba, Ibwa, Dobi, Kutunku, Tunga Maje, Gwako, Paikon-kore, Ikwa, Quarters and Central.



FIGURE 1: A Map of the Federal Capital Territory, Abuja showing the Study Area

SOURCE: MODIFIED ADMINISTRATIVE MAP OF NIGERIA, DEPARTMENT OF GEOGRAPHY, A.B.U., ZARIA

3.2 Sample Collection and Processing

In the area council, skin samples were collected from any goat with clinically suggestive lesions in goat flocks in Fulani camps, live goat markets and rural homesteads between the months of August, 2015 to January, 2016. The purposive sampling technique was used and images of sampled goats taken using a digital camera.

Using protective gloves, a sterile scalpel blade was used to obtain skin scrapings from the margins of lesions suggestive of dermatophytosis after disinfecting the area with alcohol, according to the method described by Elewski, (1998). Samples were placed in clean paper envelopes and in separate polythene bags and correctly labeled. For each sample, a different scalpel was used to avoid contamination. Collected samples were transported as dry packs to the Microbiology Laboratory of the Department of Veterinary Microbiology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria.

3.3 Laboratory Procedures

3.3.1 Direct examination

Direct examination of the samples was first carried out where small samples of each scrapping were placed on a clean slide and 1 to 2 drops of 10% potassium hydroxide (KOH) solution added. A cover slip was placed on top and the slide gently heated over a flame as described by Hainer, (2003). Each slide was examined first using 10x low power objective lens then 40x high power objective lens in order to observe for the presence of diagnostic fungal forms.

3.3.2 Culture for dermatophytes on media

The samples were then cultured on Sabouraud dextrose agar (Oxoid, U.K) with chloramphenicol (0.05 mg/ml) and cyclohexamide (5 mg/ml), which is selective media used for primary isolation of fungi (Sharma *et al.*,2011). The cultures were incubated at room temperature for one to four weeks.

3.3.3 Identification of isolates

Suspected growths were sub cultured on Potatoe dextrose agar to facilitate distinctive spore formation for identification and pigment production. They were incubated at room temperature for one to four weeks (Raymond and Pipheth, 2008). Identification was based on colonial and microscopic characteristics using the fungal colour atlas (Evans and Richardson, 1989; Baron *et al.*, 2003).

3.3.4 Slide culture preparation

In a situation where dermatophyte identification was inconclusive due to lack of sporulation, the isolates were subjected to slide cultures. This was done in order to observe the precise arrangement of the conidiophores and conidial ontogeny (i.e. the way the spores are produced). A modification of Riddel's method of slide culturing (1950) was used for the identification. Below is a brief description:

1. A sterile plate of Potatoe dextrose agar was used.

2. Another plate of Potatoe dextrose agar was used to cut out an agar block (1x1 cm) small enough to fit under a cover slip. This was cut using a sterile blade
3. The agar block was flipped onto the surface of the first sterile plate of Potatoe dextrose.
4. The four corners of the agar block was inoculated with spores or mycelia fragments of the fungi
5. The cover slip was then placed centrally on the agar block and incubated at room temperature and daily observed till growth and sporulation occurred.
6. The cover slip was then gently removed from the agar block and put on a clean glass slide with a small drop of lacto phenol cotton blue and observed for conidia.

3.3.5 Preperation of n-hexane, ethyl acetate, aqueous and methanol extracts of *B. coriacea* seed

The seeds of *B. coriacea* were purchased and a sample sent to the Herbarium, Department of Biological Sciences of the Ahmadu Bello University, Zaria for authentication and given the voucher number 6921. The seeds were cut into small pieces, dried in the shade and grinded into a coarse powder using a mortar and pestle. 600 grams of the powdered seeds of *B. coriacea* were extracted serially by cold maceration with n-hexane, ethyl acetate, distilled water and methanol as described by Alani *et al.*, (2005), with modifications where necessary. For n-hexane, ethyl acetate and methanol, the plant material (600 g) was soaked in each solvent for 72 hours each with intermittent shaking at 2 hours interval. The extract was then filtered using filter paper and later concentrated using a rotary evaporator at reduced pressure. For the aqueous extract, the

plant material (600 g) was soaked in one liter of distilled water for 24 hours. It was then sieved with a muslin cloth and the extract placed in a rotary evaporator at reduced pressure. The percentage yield for each extract was determined and the extracts kept for further use in a desiccator.

3.3.6 Preperation of the innoculum

The isolated dermatophytes were sub cultured from sterile distilled water in which they had been stored onto sterile Potatoe Dextrose Plates containing chloramphenicol (0.05 mg/ml) and cyclohexamide (5mg/ml). After 5-7 days growth, the growths were harvested using a sterile loop and mixed in 10 mls of sterile saline. The tubes were agitated using a vortex mixer to break down the hyphae. The test tube was allowed to stand so larger hyphal segments could settle and the suspension adjusted to 0.5 Macfarlands standard.

3.3.7 Testing the antifungal activity of the different extracts of *B. coriacea* seeds on the isolates

The broth macro dilution method was used for *in vitro* testing of the antifungal activity of the extracts using Clinical and Laboratory Standards Institute (CLSI) M38-A standard for moulds (CLSI document M38-A, 2002) as a guideline with modifications where necessary. Briefly, a stock solution of 1000 mg/ml of each extract was prepared a day before using distilled water as a diluent. SDA broth (4.5 mls) was added to different sets of labeled sterile test tubes for the different isolates. From the stock solution of the different extracts, 0.5 mls of each extract was removed using a sterile syringe to perform a twofold dilution. After the dilution, a drop from the prepared inoculums of the different isolates was put in each test tube. The tubes were then

incubated at room temperature and observed for 24-48 hours. A negative and positive control was set up. Any sign of cloudiness or growth was recorded as negative and signs of no growth were recorded as suspected positive. Test tubes that showed no growth were further plated on sterile plates of SDA to determine the minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC). The MIC was defined as the lowest concentration of the extract that inhibited fungal growth after the period of incubation while the MFC was defined as the lowest concentration of the extract that will kill the fungal growth.

3.3.8 Antifungal susceptibility tests

This was carried out according to the European Committee for Antimicrobial Susceptibility Testing (EUCAST) Definitive Documents E.DEF 9.1 (2008)/CLSI M-38A2 (2008) with modifications where necessary. Commercial antifungal susceptibility discs, Hexa Antimycos - 01(Himedia©) were used. Each disc contained 6 standard antifungal agents namely amphotericin B (100 units), clotrimazole (10µg), fluconazole (25µg), itraconazole (10µg) ketoconazole (10µg) and nystatin (100 units).

The agar - based disk diffusion susceptibility method for dermatophytes was used as described by Esteban *et al.*, (2005) with modifications where necessary. The inoculum prepared for testing the extracts was used. A sterile nontoxic swab stick was used to spread the standardized inoculum evenly spread on the surface of petri dishes containing Mueller Hinton agar medium with chloramphenicol and cycloheximide and exposed to air dry for 5 minutes in a safety cabinet. The antifungal discs were then applied to the plates using sterile forceps and incubated

at room temperature for up to 5 days .When growth took place, the sizes of the zones of inhibition were measured using a ruler for each antifungal agent and recorded (Pakshir *et al*, 2009).

3.3.9 Statistical analysis

The data obtained from the study was analyzed using Statistical package for social science (SPSS) version 21.0 and the results obtained were presented in tables and charts. Statistical level of significance was fixed at $P < 0.05$.

CHAPTER FOUR

4.0 RESULTS

4.1 Results of Isolation and Identification of Dermatophytes from Goats in Gwagwalada

From August 2015 to January 2016, a total number of 124 samples were collected from 124 goats showing suggestive clinical signs of dermatophytosis. After culturing and microscopy, a total of 20 dermatophytes were isolated. From the 20 isolates *Microsporum* (6) *Trichophyton* (13) and *Epidermophyton* (1) were identified. Seven species were identified namely: *Microsporum audouinii* (4), *Trichophyton verrucosum* (6), *Trichophyton tonsurans* (5), *Epidermophyton floccosum* (1), *Trichophyton mentagrophytes* (1), *Microsporum gypseum* (2) and *Trichopyton ajelloi* (1).

Other non dermatophytic fungi were also isolated from all the 124 samples namely Rhizopus, Aspergillus, Mucor, Fusarium and Yeast.

Table 4.1: Dermatophyte Isolates from Goats with Skin Lesions in Gwagwalada

DERMATOPHYTES	NO. ISOLATED (%)	
(n=124)		
<i>Microsporum</i>	6 (4.84)	65.0
<i>Trichophyton</i>	13 (10.48)	30.0
<i>Epidermophyton</i>	1 (0.81)	5.0
TOTAL	20 (16.13)	100

Table 4.2: Dermatophyte Species isolated from Goats in Gwagwalada

DERMATOPHYTES	NO. OF ISOLATES (%)	SOURCE OF SAMPLES
<i>Microsporum audouinii</i>	4 (3.23)	Fulani goat flock and Live goat markets
<i>Microsporum gypseum</i>	2 (1.61)	Live Goat Markets
<i>Trichophyton verrucosum</i>	6 (4.84)	Fulani goat flock, Live Goat markets, rural homesteads
<i>Trichophyton tonsurans</i>	5 (4.03)	Fulani goat flock, Live Goat markets ,rural homesteads
<i>Trichophyton ajelloi</i>	1 (0.81)	Rural homestead
<i>Trichophyton mentagrophytes</i>	1 (0.81)	Fulani goat flock
<i>Epidermophyton floccosum</i>	1 (0.81)	Live Goat Market
TOTAL=7	20 (16.13)	

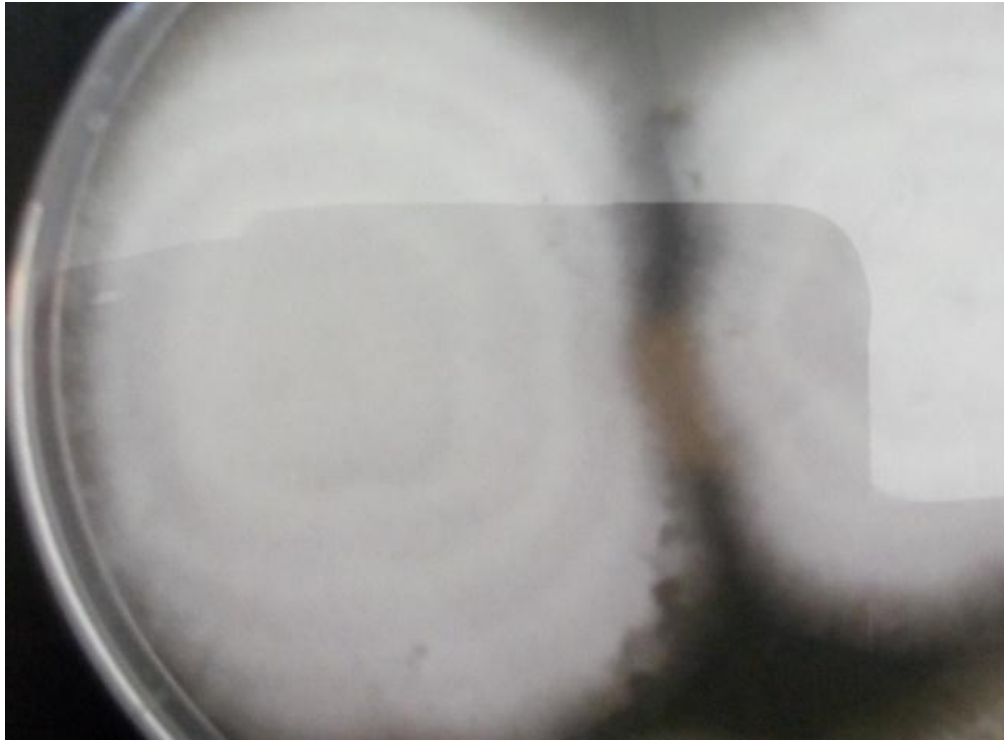


PLATE III: Colony of *Microsporium audouinii* on PDA showing white, downy texture after 12 days growth

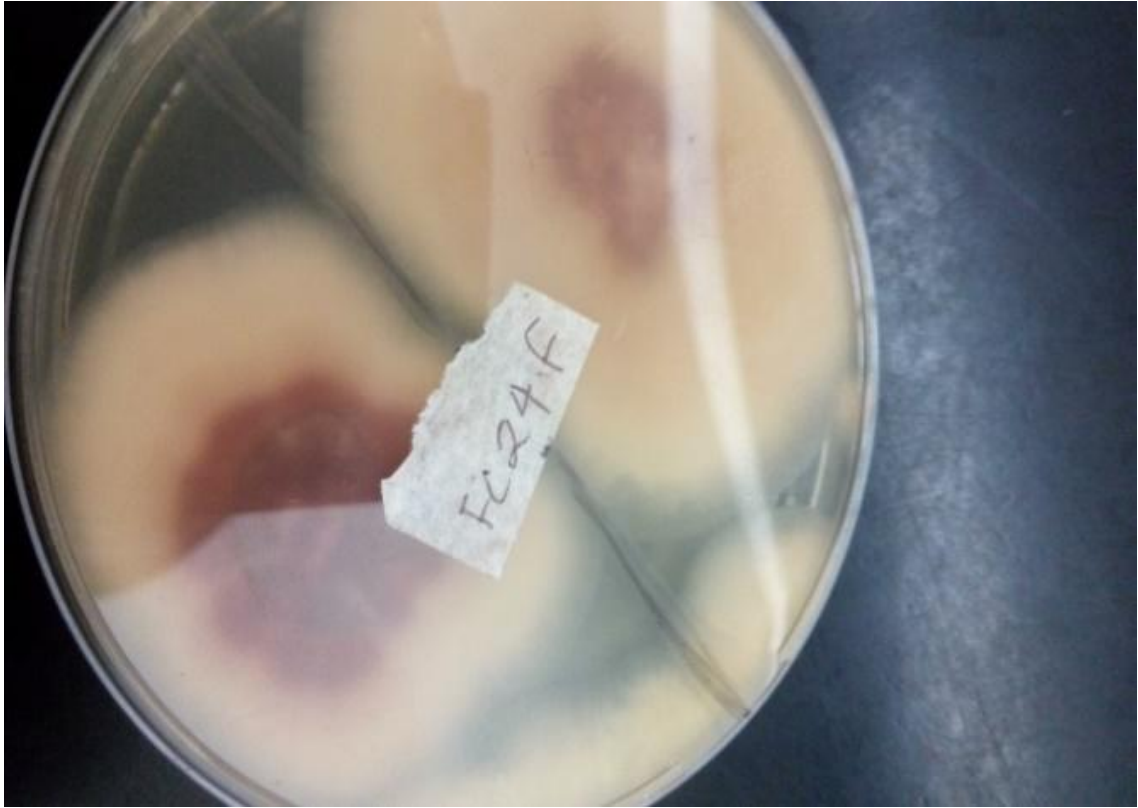


PLATE IV: Reverse of colonial growth of *Microsporium audouinii* with peach pigmentation



PLATE V: Microscopy of *Microsporium audouinii* showing deformed spindle shaped macroconidia (X400) (LCB Stain)

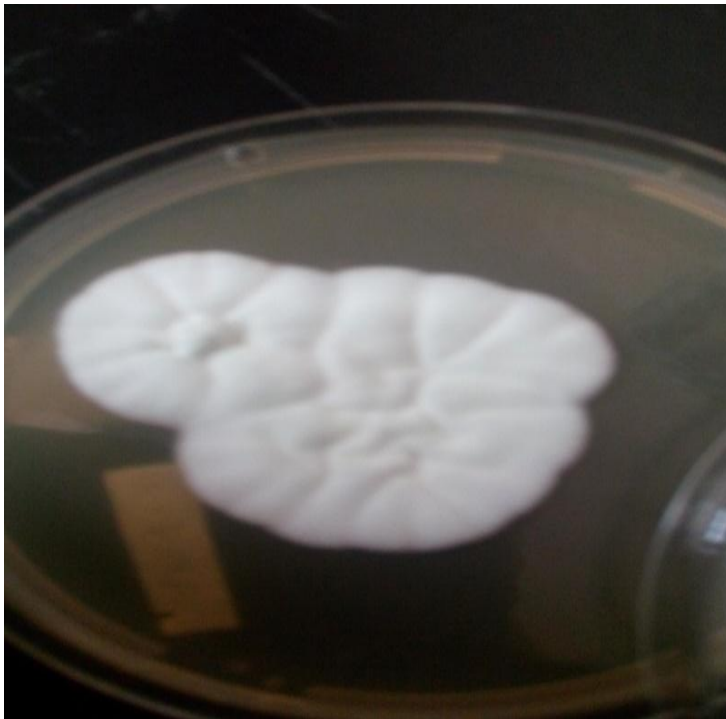


PLATE VI: Colony of *Trichophyton verrucosum* showing white velvet heaped texture, growth 14days



PLATE VII: Reverse of *Trichophyton verrucosum* colonial growth colourless

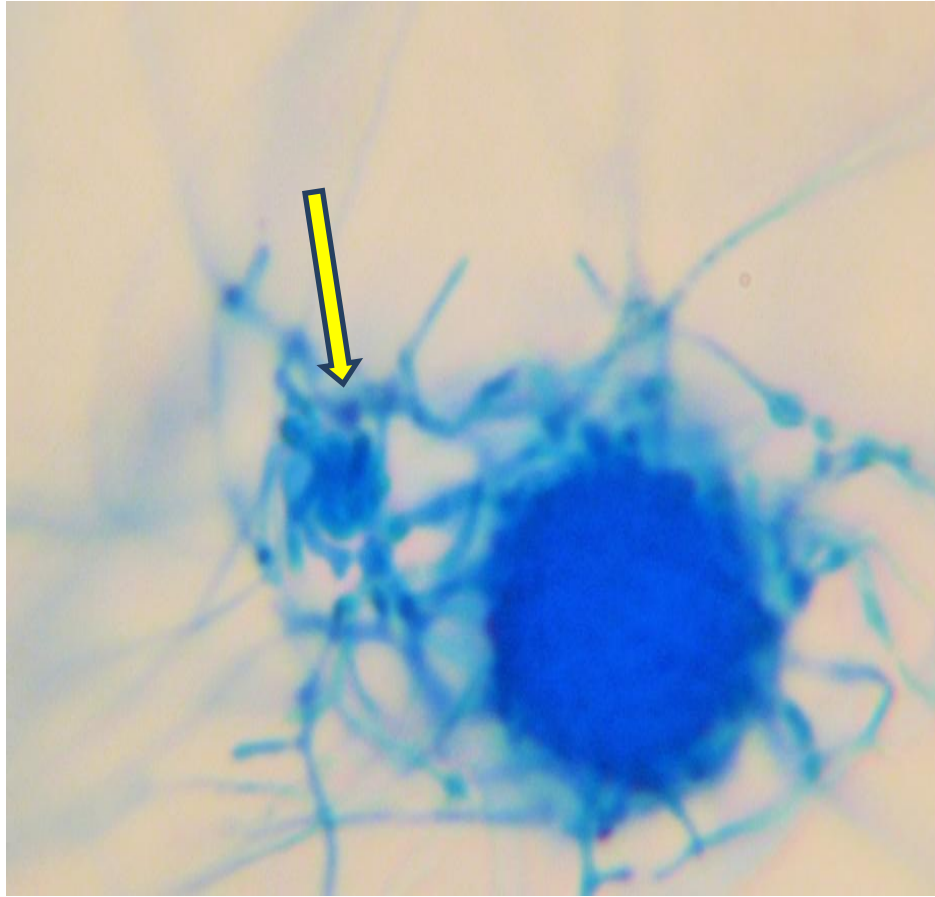


PLATE VIII: Microscopy of *Trichophyton verrucosum* with arrow pointing at chlamydiospores (x400) LCB stain



PLATE IX: Colony of *Epidermophyton floccosum* showing greenish brownish pigmentation (14days) on PDA



PLATE X: Reverse of colonial growth of *Epidermophyton floccosum* showing reddish brown pigmentation

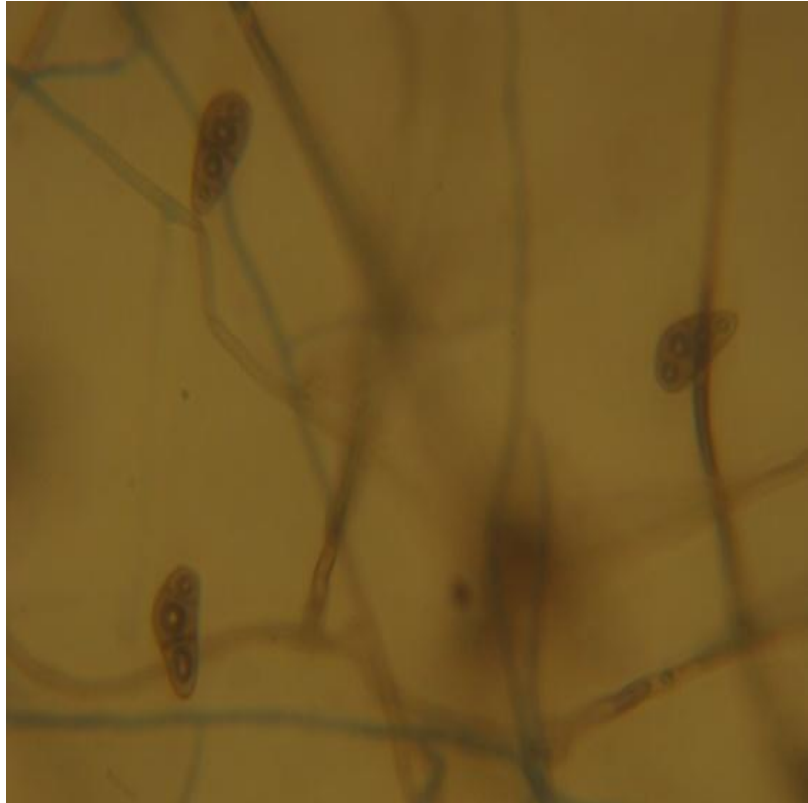


PLATE XI: Microscopy of *Epidermophyton floccosum* showing smooth walled macroconidia attached directly to hyphae singly(x400) LCB stain

Table 4.3: Non – Dermatophyte isolates from Goats in Gwagwalada, Abuja

Non-Dermatophyte	No. Isolated (%)
<i>Aspergillus spp</i>	49 (39.52)
<i>Rhizopus spp</i>	36 (29.03)
Yeast	16 (12.90)
<i>Fusarium spp</i>	3 (2.42)
TOTAL	104 (83.87)

Table 4.4: Sources of Dermatophytes isolated from Goats in Gwagwalada

Source	No.of Samples Collected	No.Positive (%)
Fulani Goat herds	36	7 (5.65)
Live Goat Markets	60	9 (7.26)
Rural homesteads	28	4 (3.23)
TOTAL	124	20 (16.13)

P=0.9604, df=2, $\chi^2=0.2982$

Table 4.5: Distribution of Dermatophytes Based on Anatomical Location

Anatomical Site	No.of Samples Collected	No.Positive (%)
Udder	15	4 (3.23)
Leg	20	4 (3.23)
Ear	35	8 (6.45)
Face	10	3 (2.42)
Neck	10	1 (0.81)
Others (shoulders,chest,stomach)	34	0 (0)
TOTAL	124	20 (16.13)

P=0.1264, df=5, $\chi^2=8.593$

Table 4.6: Seasonal Distribution of Dermatophytes in Goats in Gwagwalada, Abuja

Month of The Year	No.of Samples Collected	No.Positive (%)
August	6	0 (0)
September	35	6 (4.84)
October	21	0 (0)
November	29	9 (7.26)
December	18	0 (0)
January	15	5 (4.03)
TOTAL	124	20 (16.13)

P=0.0298, df=5, $\chi^2=12.95$

Table 4.7: Age distribution of dermatophytes in Goats from Gwagwalada

Age	No.Sampled	No. Positive (%)
Adult Goats	56	13 (10.48)
Young Goats	68	7 (5.65)
TOTAL	124	20 (16.13)

P=0.1469

Table 4.8: Sex Distribution of Dermatophytes isolated in Goats from Gwagwalada

Sex	No. Sampled	No.Positive (%)
Female	65	9 (7.26)
Male	59	11 (8.87)
TOTAL	124	20 (16.13)

P=0.6324

4.2 Results of Antifungal activity of *Buchholzia coriacea* seed on the Isolates

The aqueous extract had the highest percentage yield (4.46%) followed by the methanol extract (3.54%), hexane extract (0.64%) and finally the ethyl acetate extract (0.23%) as shown in Table 4.9. The methanol extract had 100% effect on all the isolates with MICs and MFCs ranging from 62.5 mg/ml-500 mg/ml. The hexane extract had effect on only 4 isolates with MIC and MFC ranging from 62.5 mg/ml-500 mg/ml. The ethyl acetate extract had effect on only 2 isolates of *T. tonsurans* with MIC and MFC ranging from 62.5 mg/ml-500 mg/ml. The aqueous extract did not exhibit any inhibitory activity on any of the isolates. The one isolate of *Epidermophyton floccosum* only showed activity when the methanol extract was used.

Table 4.9: Percentage Yield of Extracts of *Buchholzia coriacea* Seed

Extract	Amount (g)	Percentage Yield (%)
n-Hexane	600	0.64
Ethyl acetate	600	0.23
Aqueous	600	4.46
Methanol	600	3.54

Table 4.10: The Effect of Extracts of *Buchholzia coriacea* Seed on *Microsporium Spp* Isolated from Goats in Gwagwalada

Isolates	Extracts			
	N-hexane	Ethyl acetate	Aqueous	Methanol
1. <i>Microsporium audouinii</i>				
a.MM22m				
b.OMN5m	-	-	-	+
c.FC23f	+	-	-	+
d.FC24F	-	-	-	+
2. <i>Microsporium gypseum</i>	-	-	-	+
a.MM8m				
b.FC27f	-	-	-	+
	-	-	-	+

KEY: -:no effect

+:has effect

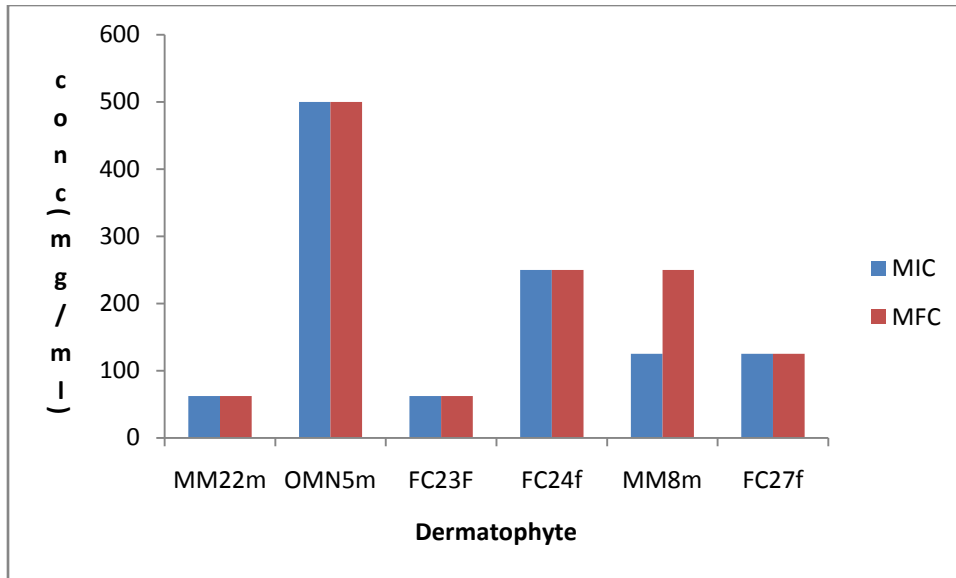


FIGURE 2: The minimum fungicidal concentration (MFC) and minimum inhibitory concentration (MIC) of the methanol extract of *Buchholzia coriacea* seed on *Microsporum* species isolated from goats in Gwagwalada

TABLE 4.11: The Effect of Extracts of *Buchholzia coriacea* Seed on *Trichophyton Spp* isolated from Goats in Gwagwalada

Isolates	Extracts			
	N-hexane	Ethyl acetate	Aqueous	Methanol
1. <i>Trichophyton verrucosum</i>				
a.FC11f	-	-	-	+
b.IB11mfpc	-	-	-	+
c.IB11m	-	-	-	+
d.MM14m	-	-	-	+
e.OMJ21m	+	-	-	+
f.FC31f	-	-	-	+
2. <i>Trichophyton tonsurans</i>				
a.IB209m	-	+	-	+
b.OMN4f	-	-	-	+
c.MM13m	+	+	-	+
d.FC13f	-	-	-	+
e.OMN5m	-	-	-	+
3. <i>Trichophyton ajelloi</i> (IB210m)				
	-	-	-	+
4. <i>Trichophyton mentagrophytes</i> (FC30f)				
	-	-	-	+

KEY: -:no effect

+:has effect

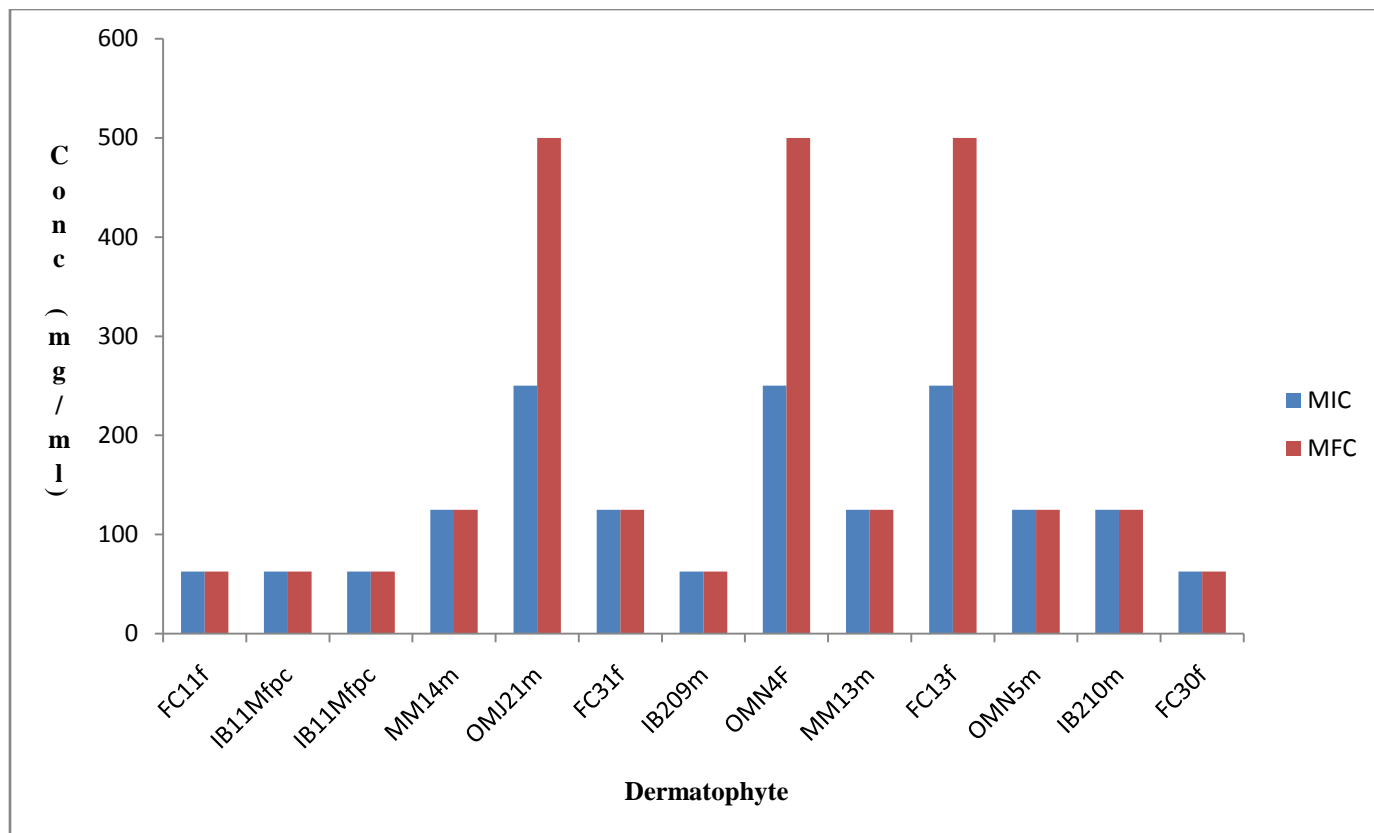


FIGURE 3: The minimum fungicidal concentration (MFC) and minimum inhibitory concentration (MIC) of the methanol extract of *Buchholzia coriacea* seed on *Trichophyton* species isolated from goats in Gwagwalada

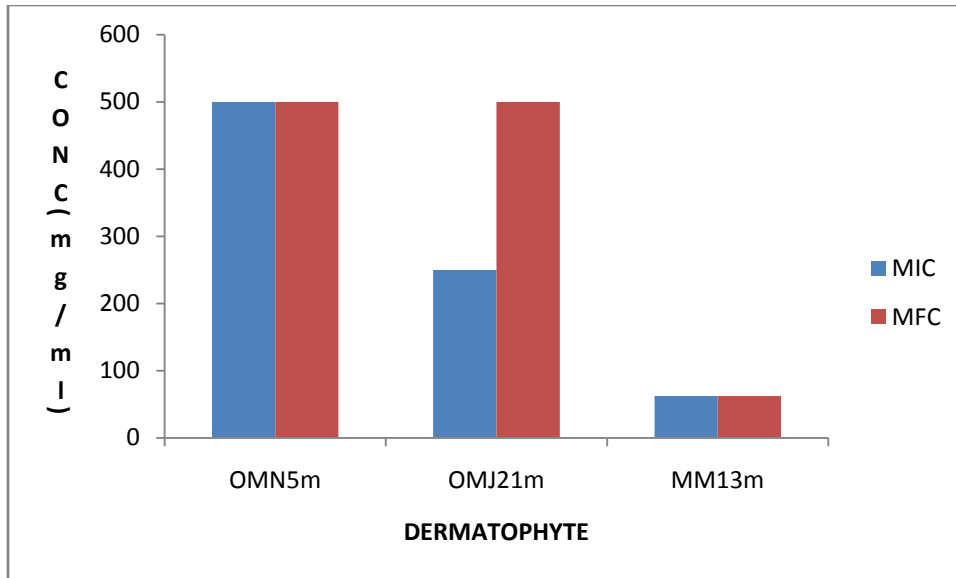


FIGURE 4: The minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of the hexane extract of *Buchholzia coriacea* seed on Dermatophyte isolates from goats in Gwagwalada

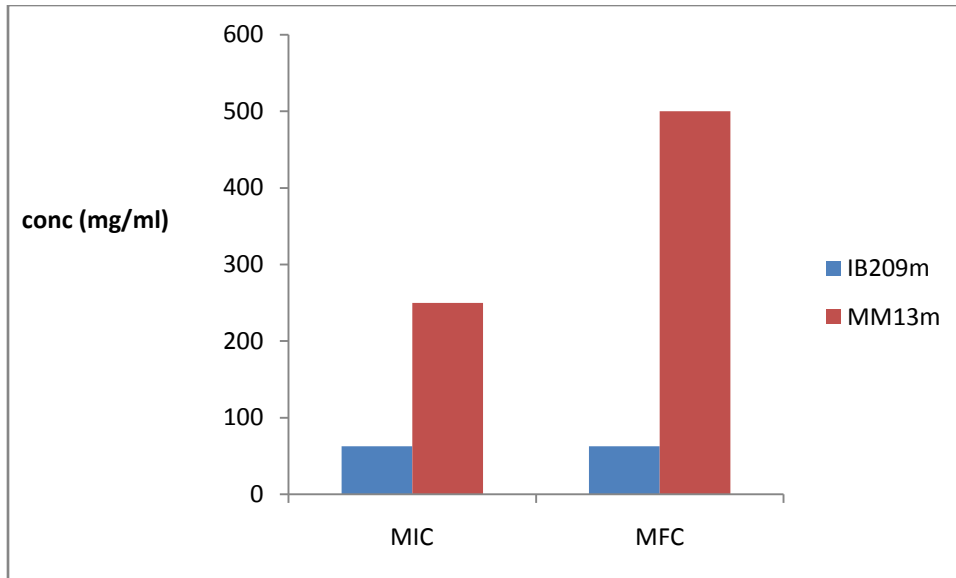


FIGURE 5: The minimum inhibitory concentraton (MIC) and minimum fungicidal concentration (MFC) of the ethyl acetate extract of *Buchholzia coriacea* seed on Dermatophyte isolates from Goats in Gwagwalada

4.3 Results of the Antifungal Activity of Commercially Standard Antifungal Agents on the Isolates

Amphotericin b was the most effective agent against all the isolates followed by nystatin, itraconazole and ketoconazole, clotrimazole and finally fluconazole which had little or no effect on a majority of the isolates. The isolates were most susceptible to Amphotericin B (70%), followed by nystatin (60%), ketoconazole (60%), itraconazole (55%), clotrimazole (30%), and finally fluconazole (15%). All *Microsporum* species were resistant to fluconazole and all but one were resistant to clotrimazole. *Trichophyton mentagrophytes* was resistant to all the antifungal agents. *Epidermophyton floccosum* was susceptible to all the agents except clotrimazole.

Table 4.12: Susceptibility profile of isolated *Microsporium species* from Goats in Gwagwalada to standard antifungal agents

SAMPLE DRUG	AP (100units)	CC (10µg)	FLC (25µg)	IT (10µg)	KT (10µg)	NS (100units)
<i>Microsporium audouinii</i> (MM22m)	S(13mm)	R	R	R	R	S(24mm)
<i>Microsporium audouinii</i> (OMN5m cotton)	S(13mm)	R	R	R	S(17mm)	S(24mm)
<i>Microsporium audouinii</i> (FC23f)	S (12mm)	R	R	R	R	R
<i>Microsporium audouinii</i> (FC24f)	R	R	R	S(30mm)	R	R
<i>Microsporium gypseum</i> (MM8m)	S (18mm)	R	R	R	S(20mm)	S(26mm)
<i>Microsporium gypseum</i> (FC27f)	S(22mm)	S(30mm)	R	S(22mm)	S(40mm)	S(23mm)

KEY: AP-Amphotericin B, **CC**-Clotrimazole, **FLC**-Fluconazole, **IT**-Itraconazole, **KT**-Ketaconazole, **NS**-Nystatin

S-Susceptible, **R**-Resistant

TABLE 4.13: Susceptibility Profile of isolated *Trichophyton* species from Goats in Gwagwalada to standard antifungal agents

SAMPLE	AP (100units)	CC (10µg)	FLC (25µg)	IT (10µg)	KT (10µg)	NS (100units)
<i>Trichophyton verrucosum</i> (FC11f)	S(23mm)	S(34mm)	R	S(32mm)	S(22mm)	R
<i>Trichophyton verrucosum</i> (IB11m fpc)	S(10mm)	R	R	S(11mm)	S(25mm)	S(20mm)
<i>Trichophyton verrucosum</i> (IB11m)	R	R	R	S(25mm)	R	R
<i>Trichophyton verrucosum</i> (MM14m)	S(12mm)	R	R	R	R	R
<i>Trichophyton verrucosum</i> (OMJ21m)	S(24mm)	S(33mm)	S(13mm)	S(23mm)	S(37mm)	S(20mm)
<i>Trichophyton verrucosum</i> (FC31f)	S(15mm)	R	R	R	R	S(20mm)
<i>Trichophyton tonsurans</i> (IB209m)	R	R	R	S(25mm)	S (15mm)	R
<i>Trichophyton tonsurans</i> (OMN4f)	R	S(14mm)	R	S(16mm)	S (20mm)	R
<i>Trichophyton tonsurans</i> (MM13m)	S(8mm)	S(15mm)	R	S(14mm)	S(22mm)	S(14mm)0
<i>Trichophyton tonsurans</i> (FC13f)	R	R	R	R	R	S(14mm)
<i>Trichophyton tonsurans</i> (OMN5m pink)	S(25mm)	R	S(11mm)	S(20mm)	S(30mm)	S(30mm)
<i>Trichophyton ajelloi</i> (IB210m)	S(26mm)	S(15mm)	R	S(28mm)	S(20mm)	S (30mm)
<i>Trichophyton mentagrophytes</i> (FC30f)	R	R	R	R	R	R

KEY: AP-Amphotericin B, **CC**-Clotrimazole, **FLC**-Fluconazole, **IT**-Itraconazole, **KT**-Ketaconazole, **NS**-Nystatin

S-Susceptible, **R**-Resistant

TABLE 4.14: Susceptibility Profile of isolated *Epidermophyton floccosum* from Goats in Gwagwalada to standard antifungal agents

SAMPLE/ DRUG	AP (100units)	CC (10µg)	FLC (10µg)	IT (10µg)	KT (10µg)	NS (100units)
Epidermophyton floccosum	S (25mm)	R	S (11mm)	S (20mm)	S (30mm)	S (30mm)

KEY: AP-Amphotericin B, CC-Clotrimazole, FLC-Fluconazole, IT-Itraconazole,

KT-Ketaconazole, NS-Nystatin, S-Susceptible, R-Resistant

CHAPTER FIVE

5.0 DISCUSSION

This study has been able to establish that dermatophytes are present in goats and cause dermatophytosis in the study area. Dermatophytes from the genera *Microsporum*, *Trichophyton* and *Epidermophyton* were isolated and identified in goats from the study area with *Trichophyton* species having the highest frequency of 13 isolates (10.5%) as seen in Table 4.1, and *Trichophyton verrucosum* being the dermatophyte with the highest frequency of 6 (4.8%) as seen in Table 4.2. This is in line with what was reported by Nweze (2011), where *T. verrucosum* was the most common species isolated in goats though at a higher frequency with a total of 10 (7.7%). The dermatophytes isolated are both of veterinary and medical importance because of the disease they cause in animals and their zoonotic potentials. *Trichophyton* and *Microsporum* species are the dominant species colonizing the animals and have often been classified as both human and animal pathogens. Animals serve as reservoirs for the zoophilic dermatophytes and the infections caused by them have a significant zoonotic importance. *Microsporum* and *Trichophyton* were also isolated from goats by Nweze (2011) whose study was carried out across seven states in Nigeria namely, Enugu, Anambra, Ebonyi, Abia, Imo, Kogi and Delta in different domestic animals.

Five out of the twenty dermatophytes isolated were *Trichophyton tonsurans* an anthropophilic fungus with a worldwide distribution. It was the second most common dermatophyte isolated in this study.

Trichophyton ajelloi was also isolated in this study .It is a geophilic fungus with a world-wide distribution which may occur as a saprophytic contaminant on humans and animals (Rippon, 1998).

Trichophyton mentagrophytes is a zoophilic fungus with a worldwide distribution and a wide range of animal hosts .There was only one species isolated in this study.

Four out of the twenty dermatophytes isolated were *Microsporum audouinii* (Plate II). *Microsporum audouinii* is an anthropophilic dermatophyte common in Africa.*Microsporum audouinii* was isolated from Fulani goat herds and markets in this study. It's very possible the infection seen in goats may be from contact with infected human handlers.

Microsporum gypseum is a geophilic fungus that infects animals that associate themselves with soil on a repeated basis (Howard, 2003) and has been isolated from ruminants.

Epidermophyton floccosum is reportedly a human pathogen but was isolated in this study (Plate IV). This is of significance showing a possibility of cross infections from humans to animals. Few studies on the isolation of *Epidermophyton* from animals are on record from work done by Efuntoye and Fashanu (2002) and Solans (1988).Philpot *et al.*, (1984) and Scott (1988) isolated *Epidermophyton floccosum* from goats along with a wide variety of other dermatophytes

including *M. gypseum*, *Trichophyton mentagrophytes*, and *T. verrucosum*, which were also isolated in this study.

More samples were isolated from markets than Fulani goat flock and rural homesteads. This is most likely because animals from different sources are pooled and housed close together, aiding the spread of the infection from infected to susceptible animals.

The ears yielded a higher number of positive isolates than any other anatomical site as seen on Table 4.5. Ellabib and Khalifa (2001) reported that the distribution of dermatophytes depends on the availability of keratin and this could explain why a large number of dermatophytes were isolated from the ears. The ear has thickened skin and is rich in keratinized tissues with minimal flesh and less hair. Also the ears are more vulnerable to contact and injuries predisposing them to infection by dermatophytes. Dermatophyte infections are known to be facilitated by breaks on the skin (Hainer *et al.*, 2003).

Skin samples were collected during both the rainy and dry seasons and the results showed a higher incidence of the infection in November followed by August and lastly January irrespective of the number of samples collected. The results show that because of the constant high temperatures and humidity in Gwagwalada, dermatophytes can readily be isolated and can cause disease at any time of the year, either rainy or dry seasons. This agrees with Scott

(1988)Macura,(1993) and Radostits *et al*(1997),who all stated that dermatophytosis is more common in tropical climates or areas having hot and humid climates.

Similarly there was a high incidence of the infection in adult goats than the young goats despite the fact that more young goats were sampled. This report disagrees with findings by Cam *et al* (2007) and Shams-Gahfarokhi *et al* (2009) who reported that young animals are particularly more susceptible to infection by ringworm fungi.This study shows that adult goats are equally susceptible to dermatophytes as young goats.This could due to the way most of the adult goats sampled were housed in close proximity to each other aiding the easy spread of dermatophytosis.

This study further showed a higher incidence of dermatophytosis in male goats than female goats.This could be due to the mating behaviour of male goats which makes them wander around more.Also male goats are more engaged in fighting which could lead to bruises that predisposes them to dermatophyte infection.

From the antifungal susceptibility test results, the methanol extract was the only extract that possessed an antifungal effect on all the isolates at the concentrations tested .The minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of the extract ranged from 62.5 mg/ml-500 mg/ml.Most of the isolates had the same MICs and MFCs with an exception of four isolates.This shows that the same concentration of an extract that can inhibit a dermatophyte can also kill it which is sign of a potentially effective antifungal agent.The

aqueous extract had a higher percentage yield than the methanol, hexane and ethyl acetate extract. Chang *et al* (1977) observed that the more polar the solvent, the better its extraction power. The seeds of *B. coriacea* are rich in phytonutrients such as alkaloids, glycosides, saponins, flavonoids, tannins and phenols both quantitatively and qualitatively (Ibrahim and Fagbohun, 2012).

The hexane extract of *B. coriacea* seed had an MIC and MFC that ranged from 62.5 to 500 mg/ml on *Microsporum audouinii*, *Trichophyton verrucosum* and *Trichophyton tonsurans* which were all isolated from goat markets. The ethyl acetate extract of *B. coriacea* seed had effect on two isolates of *T. tonsurans* with MICs and MFCs ranging from 62.5 to 500 mg/ml. This indicates that activity against the isolated dermatophytes decreased with decreasing polarity in extracts of *B. coriacea*. Despite the polarity and high percentage yield of the aqueous extract, it did not have any effect on the isolates. The variations in performance of the extracts on each test organism may be as a result of possible synergistic interactions between the active components in the extracts as well as biodiversity of the isolates.

In this study, *Microsporum* species were resistant to fluconazole but were susceptible to amphotericin B, nystatin, itraconazole and ketoconazole. Amphotericin B and nystatin are polyenes, a class of antifungal drugs usually indicated for superficial and systemic infections. It is well documented that amphotericin B is not effective against dermatophytosis (Verma and Heffernan, 2008; Hay and Ashbee, 2010; Sobera and Elewski, 2008).

The resistance seen in fluconazole and the other azoles, which are more commonly used to treat dermatophytosis, could be due to widespread use and misuse since they are relatively cheaper and more available than the polyenes (Shah *et al.*, 1988).

The one isolate of *Trichophyton mentagrophytes* was resistant to all the drugs tested. Some isolates of *T. verrucosum* and *T. tonsurans* also showed resistance to the conventional antifungal drugs. However most of *Trichophyton* isolates were still resistant to fluconazole and clotrimazole. It's interesting to note that same species of dermatophytes isolated from different sources showed different results with the different extracts used and also with the conventional antifungal drugs. This agrees with Fernández-Torres *et al.*, (2002) who stated that not all species have the same susceptibility patterns, and relative or absolute microbial resistance may occur in relation to some dermatophytes. This study has shown that differences in strains of the same species of dermatophytes affects the choice of drug used for treatment.

CHAPTER SIX

6.0 SUMMARY, CONCLUSION AND RECOMMENDATION

6.1 Summary

In this study, the incidence of dermatophyte infection from goats in Gwagwalada, F.C.T was found to be at 16.13%. The highest number of dermatophyte infections were isolated from markets (7.26%) followed by Fulani goat herds (5.64%) and finally rural households (3.23%). Males had more ringworm lesions (8.87%) than females (7.26%). More adults goats (10.48%) than young goats (5.65%) had ringworm lesions. Most lesions were found on the ears (8 out of 35), the udder and legs (4 out of 15 and 20 respectively), the face (9 out of 10) and the neck (1 out of 10). *Trichophyton* species were the highest isolated (13) followed by *Microsporum* (6) and *Epidermophyton* (1). *Trichophyton verrucosum* was the species with the highest isolates (6 out of 20) followed by *Trichophyton tonsurans* (5 out of 20), *Microsporum audouinii* (4 out of 20), *Microsporum gypseum* (2 out of 20). *Trichophyton ajelloi*, *Trichophyton mentagrophytes* and *Epidermophyton floccosum* had a frequency of 1 out of 20 each. All the isolates were susceptible to the methanol extract of *Buchholzia coriacea* with MICs and MFCs ranging from 62.5 mg/ml-500 mg/ml. The hexane extract had effect on only 3 isolates namely *Microsporum audouinii*, *Trichophyton verrucosum* and *Trichophyton tonsurans* with the MICs and MFCs ranging from 62.5 mg/ml to 500 mg/ml. The ethyl acetate extract has effect on only 2 isolates of *Trichophyton tonsurans* with MICs and MFCs within the same range. The aqueous extract had no effect on the isolates. Most of the isolates were susceptible to the range of conventional antifungal drugs used but there were cases of resistance to fluconazole and clotrimazole.

6.2 Conclusion

In this study, it has been established that a wide range of dermatophytes cause infections in goats in Gwagwalada area council, F.C.T, Abuja. All the dermatophytes isolated are potential risks to other animal species and humans who handle them as food animals, for recreational purposes and for sports. The methanol extract of *Buchholzia coriacea* seed showed *in vitro* antifungal activity against all the dermatophytes isolated while the hexane and ethyl acetate extracts showed

in vitro antifungal activity against only a few of the isolates. Thus the crude extracts of *Buchholzia coriacea* seed have a moderate effect against dermatophytes except the aqueous extract. Most of the dermatophytes isolated are susceptible to commercially available antifungal agents.

6.3 Recommendations

This study indicates that dermatophytosis is present in goats in Gwagwalada area council, F.C.T., Abuja. Goats are domestic animals that are very much a part of the average Nigerian homestead and a vital part of our economy and culture. It is therefore important that :

1. Aggressive extension veterinary services of the relevant departments of the F.C.T, Abuja is carried out to educate goat owners and farmers on the importance of dermatophytosis disease on their stock and its potential zoonotic effect.
2. Farmers and goat owners should always present their animals to the nearest veterinary clinics when ever they observe any abnormality in their goats.
3. Since there is no vaccine currently available against dermatophytosis, in the country, control should be directed towards improvement of simple management practises such as deworming, dipping and spraying and proper housing.
4. Antifungal susceptibility tests should be carried out on suspected cases of dermatophytosis to ensure the correct and proper drug with the best potential for effectiveness is used.
5. Further work is required to identify and isolate the antifungal components contained in the seeds of *Buchholzia coriacea*.
6. There is needed to further characterize the different strains of dermatophytes in the study area to ensure the correct treatment is used.

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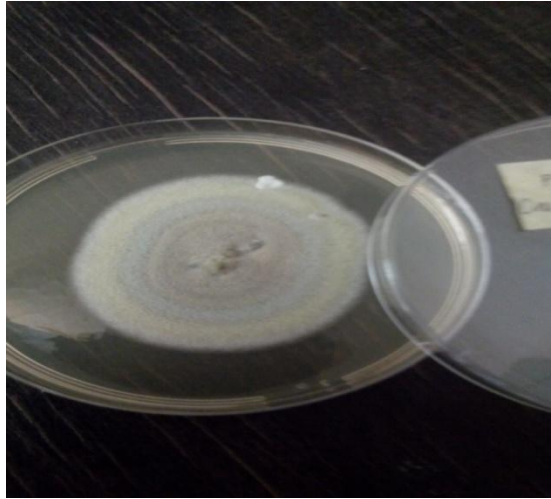
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APPENDICES

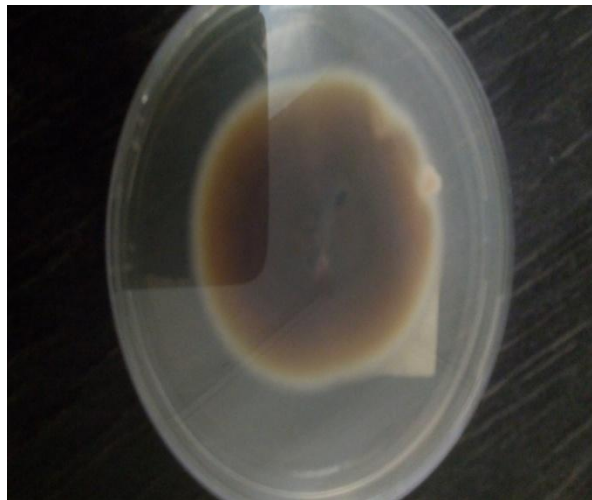
Appendix I: Plate showing flakey area of Alopecia on the Face of a Goat Sampled



Appendix II: Colonial Morphology of *Trichophyton tonsurans*



A: Colony growth (10days) on PDA

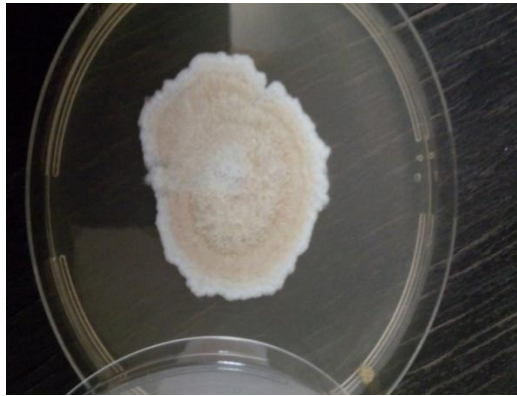


B: Reverse showing mahogany pigmentation

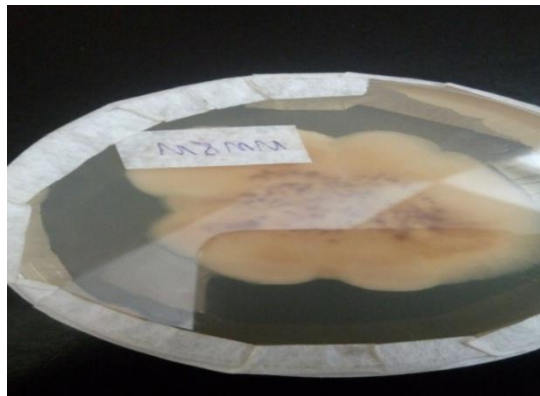
Appendix III: Microscopy of *Trichophyton tonsurans* with arrow pointed at distorted macroconidia (x400) LCB



Appendix IV: Colonial Morphology of *Microsporum gypseum*

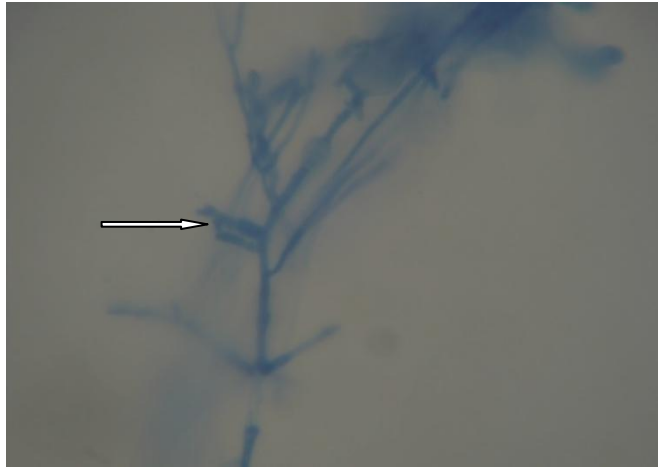


A: Colony on PDA (7 days)

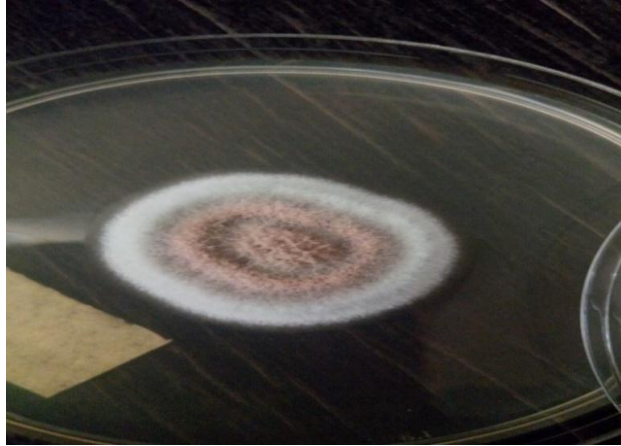


B: Reverse showing yellow brown pigmentation

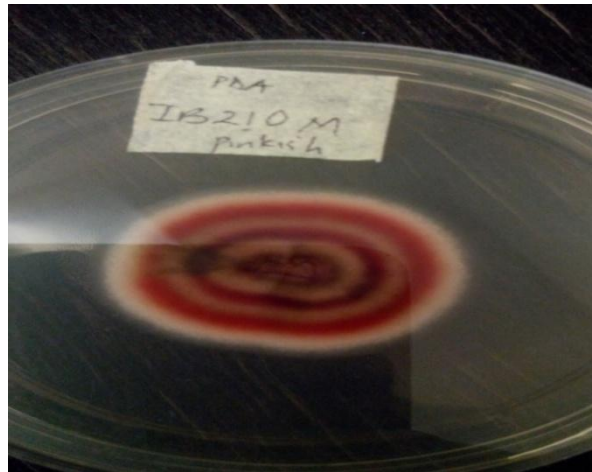
Appendix V: Microscopy of *Microsporium gypseum* with arrow pointing at macroconidia borne directly on the hyphae (x400, LCB stain)



Appendix VII: Colonial Morphology of *Trichophyton ajelloi*

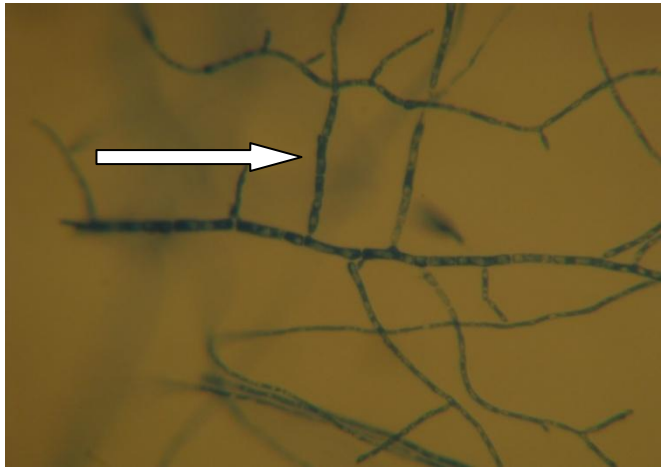


A: Colony after 9days growth on PDA

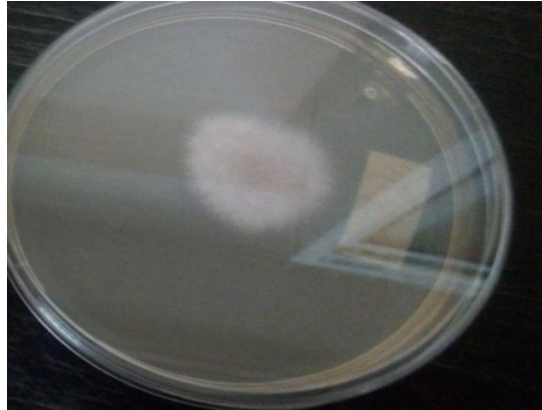


B: Reverse showing pinkish pigmentation

Appendix VIII: Microscopy of *Trichophyton ajelloi* with arrow pointing at many celled macroconidia (x400) LCB stain



Appendix IX: Colonial Morphology of *Trichophyton mentagrophytes*



A: Colony growth on PDA



B: Reverse showing yellow-pink color

Appendix X: Microscopy of *Trichophyton mentagrophytes* with arrow pointing at microconidia budding from the hyphae (x400), LCB stain

