STABILITY AND EFFICACY OF ANTI-DERMATOPHYTE CREAMS FORMULATED FROM SELECTED NIGERIAN MEDICINAL PLANTS

BY

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ABSTRACT

Dermatophytosis is a fungal infection caused by dermatophytes which invade the keratinised tissue of humans or animals. Reported cases of skin irritation and increased epidermal thickness associated with some existing antifungal drugs necessitate the search for more efficient alternatives to treat the infections. Herbal alternatives are currently being explored but their stability and efficacy have not been fully reported. The aim of this work was to evaluate the physicochemical properties, stabilities and efficacies of formulated medicinal herbal anti-dermatophyte creams.

The medicinal plants *Cassia alata* Linn, *Cassia occidentalis* Linn, *Mitracarpus villosus* Sw. Dc, *Kigelia africana* Jacq. and *Acalypha wilkesiana* Linn. were authenticated at the Herbarium of Nigeria Natural Medicine Development Agency, Lagos. The crude extracts were obtained with water, ethanol and petroleum ether separately using soxhlet extraction. Phytochemical analyses of the extracts were carried out by standard methods. Agar disc diffusion method was used to determine their antifungal activities against clinical isolates of *Microsporum audounii, Epidermophyton floccosum, Trichophyton mentagrophytes* and *Malassezia furfur*, all implicated in dermatophytic infections. Four cream formulations from triethanolamine, water, cetyl alcohol, steric acid and paraffin oil were subjected to colour, pH, spread of emulsion, rubbing-in effect and stability to centrifugation tests. The best formulation upon the determination of these characteristics was used as the cream base for anti-dermatophyte creams production. The formulated creams containing 0.5, 1.0, and 2.0% of the extracts were subjected to temperature stability test (-10, 4, 30, 37 and 45^{o}C) using standard methods. Changes in fungal morphology, skin irritability and efficacy tests using six groups of 5 Wistar rats were carried out and the effectiveness of the extracts in the formulated creams was determined by comparing with 1% clotrimazole cream as reference drug. Data were analysed using ANOVA at $\alpha_{0.05}$.

Alkaloids content was highest in *C. alata* (4.2%) and lowest in *A. wilkesiana* (0.6%). The saponins (2.1%) and flavonoids (1.6%) contents were highest in *A. wilkesiana* while the lowest was in *M.villosus* (0.9%) and (0.06%), respectively. The *C. alata* contained highest tannin (2.4%) while *M.villosus* the lowest (0.04%). Ethanol extracts of the plants showed the best anti-dermatophytic activities (9-19 mm zones of inhibition) at 10000 µg/mL. The formulated cream base was white with pH 6.9, very high spreadability of emulsion, high rubbing-in effect, and stable at 4,000 rpm for 30 minutes. The formulated creams were stable to freeze-thaw test at -10 and 45^{o}C, with pH range of 6.8 -7.3. There was no change in colour

or odour with the exception of *C. occidentalis* extract cream. The skin sensitivity test indicated no irritation. Skin biopsy efficacy test showed that the cream containing 2% *K. africana* extract significantly exhibited the best mycological inhibitory activity compared to 1% Clotrimazole in terms of changes in fungal morphology. The formulation prevented dekeratinization from 55.4±0.6 to 32.4±0.2µm of the hair follicle and also reduced the epidermal thickness from 90.3±0.2 to 51.4±0.4µm to achieve 71.9 and 75.7% efficacy, respectively.

The plant-based creams were stable and effective against dermatophytes. The clinical studies for their suitability for human use can be carried out.

Key words: Dermatophytes, Formulated cream, De-keratinisation, Skin diseases, Fungal morphology

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CERTIFICATION

I certify that this research work was carried out by Akinlolu Bankole **FAWEHINMI** under my supervision in the Department of Chemistry, University of Ibadan, Nigeria.

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ABBREVIATION

DMSO	Dimethyl sulphoxide
MIC	Minimum Inhibitory Concentration
MBC	Minimum Bactericidal Concentration
CCK	Cholecystokinin
D	Days
SD	Standard deviation

CHAPTER ONE

INTRODUCTION

1.1 Dermal creams

A dermal cream is a semi-solid emulsion prepared for topical application to the skin. Dermal creams have a lot of applications for a variety of skin conditions (dermatoses) (Günther *et al.,* 2005). They are emulsions of oil and water and can be oil-in-water (O/W) creams which are composed of small droplets of oil dispersed in a continuous

water phase or water-in-oil (W/O) creams which are composed of small droplets of water dispersed in a continuous oily phase and oil-in-oil creams (Leong *et al.,* 2009). Oil-in-water creams are more comfortable and cosmetically acceptable as they are less greasy and more easily washed off using water. They can be used to moisturize and deliver biologically active chemicals or protect the skin, from harmful effects of the environment. There are three types of dermal creams namely pharmaceutical, cosmetic and cosmeceutical.

Pharmaceutical cream or ointment is used as a means of delivering an active ingredient meant for treatment of a disease condition to the required area of the skin. Pharmaceutical preparations for treatment of conditions such as rashes, skin irritation, stings, fungal infections e.t.c are dispersed in either phase or added when the emulsion has been formed and allowed to cool (Tadros, 2005).

Cosmetic creams are products intended to be applied to the human body for cleansing, beautifying, promoting attractiveness, or altering the appearance without affecting the body's structure or functions (Marrakchi and Maibach, 2006).

Cosmeceuticals refer to creams that combine the functions of cosmetics and pharmaceuticals creams together. They are cosmetic products with biologically active ingredients which have medical benefits. Dermatological research suggests that the bioactive ingredients used in cosmeceuticals have benefits beyond traditional moisturising (Mason *et al., 2*006).

The correct use of dermal preparations falls into two parts namely skin care and decoration. The objective of skin care is the maintenance of a soft, supple and clean skin, and the prevention of effects due to causes such as excessive exposure to cold, heat, sun, wind and external or internal infections caused by microorganisms.

Dermal creams are used to produce a pleasing appearance by minimizing facial defects of colour or shape and unobtrusively enhancing and directing attention towards better points (Günther *et al.,* 2005).

1.2 Emulsion

An emulsion is a thermodynamically stable liquid consisting of an oil phase and an aqueous phase held together by an emulsifying agent (Mason *et al.,* 2006). There are

various types of emulsion. There is oil-in-water emulsion, water-in -oil emulsion and there can also be Oil-in-oil emulsion in which non polar liquid is finely dispersed in a less non polar liquid or vice – versa.

An emulsion is formed by simply applying external mechanical energy to a system which contains immiscible or partially miscible liquids. The mechanical energy will break down the two phases, dispersing one in the other, in the form of very fine droplets (Weller *et al*., 2008). When the emulsion is formed, the interfacial surface energy of the system will rise enormously, and the system will in the absence of external influences occupy the lowest energy state. Once the mechanical energy is removed, the emulsion in the absence of any other stabilising force will break down into its oil and water phases very quickly with passage of time. This is due to free surface energy of the system which is directly proportional to the interfacial area between the oil and water phases as described by the following equation:

$$\delta S = K\delta A \qquad \text{eqn 1}$$

Where S is the surface energy and A is the surface area (Esquena and Vilasau, 2013).
K is the proportionality constant.

Emulsions are used in many chemical industries. In the pharmaceutical industry, they are used to make medicines more palatable, to improve effectiveness by providing improved aesthetics for topical drugs such as ointment (Tadros, 2005).
In the agricultural industry, emulsions are used as delivery vehicles for insecticides, fungicides and pesticides. These water insoluble biocides must be applied to crops at very low levels, usually by spraying through mechanical equipment. Emulsion technology allows these chemicals to be effectively diluted and provides improved spray ability (Weller *et al*., 2008). In cosmetics, emulsions are the delivery vehicle for many hair and skin conditioning agents. Emulsions are used to deliver various oils and waxes which provide moisturization, smoothness and softness to hair and skin (Anchisi*et al*., 2001). Many food products are in the form of emulsions. An example of a naturally occurring food emulsion is milk which contains globules of milk fat (cream) dispersed in water (Bruno*et al*., 2010).

1.2.1 Analysis of emulsions

Some of the parameters used in analysing emulsion include: Particle size distribution, Specific gravity/ density and viscosity.

1.2.1.1 Particle size distribution

Particle size analysis gives useful information about the structure and stability of emulsions, which are important characteristics of these systems. It also enables the observation of the growth process of particles dispersed in emulsions (Rousi*et al*., 2014).

1.2.1.2 Specific gravity/Density

This is defined as the ratio of the mass of a given substance to the mass of an equal volume of water at a specific temperature. It is very important because if density differences of the phases are too large, the emulsion breaks and if they are close, the emulsion is stable. Density will depend on whether product is lotion or cream and v/v ratio of aqueous and oily phase.

1.2.1.3 Viscosity

Viscosity is one of the most important parameters that are measured in emulsions. It helps to ensure processabililty and product stabililty. It helps to determine whether an emulsion is milk, lotion or cream. When the viscosity is too high, there could be difficulty in the flow of the emulsion and spreadability. When it is on the low side, it could be an indication of phase reversion. However, cleansing lotions and emulsions of oil-in-oil are known to have low viscosity.

1.2.2 Emulsion Instability

Emulsions are inherently unstable. The instability can be caused by: Flocculation, creaming, sedimentation, Ostwald ripening, coalescence, phase inversion,

1.2.2.1 Flocculation

Flocculation occurs when there is no sufficient repulsion to keep the droplets apart to distances where the Van der Waals attraction is weak. Flocculation may be strong or weak depending on the magnitude of the attractive energy involved (Rousi *et al.,* 2014).

1.2.2.2 Creaming and sedimentation

The oil phase is usually less dense than the water phase. This density difference will, overtime, cause a migration of the dispersed phase particles within the continuous phase eventually resulting in partial phase separation (Esquena and Vilasau, 2013).

In an oil-in-water emulsion, the dispersed oil-phase droplets, being less dense than the continuous water phase, experience a resultant upward velocity, eventually producing an 'oil-rich' region towards the surface of the emulsion. This is known as creaming. Conversely, for a water-in-oil emulsion, the dispersed water-phase droplets exhibit a tendency to fall under gravity towards the bottom of the emulsion, a phenomenon normally referred to as sedimentation (Anne-Marie, 2008).

Therefore creaming and sedimentation describe the movement of the dispersed phase particles within the continuous phase. Creaming is only relevant to oil-in-water systems while sedimentation is relevant to water in-oil emulsions (Anne-Marie, 2008).

The rate at which either creaming or sedimentation occurs is determined by quantifying the velocity of the dispersed phased particles in the continuous phase. This velocity is described by Stoke's Law in the equation below:

$$V = \frac{2r2(\sigma\rho)}{9\eta} \qquad \text{eqn 2}$$

Where V is the falling velocity of dispersed phase droplet
r is the radius of dispersed phase droplet

σ is the density of the dispersed phase

ρ is the density of the continuous phase

g is the gravitational force

and η is the viscosity of the continuous phase.

Examination of the Stoke's Law equation predicts maximum emulsion stability for the following conditions; Minimization of the dispersed phase particle size; Reduction of the density difference between the two phases; Maximization of the continuous phase viscosity.

1.2.2.3 Ostwald ripening (Disproportionation)

This results from the finite solubility of the liquid phases. Liquids that are referred to as being immiscible often have mutual solubilities that are not negligible.Therefore in an emulsion; the smaller droplets will have larger solubilities when compared with the larger ones (due to culvature). With time, the smaller droplets disappear and their molecules diffuse to the bulk and become deposited on the larger droplets (Weller *et al*., 2008). It is Ostwald ripening that leads to coalescence.

1.2.2.4 Coalescence

This refers to the process of thinning and disruption of the liquid film between the droplets with the result of fusion of two or more droplets into larger ones, so the average droplet size increases over time due to reduced total surface area. The limiting case for coalescence is the complete separation of the emulsion into two distinct phases (Bruno*et al*., 2010).

The stability of emulsions can be characterized using techniques such as light scattering, focused beam reflectance measurement, centrifugation, and rheology. Each method has advantages and disadvantages.

1.2.2.5 Phase inversion

This refers to the process whereby there will be an exchange between the disperse phase and the medium. An oil-in-water emulsion may with time or change of condition invert to water-in-oil emulsion this may be caused by sudden changes in volume fraction as a result of drying out or mistakenly adding some chemicals or wrong use of emulsifier. In many cases, phase inversion passes through a transition state whereby multiple emulsions are produced.

1.2.3 Stability Testing

Stability testing is integral to developing new cosmetic products and to establishing their shelf life or expiry date. Some of the factors that affect the stability of an emulsion include: Electrical charge, viscosity of the continuous phase, ratio of oil phase to water phase, temperature, concentration of ions in the water phase. Therefore, stability testing includes temperature testing, cycle testing, centrifugation, pH, colour, odour and light (Esquena and Vilasau 2013).

1.2.3.1 Temperature

The determination of stability of cosmetic products are done by monitoring of samples stored at low (4°C), ambient and high (+35 to +50°C) temperatures for a period of up to six months, depending on the temperature. A good control temperature is the 4^{o}C. Most products will exhibit excellent stability at this temperature and thus the product can be compared to the high temperature samples to ascertain if any changes have taken place.

1.2.3.2 pH

Monitoring the pH value is important for determining the emulsions' stability because pH changes indicate the occurrence of chemical reactions that can compromise the quality of the final product. Emulsions produced with vegetable oils may experience a decrease in pH due to the hydrolysis of fatty acid esters into free fatty acid degradation products (Martini, 2005).

1.2.3.3 Centrifugation

The dispersed phase of an emulsion has a tendency to separate and rise to the top of the emulsion which is a first sign of impending emulsion instability and should be taken quite seriously. A good test method to predict creaming is centrifugation whereby the emulsion is heated to 50^{o}C and centrifuged for thirty minutes at 3000 rpm and then inspected for any sign of separation.

1.2.3.4 Freeze thaw cycle Testing

This stability test puts the emulsion under a tremendous stress as the product is frozen and then thawed out at room temperature. The emulsion droplets are deformed and try to break due to the swing in temperature. The test involves three cycles of placing the emulsion at -10^{o}C for 24 hours and then at room temperature for 24 hours.

1.2.3.5 Light Testing

This test is used to determine the sensitivity of the emulsion to Ultra Violet radiation for any major discolouration. There is also the possibility of oxidation taking place catalytically, change or degradation of constituents or even other reactions between contents which are mostly mixtures initially when the product is exposed to UV light. The emulsion is placed in test tubes and also in the actual packaging. The container is then put on the window sill where direct sun rays fall on them.

1.3 Dermatophytes

Dermatophytes are fungi that require keratin for growth. These fungi can cause superficial infections of the skin, hair, and nails (Ghannoum *et al*., 2010). Dermatophytes are spread by direct contact from other people (anthropophilic), animals (zoophilic), and soil (geophilic) (Kyle and Dahl, 2004).

1.4 Drugs for Treatment of Skin Diseases

Skin diseases can be treated through the use of synthetic chemical derivatives or by using medicinal plants.

1.4.1 Synthetic Chemical and Derivatives :Some synthesized chemicals are used to treat skin diseases associated with fungal infections. They include derivatives of azoles, naphthoquinones, aminosterol and morpholine. There have been reported cases of resistance by the dermatophytes with prolonged use on the skin (Phillips and Rosen, 2013).

1.4.2 Medicinal Plants

The use of medicinal plants to treat diseases is almost universal among non-industrialized societies and is often more affordable than purchasing modern pharmaceuticals. Medicinal plants have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions, and to defend against attack from predators such as insects and fungi. The metabolites discovered naturally in medicinal plants may avoid the side effect of synthetic drugs, (Weller *et al*., 2008).

Many plants synthesize substances that are useful to the maintenance of health in humans and other animals. These include aromatic substances, most of which are phenols or their oxygen-substituted derivatives such as tannins. Many are secondary metabolites, of which at least 12,000 have been isolated; a number estimated to be less than 10% of the total. In many cases, these substances (particularly the alkaloids) serve as plant defence mechanisms against predation by microorganisms, insects, and herbivores. Many of the herbs and spices used by humans to season food yield useful medicinal compounds (Dan-Bensky *et al*., 2004).

So many plants came to the attention of researchers because of their use in traditional medicine. Out of about 120 active compounds currently used in modern medicine today, over 80 percent gave a positive correlation between the traditional and modern

therapeutic use of the plants. It was reported that about two thirds of the world's plant species with medicinal value come from the developing countries (Lawal *et al.,* 2012).

Plants have always been a rich source of lead compounds such as morphine, cocaine, digitalis, quinine, tubocurarine, nicotine, and muscarine. Many of these lead compounds are useful drugs in themselves (e.g. morphine and quinine), and others have been the basis for synthetic drugs (e.g. local anesthetics developed from cocaine). Clinically useful drugs which have been recently isolated from plants include the anticancer agent paclitaxel (Taxol) from the *Taxus baccata*, and the anti-malaria agent artemisinin from *Artemisia annua* (Newman *et al.*, 2007).

1.5 Justification

There have been reported cases of skin irritation and increased epidermal thickness associated with some existing antifungal drugs. This necessitate the search for more efficient alternatives to treat dermatophytic infections. There is also reported cases of non availability and high cost of new generation antibiotics and antifungals which are not available to developing countries. The resistance of many pathogens to synthetic drug therapies can not be over-emphazised. Natural products found in plants seem to avoid toxicity associated with long-term treatment with synthetic antifungal drugs.

1.6 Aim of research study

This study is aimed at formulating antifungal herbal creams from extracts of *Kigelia africana, Cassia alata, Mitracarpus villosus, Cassia occidentalis* and *Acalypha wilkesiana* , evaluate their physicochemical properties, stability and determine their efficacy using albino rats as model experimental animals.

1.7 Specific objectives

The objectives of this study are:

1. Collection and identification of *Cassia alata, Cassia occidentalis, Acalypha wilkesiana, Mitracarpus villosus* and *Kigelia africana*.
2. Obtain Crude Extracts of the medicinal plants using Water, Ethanol and Petroleum ether.

3. Carry out phytochemical screening and antifungal properties of the extract.
4. Formulate cosmetic creams, carry out FT-IR analysis of plant extracts, emulsion and cosmeceuticals.
5. Determine the stability of the produced cosmeceutical creams.
6. Determine the efficacy of antifungal creams using animal model experiment.

CHAPTER TWO

REVIEW OF LITERATURE

2.1 Human Skin

The skin is the largest organ in the human body. It has a surface area of between 1.5-2.0 square metres (16.1 - 21.5 sq ft.), most of it between 2 – 3 mm (0.10 inch) thick. The average square inch (6.5 cm^2) of skin holds 650 sweat glands, 20 blood vessels, 60000 melanocytes, and more than 1000 nerve endings (Felipe *et al.,* 2009). It is the loose, elastic, protective outer covering of the body and is made up of cells and tissues. The nails, hair, oil glands and sweat glands are appendages of the skin.

The skin has multiple layers of ectodermal tissue and guards the underlying muscles, bones, ligaments and internal organs. Human skin is similar to that of most other mammals (Marks *et al.,* 2006). Skin plays an important immunity role in protecting the body against pathogens and excessive water loss (Proksch *et al*., 2008). Its other functions are insulation, temperature regulation, sensation, synthesis of vitamin D, and the protection of vitamin B folates (Madison, 2003).

2.1.1 Skin layer

Skin is composed of three primary layers which are the epidermis, dermis and the hypodermis.The epidermis is the outermost layer of the skin. It forms the waterproof, protective wrap over the body's surface which also serves as a barrier to infection and is made up of stratified squamous epithelium with an underlying basal lamina (Stücker *et al.,* 2002). There are no blood vessels in the epidermis, and its cells are nourished by diffused oxygen from the surrounding air and also by blood capillaries which extend to the upper layers of the dermis (Felipe *et al.,* 2009). The main type of cells which make up the epidermis are: Merkel cells, keratinocytes, melanocytes and Langerhans cells. The keratinized layer of skin is responsible for keeping water in the body and keeping other harmful chemicals and pathogens out, making skin a natural barrier to infection (Wilkinson and Millington, 2009).

The dermis is the layer of skin located beneath the epidermis. There is a basement membrane which tightly connects the dermis to the epidermis. The dermis also have many nerve endings which provide the sense of touch and heat. The sebaceous glands, apocrine glands, hair follicles, sweat glands, lymphatic vessels and blood vessels are all harboured in the dermis. Nourishment and waste removal in the dermis are carried out from its own cells and also from the stratum basale of the epidermis. The dermis is

composed of two areas namely the papillary region and the reticular region (Osinski *et al.,* 2009).

The hypodermis lies below the dermis. Its purpose is to attach the skin to underlying bone and muscle as well as supplying it with blood vessels and nerves. It consists of loose connective tissue, adipose tissue and elastin. The main cell types are fibroblasts, macrophages and adipocytes (the hypodermis contains 50% of body fat). Fat serves as padding and insulation for the body (Ryman-Rasmussen *et al.,* 2006).

2.1.2 Hygiene and Skin care

The skin supports its own ecosystems of microorganisms, including yeasts and bacteria, which cannot be completely removed by any amount of cleaning. The human skin is a rich environment for microbes. Around 1000 species of bacteria from 19 bacterial phyla have been found. Most come from only four phyla: Actinobacteria (51.8%), Firmicutes (24.4%), Proteobacteria (16.5%), and Bacteroidetes (6.3%). Propionibacteria and Staphylococci species are the main species in sebaceous areas (Grice *et al.,* 2009, Pappas, 2009). It has been estimated that the number of individual bacteria on the surface of one square inch (6.5 square cm) of human skin is about 50 million. This figure varies greatly over the average 20 square feet (1.9 m^2) of human skin. It was reported that oily surfaces may contain over 500 million bacteria per square inch (6.5 cm^2). The microorganisms keep one another in check and are part of a healthy skin. When the balance is disturbed, there may be an overgrowth and infection (Osinski *et al.,* 2009).

2.2 Review of Cosmetics

The history of cosmetics spans over a long period of years and is present in almost every society on earth (Power, 2010). Archaeological evidence of cosmetics dates from ancient Egypt and Greece. Early major developments include the use of castor oil in ancient Egypt as a protective balm and skin creams made of beeswax, olive oil, and rosewater. Men and women in Egypt use scented oils and ointments to clean and soften their skin and mask body odour. Cosmetics are an integral part of Egyptian hygiene and health. Oils and creams are used for protection against the hot Egyptian sun and dry winds (Watts, 2010). Olson (2009) reported that some women in ancient Rome invented make up including lead-based formula, to whiten the skin, and kohl was used to line the eyes. Kohl was used as black powder or smeared to darken the edges of the eyelids in

the Arab world (Oumeish, 2001). Cai (2008) said that Chinese people stained their fingernails with beeswax, gum arabic, gelatin and egg white from around 3000 BCE with the colours used representing social class. It was reported that colour gold and silver were synonymous with the Chou dynasty; later royals wore black or red while lower classes were forbidden from wearing bright colours on their nails (Power, 2010)

Lee Shu-Yueh and Naeemah Clark (2015) reported that there are so many African Americans who participated in skin bleaching in their effort to lighten their complexion and also straighten the hair to appear whiter. Skin bleaches contain hydroquinone which is known to suppress the production of melanin in the skin.

Although modern cosmetics have been used mainly by women traditionally, gradually an increasing number of males are using cosmetics usually associated with women to enhance their own facial features. Cosmetics brands are releasing cosmetic products especially tailored for men who use such products increasingly (Loden, 2003).

2.3 Review of Traditional Medicine

Traditional medicine knowledge systems developed over generations long before the advent of the era of modern medicine. The World Health Organization (WHO) defines traditional medicine as "the sum total of the knowledge, skills, and practices based on the theories, beliefs, and experiences indigenous to different cultures, whether explicable or not, used in the maintenance of health as well as in the prevention, diagnosis, improvement or treatment of physical and mental illness (WHO, 2008).

World Health Organisation reported that in some Asian and African countries, up to 80% of the population relies on traditional medicine for their primary health care needs. Practices known as traditional medicines include Ayurveda, Siddha medicine, Unani, ancient Iranian medicine, Irani, Islamic medicine, traditional Chinese medicine, traditional Korean medicine, acupuncture, Muti, Ifá, and traditional African medicine.

Traditional medicine may include formalized aspects of folk medicine, that is to say longstanding remedies passed on and practised by lay people. Folk medicine consists of the healing practices and ideas of body physiology and health preservation known to some in a culture, transmitted informally as general knowledge, and practiced or applied by anyone in the culture having prior experience (Acharya *et al.,*2008). Folk

medicine may also be referred to as traditional medicine, alternative medicine, indigenous medicine, or natural medicine.

In the written record, the study of plants dates back 5000 years to the ancient Sumerians, who described well-established medicinal uses for plants (Ansari and Inamdar, 2010). In Ancient Egyptian medicine, a list of folk remedies and magical medical practices were recorded in the Ebers papyrus from 1552 BC (Girish and Shridhar, 2007).

Acharya *et al*., (2008) reported that ancient Indian herbalists such as Charaka and Sushruta chronicled many plants and minerals used in the practice of Ayurveda. During the Han Dynasty, the first Chinese herbal book was written by Shennong Bencao Jing which was later augmented as the Yaoxing Lun (Treatise on the Nature of Medicinal Herbs) during the Tang Dynasty. Pythagoras was the early recognised Greek compiler of existing and current herbal knowledge together with his followers which include Hippocrates, Aristotle, Theophrastus, Dioscorides and Galen (David, 2003).

2.4 Dermatophytes

Dermatophytes are classified as anthropophilic (humans), zoophilic (animals) or geophilic (soil) according to their normal habitat.

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

Zoophilic organisms are found primarily in animals and cause marked inflammatory reactions in humans who have contact with infected cats, dogs, cattle, horses, birds, or other animals. This is followed by a rapid termination of the infection.

Geophilic species are usually recovered from the soil and occasionally infect humans and animals. They cause a marked inflammatory reaction, which limits the spread of the infection and may lead to a spontaneous cure but may also leave scars (Rivera *et al*., 2012).

2.5 Dermatophytosis

This is a skin disease condition caused by dermatophytes. Dermatophytes cause infections of the skin, hair and nails due to their ability to obtain nutrients from keratinized material. These anamorphic (asexual or imperfect fungi) genera are:

Microsporum, Epidermophyton and *Trichophyton*. Dermatophytosis can be identified by the appearances of enlarged raised red rings with a central area of clearing (ringworm). The ringworm may also occur on the groin (Tinea cruris), scalp *(Tinea capitis*) or beard area (*Tinea barbae*). (Lippens *et al*., 2009). *Tinea corporis* is the infection of dermatophytes on the arm and legs. In some cases there is an appearance of elevated rashes which is scaly to touch. At times the rash results in dry and flaky skin which invariably may lead to hair loss in areas of the infection (Lippens *et al*., 2009). In their own report, James *et al.,* (2005) observed that these dermatophytes naturally exist on the superficial skin surface and can induce a rash or infection when the opportunity is right. Individuals at high risk of acquiring ringworm include those who: Live in crowded, humid conditions, sweat excessively, participate in close contact sports like soccer, rugby, or wrestling, wear tight, constrictive clothing and have a weakened immune system (e.g., those infected with HIV or taking Immuno-suppressive drugs).

2.5.1 Identification

Lippens *et al.,* (2009) described a quick technique which can be done by scraping of the skin, nail, or scalp. They reported that among the epithelial cells there are unique characteristic hyphae which are visible. The causative agent of *Tinea capitis*, *Trichophyton tonsurans*, is usually seen as solidly packed arthrospores. It is found within the broken hair shafts scraped from the scalp. For the dermatophyte to be positively identified, fungal culture medium is used in which fungal growth is noted in 5 to 14 days. The most reliable identification character is to identify microscopic morphology of the micro and macro conidia, however a good slide preparation and stimulation of sporulation in some strains is also needed. Though characteristics such as topography and pigmentation are sometimes used but results obtained are not always reliable criteria for identification.

Fitzpatrick *et al.,* (2005) reported that in identifying rare non-sporulating species like *Trichophyton concentricum, Microsporum aoudini* and *Trichophyton schoenleinii,*clinical information such as geographic location, appearance of the lesion, travel history, site, animal contacts and race are also very important.

In order to grow and identify dermatophytes, a special agar called Dermatophyte Test Medium (DTM) has been formulated through which the dermatophyte is identified by a

simple color test without looking at the colony, the hyphae, or the macroconidia. To identify the dermatophyte, the specimen (scraping from skin, nail, or hair) is embedded in the DTM culture medium and is incubated at room temperature for about 10 to 14 days. If there is colour change to bright red, this indicates that the fungus is a dermatophyte and no colour change indicates that the fungus is not a dermatophyte. Keeping the test beyond 14 days may likely give false positive result (Fitzpatrick *et al.,* 2005)

2.5.2 Transmission

Dermatophytes are transmitted by direct contact with infected host (human or animal) or by direct or indirect contact with infected exfoliated skin or hair in clothing, combs, hair brushes, theatre seats, caps, furniture, bed linens, shoes, socks, towels, hotel rugs, sauna, bathhouse, and locker room floors (James *et al.,*2005). There seems to be an increased susceptibility to infection when there is a pre-existing injury to the skin or presence of excessive humidity and high temperature. When most geophilic species tries to adapt to growth on humans, this resulted in sexuality, diminished loss of sporulation and other soil-associated characteristics (Jain *et al.,* 2011).

2.5.3 Management of dermatophytosis

There are three ways through which skin diseases can be managed. These include hygienic approach, orthodox medical approach, and the traditional herbal approach.

Good hygienic practices are usually advocated such as daily baths so as to prevent the proliferation of organisms causing skin diseases. Traditional Medicines' herbal approach involves the use of medicinal plants in the fight against the microorganisms causing skin diseases (De-Benedetto *et al.,* 2009).

In the orthodox medical approach, synthetic chemicals such as creams bases, antibiotics and steroids are widely employed (Brakhage, 2005). The immuno-suppressants tacrolimus and pimecrolimus are used as topical preparation in the treatment of severe atopic dermatitis (De-Benedetto *et al.,* 2009). Also, in most cases, treating the infection requires application of topical antifungal creams to the skin. However, extensive or difficult to treat cases may require systemic treatment with oral medication. Topical antifungals are applied to the lesion twice a day for at least 3 weeks. The most commonly used antifungal creams are clotrimazole, ketoconazole, miconazole,

terbinafine, tolnaftate, and butenafine. In general, dermatophytosis responds well to topical treatment (Lippens *et al.,* 2009).

Some plants have been identified as a result of antimicrobial tests carried out on them, and several have been suggested for the management of dermatophysis. Some of these plants include *Mitracarpus villosus* (Okogun *et al.,* 2005), *Cassia alata* (Okafor *et al.,* 2001, Orafidiya *et al.,* 2002) *Cassia occidentalis* (Muyibi *et al.,* 2002), *Acalypha wilkesiana* (Adesina *et al*., 2000), *Kigelia africana* (Agyare *et al.,* 2013) *Jatropha species, Celosia argentaeae, Cnestis spp, Rauwolfa vomitora, Terminalia catapa, Vernonia amygdalina, Vitex doniana* and *Afromomium melegueta*. Several scientific researchers have recommended the use of these plants as cheap and available antibiotic raw materials for topical and systemic application as reported by Kathirvel and Sujatha, 2011.

2.6 Dermatophytes used in this study

Dermatophytes used in this study include *Microsporum aoudini, Epidermophyton floccosum, Trichophyton mentagrophytes* and *Malassezia furfur.*

2.6.1 *Microsporum aoudini*

In the 19th and early 20th centuries, it was reported that the main cause of *Tinea capitis* throughout the US and Western Europe was *M. aoudini* (Maggenti *et al*., 2005). However in the poorer parts of Africa such as Central and West Africa, *M. aoudini* has been reported to be the primary dermatophyte responsible for this disease (Rezusta *et al.,* 2011). It has been observed that the recent increase in the *M. aoudini*-related tinea capitis in Europe may be related to intercontinental migration of people infected with the disease from Africa (Jain *et al.,* 2011).

Microsporum aoudini is an anthropophilic fungus that causes *Tinea capitis* (Roque *et al.,* 2006). Maggenti *et al*., (2005) reported that skin diseases are usually found in young children and rarely affect adults. Lippens *et al.,* 2009 reported that the differences in the chemical composition and quantity of the triglycerides in hair sebum are the reasons why children are more susceptible to *M. audouinii*. However when there is a decrease in the triglyceride content of the sebum, there is also a decreases in the susceptibility of a person to the fungus (Fitzpatrick *et al.,* 2005).

However, Gupta *et al.,* (2008) and Narang *et al*., (2012) reported that infections in adult could be as a result of immune-compromised conditions such as diabetes mellitus, systemic lupus, organ transplant and HIV. They also noted that hormonal changes in postmenopausal women and animal husbandry can cause the infection in adults. Arenas *et al*., (2010), reported that where there is high immigration especially in tropical regions there is tendency of tinea capitis caused by *Microsporum aoudini* which has greatly been reduced with the advent of antimycotic agents. In general, the infection is spread is by poverty, poor hygiene and overcrowding (Gupta *et al.,* 2008).

Jain *et al*., (2011) reported that primary treatment involves griseofulvin an antimycotic agent and other drugs such as Itraconazole, fluconazole, and terbinafine. These agents have been reported to have drug-drug interactions and may cause liver damage over a period of time (Coulibaly *et al.,* 2013).

2.6.2 *Epidermophyton floccosum*

Havlickova*et al*., (2008) examined 1,305 school children, aged 3-15 years, from 21 schools located in the inner city of Barcelona and found that the infection rate caused by epidermophyton floccosum was 0.23%, distributed among the schools. Kyle*et al*., 2004 carried out studies screening 4,601 children with an average age 10.7 ± 0.16 years, and found that boys with *Tinea capitis* caused by epidermophyton floccosum were much higher than girls (63.7% and 36.3% respectively).

2.6.3 *Trichophyton mentagrophytes*

According to Rivera *et al.,* (2012), *T. mentagrophyte* has at least five different variants which make up the *Trichophyton mentagrophytes* complex. They reported that the organism has variable characteristics with anthropophilic form producing sparse aerial mycelium with numerous spores. The zoophilic isolate produces powdery or granular colonies. Microscopically the most consistent feature of *T. mentagrophytes* is the production of globose microaleurio spores arranged in grape-like clusters.

Havlickova *et al.,* (2008) explained that *T. mentagrophyte* assimilates phosphorus, potassium, sodium and calcium. It utilizes methionine but is inhibited by folic acid. They said the organism has been recovered from a variety of sources such as soil, floor of swimming pools, hairs of wild boar, cats and dogs, farm animals, foot wears, shower stalls and from human toe webs.

T. mentagrophytes breaks down keratinous substrates by both chemical and mechanical ways. Five different keratinolytic enzymes from ten strains of *T. mentagrophytes* have been isolated. These enzymes are known to play a role in pathogenesis of infections caused by this organism in both humans and animals (Oyeka, 2000).

2.6.4 *Malassezia furfur*

Malassezia spp. are dimorphic fungi which can exist both as mold and yeast. They are found on all skin surfaces in humans and animals as commensal and pathogenic organisms, and are counted among the basidiomycetous fungi, such as some *Cryptococcus* spp., *Rhodotorula* spp.and *Trichosporon* spp., which are potentially pathogenic to humans and animals (Cabañes, 2014).

Akaza *et al*., (2010) demonstrated by both the culture and non-culture method that among the *Malassezia* species, *M. restricta* predominated on the face and *M. globosa* and *M. dermatis* on the upper trunk in Japanese healthy subjects

Saunders *et al*., 2012, reported that *Malassezia* is preferentially found in seborrheic areas, as attested by the fact that most *Malassezia* spp. are lipid dependent. Due to their inability to synthesize $C_{14} - C_{16}$ saturated fatty acids. *Malassezia* spp. obtain the necessary lipids by hydrolysis of sebum triglycerides using a lipase gene product transcribed from the *Malassezia* genome. *Malassezia* is the only free-living fungus known to lack a fatty acid synthase gene.

Tragiannidis *et al.,* 2010*,* reported that *M. furfur* can contribute to nosocomial outbreaks in neonatal intensive care units and sporadically in severely immunocompromised patients.

2.7 Bioactive compounds found in plants

Most bio-active substances have been found very useful pharmacologically. They are usually large molecules with many side groups which can be variously substituted thus allowing a wide range of possible types of molecules. Many of them are for this reason, the end products of metabolic pathways. The bioactive constituents are classified as lipid, terpenoids, glycosides, phenol and related compounds, alkaloids, essential oil, flavonoids, hydroxyl acids e.t.c. They change with respect to seasons, age of the medicinal plants, time of collection and also due to climate and the type of soil. (Bruno *et a.l,* 2010).

2.7.1 Lipids

Pichon*et al.,* (2006), described lipids (Fig.1) as a group of naturally occurring molecules that include fats, waxes, sterols, fat-soluble vitamins (such as vitamins A, D, E, and K), monoglycerides, diglycerides, triglycerides, phospholipids, and others. The main biological functions of lipids include storing energy, signaling, and acting as structural components of cell membranes, (Subramaniam *et al.,* 2011). Lipids have applications in the cosmetic and food industries as well as in nanotechnology (Mashaghi *et al.,* 2013).

2.7.2 Terpenoids

Ayoola (2008) reported that plant terpenoids are used extensively for their aromatic qualities and they play a role in traditional herbal remedies. Well-known terpenoids include citral, menthol, camphor, the cannabinoids found in cannabis, ginkgolide and bilobalide found in *Ginkgo biloba*, and the curcuminoids found in turmeric and mustard seed.

2.7.3 Flavonoids

Flavonoids are plant-based compounds with powerful antioxidant properties found in many fruits and vegetables like blueberries and grapes. They serve a variety of functions such as protecting blood vessel walls in people who have heart disease or diabetes, alleviating allergies, protecting brain health against dementia and even preventing some cancer (De Sousa *et al.,* 2007).

Flavonoids have been shown to have a wide range of biological and pharmacological activities in in-vitro studies. These include anti-allergic (Yamamoto and Gaynor, 2001), anti-inflammatory (Cazarolli *et al.,* 2008; Yamamoto and Gaynor, 2001), antioxidant, antibacterial, (Cushnie and Lamb 2011), antifungal and antiviral (Cushnie and Lamb 2005; Friedman 2007), anti-cancer (Cazarolli *et al*., 2008; De Sousa *et al.,* 2007) and anti-diarrheal activities (Schuier *et al.,* 2005). Flavonoids have also been shown to inhibit topoisomerase enzymes (Esselen *et al.,* 2009; Bandele *et al.,* 2008) and to induce DNA mutations in the mixed-lineage leukemia (MLL) gene in in vitro studies (Sahar *et al .,* 2007).

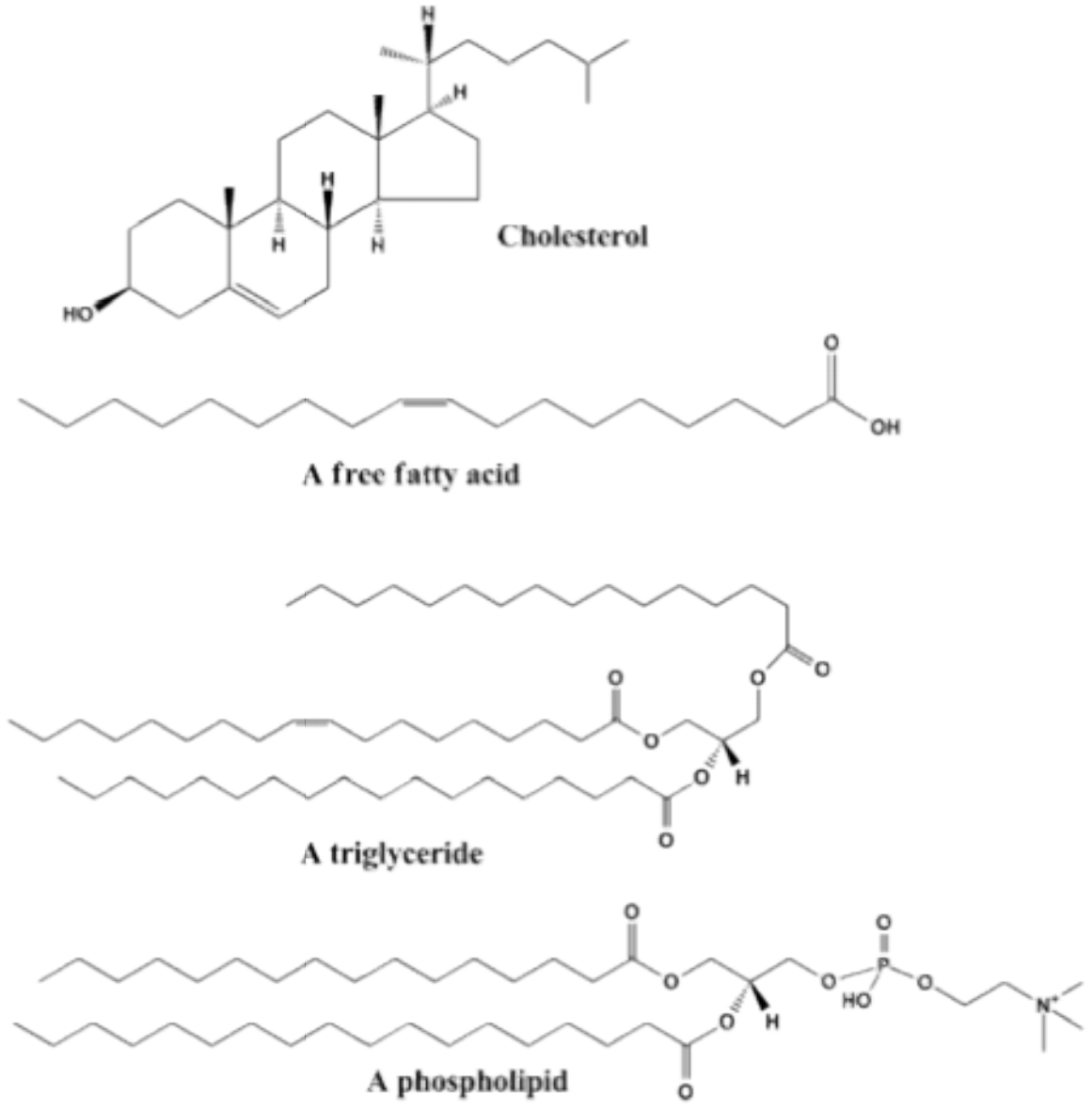


Fig.1 Some Lipids

Source- Chemolink.com

2.7.4 Alkaloids

Alkaloids are a group of naturally occurring chemical compounds that contain mostly basic nitrogen atoms. This group also includes some related compounds with neutral and even weakly acidic properties (Kittakoop *et al.,* 2014). In addition to carbon, hydrogen and nitrogen, alkaloids may also contain oxygen, sulfur and, more rarely, other elements such as chlorine, bromine, and phosphorus and generally occur as salts of various plant acids such as acetic, oxalic, citric, maleic, tartaric e.t.c. (Russo *et al.,* 2013). Alkaloids have been reported to be generated by higher plants (Qiu *et al.,* 2014). Alkaloids have complex structure, they are colourless, crystalline and non-volatile solids, however some are liquid e.g. connine and nicotine, while few are coloured e.g. berberine which is yellow in colour (Kittakoop *et al.,* 2014). The alkaloids content in plants has been found to be within a few percent. Depending on the type of plants, the maximum concentration can be observed in the leaves, fruits or seeds, root or bark (Aniszewski, 2007). Furthermore, different tissues of the same plants may contain different alkaloids (Leland, 2006).

Alkaloids have specific pharmacological activities and are used as drugs. All alkaloids have bitter taste, and they are insoluble in water but soluble in ethanol, ether and chloroform. They also form insoluble precipitate with the solution of picric acid and potassium mercuric-iodide (Faulkner *et al.,* 2006).

2.7.5 Carbohydrates

Plants synthesize carbohydrates from carbon dioxide and water by photosynthesis storing the absorbed energy internally, often in the form of starch or lipids (Eldra *et al.,* 2004). Plant components are consumed by animals and fungi, and used as fuel for cellular respiration. Energy obtained from metabolism (e.g., oxidation of glucose) is usually stored temporarily within cells in the form of ATP (Pichon, *et al.,* 2006).

2.7.6 Tannins

Katie (2006) reported that these are phenolic compounds which coagulate gelatin and other proteins. Tannins occur in trees, shrubs and plants, mostly in the bark and sometimes in the roots, leaves and fruits. They are usually in combination with other substances in the plants. Tannins and tannic acid have astringent action due to the fact that they can precipitate proteins and render them resistant to attack by Proteolytic enzymes. Tannins prevent the absorption of toxic substances and protect the inflamed

mucus membranes by internally forming a fine pellicle of coagulated protein over the lining of the alimentary tract. The irritability of the bowels is thus decreased and undue peristalsis consequently diminished (Eric, 2006).

2.7.7 Saponins

Saponins are widely understood to be plant-derived, but also reported to have been isolated from marine organisms (Jonathan *et al.,* 2004, Zentner, 2011). Saponins are indeed found in many plants (Zentner, 2011) and derive their name from the soapwort plant (genus Saponaria, family Caryophyllaceae), the root of which was historically used as a soap. They are amphipathic glycosides grouped by the soap-like foaming produced when shaken in aqueous solutions, and structurally by having one or more hydrophilic glycoside moieties combined with a lipophilic triterpene derivative (Sun *et al .,* 2009 ; Asl *et al* ., 2008).

Some plant saponins (e.g. from oat and spinach) may enhance nutrient absorption and aid in animal digestion (Akinjogunla *et al.,* 2010). One research use of the saponin class of natural products involves their complexation with cholesterol to form pores in cell membrane bilayers, e.g., in red cell (erythrocyte) membranes, where complexation leads to red cell lysis (hemolysis) on intravenous injection (Francis *et al.,* 2002). In addition, the amphipathic nature of the class gives them activity as surfactants that can be used to enhance penetration of macromolecules such as proteins through cell membranes. (Murthy *et al.,* 2010) Saponins isolated from the *Gypsophila paniculata* plant showed the ability to significantly augment the cytotoxicity of immunotoxins and other targeted toxins which are directed against human cancer cells.

2.7.8 Glycosides

Many plant glycosides are used as medications. The first glycoside ever identified was amygdalin, by the French chemists Pierre Robiquet and Antoine Boutron-Charlard, in 1830 (Brito-Arias, 2007). An example of an alcoholic glycoside is salicin which is converted in the body into salicylic acid. It has analgesic, antipyretic, and anti-inflammatory effects. Anthraquinone glycosides have laxative effect and flavonoids glycosides have antioxidant effect. Arbutina phenolic glycosides found in the common bearberry has a urinary antiseptic effect. Some heart diseases such as congestive heart

failure are managed with cardiac glycosides found in some plant genera such as Digitalis, Scilla, and Strophanthus (Lindhorst, 2007).

2.8 Pharmacological Studies of Medicinal Plants used

Quite a number of pharmacological researches on the medicinal plants used have been carried out.

2.8.1 *Cassia alata*

Ethanolic extracts of the leaves of *Cassia alata, Baphia nitida, Ficus exasperate* and *Gossypium arboretum* were analyzed by Okafor *et al.,* (2001), for their antifungal activities against four pathogenic fungi namely: *Trichophyton rubrum, Epidermophyton floccosum, Basidiobolus haptosporus* and *Candida albicans*. The extracts inhibited the growth of the fungal pathogens except that of *Basidiobolus haptosporus*.

The effect of mineral contents and time of harvest of *Cassia alata* for antifungal activities was investigated by Orafidiya *et al.,* (2002). In their study, leaves of *Cassia alata* were collected at different periods of the day. Calcium and magnesium were found in concentration range of 0.00485-0.10192 mg/mL and 0.00371-0.00477mg/mL for Ca and Mg respectively using atomic absorption spectrophotometry. *Cassia alata* extracts from the leaves collected in the morning and afternoon clearly showed greater anti-fungal activities on *Epidermophyton glyccosum* than those collected in the evening.

In vitro antimicrobial activity of ethanol and water extracts of *Cassia alata* leaves and barks were evaluated in vitro against the fungi, (*Aspergillus fumigatus* and *Microsporumcanis*), yeast (*Candida albicans*) and bacteria (*Staphylococcus aereus* and *Escherichia coli*) by Somchit *et al.,* (2003). Water extract exhibited higher antibacterial activity than the ethanol extract from leaves (inhibition zones of 11–14 and 9–11 mm, respectively) but *E. coli* showed resistance to all types of extracts. It was therefore concluded that the plant has antimicrobial activity, which is as potent as standard antimicrobial drugs against certain microorganisms.

Preliminary investigation on the phytochemistry and antimicrobial activity of *Cassia alata* leaves was carried out by Idu *et al.,* (2006). The leaves contained chrysoeriol, kaempferol, quercetin, 5,7,4'-trihydroflavanone, kaempferol-3-O-beta-D-glucopyranosyl - (1,6) - beta - D-glucopyranoside, 17-hydrotetratriacontane, n-

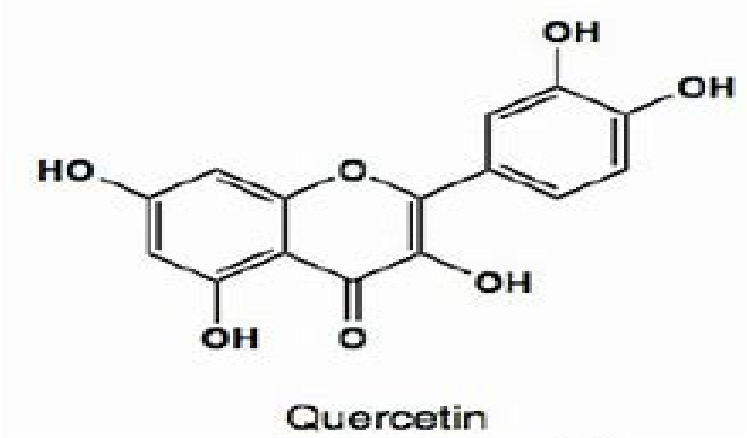
dotriacontanol, n-triacontanol, palmitic acid, ceryl ester, rutin , stearic acid and palmitic acid (Fig.2).

Makinde *et al.,* (2007) evaluated antibacterial and antifungal activity of the aqueous and methanol extract of *Cassia alata* leaves. In their studies, the extract of the plant was more active against fungi than the bacteria with 20 – 30 mm diameter of zones of inhibition.

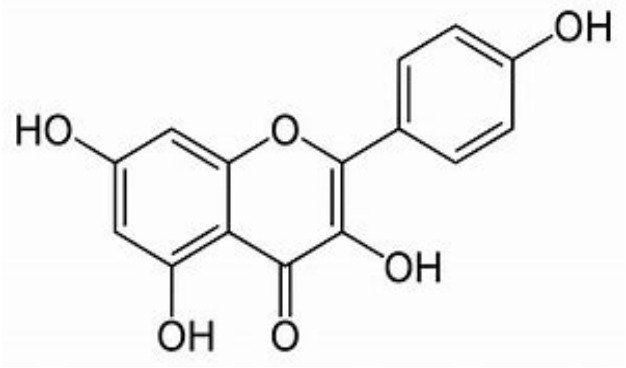
Cassia alata leaves are well known for their application in the treatment of fungal infections. This necessitated Abubaker and his co-workers in 2008 to investigate the antifungal activity of the aqueous flower extract of the plant against three distinct groups of fungi which are aflatoxin producing fungi, plant pathogenic fungi and human pathogenic fungi. In their study, total inhibition was observed at 10 and 15 mg/mL for aflatoxins producing strains, whereas for plant and human pathogens, total inhibition was at 15 mg/mL. Minimum Inhibitory Concentration (MIC) varied from 5.6 – 8.0 mg/mL. It was concluded that the extract can be used as a potential antifungal agent for these three groups of fungi. Aiyelola *et al.,* 2006, investigated the primary and secondary metabolite composition of vegetative and reproductive plant parts and cell cultures with emphasis on potent phenolic antioxidants such as anthraquinones, flavonoids and flavan-3-ol derivatives Proanthocyanidins (PA) in *Cassia alata* leaves by thiolysis with benzyl mercaptan, LC–MS and NMR. Mohammed *et al.,* 2015 reported that *Cassia alata* leaves consisted of almost pure propelargonidins with < 6% procyanidins, had B-type linkages and a mean degree of polymerisation of three. Epiafzelechin was the major flavan-3-ol subunit (> 94%) and epicatechin a minor constituent (6.4%) in residual PA was mainly detected as an extension unit.

Baljinder *et al*., 2012, investigated the anti-allergic activities of hydro-methanolic extract of *Cassia alata* (Linn.) and its constituents rhein and kaempferol on triple antigen/sheep serum-induced mast-cell degranulation in rats. Its major compound, rhein, exhibits antiallergic activity *via* mast cell stabilization and lipoxygenase inhibition.

Mohammed *et al*., 2015 isolated three anthraquinones (rhein, emodin and aloeemodin) from Chloroform extract of *Cassia alata* leaves. These phytochemicals are all known to have useful bioactivities including anticancer activities. On the other hand, the fatty acids and the unsaponifiable matter were fractionated and identified by GC/MS. It was



Quercetin



kaempferol

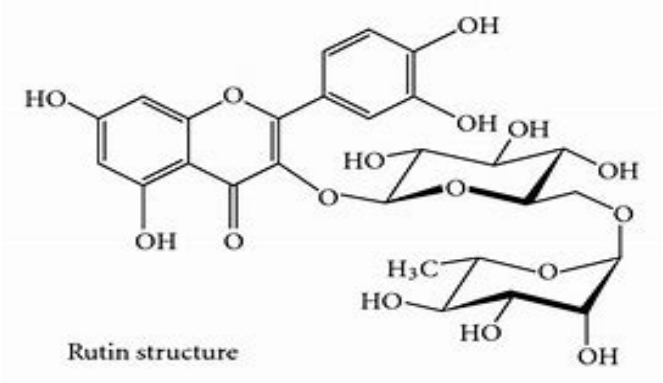


Fig. 2 Some phytochemicals present in *Cassia alata* leaves.

Source- Chemolink.com.

concluded that the findings showed the importance of screening *Cassia alata* as medicinal plant for various cancers cell lines and the responsible active compounds.

2.8.2 *Cassia occidentalis*

Phytochemical analysis of the aqueous extracts of *Cassia occidentalis* leaf by Muyibi *et al.*,(2002) indicated that the leaves contained tannins, anthraquinones, sterols, glycosides, saponins and alkaloids, all biochemicals needed for treating skin related diseases.

Vedpriya *et al.,* (2010), screened different organic and aqueous extracts of leaves of *Cassia occidentalis* for their antimicrobial activity against seven human pathogenic bacteria and two fungal strains by disk diffusion assay. Among these extracts, methanol and aqueous extracts showed significant antimicrobial activity against most of the tested microbes. The most susceptible microorganism was *P. aeruginosa* (18 mm zone of inhibition in aqueous extract) followed by *P. mirabilis* (15 mm zone of inhibition in methanol extract) and *Candida albicans* (8mm zone of inhibition in methanol extract). The presence of anthraquinones, carbohydrates, glycosides, cardiac glycosides, steroids, flavanoids, saponins, phytosterols, gums and mucilages were confirmed, while alkaloids were absent in all the tested extracts.

The phytochemical screening of petroleum ether, chloroform and methanolic extracts of *Cassia occidentalis* were performed by Muyibi *et al.,* 2002. The chloroform and methanolic extracts of both flower and seed were found to contain flavonoids, alkaloids, phenolics tannins, steroids, glycosides and anthraquinones. The antioxidant potential of flowers and seeds in the different solvents was evaluated. Their SC_{50} and EC_{50} values were determined to evaluate the therapeutic potential, in which seeds were found to have higher antioxidant activity revealed by lower SC_{50} and EC_{50} value. The total phenol, flavonoid, flavonol and tannin content were determined to study the free radical scavenging property. It was therefore concluded that the seeds were found to have higher antioxidant activity when compared to flowers in various solvent extracts indicating their pharmacological property.

Sathya *et al.,* (2012) determined the phytochemistry of aqueous extract of *Cassia occidentalis*. The antimicrobial activity and antioxidant properties of the plant on *Escherichia coli*, *Pseudomonas* aeruginosa and *Staphylococcusaureus* was determined. Alkaloids, tannins, saponins, carbohydrate, glycoside, phytosterols, Oils and Fats,

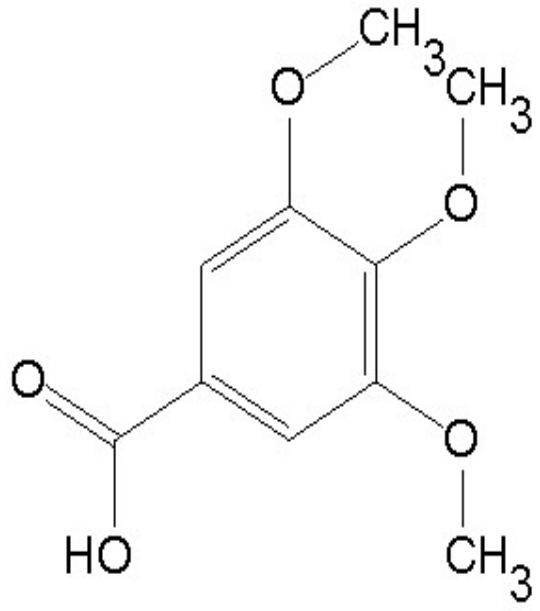
phenol, flavonoids, protein and amino acid were present in the leaves and seeds of the plant. It was concluded that the aqueous plant extract can be used as antimicrobial agent, antioxidant and also used as herbal medicine for curing number of disease in the form of pellets or paste.

2.8.3 *Mitracarpus villosus*

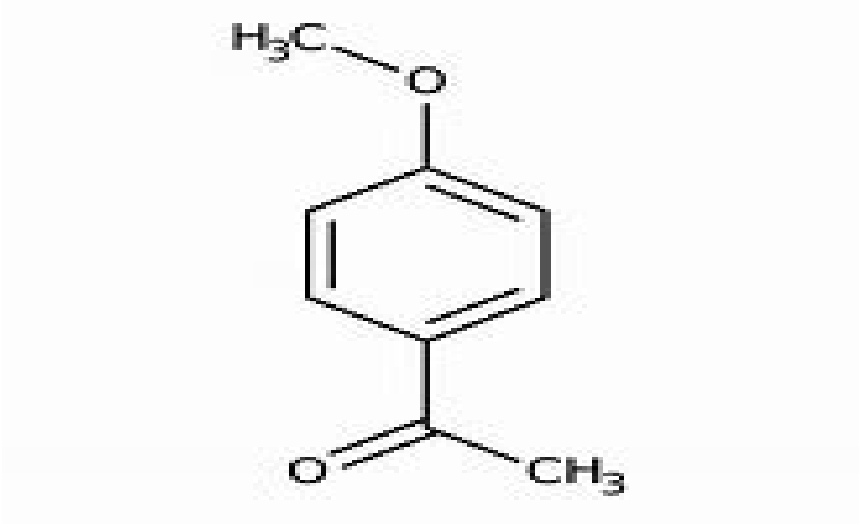
Bisignano *et al.,* 2000 examined the antimicrobial activity of methanol extract of *Mitracarpus villosus* against *Staphylococcus aureus* and *Candida albicans* strains. It was observed that the extract possesses both antibacterial and antimycotic activities (minimum inhibitory concentration—MIC 31·25 and 62·50µg/ mL, respectively). Some of the phytochemicals isolated include gallic acid and 3, 4, 5-trimethoxybenzoic acid which they found inhibited the growth of *Staphyloccocus aureus* (MIC 3·90 and 0·97 µg/mL), 4-Methoxyacetophenone and 3, 4, 5-trimethoxyacetophenone which effectively inhibited *Candida albicans* (MIC 1·95µg/mL). Other compounds isolated include kaempferol-3-O-rutinoside, rutin and psoralen which showed low antibacterial and antimycotic activities (125–500µg/mL)(Fig.3).

Okogun and his co-workers in 2005 also tested the bactericidal properties of 95% ethanolic crude extracts of *Mitracarpus villosus* against *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis* and *Staphylococcus feacalis* and they were all inhibited. However, *Pseudomonas aeruginosa* and *Proteus mirabilis* were not inhibited. It was reported that the minimum inhibitory concentrations (MIC) of the extract were in the range of 0.06-8.0 mg/mL, and minimum bactericidal concentrations (MBC) of 0.06-32.0 mg/mL.

Jegede *at al*., 2005 carried out Pharmacognostic investigation on fresh, powdered and anatomical sections of leaf of *Mitracarpus villosus* (S.W) D.C to determine its macromorphological, micromorphological and chemo-micromorphological profiles. Qualitative and quantitative studies indicated presence of amphicribal vascular bundle arrangement, characteristic asperites, cone-shaped clothing trichomes, simple leaf arrangement lanceolate shape, entire margin, cuneate base, parallel venation and opposite/decussate arrangement. Presence of calcium oxalate crystals, lignin and oil globules with palisade ratio of 4 – 7 and stomatal number of 13.5 were also reported.



3, 4, 5-trimethoxybenzoic acid



3, 4, 5-trimethoxyacetophenone

Fig. 3 Some phytochemicals present in *Mitracarpus villosus* leaves

Source- Chemolink.com

The antifungal activity of cream formulation of *Mitracapus villosus* crude extract was reported by Arhewoh *et al*., 2011 against *Microsporum aoudini, Trichophyton mentagrophyte and Candida albicans*. The minimum inhibitory concentrations (MIC) obtained for the crude extract was 5% w/v. The zones of inhibition of the formulated cream at 5 and 20% w/v ranged from 2 – 6 and 7.5 – 12 mm, respectively.

Mitracarpus villosus, *Euphorbia hirta* and *Spermacoce ocymoides* were investigated for some of their components and antimicrobial activities of their leave extracts against bacteria and fungi by Onawunmi *et al*.,2012. Presence of saponins, tannins and volatile oils were reported. Infrared and ultraviolet-visible (U.V) analysis carried out by them suggested that the plants contain Benzene ring, hydroxyl group and aniline in their structure. It was observed that the plants extract exhibited antimicrobial activities at a concentration of 25mg/ml. The zones of inhibition of the plants extract against fungi and bacteria ranges between 10 – 35mm with minimum inhibitory concentration (MIC) ranges between 25 – 50mg/ml for fungi while that of bacteria ranges between 25 – 100 mg/mL with *S. Ocymoides* less effective against bacteria. It was concluded that the ethanolic extracts of both dried and fresh leaves of the plants showed more antimicrobial activity against the tested organisms than n-hexane extracts of the plants.

Ogenejobo *et al*., 2013, reported that the phytochemistry screening carried out showed that there was presence of secondary metabolites such as steroids, reducing sugars, alkaloids, tannins, saponins, terpenoids, saponins and cardiac glycosides. It was suggested that the plant could be used as herbal mixtures for the management of skin infections caused by some bacteria and fungi.

Aboh *et al.,* 2014, investigated the antifungal activities of phyto compounds from *Mitracarpus villosus* against clinical isolates of *Candida albicans*, *Candida krusei, Trichophyton verrucosum*, *Trichophyton mentagrophytes*, *Aspergillus fumigatus* and *Aspergillus niger* using agar diffusion and micro broth dilution methods. In their results, it was observed that crude tannin extract produced the strongest antifungal activity against the yeasts with diameter zones of inhibition ranging from 21.67 – 23.67 mm while the crude saponin extract exhibited the strongest antifungal activity against the

moulds with diameter zones of inhibition ranging from 21.00 – 24.67 mm. It was concluded that the plant has good potentials for development of new antifungal drugs.

Kenneth *et al*., 2017, isolated and idenified a new benzophenone glycoside, mitraphenone A, together with three known compounds from the leaves of *Mitracarpus villosus* using a combination of one- and two-dimensional NMR spectroscopy. The isolated compounds were screened for their antibacterial activity against several Gram-positive and Gram-negative bacteria and concluded that the Compounds allexhibited moderate activity against *Enterococcus faecium* (strains ATCC 35667 and ATCC 700221) and *Staphylococcus aureus* ATCC 25923 with MIC values ranging from 25 to 50 μM.

Jato *et al*,. 2018, reported that *M. villosus* leaves contains phenolics, saponins, flavonoids, cardiac glycosides and tannins but no alkaloids. The quantitative phytochemical analysis showed varied percentage content of these compounds with saponins being highest (14.0%) and tannins lowest (1.41%).

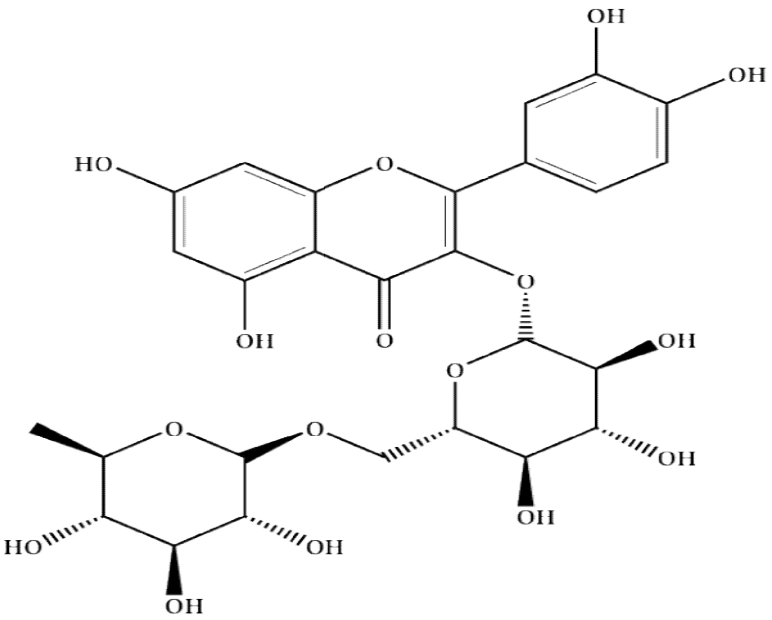
Gbala and Anibijuwon (2018) worked on the ethanol and methanol extract of *Mitracarpus villosus* and two other medicinal plants namely *Terminalia glaucenses* and *Mangiferaindica* on Carbapenem-resistant enterobacteriaceae. The ethanol extract of *Mitracarpus villosus* exhibited considerable higher antibacterial activity compared to methanol extract (10 - 26.5 mm).

2.8.4 *Acalypha wilkesiana*

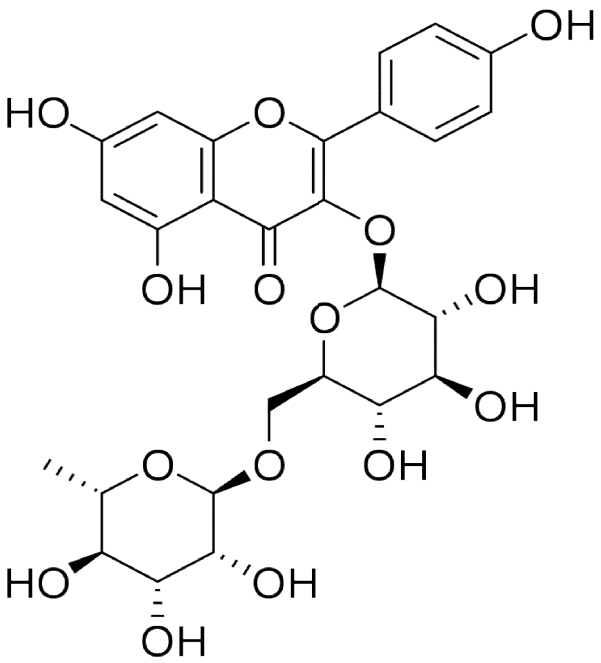
Adesina *et al.,* (2000) carried out an activity directed fractionation of a 50% aqueous ethanol extract of *Acalypha wilkesiana* and *Acalypha hispida* leaves which resulted in the isolation of gallic acid, corilagin and geraniin as the compounds responsible for the observed antimicrobial activity. Quercetin 3-O-rutinoside and kaempferol 3-O-rutinoside were also isolated from the inactive fraction of *A. wilkesiana* (Fig.4)

The antimicrobial activity of methanolic leaf extracts and crude alkaloid extracts of *A. wilkesiana* was evaluated by Ezekiel *et al.,* (2009) against clinical strains of *Escherichia coli, Salmonella typhi, Streptococcus pyogenes, Strept. pneumoniae, Methicillin-Resistant Staphylococcus aureus* (MRSA), non-methicillin resistant *Staph. aureus*, *Candida albicans*, *Aspergillus fumigatus* and *A. flavus*. The crude alkaloid extracts inhibited only the Gram-negative bacteria with mean inhibition zones of 10.0 ± 0.00 to

12.3 ± 0.03mm while the methanol extracts inhibited all other test organisms showing a broad spectrum activity. The water extracts had no activity against the non-MRSA



Quercetin 3-O-rutinoside



kaempferol 3-O-rutinoside

Fig. 4 Some phytochemicals present in *Acalypha wilkesiana* leaves

Source- Chemolink.com

strains. The MIC was 0.4 mg mL-1 for all unicells except strains of *C. albicans* which both had MICs of 0.8 mg/mL. The MBC was 0.4 mg/mL for tested isolates except the non-MRSA and *C. albicans* which had MBCs of >12.0 mg/mL and 1.0 mg/mL, respectively. It was concluded that the methanolic extract totally inhibited all tested *aspergilli* while the water extract had a varying inhibitory effect (63.0±2.50 to 81.0±2.90%) on the tested fungi strains.

Phytochemical and Elemental determination of *Acalypha wilkesiana* was conducted by Madziga and his co-workers in 2010. Phytochemical analysis of the aqueous leaf extract of *Acalypha wilkesiana* revealed a high presence of carbohydrates, tannins and flavonoid, a moderate presence of Phlobatannins, saponins, alkaloids, cardiac glycosides and minute quanity of Terpenes and Steroids. Anthraquinone derivatives were not present. The elemental analysis showed presence of chloride, sodium, potassium, calcium, iron, magnesium, zinc copper and mangenese in moderate quantity while cadmium and lead were not detected.

2.8.5 *Kigelia africana*

Akunyili *et al*.,1991 carried out chemical investigation on the aqueous extracts of the stembark of *Kigelia pinnata*. It was observed that iridoids were the major components. Antimicrobial determination of the aqueous extracts and two major iridoids against *Bacillus subtilis. Escherichia coli, Pseudomonas aeruginosa, Stuphylococcus aureus* and *Candida albicans* showed significant activity.

Antibacterial studies carried out by Grace *et al.,* (2001) on the fruit and stem bark of *Kigelia africana* shows that the fruit and stem bark contain a mixture of three fatty acids which were very active against gram-negative and gram-positive bacteria.

The effect of ethanolic stem bark of *Kigelia africana* on central nervous system was carried out by Owolabi *et al.,* (2007). In their studies, the extracts at all doses reduced the duration of sleeping time when compared to the control group that received distilled water. The difference in sleeping time was significant ($p<0.0001$ at all doses tested) which was also found to be dose dependent.

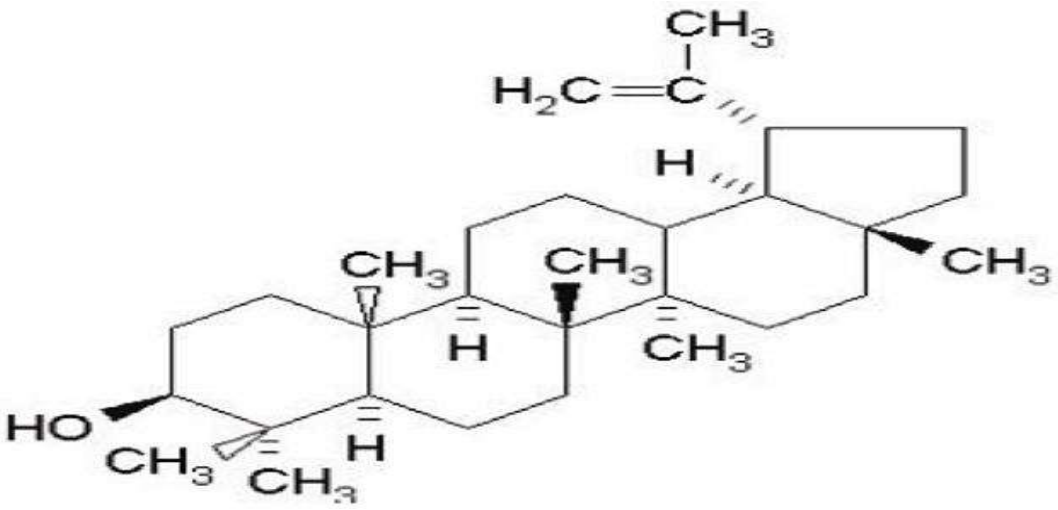
Olayinka *et al*., 2007 isolated volatile constituents of the oil from the leaves and flowers of *Kigelia africana* by hydrodistillation and analysed using GC and GC-MS. The leaf oil was found to contain 25 components, while the flower oil contained nine. Both oils were rich in non-terpenoids; hexadecanoic acid (21.91%, leaf oil; 57.00%, flower oil) was the most abundant in both oils. The other major components also isolated were ethyl linoleate (21.73%) and α-pinene (12.28%) in leaf oil and terpenolene (8.26%), myristic acid (7.95%) and linalool (6.71%) in the flower oil.

Olatunji and Atolani (2009) in their work reported the various chemical constituents such as the naphthaquinones, iridoids, fatty acids, norviburtinal, sterols, lignans, terpenoid, and flavonoids present in the medicinal plant (Fig.5).

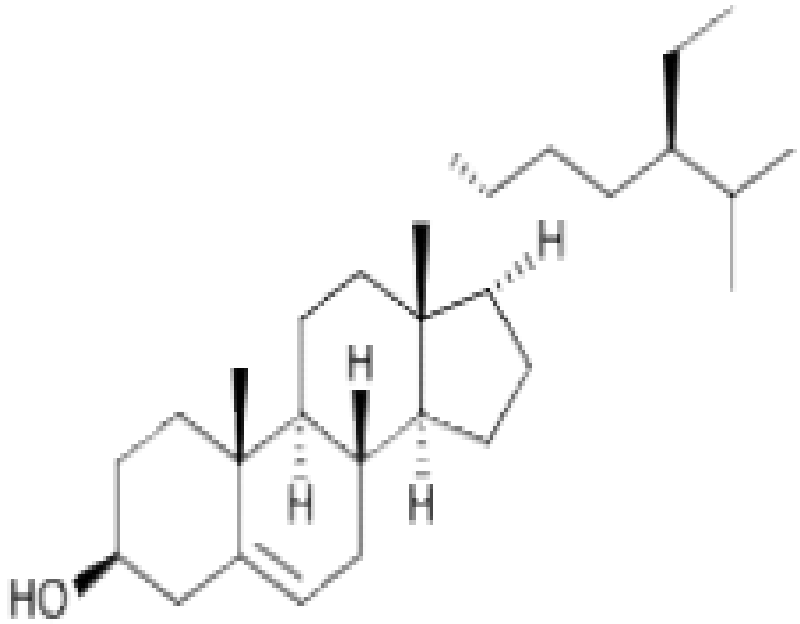
Oyedeji and Bankole-Ojo (2012) carried out Quantitative evaluation of the anti-psoriatic activity of sausage tree (*Kigelia africana*) using methanol and hexane extracts of the stem, bark, leaves and fruit of the plant. The results of their studies showed that the topical application of *K. africana* exhibited an important anti-psoriatic activity with *K. africana* stem methanol extracts exhibiting the highest anti-psoriatic activity with little irritation potential. Agyare *et al.,* (2013) investigated the antimicrobial and antioxidant properties of methanol leaf and stem bark extracts of *Kigelia africana* and methanol leaf and root extracts of *Strophanthus hispidus,* and also determined the wound healing properties of the extracts. The antimicrobial activities of the methanol extracts were determined against two Gram-positive and two Gram-negative bacteria and a fungus using agar diffusion and micro-dilution methods. *K. africana* extracts (7.5% w/w) showed significant wound contraction at day 7 with 72% of wound closure whiles significant wound contractions were observed on day 11 for stem bark of *K. africana*, leaf and root extracts of *S. hispidus*. It was observed that wound tissues treated with the extracts showed improved collagenation, re-epitheliazition and rapid granulation formation compared with untreated wound tissues. The extracts were found to contain alkaloids, saponins, tannins, flavonoids, carbohydrates, and sapogenetic glycosides.

Chemical investigation of the methanol/dichloromethane(1:1 *v/v*) extract of the leaves and fruits of *Kigelia africana* carried out by Lazare *et al*., 2015, usingspectroscopic analysis indicated presence of lupeol, *β*-sitosterol, *β*-D-glucoside, canophyllol,

fibrarecisin, pomolic acid, hydroxy-pomolic acid, *β*-friedelinol, sesamin, and paulownin (Fig.5).



Lupeol



β-sitosterol

Fig.5 Some phytochemicals present in*Kigelia africana* leaves

Source - Wikipedia

2. 9 Synthetic Chemical Derivatives for Skin Infection Treatment

Some synthesized chemicals used to treat skin diseases associated with both fungal and bacterial infections include:

2.9.1 Imidazole

Kumar *et al.,* (2010) described imidazole as an organic compound with the formula $C_3H_4N_2$ and classified as an alkaloid. They are synthesized from the condensation reaction of glyoxalin, formaldehyde and ammonia. Some of the derivatives of imidazole are Clotrimazole and Ketoconazole (Fig.6).

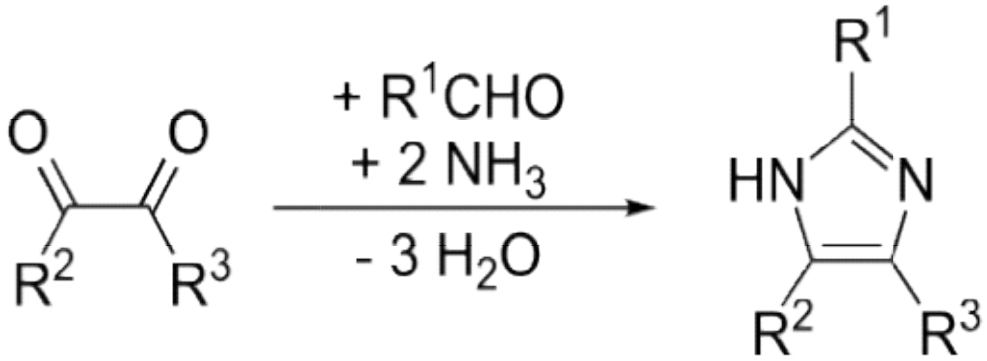
2.9.1.1 Clotrimazole

Shahin *et al.,* (2011) evaluated an emulsion containing clotrimazole. It was found that the incorporated clotrimazole showed higher antifungal activity against *Candida albicans* compared to the commercially available formulation using cylinder and plate method. Effect of in-vivo chronic exposure to clotrimazole on zebra fish testis function was carried out in 2013 by Baudiffier *et al*.,. In their study, they acknowledged that a direct mode of action was not yet established. Nevertheless, major adverse effects in terms of developmental and reproductive system in teleost, such as masculinization during gonadal differentiation, advanced germ cell development, increased gonadosomatic, proliferation of Leydig cells and decreased egg production were observed as a result of disturbance in CYP450-regulated steroidogenesis by the fungicide. Therefore, it was concluded that there was sufficient evidence to support that the fungicide was endocrine disrupting chemical (EDC).

2.9.1.2 Ketoconazole

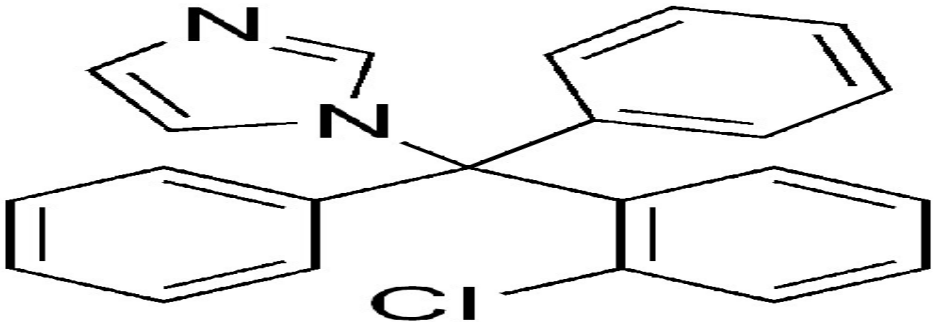
Proksch *et al*., 2008, carried out two dermal irritancy studies, a human sensitization test, a photo- toxicity study and a photo-allergy study conducted on 38 male and 62 female volunteers. It was observed that there was no irritation, no phototoxicity and no photoallergenic potential due to ketoconazole cream, 2%.When ketoconazole cream, 2% was applied dermally to intact or abraded skin of beagle dogs for 28 consecutive

days at a dose of 80 mg, there were no detectable plasma levels using an assay method having a lower detection limit of 2 mg/mL.

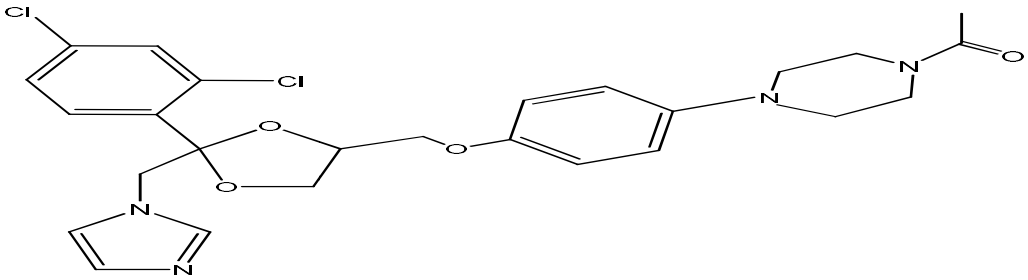


Glyoxal Imidazole

Synthesis of Imidazole



Clotrimazole



Ketoconazole

Fig. 6. Some synthetic chemicals used in the management of dermatophytosis

Source- Chemolink.com

Phillips and Rossi, (2013) highlighted the use of ketoconazole where it was used for different antifungal medication purposely for the treatment of fungal infections and also for topical administration in creams which are used to treat tinea, cutaneous candidiasis and shampoos which are used primarily to treat dandruff.

In vitro studies suggest that ketoconazole impairs the synthesis of ergosterol, which is a vital component of fungal cell membranes. It is postulated that the therapeutic effect of ketoconazole in seborrheic dermatitis is due to the reduction of *M. ovale*, but this has not been proven (Phillips and Rossi, 2013).

2.9.2 Terbinafine

Mukherjee *et al.,*(2003) carried out in vitro antifungal susceptibilities of six clinical *Trichophyton rubrum* isolates obtained sequentially from a single onychomycosis patient who failed oral terbinafine therapy. It was observed that the terbinafine-resistant isolates exhibited normal susceptibilities to clinically available antimycotics including itraconazole, fluconazole, and griseofulvin. This was the first confirmed report of terbinafine resistance in dermatophytes.

Jaiswa *et al.,* (2007) carried out an open randomized comparative study to test the efficacy and safety of oral terbinafine (Fig.7) pulse as a monotherapy and in combination with topical ciclopirox-olamine 8% or topical amorolfine hydrochloride 5% in the treatment of onychomycosis. It was observed that there was clinical cure in 71.73, 82.60 and 73.91% patients in groups A, B and C, respectively. Mycological cure rates against dematophytes were 88.9, 88.9 and 85.7 in groups A, B and C, respectively. It was therefore concluded that terbinafine is effective and safe alternative in treatment of onychomycosis due to dermatophytes; and combination therapy with topical ciclopirox or amorolfine do not show any significant difference in efficacy in comparison to monotherapy with oral terbinafine.

Terbinafine hydrochloride is a synthetic allylamine antifungal. It is highly hydrophobic in nature and tends to accumulate in skin, nails, and fatty tissues. Terbinafine inhibits ergosterol synthesis by inhibiting squalene epoxidase, an enzyme that is part of the

fungal cell membrane synthesis pathway. Terbinafine prevents conversion of squalene to lanosterol, therefore ergosterol cannot be synthesized. This is thought to change cell membrane permeability, causing fungal cell lysis. It was reported that terbinafine

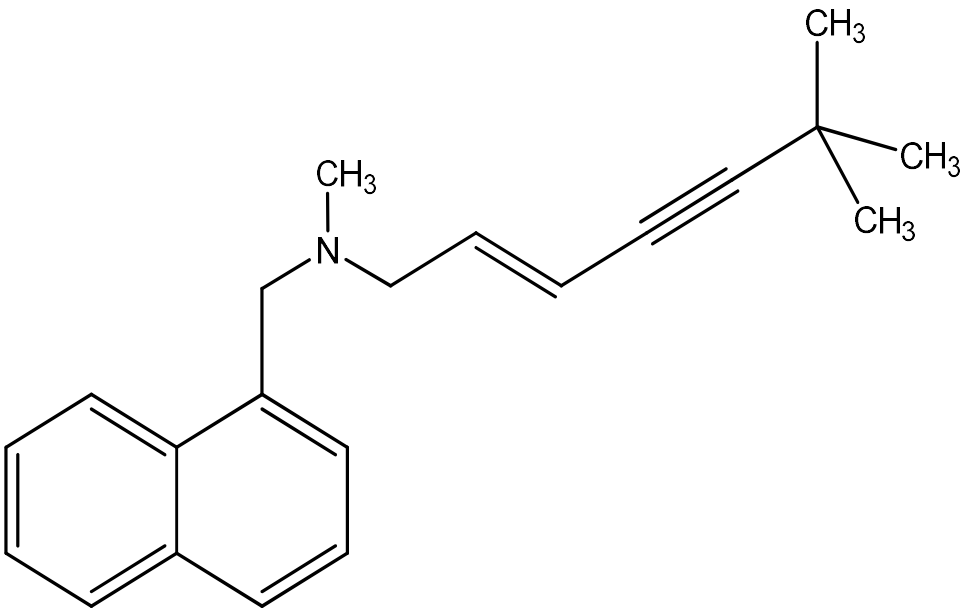


Fig. 7 Terbinafine

Source- Chemolink.com.

hydrochloride can induce or in some cases triger subacute cutaneous lupus erythematosus (Phillips and Rosen, 2013).

Kaur *et al.,* (2014) carried out an experiment of a formulated terbinafine hydrochloride gel and evaluated it for the treatment of fungal infections. All formulations were evaluated for pH, drug content, viscosity, spread ability, extrudability and in-vitro permeation study. On the basis of evaluation parameters, it was found that the formulation prepared with Carbopol 934 and 3% oleic acid showed better permeation as compared to others. They concluded that the highest antifungal inhibitory activity was against *Candida* species.

2.9.3 Naftifine hydrochloride

Ghannoum *et al.,* (2013) evaluated the in vitro antifungal activity of naftifine hydrochloride, the active ingredient in naftifine hydrochloride cream and gel against dermatophytes. The MICs and minimum fungicidal concentrations (MFCs) of naftifine

hydrochloride against 350 clinical strains, including *Trichophyton rubrum*, *T. mentagrophytes, T. tonsurans, Epidermophyton floccosum,* and *Microsporum* canis, were determined using the CLSI methodology.

The MIC range against the dermatophyte isolates tested was 0.015 - 1.0 µg/mL. There was no increase in MIC for any strains following repeated exposure to naftifine hydrochloride. It was concluded that naftifine hydrochloride demonstrated potent activity against all dermatophytes tested, and none of the isolates within this test panel demonstrated the potential for the development of resistance.

In a review carried out by Gupta *et al*., (2008), it was reported that Naftifine causes interruption of fungal ergosterol synthesis and accumulation of squalene in fungal organisms. It demonstrated anti-inflammatory properties such as a reduction in superoxide production and a reduction in polymorphonuclear leukocyte chemotaxis/endothelial adhesion. Naftifine showed good efficacy and safety for a variety of conditions and is a useful treatment that provides both antifungal action and

relief of inflammatory signs and symptoms and few adverse events have been noted with naftifine use, the most frequent being mild and transient burning, stinging, or itching in the application area. It was observed that Naftifine remains a reliable multifunctional agent for a variety of superficial infections.

2.10 Stability Studies on Cosmetic Emulsions

Anchisi *et al.,* (2001) carried out stability study on a newly formulated oil-in-water emulsion with increasing amounts of Sepigel 305 as an emulsifier in the presence of vegetable extracts using centrifugation and accelerated ageing tests. The results obtained were compared with those from formulations described in the National Formulary of Italian Pharmacopoeia X. The study emphasized that the new gel emulsions have a greater stability compared to the other formulations.

Formulation of emulsions capable of breaking and releasing inner aqueous phase under shear rates compatible with agro-alimentary, pharmaceutical and cosmetic applications were carried out by Muguet *et al.,* (2001). The formulated emulsions were studied : one with a high concentration of primary emulsion, not viscosified in the external aqueous phase; emulsion gelified with a synthetic polymer (Carbopol 974P®); and other emulsions thickened with chemically modified cellulose (hydroxy-propyl-cellulose). The results of this study showed the influence of the composition of the external aqueous phase of the emulsions on their fragmentation and release as a function of the shear rate.

Emulsion stability by evolution of backscattering with time multiple lights scattering technique was carried out by Porass *et al.,* (2012). In their studies, droplet sizes were measured by dynamic light scattering (DLS) and mean sizes between 30 and 120 nm were obtained. They concluded that for low water concentration, emulsions breakdown could be attributed to Ostwald ripening and for high water concentration, emulsions breakdown could be attributed to coalescence.

Masmoudi *et al.,* (2005) investigated the utilization of spectrometric method using Fourier Transform Infrared (FTIR) spectroscopy compared to Conductimetry method in stability study of cosmetic “oil in water” (O/W) emulsions. It was concluded that Conductimetry was a useful technique to predict emulsion destabilization while FTIR

allowed the measurement of chemical modifications and helped to understand the chemical mechanisms which occured during the oxidation.

2.11 Assesment of Cosmetic Emulsion efficacy

Tadros (2013) carried out sensitivity and efficacy test on a developed moisturizing emulsion on dry skin. It was reported that Skin hydration (corneo-metry) and trans-epidermal water loss (TEWL) studies with a single application in 18 volunteers confirmed its efficacy ($p<0.0001$) and showed that it was superior to Ultrabase® and Diprobase® ($p<0.001$).Irritation tests in 74 eczema-prone patients resulted in only one mild reaction, and allergy tests in 99 healthy volunteers elicited no positive reactions.

In 2012, Pathan *et al.,* investigated the effect of a formulated poly herbal cream with *Hibiscus rosasinensis,* and *Calotropis gigantea* on some groups of rats to determine the prevention of hair loss and hair growth activity of the plant extract. It was observed that their formulation contributed most significant hair growth activity with $p<0.05$. In conclusion, it was reported that their formulation did not induce any signs of erythema and edema.

Ratz-lyko *et al.,* (2016) evaluated the in vivo moisturizing and anti-inflammatory properties of cosmetic formulations (oil-in-water emulsion cream and hydrogel) containing different concentrations of *Centella asiatica* extract on human skin. The study was carried out over four weeks on a group of 25 volunteers after twice a day application of cosmetic formulations with *Centella asiatica* extract (2.5 and 5%, w/w) on their forearms. It was concluded that In vivo tests formulations containing 5% of *Centella asiatica* extract showed the best efficacy in improving skin moisture by increase of skin surface hydration state and decrease in trans-epidermal water loss.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Sample Collection, Identification and Preparation

3.1.1 Medicinal Plants

Four plant samples namely *Mitracarpus villosus* (Fig.1)*, Cassia occidentalis* (Fig.2), *Cassia alata* (Fig.3),and *Acalypha wilkesiana* (Fig.4)were collected at the Botanical Garden of Nigeria Natural Medicine Development Agency, Lagos between 10am -12 noon, while *Kigelia africana* (Fig.5) was collected at the Botanical Garden of the University of Ibadan at about 11am in the morning. They were identified by the taxonomist Mr Alfred Ozioko and samples deposited at Medicinal Plants of Nigeria Herbarium of NNMDA with voucher numbers assigned (MPNH/2013/1252, MPNH/2013/1253, MPNH/2013/1254, MPNH/2013/1255 and MPNH/2013/1256) respectively. The plants were air dried in the shade and pulverized to fine-sized particles for solvent extraction processes.

3.1.2 Materials for Emulsion Formulation

Cosmetic grade Stearic acid (Wilmar International Ltd,Singapore), Cetyl alcohol (Wego Chemical Group Inc. NY) and Parafin oil were purchased. Triethanolamine (Intratrade Chemicals GmbH Germany) was obtained from Department of Chemistry University of Ibadan, while deionized water was obtained from International Institute of Tropical Agriculture (IITA) Ibadan, Oyo State, Nigeria.

3.1.3 Micro-organisms

Clinical isolates of *Microsporium aoudini, Epidermophyton floccosum, Trychophyton mentagrophyte and Malassezia furfur* were obtained from Spectralab Medical and Diagnostic Services, Sagamu, Ogun State, Nigeria.

3.1.4 Laboratory Animals

Albino rats weighing between 150-200 g were obtained from the Experimental Animal Unit of the Faculty of Veterinary Medicine of the University of Ibadan. They were kept

Plate 1 ***Mitracarpus villosus***

Plate 2 ***Cassia occidentalis***

Plate 3 ***Cassia alata***

Plate 4 ***Acalypha wilkesiana***

Plate 5 ***Kigelia africana***

in well ventilated rat cages with free access to water and feed and were left in this environment for two (2) weeks to acclimatize (Pathan *et al.,* 2012).

3.2 Extraction of Bioactive Materials

3.2.1 Ethanol/Petroleum ether extract preparation

The pulverized plant samples (200 g) of *Mitracarpus villosus, Cassia occidentalis, Cassia alata, Acalypha wilkesiana* and *Kigelia africana* each were soaked in 500 mL of the solvents. These mixtures were kept on the rotator shaker for 72 hours for accelerated agigated extraction. The mixtures were then filtered using whatman filter paper grade 1. The filterate were concentrated using water bath at 60^{o}C until the solvent was completely removed.

3.2.2 Hot water extract

The plant samples were pulverized and placed in a blender and 1litre of hot distilled water was added to it. The mixture was then thoroughly blended for five minutes and then filtered using a Whatman filter paper grade 1. Excess water was removed using hot water bath.

3.3 Microbiological Assay

3.3.1 Sterilization of bottles, petri -dishes and experimental discs

All the bottles used were washed and thoroughly rinsed with distilled water. They were subsequently autoclaved at 121^{o}C. The experimental discs were also sterilized for about 15 minutes at the same temperature.

3.3.2 Preparation of Sabouraud dextrose agar

Sabouraud dextrose agar powder (65 g) (Titan Biotech Ltd, India) was weighed and dissolved in 1 L of distilled water. 400 mg Cycloheximide, 50 mg Chloramphenicol and 40 mg Gentamycin were added to prevent saprophytic fungi and bacteria contaminant respectively. The media were autoclaved at 121^{o}C for 15 minutes, poured into the Petri dishes, after which they were allowed to solidify and then inverted.

3.3.3 Streaking of test organism

The bottom of each of the Petri dishes was marked into 4 quadrants while they were still in inverted position. The loop full of appropriate test organism was then streaked in a zigzag pattern on each plate using cotton bud.

3.3.4 Preparation of Antifungal solution

Certiofur Sodium powder (10 g) (Titan Biotech Ltd, India) was dissolved in sterile water and different concentrations were made to the final working concentration of 1.0 mg/ml which is 1000 µg/1000 µL according to Finegold and Martin (1982). Ketoconazole (5 µg / disc) was prepared from stock powder of 200 mg by dissolving into 10 mL of distilled water and then serially diluted to 0.2 mg/mL. For negative control, 10 mL of DMSO mixed with 10 mL of N-Hexane was used.

3.3.5 Preparation of experimental discs

Filter paper was perforated using office perforator to produce well rounded 0.6 cm diameter discs. The small sterilized bottles were arranged in quadruplicate with the samples to be assayed in different concentrations that has been serially diluted from 10000 µg/mL, 1000 µg/mL, 100 µg/mL, to 10 µg/mL with each bottle containing 20 discs.

3.3.6 Preparation of Extracts Stock Solutions

Weighed extracts (0.2 g) and 2.0 mL of mixture of DMSO and N-Hexane were used in preparing each stock of 10000 µg/mL. The stocks were then serially diluted to obtain 1000 µg/mL, 100µg/mL and 10µg/mL respectively.

3.3.7 Application of experimental discs from the dilution series

Different experimental discs were applied upon each inoculated dish of each organism in triplicates at the appropriate quadrant positions giving room for 10000 µg/mL, 1000 µg/mL 100 µg/mL to 10 µg/mL. Positions for the positive and negative control discs were also created. Thereafter, the set up were allowed to incubate for 72 hours.

3.3.8 Reading the plates and scoring the results

The zone of inhibition were measured in mm and recorded. The zones of inhibition produced by the solvents alone were subtracted from those of the extracts in order to obtain the zones of inhibition due to the extracts alone.

3.4 Preparation of Emulsions

All oil soluble substances (A) were placed in a stainless steel container and heated to between 70 – 75^{o}C. All water-soluble substances (B) were placed in another stainless steel container and heated to the same temperature. The oil phase was then added to the

aqueous phase slowly with stirring. Heating was continued at the same temperature for about 10 - 15 minutes. The coarse emulsions formed were then cooled to about 35^{o}C gradually. The emulsions were allowed to stay at room temperature for twelve hours and then homogenized with the aid of a mechanical stirrer. The samples were then poured into labeled containers. Four emulsions were formulated based on four different formularies and labeled FA-FD (Table 1). Emulsions containing 0.5%, 1% and 2%, of *Kigelia africana, Cassia alata, Mitracarpus villosus, Cassia occidentalis and Acalypha wilkesiana* ethanolic extracts were produced. The prepared herbal emulsions were then vigorously homogenized.

3.5 Qualitative Phytochemical Analysis of Pulverized Medicinal Plants

3.5.1 Tannins

Plant material (200 mg) was weighed and 10 mL of hot distilled water added and then filtered. 2 mL of $FeCl_3$ was added to the filtrate. A brownish green or a blue black colouration indicated presence of tannin.

3.5.2 Alkaloids

Each sample (200 mg) of the medicinal plants was weighed and 10 mL of methanol was added and then filtered. 1% HCl with 4 mL of Dragendroff reagent was added to 2 mL of the filtrate. A precipitation of orange colour infers the presence of alkaloid.

3.5.3 Saponins (frothing test)

Distilled water (5 mL) was added to 0.5 mL of plant material filtrate and the mixture vigorously shaken. Frothing indicated presence of saponin.

3.5.4 Cardiac glycosides (Keller-Kiliani test)

Filterate of medicinal plant material (2 mL) was added to the mixture of 1 mL glacial acetic acid, 1 mL $FeCl_3$ and 1 mL Conc.H_2So_4. A brick-red colouration on heating indicated positive result.

3.5.5 Flavonoids

About 200 mg of plant materials was weighed. 10 mL of ethanol was added and filtered. Concentrated HCl and magnesium ribbon were added to 2 mL filtrate of the medicinal plant material. Appearance of yellow colouration which disappeared on standing showed the presence of flavonoids (Ogunyemi, 1979).

Table 1. Emulsion formulations

A		% w/w			
	SF	FA	FB	FC	FD
Stearic acid	4	3	3	2	4.5
Cetyl alcohol	2	1	1	2	1
Parafin oil	9	9	9	2	-
B					
De-inonised water	84.7	86.6	86.4	93.5	94.0
Triethanolamine	0.3	0.2	0.2	0.5	0.3

SF – Standard formulation, FA – Formulation A, FB- Formulation B, FC - Formulation C and FD - Formulation D

3.6 Quantitative Determination of Chemical Constituents

3.6.1 Determination of alkaloid content (Harborne, 1973)

Plant sample (5 g) was weighed into 250 mL beaker and 200 mL of 10% acetic acid in ethanol was added and covered and allowed to stand for 4 hrs. This was filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was removed and washed with 1% ammonium hydroxide and then filtered. The residue is the alkaloid and this was oven dried for 30 mins at 60^{o}C and reweighed. The alkaloid content of the samples was determined by difference using the equation:

$$\text{Percentage alkaloid} = \frac{W_2 - W_1}{} \text{ X } 100 \qquad \text{eqn 4}$$

Where W = weight of sample;

W_1 = weight of empty filter paper;

W_2 weight of paper + precipitate

3.6.2 Determination of saponin content (Obadoni and Ochuko, 2001)

Plant samples (20 g) each were put into conical flask and 100 mL of 20% aqueous ethanol was added. The samples were heated over a hot water bath for 4 hours with continuous stirring at about 55^{o}C. The mixture was filtered and the re-extracted with another 200 mL 20% ethanol. The combined extracts were reduced to 40 mL over water bath at about 90^{o}C. The concentrate was transferred into a 250 mL separatory funnel and 20 mL of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 60 mL n-butanol was then added. The combined n-butanol extracts were washed twice with 10 mL of 5% aqueous sodium chloride. The remaining solution was heated on a water bath. After evaporation, the samples were dried in the oven to a constant weight. The saponins content was calculated thus:

$$\% \text{ Saponin} = \frac{\text{Weight of Saponin}}{\text{Weight of Sample}} \text{ X } 100 \qquad \text{eqn 5}$$

3.6.3 Determination of flavonoid content (Bohm and Kocipai-Abyazan, 1994)

Plant samples weighing 10 g were extracted repeatedly with 100 mL of 80% aqueous methanol at room temperature. The whole solution was filtered through Whatman filter paper no. 2. The filtrates was later transferred into a crucible and evaporated into dryness over a water bath and weighed to constant weight.

$$\% \text{ Flavonoid} = \frac{\text{Weight of Flavonoid}}{\text{Weight of Sample}} \times 100 \qquad \text{eqn 6}$$

3.6.4 Determination of tannin content

Plant sample (500 mg) was weighed into a 50 mL plastic bottle. 50 mL of distilled water was added and shaken for 1 hour in a mechanical shaker. This was filtered into a 50 mL volumetric flask and made up to the mark. Then 5 mL of the filtrate was pipetted out into a test tube and mixed with 2 mL of 0.1 M $FeCl_3$ in 0.1 N HCl and 0.008 M Potassium ferrocyanide. The absorbance was measured at 120 nm within 10 min (Van-Burden and Robinson, 1981)

3.7 Fourier Transformation Infra-red Spectroscopy (FTIR)

The FT-IR analysis was carried out on the cream base (Placebo) and the cosmeuticals. They were placed in the sample chamber of the equipment and the spectra were recorded in the range of 3600-600 cm^{-1} on Nicolet Avatar 330 FTIR Spectrophotometer.

3.8 Physicochemical Analysis of Formulated Herbal Emulsions

3.8.1 Determination of emulsion type

Dye Uptake method

Two 10 cm^3 beakers were taken and some quantity of the sample emulsion put into each of them. A drop or two of methyl orange indicator was put into one of the sample, while a drop or two of Sudan III indicator was put into the other one. Each beaker's content was stirred with the aid of a glass rod. The observation was noted.

3.8.2 pH

This was determined using pH Meter Model 290 Mk 2 in the Laboratory of the Nigeria Natural Medicine Development Agency, Lagos. The pH meter was calibrated with buffer solutions 4 and 7 after which the sample solution pH was then taken.

3.8.3 Viscosity

This was determined using Brookfield's Viscometer. The sample was put in a beaker and the viscometer spindle inserted into the sample and allowed to rotate for five minutes at a pre-determined speed after which the viscometer dial reading is converted to a viscosity value in units of centipoise using tables applicable to Brookfield viscometer Models LV

3.8.4 Colour

Colour was determined by visual observation.

3.8.5 Odour

This was evaluated making use of the olfactory organ.

3.9 Stability

Stability tests were carried out on the emulsions following the methods as described by Cannel (1992).

3.9.1 Temperature variation

1. Samples were stored at -10^{o}C
2. Samples were stored at 4^{o}C and ambient humidity
3. Samples were stored at 28^{o}C and ambient humidity
4. Samples were stored at elevated temperatures (37^{o}C and 45^{o}C)

All observations were noted and recorded.

3.9.2 Freeze thaw cycles testing

The samples were made to pass three cycles of temperature testing by placing the samples at -10^{o}C for 24 hours and then at room temperature for 24 hours.

3.9.3 Centrifugation

The emulsions were heated to 50^{o}C and then centrifuged for thirty minutes at 2000, 2500, 3000 and 4000 rpm. The emulsions were inspected for signs showing that they were breaking and the two phases were separating.

3.9.4 Light testing

The emulsions were placed in test tubes and also in the actual package. The containers were then put on the window sill where direct sun rays fell on them .This method is used to determine the sensitivity of the emulsions to the Ultra Violet radiation.

3.9.5 Moisture loss on drying/ residue content at 105^{o}C

Cosmetic emulsion of about 2.0 g was weighed into a dried Petri dish and kept in the oven at 105^{o}C for 2 hours. It was taken and stored in a desiccator to cool. After cooling to room temperature, the sample was weighed. The procedure was repeated until the weight of the sample was constant. Moisture loss and residue contene was calaulated as below:

$$\text{Moisture Loss on drying \%} = \frac{(A-B)}{W} \times 100 \qquad \text{eqn 7}$$

Where A = Weight of dish + Product before drying in g

B = Weight of dish + Product after drying in g

W = Weight of sample taken in g

Residue Content = 100 - Loss on Drying

3.10 Animal Model/Efficacy

3.10.1 Ethics approval

The experimental animal study design was submitted to the University of Ibadan ethics committee and was approved with Approval number UI ACUREC /App / 12 / 2016 / 06 (see Appendix).

3.10.2 Skin Sensitivity

All the emulsion preparations with plant extracts and the placebo were tested on the shaved skin of five albino rats. The test samples were applied in the morning on square sites (2 x 2 cm) on the skin and allowed to remain there until the following morning. The treated surface was then rinsed with water and patted dry with absorbent paper. The site was examined 24 hours later for signs of irritation.

3.10.3 The Experimental Animal Study Design

Fourty five (45) animals were required per batch and were grouped into 6 groups of 5 rats.

Group 1 – Animals without any treatment – Negative control

Group 2 – Animals treated with cream containing 1% Clotrimazole (Positive Control - a).

Group 3 – Animals treated with placebo (Emulsion base only/Positive control - b)

Group 4a – Animals treated with 0.5% medicinal plant extract alone

Group 4b – Animals treated with emulsion containing 0.5% medicinal plant extract

Group 5a – Animals treated with 1% medicinal plant extract alone

Group 5b – Animals treated with emulsion containing 1% medicinal plant extract

Group 6a- Animals treated with 2% medicinal plant extract alone

Group 6b- Animals treated with emulsion containing 2% medicinal plant extract

Group 7a – Un-infected animals (normal skin condition – control)

Group 7b – Uninfected animals with patch rubbed with emulsion base

3.10.4 Animal study

The animals were randomly allocated to six (6) groups (5rats/group) such that the difference in average weight did not exceed 5 g. Each animal was used once in the experiment. The rats were then inoculated with the dermatophytes. One week after inoculation of the animals with the dermatophytes, the inoculated skin area of 2 cm^2 were treated with the plants extracts and the formulated emulsions with plants extracts for seven days. At the end of seven days, the animals were euthanized by cervical dislocation and the 2 cm^2 skin areas cut and put in 10% formalin for histopathological analysis. Skin biopsy samples were examined for presence of fungal hyphae, hair follicles, sebaceous gland, inflammation and tissue destruction using light microscope. Also epidermal thickness and keratin layer were obtained with the aid of calibrated Toupview® software.

3.10.5 Histopathological procedure

The skin tissues were fixed in 10% formalin before castifying into 30% phenol acid for 24 hours. The tissues were trimmed into tissue cassettes for processing. The tissues were dehydrated and were processed gradually through graded ethanol starting from 70,

80, 90, 95 and 100% ethanol. Time frame for the tissues in each grade was 1 hour while it was 2 hours in absolute ethanol. Xylene was added to remove the ethanol and at the same time rendered the tissues transparent. The skin tissues were impregnated with paraffin wax which had been heated in the oven for 1 hour at 50^{o}C after which the tissues went through sectioning at 4µ with microtome in water bath. The section was placed on microslide and put in oven for dewaxing. It was thereafter stained with heamatoxylin for 15minutes and excess stain washed off and differentiated in 2% acid alcohol for 3 minutes and excess acid washed off and later counter stained in eosin for 3 minutes. The tissues were dehydrated using ethanol and the slide remain in the xylene until it was mounted using DPX.

3.11 Statistical Analysis of Data

Statistical analysis of data was carried out by one way ANOVA comparing all test groups vs control followed by Tukeys post hoc analysis. Data is reported as mean ± SEM.

CHAPTER FOUR
RESULTS AND DISCUSSION

4.1 Solvent Extraction

The aqueous extracts yield of the medicinal plants were in the range of 1.5 - 7.5% with *Mitracarpus villosus* having the least value and *Kigelia africana* having the highest (Table 2). The petroleum ether extracts yield were *Mitracarpus villosus* (1.3%), *Acalypha wilkesiana* (5.1%), *Cassia occidentalis* (5.7%), *Cassia alata* (7.1%), *Kigelia africana* (7.6%) *respectively* (Table 2). The ethanol extracts yield of the medicinal plants were from 2.1 - 8.5% with *Mitracarpus villosus* giving the lowest yield while both *Cassia alata* and *Kigelia africana* gave the highest yields (Table 2). The high yield obtained for ethanol extracts could be due to the fact that as a solvent it was able to extract polar and some non-polar components of the medicinal plants. The least yield for all solvents was recorded for *Mitracarpus villosus*. The results agree with work done by Okogun *et al*., 2005 who reported the ethanol yield of *Mitracarpus villosus* to be 2.0%.

4.2 Phytochemical analysis

The alkaloid contents of the medicinal plants ranged from 0.58 - 4.24% with *A.wilkesiana* being the lowest while *C.alata* contained the highest (Table 3). These values are in agreement with values obtained by Obdoni *et al.,* (2001) who reported alkaloid values of 4.22 and 3.52% for *C.alata* and *C. occidentalis* respectively and Edeoga *et al.,* (2005) who reported 4.0 and 0.55% for *K. africana and A. wilkesiana* respectively. In their studies, they reported that the high content of alkaloid in some medicinal plants justify their use for therapeutic purposes. The saponin contents of the plants were in the range 0.96 - 2.10% with *M.villosus* being the lowest while *A. wilkesiana* was the highest. The values are in agreement with the results obtained by

Eleazu *et al.,* 2011. They obtained 1.39% for *C.alata*, 2.14% for *A.wilkesiana* and 0.98% for *C. occidentalis* and reported that saponin at moderate level has the property of precipitating and coagulating red blood cells and also has haemolytic activity. The moderate amount contained in the plants further suggests that the plants may not be deleterious to the user (Eleazu *et al.,* 2010).

Table 2 Percentage yield of Plant extracts

Extracts	*Cassia alata*	*Cassia occidentalis*	*Mitracarpus villosus*	*Acalypha wilkesiana*	*Kigelia africana*
Aqueous	7.2	6.4	1.5	6.9	7.5
Petroleum ether	7.1	5.7	1.3	5.1	7.6
Ethanol	8.5	7.3	2.1	7.9	8.5

Table 3 Phytochemical Composition of the Medicinal Plants

Medicinal plants	Alkaloid (%)	Saponin (%)	Flavonoid (%)	Tannin (%)
C. alata	4.24±0.24	1.35±0.39	0.45±0.29	2.42±0.32
C. occidentalis	3.49±0.01	1.02±0.05	0.48±0.06	2.31±0.44
M. villosus	1.06±0.04	0.96±0.07	0.06±0.02	0.04±0.01
A. wilkesiana	0.58±0.01	2.10±0.23	1.61±0.04	0.61±0.02
K. Africana	3.97±0.11	1.48±0.31	0.81±0.62	2.05±0.08

Each value in the table is the average ± standard deviation of triplicate experiments

The flavonoid contents of the medicinal plants range from 0.06 - 1.6% with *M.villosus* being the lowest while *A. wilkesiana* containing the highest. These values agrees with those reported by Akinmoladun *et al*., 2007 who said that flavonoid content of *C.alata* was 0.45% and *C.occidentalis* was 0.49% respectivelywhile Okwu 2004 in his work reported that *M.villosus* and *A. wilkesiana* had flavonoid contents of 0.08 and 1.56% respectively. The tannin contents of the plants range from 0.04 - 2.42 % with *M.villosus* being the lowest while *C.alata* containing the highest. Previous studies by Eleazu *et al.,* 2010 and Eleazu 2011, justified the use of medicinal plants containing tannin because of its astringent property and ability to bind proteins and carbohydrate (Table 3).

4.3 Emulsion Type

The emulsions bases took up colour of methyl orange and therefore are of the oil in water type. Oil- in- water emulsions are ideal for topical preparations as they are easily washed from the skin and their non-greasy texture is good for tropical weather. This view agrees with studies carried out by Ratz-lyko *et al.,* (2016). In their study, it was showon that oil-in-water emulsion can improve skin moisture by increase of skin surface hydration state and decrease in transepidermal water loss. This type of emulsion is best used in pharmaceutics, hairstyling, personal hygiene and cosmetics (Ratz-lyko *et al.,* 2016.)

4.3.1 Properties of the emulsions

Properties studied included colour, pH, spreading of emulsion, rubbing in effect, and stability to centrifugation (Table 4). All the formulated emulsions had white colour. The pH of the samples range from 7.02 - 7.26. Sample FA had good rheology and good spread of emulsion resulting in better rubbing–in effect due to the right amount of stearic acid (3 g). However, sample FB was more viscous than sample FC due to the amount of stearic acid 2.5 and 2 g respectively. Therefore, the spread of emulsion and rubbing–in effect of sample B is better that sample FC. The higher percentage of stearic

acid in sample FD (4.5 g) produced an emulsion with high viscosity but reduced spread of emulsion and rubbing-in effect. Sample A has the best moisturizing effect, while absence of mineral oil in sample FD could not make the sample retain moisture.

Table 4 Properties of the emulsion bases

Sample	Colour	pH	Spread of emulsion	Rubbing in effect	Stability to centrifugation
FA	White	7.02	Very high	Very high	Very high
FB	White	7.04	High	Very high	Very high
FC	White	7.21	Medium	Medium	High
FD	White	7.26	Medium	Low	High

4.4 Antimicrobial Screening of Medicinal Plants Extracts

4.4.1 Effect of aqueous extracts of medicinal plants against *T. mentagrophytes*

At 10000 µg/mL, there were significant inhibition activities of the extracts of the medicinal plants against *T. mentagrophytes* with zones of inhibition in the range from 9 - 12 mm with *M. villosus* having the lowest and *Cassia alata* having the highest. At 1000 µg/mL, the activities reduced with range from 8 – 10 mm with *C.alata* having the lowest value, while the value obtained for *C.occidentalis* and *M. villosus* were the same. The values obtained for *A. wilkesiana* and *K. africana* were equally the same. Activities were only noticed for extracts of *A. wilkesiana* and *K.africana* at lower concentrations of 100 and 10 µg/mL where the zones of inhibition were 7 and 5 mm respectively (Table 5). This result agrees with what Somchit *et al.,* (2003) observed in their studies against some dermatophytes with observed zones of inhibition of 11 – 14 mm. Also the conclusion by Abubaker *et al.,* (2008) that the aqueous extracts of the plants has powerful activity against plant pathogenic fungi and human pathogenic fungi were confirmed by this study. It was observed that the aqueous extract activity is concentration dependent. The antimicrobial activity against seven human pathogenic bacteria and two fungal strains carried out by Vedpriya *et al*., (2010) clearly supported the effectiveness of the aqueous extract of the plant with higher zones of inhibition compared to the methanol extract.

4.4.2 Effect of ethanol extracts of medicinal plants against *T. mentagrophytes*

The activities of ethanol extracts of the medicinal plants against *T.mentagrophytes* at 10000 µg/mL range from 10 – 15 mm (Table 5) with *C. occidentalis* extract being the least activewhile *K. africana* extract was the most active. There was noticeable

reduction in activities of the extracts as the concentration reduced to 1000 µg/mL with zones of inhibition in the range 8 – 14 mm. At this concentration, *C. occidentalis* was also the least active while *K. africana* was the most active. At 100 µg/mL, activities were only observed for three of the medicinal plants namely *M. villosus* , *A. wilkesiana* and *K. africana* with zones of inhibition in the range 7 – 10 mm. However, no activities were recorded for *C. alata, C. occidentalis* and *M. villosus*. At 10 µg/mL, only *A. wilkesiana* (7 mm) and *K. africana* (9 mm) (Table 5) had activities.

Previous study by Okafor *et al.,* (2001) against the pathogens seems to agree with the trend observed in this study. The study also confirmed the results obtained by Makinde

Table 5. Zones of inhibition (in mm) of solvent extracts of medicinal plants against *T. mentagrophytes*

	AW				CA				CO				MV				KA			
Conc.(µg/mL)	10	100	1000	10000	10	100	1000	10000	10	100	1000	10000	10	100	1000	10000	10	100	1000	10000
Aq	5±1	7±0.58	10±0.33	11±1	-	-	8±0.2	12±0.1	-	-	9±0	11.3±0.58	-	-	9±0	9.3±0.58	5±0	7.3±0.58	10±0.33	10±1
Et	7.3±0.8	8.3±.06	10±0.33	13±0	-	-	11±0.24	14±0.	-	8±0	8±0	10±0.	-	7.6±0.58	12±0	13±1	8.6±0.58	10±0	14±1	15±0.
Pet.E	-	-	7±0.24	8±0.2	-	-	7±0	11±0	-	-	8±0.2	12±1	-	-	8±0.24	12±0.24	4±0.24	7±0.24	10±0.58	10±0.

Aq - Aqueous extracts of medicinal plants

Et – Ethanol extracts of medicinal plants

Pet.E – Petroleum ether extracts of medicinal plants

AW- *Acalypha wilkesiana*

CA - *Cassia alata*

CO – *Cassia occidentalis*

MV- *Mitracarpus villosus*

et al., (2007) who compared the activities of the extract against some fungi and bacteria and concluded that the extract was more active against the fungi. This suggests that the extract can be a good candidate for drug development. Aboh *et al.,* (2014) in their study of the *M.villosus* ethanol extract against clinical isolates of *Candida albicans*, *Candida* krusei, *Trichophyton mentagrophytes*, *Trichophyton verrucosum*, *Aspergillus fumigates* and *Aspergillus niger* showed that it was very active. The results obtained here, corroborates their results.

4.4.3 Effect of petroleum ether extracts of medicinal plants against *T. mentagrophytes*

The activities of petroleum ether extracts of the medicinal plants against *T.mentagrophytes* at 10000 µg/mL range from 8 – 12 mm (Table 5) with *A. wilkesiana* having the least value while the activity was highest in *C. occidentalis* and *M. villosus.* However, at 1000 µg/mL, the activities reduced with range from 7 – 10 mm. No activity was observed for all the medicinal plants at the lower concentrations of 100 and 10 µg/mL except for *K. africana*. Kathirvel and Sujatha, (2011) reported the presence of some phytochemicals in the petroleum ether extract of *C.occidentalis*, but their presence is small compared to the quantity obtained from aqueous and ethanol extracts. This probably explains the low activities observed for the extract against the dermatophyte. Reports on activity of petroleum extracts of these plants are not common.

4.4.4 Effect of aqueous extracts of medicinal plants against *M.aoudinin*

The activities of aqueous extracts of the medicinal plants against *M.aoudini,* at 10000 µg/mL are in the range 7 – 12 mm (Table 6), with *M.villosus* having the least zone of inhibition of 7.3 mm while *C.alata* had the highest of 12 mm. However, at 1000 µg/mL, the activities reduced with range from 5 – 9 mm while no activity was observed for all the medicinal plants except for *K. africana* (6 mm) at 100 µg/mL. None of the extracts was observed to be effective at 10 µg/mL. Though local usage of *M.villosus* water extract has been reported for treatment of skin diseases, available scientific data according to Onawunmi *et al*., 2012, indicated that there was no inhibition against *Trichophyton rubrum*, *Microsporum gypseum*, *Candida albicans*, *Aspergillus niger* and *Fusarium solani*. Arti and Kanika (2014) reported that antimicrobial activity of the plant extracts depends on the chemical nature of compounds present in them.

Table 6. Zones of inhibition (in mm) of medicinal plants extracts against *M.aoudini*

	AW				CA				CO				MV				KA			
Conc.(µg/mL)	10	100	1000	10000	10	100	1000	10000	10	100	1000	10000	10	100	1000	10000	10	100	1000	10000
Aq	-	-	7±1	9±0.33	-	-	7±0.2	12±0.1	-	-	7±0.2	11.3±0.58	-	-	5±0.2	7.3±0.58	-	6±0.58	9±0.33	10±1
Et	5±0.8	6±1	9±0.33	11	-	-	10±0.14	12±0.	-	-	7±0	9±1	-	5±0.58	9±0	12±1	-	10±0	12±0.33	14±0.5
Pet.E	-	-	8±0.33	10±0.2	-	-	6±0	10±0	-	-	7±0.1	12±0	-	4	10±0	11±1	-	7±0.33	10±08	10±1

They concluded that various physical factors such as pH, temperature, exposure to sunlight may bring about a change in chemical nature of these compounds thereby affecting the antimicrobial property of the extract.

4.4.5 Effect of ethanol extracts of medicinal plants against *M.aoudini*

The activities of ethanol extracts of the medicinal plants against *M. aoudini* at 10000 µg/mL range from 9 -14 mm (Table 6) with *C. occidentalis* having zone of inhibition of 9mm while *K. africana* had the highest(14 mm). There was noticeable reduction in activities of the extracts as the concentration reduced to 1000µg/mL. At 100 µg/mL, activities were only observed for three of the medicinal plants namely *M. villosus* (5 mm),*A. wilkesiana* (5 mm) *and K. africana* (10 mm). It was only *A. wilkesiana* that has activity at 10 µg/mL. It was observed that the *A. wilkesiana* ethanol extract exerted greater antibacterial activity than corresponding water extract. Akinyemi *et al.,* (2005) reported this observation. They suggested that the nature of biologically active components were possibly enhanced in the presence of ethanol and the stronger extraction *c*apacity of ethanol may results in greater number of active constituents and might be responsible for greater antibacterial activity.

4.4.6 Effect of petroleum ether extracts of medicinal plants against *M.aoudini*

At 10000 µg/mL the activities of petroleum ether extracts of the medicinal plants against *M. aoudinin* range from 10 – 12 mm (Table 6) with *A. wilkesiana, C. alata* and *K. africana* having the same zone of inhibition of 10 mm. The zone of inhibition of *M. villosus* was 11 mm while *C. occidentalis* was 12 mm.At 1000 µg/mL, the activities of the extracts reduced against the dermatophyte with zones of inhibition in the range 6 – 10 mm with *C.alata* having the least activity(6 mm) and *M. villosus* having 10 mm same as *K. africana*. At 100 µg/mL, activities were observed for two of the medicinal plants namely *K. africana* (7 mm) and *M. villosus* (4 mm). None of the extracts were active at 10 µg/mL.

4.4.7 Effect of aqueous extracts of medicinal plants against *E.floccossum*

The activities of aqueous extracts of the medicinal plants against *E. floccossum* at 10000 µg/mL range from 9 – 12 mm (Table 7). *A. wilkesiana* and *C. occidentalis* had 9 mm while *C. alata* had 12 mm. However, at 1000 µg/mL, the activities reduced with range from 7 - 8 mm with *A. wilkesiana* and *C. alata* having the same activity.

Table 7. Zones of inhibition (in mm) of medicinal plants extracts against *E. floccossum*

	AW				CA				CO				MV				KA			
Conc.(µg/mL)	10	100	1000	10000	10	100	1000	10000	10	100	1000	10000	10	100	1000	10000	10	100	1000	10000
Aq	-	-	7±1	9±0.33	-	-	7±0.2	12±0.1	-	-	8±0.2	9.3±0.58	-	-	8±0.2	10±0.58	4±1	5±0.58	8±0.33	10±1
Et	-	5±0.33	10±0.33	11±1	-	5±0.2	10±0	14±0.5	-	-	6±0	10±1	-	3±0.2	8±0.33	9±1	8±1	10±0	11±0.33	13±0.5
Pet.E	-	4	9±0.2	10±1	-	5	8±1	13±0	-	-	9±0.1	12±0	-	-	10±1	11±0.5	4±1	8±0.3	10±1	10±1

The activities of the remaing extracts were the same. At 100 and 10 µg/mL, no activity was observed for all the medicinal plants except for *K.africana* with zones of inhibition of 5 and 4 mm respectively. There have been various scientific reports concerning the use of *A. wilkesiana* aqueous extract against microorganisms. The result obtained agrees with Akinyemi *et al.,* (2005) who reported that water extract of *Acalypha wilkesiana* was not very active at low concentration against methicillin resistant Staphylococcus aureus. Their conclusion also agrees with the observed inactivity of the extract at lower concentrations.

4.4.8 Effect of Ethanol extracts of Medicinal plants against *E.floccossum*

The activities of ethanol extract of the medicinal plants against *E. floccossum* at 10000 µg/mL range from 9 -14 mm (Table 7) with *M. villosus* having the least activity while *C. alata* had the highest. At 1000 µg/mL, the zones of inhibition were in the range 6 – 11 mm. *C. occidentalis* had the lowest activity while *K. africana* had the highest. At 100 µg/mL, activities were observed for four of the medicinal plants namely *M. villosus* (3) *A. wilkesiana* (5), *C. alata* (5), and *K. africana* (10). No activity was recorded for *C. occidentalis*. It was only *K. africana* that has activity at 10 µg/mL with 8 mm zone of inhibition. The ethanol extract of *K. africana* exbited more activity as compared to ethanol extracts of other medicinal plants. Owolabi *et al.,* (2007) repoted the fact that the extract was active against bacteria and fungi. They also found that the ethanolic extract activity was comparable with that of ampicillin, gentamicin and ciprofloxacin. Similar conclusion was drawn by Jeyachandran and Mahesh (2007).

4.4.9 Effect of petroleum ether extracts of medicinal plants against *E.floccossum*

The activities of petroleum ether extracts of the medicinal plants against *E. floccossum* at 10000 µg/mL range from 10 – 13 mm (Table 7). *K. africana* and *A. wilkesiana* had the least activity while *C. alata.*had the highest. At 1000 µg/mL, the zones of inhibition were in the range 8 – 10 mm with *C. alata* having the least while both *K. africana* and *M. villosus* had the highest. At 100 µg/mL, activities were observed for three of the medicinal plants namely *A. wilkesiana, C. alata* and *K. africana* with zones of inhibition in the range 4 -8 mm. No activity was recorded for *C. occidentalis* and *M. villosus*. It was only *K. africana* that had activity at 10µg/mL with 4mm zone of inhibition. Okogun *et al.,* (2005) were able to show that the petroleum ether extract of

M. villosus was more active than the methanol extract when tested against some bacteria and fungi. The effectiveness of the extract against clinical isolates of *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Candida albicans* and *Sarcinalutea* also carried out by Abere *et al.,* (2007) showed that the petroleum ether extract was very active but have low activity against *Pseudomonas aeruginosa*.

4.4.10. Effect of aqueous extracts of medicinal plants against *M.furfur*

The activities of aqueous extracts of the medicinal plants against *M. furfur* at 10000 µg/mL range from 9 – 14 mm (Table 8). *C. occidentalis* had the least activity while *M. villosus* had the highest.. At 1000 µg/mL, the zones of inhibition were in the range 7 – 12 mm with *C. occidentalis* having the least activity while *M. villosus* had the highest. At 100 µg/mL, activities were observed for four of the medicinal plants namely *K. africana*, *C. alata*, *A. wilkesiana*,and *M. villosus* with zones of inhibition in the range 5 – 10 mm. No activity was recorded for *C. occidentalis*. The same observation was recorded for the medicinal plants extracts at 10 µg/mL with zones of inhibition in the range 4 – 7 mm while *C. occidentalis* exhibited no activity (Table 13).

Though Owolabi *et al.,* (2007) reported that the aqueous extract of *Kigelia africana* water extract exhibited no antibacterial or antifungal activity at low concentrations, but the low activity observed here could be attributed to the conclusion drawn by Arti and Kanika (2014) who submitted that phytochemicals in little quantities have definite physiological action on the human body to fight microbes.

4.4.11 Effect of ethanol extracts of medicinal plants against *M.furfur*

The activities of ethanol extracts of the medicinal plantsat 10000 µg/mL range from 9 – 15 mm (Table 8) with *C. occidentalis* having the least activity while *K. africana and M. villosus had the highst.* At 1000µg/mL, the zones of inhibition were in the range 6 – 15. At 100 µg/mL, activities were observed for four of the medicinal plants namely *C.alata, K. africana*, *A. wilkesiana* and *M. villosus* with zones of inhibition in the range 7 – 12 mm. No activity was recorded for *C. occidentalis*. Four of the medicinal plants extracts also have activity at 10 µg/mL with zones of inhibition in the range 5 – 9 mm with *C. alata* having the least activity and *A. wilkesiana* the highest.

Table 8. Zones of Inhibition (in mm) of medicinal plants extracts against *M.furfur*

			AW				CA				CO				MV				KA	
Conc.(µg/mL)	10	100	1000	10000	10	100	1000	10000	10	100	1000	10000	10	100	1000	10000	10	100	1000	10000
Aq	7±0.5	8±1	11±0	12±0.33	5±1	7±24	8±0.2	10±0.11	-	-	7±0.2	9.4±0.58	4±1	10±06	12±0	14±0.1	4±0.33	5±0.58	8±0.33	10±1
Et	9±1	10±0.33	12±0.33	14±0.5	5	7±0.2	13±0	13±1	-	-	6±0	9±1	6	12±0.2	15±0.33	15±1	7±1	8±0	12±0.33	15±0.3
Pet.E	-	-	10±0.2	10±1	-	6±1	8±1	10±0	5±0	7±1	8±0.1	10±0	-	7.1±1	10±1	11±0	3±1	5±0.3	11±0.33	12±0.3

Aq - Aqueous extracts of medicinal plants

Et – Ethanol extracts of medicinal plants

Pet.E – Petroleum ether extracts of medicinal plants

AW- *Acalypha wilkesiana*

CA - *Cassia alata*

CO – *Cassia occidentalis*

MV- *Mitracarpus villosus*

KA- *Kigelia aficana*

This result is in agreement with what Odima *et al.,* (2014) observed when they tested the *M.villosus* ethanol plant extract against some microorganisms. They concluded that the phytochemicals was of medical importance and their effect affected by the mineral and organic composition of the soil on which they grow.

4.4.12 Effect of petroleum ether extracts of medicinal plants against *M.furfur*

The activities of petroleum ether extracts of the medicinal plants against *M. furfur* at 10000 µg/mL range from 10 – 12 mm (Tabel 8). The activity of *A.wilkesiana*, *C.alata* and *C.occidentalis* were the same while *K. africana.*had the highest actibity. At 1000 µg/mL, the zones of inhibition were in the range 8-11mm. At 100µg/mL, activities were observed for four of the medicinal plants namely *K. africana,C. alata,C. occidentalis* and *M. villosus* with zones of inhibition in the range 5 – 7 mm. No activity was recorded for *A.wilkesiana*. Two of the medicinal plants extracts have activity at 10 µg/mL with zones of inhibition 3 and 5 mm for *K. africana* and *C. occidentalis* respectively. However, *A. wilkesiana, C. alata* and *M. villosus* exhibited no activity. The activity of *K. africana* petroleum ether extract was more against the dermatophyte. Similar conclusions were drawn by John Britto, 2001 and Singh and Singh, 2000 in their studies.

4.5 Fourier Transform Infra-red Spectrocopy analysis

Results of FTIR spectroscopic studies revealed the presence of various functional groups in the cream and the test samples (Table 9). The infra red spectrum of the placebo (Fig 48. See Appendix), showed strong O-H stretch at 3373.00 cm^{-1} of amines.N=H bending at 1640.00 cm^{-1} indicating that the placebo contains an unsaturated amide group. O-H bending of phenyl group at 1409.00 cm-1 indicated the presence of carboxylic acid group in the sample, S=O stretch at 1040.00 cm^{-1} indicated presence of sulphoxide. The Ca cream contains S-S stretch at 470.00 cm^{-1} indicating presence of polysulphides (Fig.49 see Appendix). N=H bend at 1637.00 cm^{-1} shows presence of amine group. The spectrum also shows NH stretch of aliphatic amine at 3376.00 cm^{-1}. The spectrum also shows presence of S=O stretch at 1040.00 cm^{-1}. In figure 50, the spectrum of Co cream shows C-N stretch of pyridines at 1559.00 cm^{-1} and also polymeric O-H stretch at 3158.00 cm^{-1}.CH_2 of ketones at 1409.00 cm^{-1} was observed for Mv cream (Fig.51 see Appendix). C=C stretch of aromatic ring at 1641.00 cm^{-1} was also observed. These peaks confirm the presence of aromatic structure.

Table 9. Comparison of FTIR values in placebo and cosmeceuticals

Functional groups and properties	PL(cm^{-1}	Ca(cm^{-1})	Co(cm^{-1})	Mv(cm^{-1})	Aw($cm^{-1)}$	Ka(cm^{-1})
C-C	1409.00	1409.00	1409.00	1409.00	1409.00	1409.00
C=C stretch		1675.00		1641.00	1656.00	1671.00
C-N stretch			1559.00	1182.00	1184.00	1091.00
C-H stretch	2095.00	2092.00	2097.00	2857.00	2920.00	2920.00
S-S stretch	458.33	470.00	461.00	461.00	411.00	431.00
N-H bend	1640.00	1637.00	1639.00	1635.00	1641.00	1639.00
N-H stretch		3376.00		3431.00	3436.00	3431.00
O-H Stretch	3373.00	3376.00	3158.00	3372.00	3493.00	3254.00
S-O Stretch	1040	1040.00		1043.00	1042.00	1040.00

PL = Cream base
Ca= *Cassia alata*
Co= *Cassia occidentalis*
Mv= *Mitracarpus villosus*
Aw= *Acalypha wilkesiana*
Ka= *Kigelia africana*

An aromatic C-N stretch peak at 1182.00 cm^{-1} was also observed. The peak of S=O stretch observed at 1043.00 cm^{-1} indicated presence of sulphoxide and peak of S-S stretch of Aryl observed at 461.00 cm^{-1}. Spectrum of Aw cream (Fig.52 see Appendix) shows a peak of dimeric O-H stretch at 3493.00 cm^{-1}. The presence of a new peak at 3493.00 $cm^{-1.}$ is attributed to the ether group ROR stretching, indicating that the epoxy group might be opened. The result is in tune with what Saremi *et al*., 2012 reported for epoxides and polysulphide S-S peak at 411.00 cm^{-1}. There is a spectrum of C-N stretch which peaks at 1019.00 cm^{-1} (Fig 53 see Appendix) for Ka cream, this peak serves as good evidence for the homopolymerization reaction. Also, an O-H stretch peak was observed at 3254.00 cm^{-1}. Some of these functional groups found in the herbal cream formulations which are not in the cream base maybe responsible for the plants effectiveness in the management of dermatophytosis.

4.6 Stability

4.6.1 Effect of temperature on aqueous extracts emulsions

The results of changes in colour, odour and pH from date of production through 2 - 16th week of production and stored at -10^{o}C is presented in Table 10. At production, the pH of control was 7.02, Mv.W was 7.15, Aca.W is 7.17, Ca.W was 7.20, Co.W was 7.18 and Ka.W was 7.21. After two weeks of production through 4,8,12 and 16th week, the colour, odour and pH of the samples of the extracts emulsions remain stable.

At 4^{o}C, there was no change in the colour, odour and pH of the control, Mv.W and Aw.W from the production day through the sixteen weeks of tests (Table 11). No change was observed for both Ca.W and Co.W from production day to 16th week. No change in colour and odour was observed for Ka.W, the pH was 7.21 and remained constant throughout the test period. However, no change in colour and odour of the samples were observed. There was no change in colour, odour and pH of the control between the production day and the first 2 weeks with the pH of 7.02 at 30^{o}C.

The pH dropped to 7.01 at 4th week and the same value was measured at 8th week. However, at 12th and 16th week, the pH was 7.00. No change was noticed for Mv.W at 2 weeks of production, but it was observed that the pH decreased from 7.15 to 7.13 from 4th week to 16th week. From the day of production to 4th week of test, no change was observed for Aca.W. Between 8th and 12th week, there was a decrease of the pH to 7.16 from 7.17 and at 16th week it became 7.19.

Table 10 Effect of (-10^{o}C) Temperature on aqueous extracts emulsion samples after production

product	Day 1			2 weeks			4 weeks			8 weeks			12 weeks			16 weeks		
	pH	Colour	odour	pH	Colour	odour	PH	colour	odour	pH	colour	odour	pH	colour	odour	pH	colour	odour
Control	7.02	NCC	NCIO	7.02	NCC	NCIO	7.02	NCC	NCIO	7.02	NCC	NCIO	7.02	NCC	NCIO	7.02	NCC	NCIO
Mv.W	7.15	NCC	NCIO	7.15	NCC	NCIO	7.15	NCC	NCIO	7.15	NCC	NCIO	7.15	NCC	NCIO	7.15	NCC	NCIO
Aca.W	7.17	NCC	NCIO	7.17	NCC	NCIO	7.17	NCC	NCIO	7.17	NCC	NCIO	7.17	NCC	NCIO	7.17	NCC	NCIO
Ca.W	7.20	NCC	NCIO	7.20	NCC	NCIO	7.20	NCC	NCIO	7.20	NCC	NCIO	7.20	NCC	NCIO	7.20	NCC	NCIO
Co.W	7.18	NCC	NCIO	7.18	NCC	NCIO	7.28	NCC	NCIO	7.18	NCC	NCIO	7.18	NCC	NCIO	7.18	NCC	NCIO
Ka.W	7.21	NCC	NCIO	7.21	NCC	NCIO	7.21	NCC	NCIO	7.21	NCC	NCIO	7.21	NCC	NCIO	7.21	NCC	NCIO

NCC - No change in colour

NCIO - No change in odour

CC - Change in colour

CIO - Change in odour

Mv.W – *Mitracarpus villosus* water extract

Aca.W – *Acalypha wilkesiana* water extract

Ca.W – *Cassia alata* water extract

Co.W – *Cassia occidentalis* water extract

Ka.W – *Kigelia africana* water extract

product	Day 1			2 weeks			4 weeks			8 weeks			12 weeks			16 weeks		
	pH	Colour	odour	pH	colour	odour	PH	colour	odour	pH	colour	odour	pH	colour	odour	pH	colour	odour
Control	7.02	NCC	NCIO	7.02	NCC	NCIO	7.02	NCC	NCIO	7.02	NCC	NCIO	7.02	NCC	NCIO	7.02	NCC	NCIO
Mv.W	7.15	NCC	NCIO	7.15	NCC	NCIO	7.15	NCC	NCIO	7.15	NCC	NCIO	7.15	NCC	NCIO	7.15	NCC	NCIO
Aca.W	7.17	NCC	NCIO	7.17	NCC	NCIO	7.17	NCC	NCIO	7.17	NCC	NCIO	7.17	NCC	NCIO	7.17	NCC	NCIO
Ca.W	7.20	NCC	NCIO	7.20	NCC	NCIO	7.20	NCC	NCIO	7.20	NCC	NCIO	7.21	NCC	NCIO	7.21	NCC	NCIO
Co.W	7.18	NCC	NCIO	7.18	NCC	NCIO	7.18	NCC	NCIO	7.18	NCC	NCIO	7.18	NCC	NCIO	7.18	NCC	NCIO
Ka.W	7.21	NCC	NCIO	7.21	NCC	NCIO	7.21	NCC	NCIO	7.21	NCC	NCIO	7.21	NCC	NCIO	7.21	NCC	NCIO

Table 11 Effect of (4^{o}C) Temperature on aqueous extracts emulsion samples after production

NCC - No change in colour

NCIO - No change in odour

CC - Change in colour

CIO - Change in odour

Mv.W – *Mitracarpus villosus* water extract emulsion

Aca.W – *Acalypha wilkesiana* water extract emulsion

Ca.W – *Cassia alata* water extract emulsion

Co.W – *Cassia occidentalis* water extract emulsion

Ka.W – *Kigelia africana* water extract emulsion

The same marginal trend in pH was noticed for Ca.W which at production to 4th week of test, it was constant and remain stable at 7.20 But at 8th week it decreased to 7.19 and at 12th week through 16th week, the pH is 7.24

It was also observed that though the pH for Co.W was stable within the first two weeks of production at 7.18, but decreased to 7.15 at 16th week of test. Same goes for Ka.W with decrease in pH from 7.21 at the day of production to 7.18 at 16th week of production (Table 12). The results of changes in colour, odour and pH from date of production through 2, 4, 8, 12 and 16th week of production and stored at 37°C is presented in Table 13. No noticeable changes in colour and odour were observed for all the samples. The pH of control sample decreases from7.02 to 6.99 as the week increases. However, the pH of the emulsions with medicinal plants extracts decreases as the week increases. Mv.W decreased from 7.15 at day of production to 7.12 at 16th week. Aca.W decreased from 7.17 to 7.16, Ca.W decreased from 7.20 to 7.17, Co.W from 7.18 to 7.17 and Ka.W decreased from 7.21 to 7.19. At 45°C, no noticeable changes in colour and odour were observed for all the samples except for *Cassia occidentalis* emulsion sample at 12th and 16th week. There was decrease in the pH of control sample from 7.02 to 6.98 by the 16th week. pH of Mv.W decreased marginally at date of production 7.15 to 7.13. Also, Aca.W from 7.17 to 7.14. Ca.W from 7.20 to 7.18, Co.W from 7.18 to 7.14 and Ka.W from 7.21 to 7.18 (Table 14).

It was observed that at lower temperatures of -10 and 4°C, there were no changes in the pH. Also, no change was observed for colour and odour. But as the temperature increases, there is very slight decrease in the pH of the control which may be due to more production of the hydrogen ions from the stearic acid component of the emulsion. The decrease in the pH of the emulsions containing medicinal plants extracts may be due to alkaloids released. The change in colour and odour observed for *Cassia occidentalis* emulsion at 12th and 16th week of test may be due to the presence of some phytochemicals in the extracts which were released at that high temperature.

4.6.2 Effect of Temperature on Ethanol Extracts Emulsions

The results of changes in colour, odour and pH from date of production through 2, 4,8,12 and 16th week of production and stored at -10°C is presented in Tables 15. At production, the pH of control is 7.02, Mv.E is 7.20, Aca.E is 7.23, Ca.E is 7.26, Co.E

Table 12 Effect of (30^{o}C)Temperature on aqueous extracts emulsion samples after production

product	Day 1			2 weeks			4 weeks			8 weeks			12 weeks			16 weeks		
	pH	Colour	odour	pH	colour	odour	PH	colour	odour	pH	colour	odour	pH	colour	odour	pH	colour	odour
Control	7.02	NCC	NCIO	7.02	NCC	NCIO	7.01	NCC	NCIO	7.01	NCC	NCIO	7.00	NCC	NCIO	7.00	NCC	NCIO
Mv.W	7.15	NCC	NCIO	7.15	NCC	NCIO	7.15	NCC	NCIO	7.13	NCC	NCIO	7.13	NCC	NCIO	7.13	NCC	NCIO
Aca.W	7.17	NCC	NCIO	7.17	NCC	NCIO	7.17	NCC	NCIO	7.16	NCC	NCIO	7.16	NCC	NCIO	7.16	NCC	NCIO
Ca.W	7.20	NCC	NCIO	7.20	NCC	NCIO	7.19	NCC	NCIO	7.19	NCC	NCIO	7.19	NCC	NCIO	7.19	NCC	NCIO
Co.W	7.18	NCC	NCIO	7.18	NCC	NCIO	7.15	NCC	NCIO	7.15	NCC	NCIO	7.15	CC	CIO	7.15	CC	CIO
Ka.W	7.21	NCC	NCIO	7.21	NCC	NCIO	7.18	NCC	NCIO	7.18	NCC	NCIO	7.18	NCC	NCIO	7.18	NCC	NCC

NCC - No change in colour

NCIO - No change in odour

CC - Change in colour

CIO - Change in odour

Mv.W – *Mitracarpus villosus* water extract emulsion

Aca.W – *Acalypha wilkesiana* water extract emulsion

Ca.W – *Cassia alata* water extract emulsion

Co.W – *Cassia occidentalis* water extract emulsion

Ka.W – *Kigelia africana* water extract emulsion

Table 13 Effect of (37^{o}C) Temperature on aqueous extracts emulsion samples after production

product	Day 1			2 weeks			4 weeks			8 weeks			12 weeks			16 weeks		
	pH	Colour	odour	pH	colour	odour	PH	colour	odour	pH	colour	odour	pH	colour	odour	pH	colour	odour
Control	7.02	NCC	NCIO	7.00	NCC	NCIO	7.00	NCC	NCIO	6.99	NCC	NCIO	6.99	NCC	NCIO	6.99	NCC	NCIO
Mv.W	7.15	NCC	NCIO	7.15	NCC	NCIO	7.12	NCC	NCIO	7.12	NCC	NCIO	7.12	NCC	NCIO	7.12	NCC	NCIO
Aca.W	7.17	NCC	NCIO	7.16	NCC	NCIO	7.16	NCC	NCIO	7.16	NCC	NCIO	7.16	NCC	NCIO	7.16	NCC	NCIO
Ca.W	7.20	NCC	NCIO	7.17	NCC	NCIO	7.17	NCC	NCIO	7.17	NCC	NCIO	7.17	NCC	NCIO	7.17	NCC	NCIO
Co.W	7.18	NCC	NCIO	7.18	NCC	NCIO	7.17	NCC	NCIO	7.17	NCC	NCIO	7.17	CC	CIO	7.17	CC	CIO
Ka.W	7.21	NCC	NCIO	7.20	NCC	NCIO	7.19	NCC	NCIO	7.19	NCC	NCIO	7.19	NCC	NCIO	7.19	NCC	NCIO

NCC - No change in colour

NCIO - No change in odour

CC - Change in colour

CIO - Change in odour

Mv.W – *Mitracarpus villosus* water extract emulsion

Aca.W – *Acalypha wilkesiana* water extract emulsion

Ca.W – *Cassia alata* water extract emulsion

Co.W – *Cassia occidentalis* water extract emulsion

Ka.W – *Kigelia africana* water extract emulsion

Table 14 Effect of (45^{o}C) Temperature on aqueous extracts emulsion samples after production

product	Day 1			2 weeks			4 weeks			8 weeks			12 weeks			16 weeks		
	pH	Colour	odour	pH	colour	odour	PH	colour	odour	pH	colour	odour	pH	colour	odour	pH	colour	odour
Control	7.02	NCC	NCIO	6.99	NCC	NCIO	6.99	NCC	NCIO	6.98	NCC	NCIO	6.98	NCC	NCIO	6.98	NCC	NCIO
Mv.W	7.15	NCC	NCIO	7.13	NCC	NCIO	7.13	NCC	NCIO	7.13	NCC	NCIO	7.13	NCC	NCIO	7.13	NCC	NCIO
Aca.W	7.17	NCC	NCIO	7.15	NCC	NCIO	7.14	NCC	NCIO	7.14	NCC	NCIO	7.14	NCC	NCIO	7.14	NCC	NCIO
Ca.W	7.20	NCC	NCIO	7.18	NCC	NCIO	7.18	NCC	NCIO	7.18	NCC	NCIO	7.18	NCC	NCIO	7.18	NCC	NCIO
Co.W	7.18	NCC	NCIO	7.14	NCC	NCIO	7.14	NCC	NCIO	7.14	NCC	NCIO	7.14	CC	CIO	7.14	CC	CIO
Ka.W	7.21	NCC	NCIO	7.19	NCC	NCIO	7.18	NCC	NCIO	7.18	NCC	NCIO	7.18	NCC	NCIO	7.18	NCIO	NCIO

NCC - No change in colour

NCIO - No change in odour

CC - Change in colour

CIO - Change in odour

Mv.W – *Mitracarpus villosus* water extract emulsion

Aca.W – *Acalypha wilkesiana* water extract emulsion

Ca.W – *Cassia alata* water extract emulsion

Co.W – *Cassia occidentalis* water extract emulsion

Ka.W – *Kigelia africana* water extractemulsion

Table 15 Effect of (-10^{o}C) Temperature on ethanol extracts emulsion samples after production

product	Day 1			2 weeks			4 weeks			8 weeks			12 weeks			16 weeks		
	pH	Colour	odour	pH	colour	odour	PH	colour	odour	pH	colour	odour	pH	colour	odour	pH	colour	odour
Control	7.02	NCC	NCIO	7.02	NCC	NCIO	7.02	NCC	NCIO	7.02	NCC	NCIO	7.02	NCC	NCIO	7.02	NCC	NCIO
Mv.E	7.20	NCC	NCIO	7.20	NCC	NCIO	7.20	NCC	NCIO	7.20	NCC	NCIO	7.20	NCC	NCIO	7.20	NCC	NCIO
Aca.E	7.23	NCC	NCIO	7.23	NCC	NCIO	7.23	NCC	NCIO	7.23	NCC	NCIO	7.23	NCC	NCIO	7.23	NCC	NCIO
Ca.E	7.26	NCC	NCIO	7.26	NCC	NCIO	7.26	NCC	NCIO	7.26	NCC	NCIO	7.26	NCC	NCIO	7.26	NCC	NCIO
Co.E	7.24	NCC	NCIO	7.24	NCC	NCIO	7.24	NCC	NCIO	7.24	NCC	NCIO	7.24	NCC	NCIO	7.24	NCC	NCIO
Ka.E	7.25	NCC	NCIO	7.25	NCC	NCIO	7.25	NCC	NCIO	7.25	NCC	NCIO	7.25	NCC	NCIO	7.25	NCC	NCIO

NCC - No change in colour

NCIO - No change in odour

CC - Change in colour

CIO - Change in odour

Mv.E – *Mitracarpus villosus* ethanol extract emulsion

Aca.E – *Acalypha wilkesiana* ethanol extract emulsion

Ca.E – *Cassia alata* ethanol extract emulsion

Co.E – *Cassia occidentalis* ethanol extract emulsion

Ka.E – *Kigelia africana* ethanol extract emulsion

is 7.24 and Ka.E is 7.25. After two weeks of production through 4th, 8th, 12th and 16th week, the colour, odour and pH of the samples of the extracts emulsions remain stable.

At 4°C, there was no change in the colour, odour and pH of the control and all the samples (Tables 16). At 30°C, there was no change in colour and odour of all the samples throughout the weeks of test. However, the pH of control dropped from 7.02 to 7.00 after 2 weeks of production and remained stable till 4th week. At 8th week, the pH further dropped by 0.01 to 6.99 and remained stable till 16th week. There was a minor pH decrease of 0.02 noticed for Mv.E at 2 weeks and 4weeks after production to 7.18. From 8th week to 16th week, the pH was 7.17. For Aca.E, the pH at the point of production of the sample was 7.23 and it remained stable till 4 weeks after production. At 8thweek, the pH was 7.21 and stable till 16th week. The same marginal trend in pH was noticed for Ca.E which at production was 7.26 and decreased to 7.22 at 16th week. At production, the pH of Co.E sample was 7.24 and by 2nd week after production, it has decreased to 7.22 and remained unchanged at 4th week. There was a decrease to 7.19 at 8th week through 16th week. pH of 7.25 was measured for Ka.E sample on the day of production. However, after 2 weeks of production, the pH decreased to 7.22 and remain stable till 16th week of test (Table 17).

There were changes in colour and odour of Co.E sample at 12th and 16th of test when stored at 37°C which may be due to the release of some phytochemicals from the ethanol extracts component of the emulsion at this temperature. The pH of control on the day of production is 7.02. It was observed that between the day of production and 4th week of test, the pH dropped to 7.00 and between the 8th week and 16th week, the pH was 6.99. The pH of Mv.E sample decreased from 7.20 at day of production to 7.18 within the first 4 weeks and then 7.15 at 8th through 16th week. The pH of Aca.E sample at the day of production was 7.23 which decreased to 7.20 at 4th week and 7.18 at 8th week but remained stable with pH of 7.31 at 12th and 16th week. It was observed for Ca.E sample that the pH decreases as the week increases. The pH was 7.26 at production but by the 2nd week, it was 7.25. Between 4th and 8th week it was 7.22 and at 12th and 16th week it was 7.19. For Co.E sample, the pH decreased from 7.24 at production to 7.17 at the 16th week after production. The same observation was made for pH of Ka.E sample which decreased from 7.25 at production to 7.21 after 16 weeks of production (Table 18)

Table 16 Effect of (4^{o}C) Temperature on ethanol extracts emulsion samples after production

product	Day 1			2 weeks			4 weeks			8 weeks			12 weeks			16 weeks		
	pH	Colour	odour	pH	colour	Odour	PH	colour	odour	pH	colour	odour	pH	colour	odour	pH	colour	odour
Control	7.02	NCC	NCIO	7.02	NCC	NCIO	7.02	NCC	NCIO	7.02	NCC	NCIO	7.02	NCC	NCIO	7.02	NCC	NCIO
Mv.E	7.20	NCC	NCIO	7.20	NCC	NCIO	7.20	NCC	NCIO	7.20	NCC	NCIO	7.20	NCC	NCIO	7.20	NCC	NCIO
Aca.E	7.23	NCC	NCIO	7.23	NCC	NCIO	7.23	NCC	NCIO	7.23	NCC	NCIO	7.23	NCC	NCIO	7.23	NCC	NCIO
Ca.E	7.26	NCC	NCIO	7.26	NCC	NCIO	7.26	NCC	NCIO	7.26	NCC	NCIO	7.26	NCC	NCIO	7.26	NCC	NCIO
Co.E	7.24	NCC	NCIO	7.24	NCC	NCIO	7.24	NCC	NCIO	7.24	NCC	NCIO	7.24	NCC	NCIO	7.24	NCC	NCIO
Ka.E	7.25	NCC	NCIO	7.25	NCC	NCIO	7.25	NCC	NCIO	7.25	NCC	NCIO	7.25	NCC	NCIO	7.25	NCC	NCIO

NCC - No change in colour

NCIO - No change in odour

CC - Change in colour

CIO - Change in odour

Mv.E – *Mitracarpus villosus* ethanol extract emulsion

Aca.E – *Acalypha wilkesiana* ethanol extract emulsion

Ca.E – *Cassia alata* ethanol extract emulsion

Co.E – *Cassia occidentalis* ethanol extract emulsion

Ka.E – *Kigelia africana* ethanol extract emulsion

Table 17 Effect of (30^{o}C) Temperature on ethanol extracts emulsion samples after production

product	Day 1			2 weeks			4 weeks			8 weeks			12 weeks			16 weeks		
	pH	Colour	odour	pH	colour	odour	PH	colour	odour	pH	colour	odour	pH	colour	odour	pH	colour	odour
Control	7.02	NCC	NCIO	7.00	NCC	NCIO	7.00	NCC	NCIO	6.99	NCC	NCIO	6.99	NCC	NCIO	6.99	NCC	NCIO
Mv.E	7.20	NCC	NCIO	7.18	NCC	NCIO	7.17	NCC	NCIO	7.17	NCC	NCIO	7.17	NCC	NCIO	7.17	NCC	NCIO
Aca.E	7.23	NCC	NCIO	7.21	NCC	NCIO	7.21	NCC	NCIO	7.21	NCC	NCIO	7.21	NCC	NCIO	7.21	NCC	NCIO
Ca.E	7.26	NCC	NCIO	7.22	NCC	NCIO	7.22	NCC	NCIO	7.22	NCC	NCIO	7.22	NCC	NCIO	7.22	NCC	NCIO
Co.E	7.24	NCC	NCIO	7.22	NCC	NCIO	7.22	NCC	NCIO	7.19	NCC	NCIO	7.19	CC	CIO	7.19	CC	CIO
Ka.E	7.25	NCC	NCIO	7.22	NCC	NCIO	7.22	NCC	NCIO	7.22	NCC	NCIO	7.22	NCC	NCIO	7.22	NCC	NCIO

NCC - No change in colour

NCIO - No change in odour

CC - Change in colour

CIO - Change in odour

Mv.E – *Mitracarpus villosus* ethanol extract emulsion

Aca.E – *Acalypha wilkesiana* ethanol extract emulsion

Ca.E – *Cassia alata* ethanol extract emulsion

Co.E – *Cassia occidentalis* ethanol extract emulsion

Ka.E – *Kigelia africana* ethanol extract emulsion

Table 18. Effect of (37^{o}C) Temperature on ethanol extracts emulsion samples after production

product	Day 1			2 weeks			4 weeks			8 weeks			12 weeks			16 weeks		
	pH	Colour	Odour	pH	colour	odour	PH	colour	odour	pH	colour	odour	pH	colour	odour	pH	colour	Odour
Control	7.02	NCC	NCIO	7.00	NCC	NCIO	7.00	NCC	NCIO	6.99	NCC	NCIO	6.99	NCC	NCIO	6.99	NCC	NCIO
Mv.E	7.20	NCC	NCIO	7.18	NCC	NCIO	7.18	NCC	NCIO	7.15	NCC	NCIO	7.15	NCC	NCIO	7.15	NCC	NCIO
Aca.E	7.23	NCC	NCIO	7.20	NCC	NCIO	7.20	NCC	NCIO	7.18	NCC	NCIO	7.18	NCC	NCIO	7.18	NCC	NCIO
Ca.E	7.26	NCC	NCIO	7.25	NCC	NCIO	7.22	NCC	NCIO	7.21	NCC	NCIO	7.21	NCC	NCIO	7.21	NCC	NCIO
Co.E	7.24	NCC	NCIO	7.17	NCC	NCIO	7.17	NCC	NCIO	7.16	NCC	NCIO	7.16	CC	CIO	7.16	CC	CIO
Ka.E	7.25	NCC	NCIO	7.21	NCC	NCIO	7.21	NCC	NCIO	7.21	NCC	NCIO	7.21	NCC	NCIO	7.21	NCC	NCIO

NCC - No change in colour

NCIO - No change in odour

CC - Change in colour

CIO - Change in odour

Mv.E – *Mitracarpus villosus* ethanol extract emulsion

Aca.E – *Acalypha wilkesiana* ethanol extract emulsion

Ca.E – *Cassia alata* ethanol extract emulsion

Co.E – *Cassia occidentalis* ethanol extract emulsion

Ka.E – *Kigelia africana* ethanol extract emulsion

At 45^{o}C, no noticeable changes in colour and odour were observed for all the samples except for *Cassia occidentalis* emulsion sample at 12th and 16th week. There was decrease in the pH of control sample from 7.02 to 6.98 by the 16th week. pH of Mv.E was 7.20 at production but decreased to 7.14 at 2 weeks after production. The pH thereafter remained unchanged from 2 weeks to 16th week of test. Also, the pH of Aca.E was 7.23 at production but decreased as the week increased to 7.18 at 16th week.The same trend was observed for Ca.E from 7.26 to 7.21, Co.E from 7.24 to 7.16 and Ka.E from 7.25 to 7.20 (Table 19).

The general observation was that at lower temperatures of -10 and 4^{o}C, there were no movement of ions within the molecular structures of the samples, therefore the pH remain the same as well as no change observed for colour and odour. But with increase in the temperature, the pH of the control decreased. This may be due to more production of hydrogen ions from the stearic acid component of the emulsion. The increase in the pH of the emulsions containing medicinal plants extracts may also be due to alkaloids released which are alkaline in nature. The change in colour and odour observed *for Cassia occidentalis* emulsion at 12th and 16th week of test may probably be due to the presence of some phytochemicals in the extracts which were released at that high temperature.

4.6.3 Effect of Temperature on Petroleum ether Extracts Emulsions

At 10^{o}C and 4^{o}C, there was no change in colour, odour and pH from date of production of control and test samples throughout the 16 weeks of test. This may be due to the fact that at low temperatures, the ions remain immobile as there is no external energy to induce dissociation that may lead to increase or decrease in the pH and changes in the colour or odour of the test sample (Tables 20 and 21). At 30^{o}C, there was no change in colour and odour of all the samples throughout the weeks of test. However, the pH of control dropped from 7.02 to 6.98 by the 16th week of test. There was a neglible pH decrease of 0.01 noticed for Mv.Pet. at 2 weeks through the 16th week. For Aca.Pet.sample, the pH at the point of production of the sample was 7.15 and there was a decrease of to 7.14 at 2 weeks after production which remain stable till the 16th week. There was a decrease of 0.03 in pH noticed for Ca.Pet.and Co.Pet.samples from day of production to 16th week.

Table 19. (45^{o}C) Effect of Temperature on ethanol extracts emulsion samples after production

product	Day 1			2 weeks			4 weeks			8 weeks			12 weeks			16 weeks		
	pH	Colour	odour	pH	colour	odour	PH	colour	odour	pH	colour	odour	pH	colour	odour	pH	colour	odour
Control	7.02	NCC	NCIO	6.99	NCC	NCIO	6.99	NCC	NCIO	6.98	NCC	NCIO	6.98	NCC	NCIO	6.98	NCC	NCIO
Mv.E	7.20	NCC	NCIO	7.14	NCC	NCIO	7.14	NCC	NCIO	7.14	NCC	NCIO	7.14	NCC	NCIO	7.14	NCC	NCIO
Aca.E	7.23	NCC	NCIO	7.21	NCC	NCIO	7.21	NCC	NCIO	7.21	NCC	NCIO	7.21	NCC	NCIO	7.21	NCC	NCIO
Ca.E	7.26	NCC	NCIO	7.21	NCC	NCIO	7.21	NCC	NCIO	7.21	NCC	NCIO	7.21	NCC	NCIO	7.21	NCC	NCIO
Co.E	7.24	NCC	NCIO	7.16	NCC	NCIO	7.16	NCC	NCIO	7.16	NCC	NCIO	7.16	CC	CIO	7.16	CC	CIO
Ka.E	7.25	NCC	NCIO	7.20	NCC	NCIO	7.20	NCC	NCIO	7.20	NCC	NCIO	7.20	NCC	NCIO	7.20	NCC	NCIO

NCC - No change in colour

NCIO - No change in odour

CC - Change in colour

CIO - Change in odour

Mv.E – *Mitracarpus villosus* ethanol extract emulsion

Aca.E – *Acalypha wilkesiana* ethanol extract emulsion

Ca.E – *Cassia alata* ethanol extract emulsion

Co.E – *Cassia occidentalis* ethanol extract emulsion

Ka.E – *Kigelia africana* ethanol extract emulsion

Table 20 Effect of (-10^{o}C) Temperature on petroleum ether extracts emulsion samples after production

product	Day 1			2 weeks			4 weeks			8 weeks			12 weeks			16 weeks		
	pH	Colour	odour	pH	colour	odour	PH	colour	odour	pH	colour	odour	pH	colour	odour	pH	colour	odour
Control	7.02	NCC	NCIO	7.01	NCC	NCIO	7.02	NCC	NCIO	7.02	NCC	NCIO	7.02	NCC	NCIO	7.01	NCC	NCIO
Mv.Pet	7.12	NCC	NCIO	7.16	NCC	NCIO	7.16	NCC	NCIO	7.16	NCC	NCIO	7.16	NCC	NCIO	7.16	NCC	NCIO
Aca.Pet	7.15	NCC	NCIO	7.15	NCC	NCIO	7.15	NCC	NCIO	7.15	NCC	NCIO	7.15	NCC	NCIO	7.15	NCC	NCIO
Ca.Pet	7.18	NCC	NCIO	7.18	NCC	NCIO	7.18	NCC	NCIO	7.18	NCC	NCIO	7.18	NCC	NCIO	7.18	NCC	NCIO
Co.Pet	7.16	NCC	NCIO	7.16	NCC	NCIO	7.16	NCC	NCIO	7.16	NCC	NCIO	7.16	NCC	NCIO	7.16	NCC	NCIO
Ka.Pet	7.19	NCC	NCIO	7.19	NCC	NCIO	7.19	NCC	NCIO	7.19	NCC	NCIO	7.19	NCC	NCIO	7.19	NCC	NCIO

NCC - No change in colour

NCIO - No change in odour

CC - Change in colour

CIO - Change in odour

Mv.Pet – *Mitracarpus villosus* Petroleum ether extract emulsion

Aca.Pet – *Acalypha wilkesiana* Petroleum ether extract emulsion

Ca.Pet– *Cassia alata* Petroleum ether extract emulsion

Co.Pet – *Cassia occidentalis* Petroleum ether extract emulsion

Ka.Pet – *Kigelia africana* Petroleum ether extract emulsion

Table 21 Effect of (4^{o}C) Temperature on petroleum ether extracts emulsion samples after production

product	Day 1			2 weeks			4 weeks			8 weeks			12 weeks			16 weeks		
	pH	Colour	odour	pH	colour	odour	PH	colour	odour	pH	colour	odour	pH	colour	odour	pH	colour	odour
Control	7.02	NCC	NCIO	7.01	NCC	NCIO	7.01	NCC	NCIO	7.01	NCC	NCIO	7.01	NCC	NCIO	7.01	NCC	NCIO
Mv.Pet	7.12	NCC	NCIO	7.12	NCC	NCIO	7.12	NCC	NCIO	7.12	NCC	NCIO	7.12	NCC	NCIO	7.12	NCC	NCIO
Aca.Pet	7.15	NCC	NCIO	7.15	NCC	NCIO	7.15	NCC	NCIO	7.15	NCC	NCIO	7.15	NCC	NCIO	7.15	NCC	NCIO
Ca.Pet	7.18	NCC	NCIO	7.18	NCC	NCIO	7.18	NCC	NCIO	7.18	NCC	NCIO	7.18	NCC	NCIO	7.18	NCC	NCIO
Co.Pet	7.16	NCC	NCIO	7.16	NCC	NCIO	7.16	NCC	NCIO	7.16	NCC	NCIO	7.16	NCC	NCIO	7.16	NCC	NCIO
Ka.Pet	7.19	NCC	NCIO	7.19	NCC	NCIO	7.19	NCC	NCIO	7.19	NCC	NCIO	7.19	NCC	NCIO	7.19	NCC	NCIO

NCC - No change in colour

NCIO - No change in odour

CC - Change in colour

CIO - Change in odour

Mv.Pet – *Mitracarpus villosus* Petroleum ether extract emulsion

Aca.Pet – *Acalypha wilkesiana* Petroleum ether extract emulsion

Ca.Pet– *Cassia alata* Petroleum ether extract emulsion

Co.Pet – *Cassia occidentalis* Petroleum ether extract emulsion

Ka.Pet – *Kigelia africana* Petroleum ether extract emulsion

pH of 7.19 was measured for Ka.Pet.sample on the day of production. However, after 4 weeks of production, the pH deceased to 7.14 and remain stable till 16th week of test (Table 22). There were changes in colour and odour of Co.Pet. sample at 12th and 16th of test when stored at 37^{o}C which may be due to the release of some phytochemicals from the petroleum ether extracts component of the emulsion at this temperature. However it was observed that the pH of control on the day of production is 7.02 and with increase in the number of weeks, it dropped to 6.98. The pH of Mv. Pet. sample decreased from 7.12 at day of production to 7.09 by the 16th week. The pH of Aca.Pet. sample at the day of production was 7.15 which decreased to 7.13 by 16th week. It was also observed for Ca.Pet. sample that the pH decreases as the week increases. The pH was 7.18. at production and it decreased to 7.14 by 16th week of test. For Co.Pet. sample, the pH decreased from 7.16 at production to 7.12 at the 16th week after production. The same observation was made for pH of Ka.Pet. sample which decreased from 7.19 at production to 7.16 after 16 weeks of production (Table 23).

No noticeable changes in colour and odour were observed for all the samples at 45^{o}C except for *Cassia occidentalis* emulsion sample at 12th and 16th week. There was decrease in the pH of control sample from 7.02 to 6.98 by the 16th week. pH of Mv.Pet.sample was 7.12 at production but decreased to 7.08 at 2 weeks after production and remain stable till 16th week of test. Also, the pH of Aca.E was 7.15 at production and decreased as the week increased to 7.11 at 16th week.The same trend was observed for Ca.Pet. from 7.18 to 7.15, Co.Pet. from 7.16 to 7.13 and Ka.Pet. from 7.19 to 7.15 (Table 24). It was observed was that at lower temperatures of -10 and 4^{o}C, the pH remain the same as well as no change observed for colour and odour.

But with increase in the temperature, the pH of the control decreased. This may be due to more production of hydrogen ions from the stearic acid component of the emulsion. The decrease in the pH of the emulsions containing medicinal plants extracts may also be due to alkaloids released which are alkaline in nature. The change in colour and odour observed for *Cassia occidentalis* emulsion at 12th and 16th week of test may probably be due to the presence of some phytochemicals in the extracts which were also released at that high temperature.

Table 22 Effect of (30^{o}C) Temperature on petroleum ether extracts emulsion samples after production

product	Day 1			2 weeks			4 weeks			8 weeks			12 weeks			16 weeks		
	pH	Colour	odour	pH	colour	odour	PH	colour	odour	pH	colour	odour	pH	colour	odour	pH	colour	odour
Control	7.02	NCC	NCIO	7.01	NCC	NCIO	6.99	NCC	NCIO	6.98	NCC	NCIO	6.98	NCC	NCIO	6.98	NCC	NCIO
Mv.Pet	7.12	NCC	NCIO	7.11	NCC	NCIO	7.11	NCC	NCIO	7.11	NCC	NCIO	7.11	NCC	NCIO	7.11	NCC	NCIO
Aca.Pet	7.15	NCC	NCIO	7.14	NCC	NCIO	7.14	NCC	NCIO	7.14	NCC	NCIO	7.14	NCC	NCIO	7.14	NCC	NCIO
Ca.Pet	7.18	NCC	NCIO	7.16	NCC	NCIO	7.15	NCC	NCIO	7.15	NCC	NCIO	7.15	NCC	NCIO	7.15	NCC	NCIO
Co.Pet	7.16	NCC	NCIO	7.13	NCC	NCIO	7.13	NCC	NCIO	7.13	NCC	NCIO	7.13	CC	CIO	7.13	CC	CIO
Ka.Pet	7.19	NCC	NCIO	7.14	NCC	NCIO	7.14	NCC	NCIO	7.14	NCC	NCIO	7.14	NCC	NCIO	7.14	NCC	NCIO

NCC - No change in colour

NCIO - No change in odour

CC - Change in colour

CIO - Change in odour

Mv.Pet – *Mitracarpus villosus* Petroleum ether extract emulsion

Aca.Pet – *Acalypha wilkesiana* Petroleum ether extract emulsion

Ca.Pet– *Cassia alata* Petroleum ether extract emulsion

Co.Pet – *Cassia occidentalis* Petroleum ether extract emulsion

Ka.Pet – *Kigelia africana* Petroleum ether extract emulsion

Table 23 Effect of (37^{o}C) Temperature on petroleum ether extracts emulsion samples after production

product	Day 1			2 weeks			4 weeks			8 weeks			12 weeks			16 weeks		
	pH	Colour	odour	pH	colour	odour	PH	colour	odour	pH	colour	odour	pH	colour	odour	pH	colour	odour
Control	7.02	NCC	NCIO	7.00	NCC	NCIO	6.98.	NCC	NCIO	6.98	NCC	NCIO	6.98	NCC	NCIO	6.98	NCC	NCIO
Mv.Pet	7.12	NCC	NCIO	7.09	NCC	NCIO	7.09	NCC	NCIO	7.09	NCC	NCIO	7.09	NCC	NCIO	7.09	NCC	NCIO
Aca.Pet	7.15	NCC	NCIO	7.14	NCC	NCIO	7.13	NCC	NCIO	7.13	NCC	NCIO	7.13	NCC	NCIO	7.13	NCC	NCIO
Ca.Pet	7.18	NCC	NCIO	7.14	NCC	NCIO	7.14	NCC	NCIO	7.14	NCC	NCIO	7.14	NCC	NCIO	7.14	NCC	NCIO
Co.Pet	7.16	NCC	NCIO	7.15	NCC	NCIO	7.12	NCC	NCIO	7.12	NCC	NCIO	7.12	CC	CIO	7.12	CC	CIO
Ka.Pet	7.19	NCC	NCIO	7.16	NCC	NCIO	7.16	NCC	NCIO	7.16	NCC	NCIO	7.16	NCC	NCIO	7.16	NCC	NCIO

NCC - No change in colour

NCIO - No change in odour

CC - Change in colour

CIO - Change in odour

Mv.Pet – *Mitracarpus villosus* Petroleum ether extract emulsion

Aca.Pet – *Acalypha wilkesiana* Petroleum ether extract emulsion

Ca.Pet– *Cassia alata* Petroleum ether extract emulsion

Co.Pet – *Cassia occidentalis* Petroleum ether extract emulsion

Ka.Pet – *Kigelia africana* Petroleum ether extract emulsion

Table 24 Effect of(45^{o}C) Temperature on petroleum ether extracts emulsion samples after production

product	Day 1			2 weeks			4 weeks			8 weeks			12 weeks			16 weeks		
	pH	Colour	odour	pH	colour	odour	PH	colour	odour	pH	colour	odour	pH	colour	odour	pH	colour	odour
Control	7.02	NCC	NCIO	6.99	NCC	NCIO	6.98	NCC	NCIO	6.98	NCC	NCIO	6.98	NCC	NCIO	6.98	NCC	NCIO
Mv.Pet	7.12	NCC	NCIO	7.08	NCC	NCIO	7.08	NCC	NCIO	7.08	NCC	NCIO	7.08	NCC	NCIO	7.08	NCC	NCIO
Aca.Pet	7.15	NCC	NCIO	7.11	NCC	NCIO	7.11	NCC	NCIO	7.11	NCC	NCIO	7.11	NCC	NCIO	7.11	NCC	NCIO
Ca.Pet	7.18	NCC	NCIO	7.15	NCC	NCIO	7.15	NCC	NCIO	7.15	NCC	NCIO	7.15	NCC	NCIO	7.15	NCC	NCIO
Co.Pet	7.16	NCC	NCIO	7.13	NCC	NCIO	7.13	NCC	NCIO	7.13	NCC	NCIO	7.13	CC	CIO	7.13	CC	CIO
Ka.Pet	7.19	NCC	NCIO	7.15	NCC	NCIO	7.15	NCC	NCIO	7.15	NCC	NCIO	7.15	NCC	NCIO	7.15	NCC	NCIO

NCC - No change in colour

NCIO - No change in odour

CC - Change in colour

CIO - Change in odour

Mv.Pet – *Mitracarpus villosus* Petroleum ether extract emulsion

Aca.Pet – *Acalypha wilkesiana* Petroleum ether extract emulsion

Ca.Pet– *Cassia alata* Petroleum ether extract emulsion

Co.Pet – *Cassia occidentalis* Petroleum ether extract emulsion

Ka.Pet – *Kigelia africana* Petroleum ether extract emulsion

4.7 Centrifugation

4.7.1 Centrifugation Of Aqueous Samples

At 2000 and 2500 revolution per minute, no phase separation was detected for all the test samples (Table 25). Also at 3000 and 4000 rpm, no separation was noticed but there was very marked phase separation for *Cassia occidentalis* aqueous extract at 3000 and 4000 rpm.

4.7.2 Centrifugation Of Ethanol Samples

No phase separation was detected in all the test samples at 2000, 2500 and 3000rpm (Table 25). But slight separation was noticed for *Cassia occidentalis* at 4,000 rpm

4.7.3 Centrifugation Of Petroleum Ether Samples

No phase separation in all the test samples at 2000 – 4000 rpm except for *Cassia occidentalis* at 4,000 rpm (Table 25).

4.8 Light Testing

No changes were noticed for all the samples except for *Cassia occidentalis* aqueous, ethanol and *Cassia occidentalis* petroleum ether samples which indicated that they were sensitive to ultra violet light (Table 26)

4.9 Freeze-thaw

There was no change observed in all the test samples during the cycle testing except for *Cassia occidentalis* samples where changes were observed (Table 26).

4.10 Moisture loss on drying/residue content for medicinal plants extracts emulsions

Moisture loss on drying ranged between 6.52 – 6.33 (Table 27) with *Mitracarpus villosus* aqueous extract emulsion having the highest and *Cassia alata* ethanol extract emulsion the least. This implies that *Mitracarpus villosus* aqueous extract emulsion would be more susceptible to microbial attack because of the high moisture content compared to the other products. The residue content was highest in *Cassia alata* ethanol extract emulsion (93.67) and lowest in *Mitracarpus villosus* aqueous extract emulsion (93.48). This implies that *Cassia alata* ethanol extract emulsion may be more stable to microbial attack compared to other products because of less moisture content.

Table 25 Centrifugation of anti-dermatophyte creams

Sample	2000rpm	2500rpm	3000rpm	4000rpm
Control	NPS	NPS	NPS	NPS
Mv.W	NPS	NPS	NPS	NPS
Aca.W	NPS	NPS	NPS	NPS
Ca.W	NPS	NPS	NPS	NPS
Co.W	NPS	NPS	PS	PS
Ka.W	NPS	NPS	NPS	NPS
Mv.E	NPS	NPS	NPS	NPS
Aca.E	NPS	NPS	NPS	NPS
Ca.E	NPS	NPS	NPS	NPS
Co.E	NPS	NPS	PS	PS
Ka.E	NPS	NPS	NPS	NPS
Mv.Pet	NPS	NPS	NPS	NPS
Aca.Pet	NPS	NPS	NPS	NPS
Ca.Pet	NPS	NPS	NPS	NPS
Co.Pet	NPS	NPS	NPS	PS
Ka.Pet	NPS	NPS	NPS	NPS

NPS= No phase separation, PS = Phase separation

Mv.W – *Mitracarpus villosus* water extract emulsion

Mv.E – *Mitracarpus villosus* ethanol extract emulsion

Mv.Pet – *Mitracarpus villosus* petroleum ether extract emulsion.

Aca.W - *Acalypha wilkesiana* water extract emulsion

Aca. E – *Acalypha wilkesiana* ethanol extract emulsion

Aca.Pet.- *Acalypha wilkesiana* Petroleum ether extract emulsion

Ca.W - *Cassia alata* water extract emulsion

Ca.E - *Cassia alata* ethanol extract emulsion

Ca.Pet.- *Cassia alata* Petroleum ether extract emulsion

Co.W - *Cassia occidentalis* water extract emulsion

Co.E - *Cassia occidentalis* ethanol extract emulsion

Co.Pet. *Cassia occidentalis* Petroleum ether extract emulsion

Ka.W- *Kigelia africana* water extract emulsion

Ka.E - *Kigelia africana* ethanol extract emulsion

Ka.Pet.- *Kigelia africana* Petroleum ether extract emulsion

Table 26. Light and Freeze-thaw testing of anti-dermatophyte creams

	Light	Freeze-thaw
Control	NCC	NPS
Mitracarpus villosus water emulsion	NCC	NPS
Mitracarpus villosus ethanol extract emulsion	NCC	NPS
Mitracarpus villosus Pet.ether extract emulsion	NCC	NPS
Acalypha wilkesiana water extract emulsion	NCC	NPS
Acalypha wilkesiana ethanol extract emulsion	NCC	NPS
Acalypha wilkesiana petroleum ether extract emulsion	NCC	NPS
Cassia alata water extract emulsion	NCC	NPS
Cassia alata ethanol extract emulsion	NCC	NPS
Cassia alata petroleum ether extract emulsion	NCC	NPS
Cassia occidentalis water extract emulsion	CC	PS
Cassia occidentalis ethanol extract emulsion	CC	PS
Cassia occidentalis petroleum extract emulsion	CC	PS
Kigelia africana water extract emulsion	NCC	NPS
Kigelia africana ethanol extract emulsion	NCC	NPS
Kigelia africana petroleum ether extract emulsion	NCC	NPS

NCC –No colour change NPS- No phase separation

CC – Colour change PS – Phase separation

Table 27 Moisture loss on drying/residue content of medicinal plants extracts emulsions.

Extract	Moisture loss on drying%	Residue content %
Mitracarpus villosus aqueous	6.52	93.48
Acalypha wilkesiana aqueous	6.49	93.51
Cassia alata aqueous	6.35	93.65
Cassia occidentalis aqueous	6.39	93.61
Kigelia africana aqueous	6.37	93.63
Mitracarpus villosus ethanol	6.39	93.61
Acalypha wilkesiana ethanol	6.40	93.60
Cassia alata ethanol	6.33	93.67
Cassia occidentalis ethanol	6.36	93.64
Kigelia africana ethanol	6.39	93.61
Mitracarpus villosus pet.ether	6.40	93.60
Acalypha wilkesiana pet. Ether	6.44	93.56
Cassia alata pet.ether	6.40	93.60
Cassia occidentalis pet.ether	6.41	93.59
Kigelia africana pet.ether	6.40	93.60

4.11 Skin sensitivity

It was observed that all the herbal emulsion preparations had no adverse effect on animals tested. There was no irritation observed during the study.

4.12 Animal studies

Histopathology of skin biopsies of uninfected rats and placebo treated rats indicated that there were no fungal hyphae as they were not infected with the dermatophytes. Numerous hair follicles were seen in the uninfected group while moderate amount were seen in the placebo treated group. Numerous sebaceous glands were seen in the two groups. No inflammation and discontinuity of the epidermis or tissue destruction were also observed. The average keratin values for the uninfected and placebo treated groups were 55.42 and 51.62µm respectively while the epidermal thickness was 31.24 and 31.58µm respectively. The results obtained show that application of placebo on the uninfected rats had little or no effect on the mycology, keratin and epidermal layers.

4.12.1 Effect of *Kigelia africana* extracts on dermatopytes

4.12.1.1 *Microsporium aoudini*

Marked inflammation of the skin and foci of discontinuity of the epidermis were observed in the untreated group (group 1) which confirms successful infection (Table 28). There was marked presence of fungal hyphae as shown in plate 8. There were few hair follicles enmeshed within the sparse connective tissue in the dermis which indicated that the dermatophyte was feeding on the follicles. Very few sebaceous glands were seen showing that the oil in the sebaceous gland had been used up by the dermatophytes. Marked tissue destruction was observed.

No inflammation and tissue destruction was however observed in group 2, which is the group treated with 1% clotrimazole. No fungal hyphae were seen in the group which indicated that the drug was able to prevent the growth of the hyphae therefore the dermatophyte was not able to attack the hair follicles and sebaceous glands in the skin. There were numerous hair follicles in the dermis and also abundant sebaceous glands (Plate.6). In the group treated with emulsion alone (group 3), Inflammation and tissue destruction of the skin were moderate in the group. There was marked presence of fungal hyphae which indicated that the cream alone has no medicinal effect on the dermatophyte infection and reduction of numbers of hair follicles also occured as the dermatophye was able to attack unhindered.

Table 28. Mycological efficacy of *Kigelia africana* formulations on *Microsporium aoudini*

Group	Fungal hyphae	Hair follicles	Sebaceous gland	Inflammation	Continuity/Tissue destruction
1	+++	+	+	+++	+++
2	-	+++	+++	-	-
3	+++	+	++	++	++
4a	++	+	++	++	++
4b	++	+	++	++	++
5a	+	++	++	+	+
5b	+	++	++	+	+
6a	-	++	++	-	+
6b	-	++	+++	-	+

Absent = -, Mild = +, Moderate = ++, Marked = +++,

However the group has moderate number of sebaceous glands in the dermis showing the content of the emulsion provided alternative source for the dermatophyte thus reducing the effect on the glands.

In group 4a (0.5% plant extract alone), Inflammation and tissue destruction of the skin were moderate in the group (Table 28). Presence of fungal hyphae was also moderate while there were moderate number of hair follicles enmeshed within the sparse connective tissue in the dermis. There are moderate number of sebaceous glands in the dermis. Presence of fungal hyphae was moderate in group 4b (0.5% plant extract in emulsion). There are moderate hair follicles enmeshed within the sparse connective tissue in the dermis. There were also moderate numbers of sebaceous glands in the dermis. Inflammation and tissue destruction of the skin were also moderate in the group (Table 28). In groups 5a (1% plant extract alone), the inflammation and tissue destruction of the skin were moderate and same observation were recorded for 5b (1% plant extract in emulsion). Few numbers of fungal hyphae were seen with moderate amount of hair follicle and sebaceous gland.

In group 6a (2% plant extract alone), no inflammation of the skin was seen but mild tissue destruction was however observed in the group. No fungal hyphae were seen which indicated that the extract was able to prevent the growth of the hyphae by the dermatophyte. There were moderate hair follicles in the dermis and also moderate amount of sebaceous glands. This may be as a result of the bioactives in the extracts not been able to stop the growth of the fungal hyphae until after several applications.

Same observation was made for group 6b (2% plant extract in emulsion) except for the abundance of sebaceous glands in the dermis (Plate 7) which may have been on account of the fat in the emulsion been available as alternative to the oil in the sebaceous glands. This shows that 2% plant extract alone and 2% plant extract in emulsion are significantly effective as the standard drug in preventing the secretion of keratinase which plays a vital role in the process of infection by *Microsporium aoudini*. From the results above, there is clear indication that efficacy of the formulations is concentration dependent.

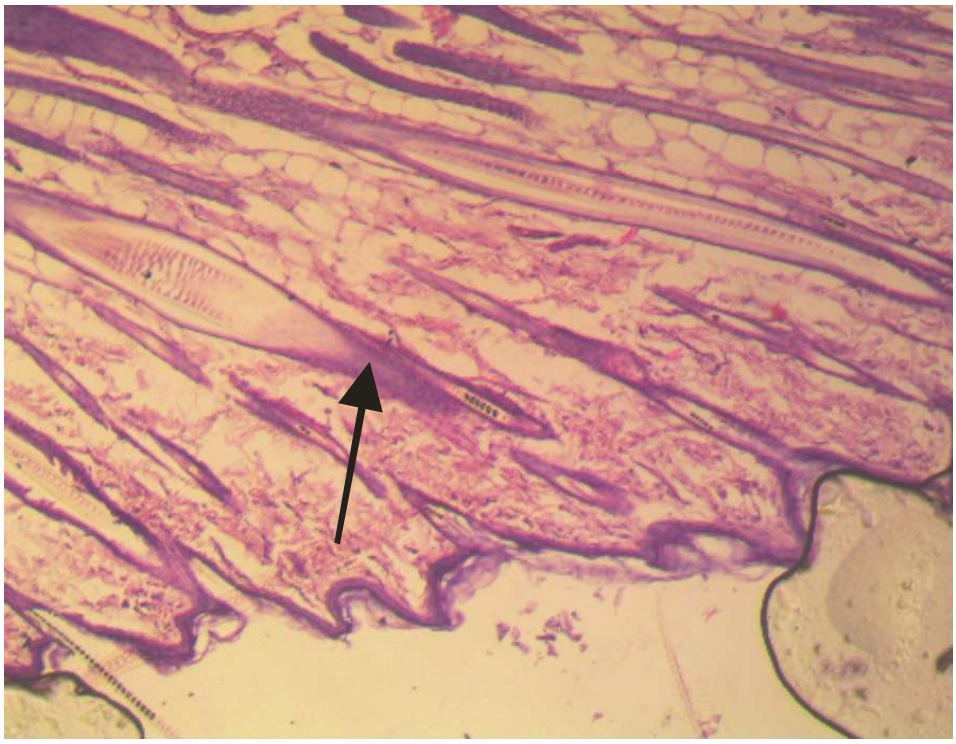


Plate 6. There is hair follicular hyperplasia (arrow). HE x400

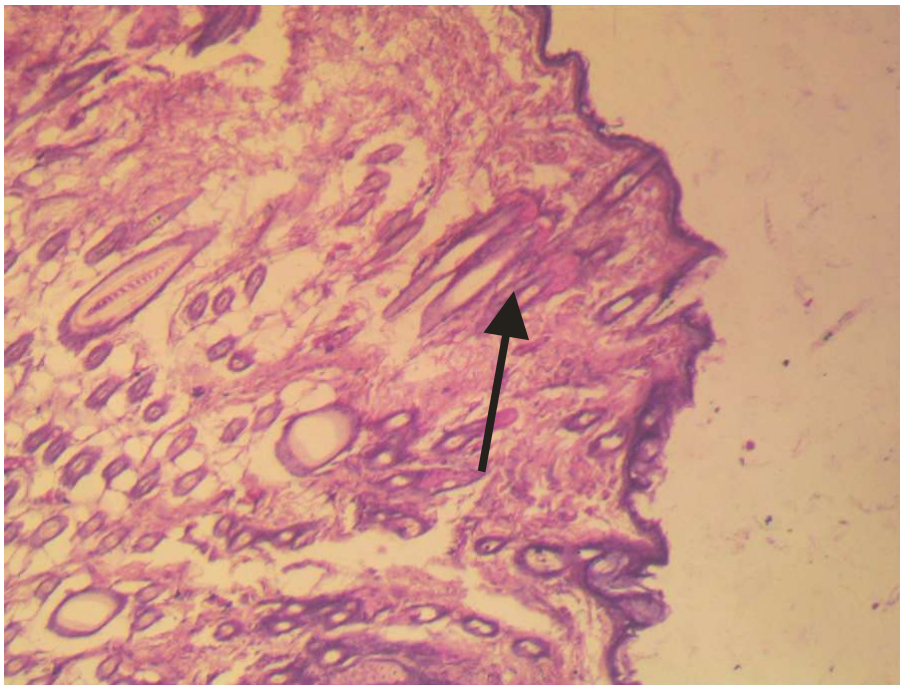


Plate 7 There is hair follicular hyperplasia (arrow). HE x100

Table 29 shows the effect of the formulations on dekeratinization. Higher scores indicated improved efficacy. The untreated group 1 with the value 16.20 ± 0.76 µm indicated significant dekeratinization. Group 2 which is the standard drug (1% clotrimazole cream) showed high efficacy (39.45 ± 0.82 µm) indicating that it was able to reduce dekeratinization significantly by the dermatophyte. Emulsion alone (placebo), 0.5% *Kigelia africana* extract and 0.5% *Kigelia africana* extract emulsion cannot be said to be mycologically effective compared to the values obtained for the other formulations. However, 1% *Kigelia africana* extract alone and 1% *Kigelia africana* extract emulsion showed slight efficacy, but not as effective as the 2% *kigelia africana* extract alone and 2% *kigelia africana* extract emulsions which showed comparative efficacy with the standard drug (Fig. 8).

Table 88 and Plate Fig.54 (See Appendix) shows that there is significant difference in keratinization within the groups as determined by one way Anova. ($F_{6,28}$ = 159.174, $p<0.05$). Tukeys post hoc test shows significant differences when the control is compared with other groups.

Epidermal thickness of the skin biopsies ranged from 41.62±0.58 – 92.37±1.76 µm (Table 30). Higher value indicates significant infection of the dermis with attendance inflammation of the skin and tissue destruction. The untreated group 1 with the value of 92.37±1.76µm indicated significant infection. Group 2 which is the standard drug (1% clotrimazole cream) showed that the drug was able to reduce the epidermal thickness significantly (36.52 ± 0.54µm). The values obtained for emulsion alone (89.38±0.56µm) was not different from the untreated group. However, the efficacy of 0.5% *Kigelia africana* extract, 0.5% *Kigelia africana* extract emulsion, 1% *Kigelia africana* extract and 1% *Kigelia africana* emulsion in reducing the epidermal thickness is not significant compared with the untreated control. In contrast, there was significant efficacy of 2% *Kigelia africana* extract and 2% *Kigelia africana* emulsion compared with the values obtained for the standard drug (Fig.9).

Data showed in Table 89 and Fig.55 (See Appendix) reveals that there is significance difference in epidermal thickness between groups as determined by one way anova ($F_{6,28}$=373.418 , $p<0.05$). A tukey post hoc test revealed that there is significance difference in epidermal thickness between the control and the test groups.

Table 29 Effect of *Kigelia africana* formulations on keratinization (in µm) on skin infected by *Microsporium aoudini*

Group 1	16.52	15.48	15.96	16.24	16.81
Group 2	36.46	41.11	39.24	40.24	40.44
Group 3	19.73	18.22	20.46	17.0	19.45
Group 4a	18.72	20.43	19.46	21.48	19.82
Group 4b	20.16	17.21	19.62	20.24	18.24
Group 5a	26.92	27.84	24.52	25.06	26.08
Group 5b	23.01	21.35	22.31	24.12	20.64
Group 6a	31,42	30.89	31.01	29.35	30.62
Group 6b	32.61	31.46	32.30	30.77	29.71

Group 1 – Animals without any treatment – Negative control

Group 2 – Animals treated with cream containing 1% Clotrimazole (Positive Control - a).

Group 3 – Animals treated with placebo (Emulsion base only/Positive control - b)

Group 4a – Animals treated with 0.5% medicinal plant extract alone

Group 4b – Animals treated with emulsion containing 0.5% medicinal plant extract

Group 5a – Animals treated with 1% medicinal plant extract alone

Group 5b – Animals treated with emulsion containing 1% medicinal plant extract

Group 6a- Animals treated with 2% medicinal plant extract alone

Group 6b- Animals treated with emulsion containing 2% medicinal plant extract

Table 30. Effect of *Kigelia africana* formulations on Epidermal thickness (in µm) of skin infected by *Microsporium aoudini*

Gpr 1	96.11	86.42	94.48	90.43	94.41
Grp 2	37.95	36.48	34.64	36.62	36.90
Grp 3	89.51	90.42	87.74	88.52	90.71
Grp 4a	85.42	87.26	80.25	83.24	81.32
Grp 4b	82.61	80.71	84.31	76.64	80.41
Grp 5a	80.84	80.51	75.72	78.62	80.46
Grp 5b	80.44	74.11	79.81	81.23	83.44
Grp 6a	43.27	44.64	45.83	41.67	43.59
Grp 6b	46.31	40.32	44.71	45.32	43.90

Group 1 – Animals without any treatment – Negative control

Group 2 – Animals treated with cream containing 1% Clotrimazole (Positive Control - a).

Group 3 – Animals treated with placebo (Emulsion base only/Positive control - b)

Group 4a – Animals treated with 0.5% medicinal plant extract alone

Group 4b – Animals treated with emulsion containing 0.5% medicinal plant extract

Group 5a – Animals treated with 1% medicinal plant extract alone

Group 5b – Animals treated with emulsion containing 1% medicinal plant extract

Group 6a- Animals treated with 2% medicinal plant extract alone

Group 6b- Animals treated with emulsion containing 2% medicinal plant extract

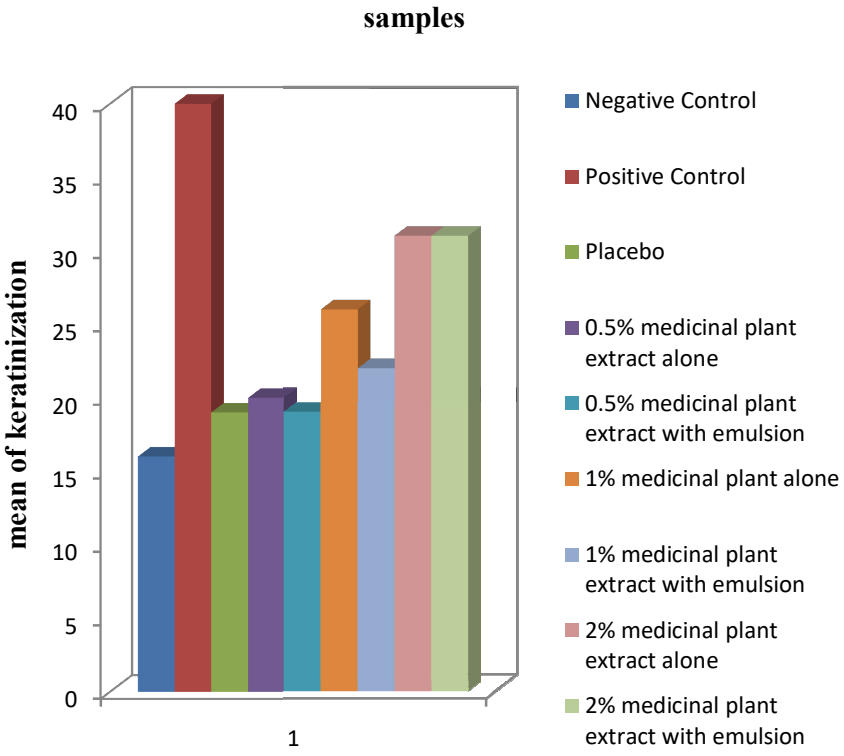


Fig. 8 Effect of *Kigelia africana* extracts on keratinization in *Microsporium aoudini* infected skin

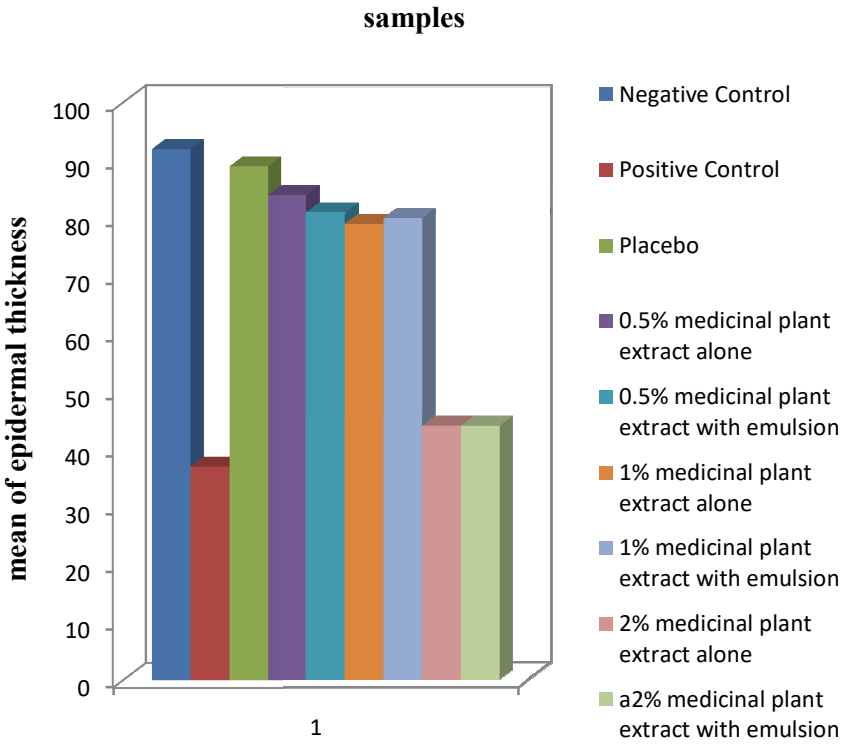


Fig.9 Effect of *Kigelia africana* extracts on epidermal thickess in *Microsporium aoudini* infected skin

4.12.1.2 *Epidermophyton floccosum*

Group 1 animals which are the untreated infected group showed marked inflammation of the skin and foci of discontinuity of the epidermis (Table 31). Marked tissue destruction was also observed in the group compared to the other groups which were treated with various formulations. There was marked presence of fungal hyphae which implies successful infection by the dermatophyte as shown in Plate 8. No hair follicles were seen this implies that the dermatophyte fed on the follicles. There were very few sebaceous glands.

No inflammation and tissue destruction was observed in the group treated with 1% clotrimazole (group 2) showing that the drug was able to prevent the growth of the hyphae by the dermatophyte. No fungal hyphae were seen in the group. There were numerous hair follicles in the dermis and also moderate amount of sebaceous glands.

Group 3 animals were treated with emulsion alone. Skin inflammation was marked while tissue destruction of the skin was moderate (Table 31). There was marked presence of fungal hyphae which indicated that the cream alone has no medicinal effect on the dermatophyte infection which a reduction of numbers in hair follicles. However there are few sebaceous glands in the dermis.

In group 4a, the animals were treated with 0.5% plant extract alone. Inflammation and tissue destruction of the skin were moderate in the group (Table 31). Presence of fungal hyphae was moderate while there were few hair follicles enmeshed within the sparse connective tissue in the dermis. There were few sebaceous glands in the dermis.

Skin inflammation was moderate and tissue destruction of the skin were also mild (Table 31) in group 4b (0.5% plant extract in emulsion). Presence of fungal hyphae was few. There were few hair follicles enmeshed within the sparse connective tissue in the dermis. There are also few sebaceous glands in the dermis.

In group 5a (1% plant extract alone), the inflammation and tissue destruction of the skin were mild. Few amounts of fungal hyphae were seen with few amounts of hair follicle and sebaceous gland. Same observation were recorded for group 5b (1% plant extract in emulsion).

Table 31 Mycological efficacy of *Kigelia africana* formulations on *Epidermophyton floccosum* infected skin

Group	Fungal hyphae	Hair follicles	Sebaceous gland	Inflammation	Continuity/Tissue destruction
1	+++	-	+	+++	+++
2	-	+++	++	-	-
3	+++	+	+	+++	++
4a	++	+	+	++	++
4b	+	+	+	++	+
5a	+	+	+	+	+
5b	+	+	+	+	+
6a	-	++	++	-	-
6b	-	++	++	-	-

Absent = -, Mild = +, Moderate = ++, Marked = +++,

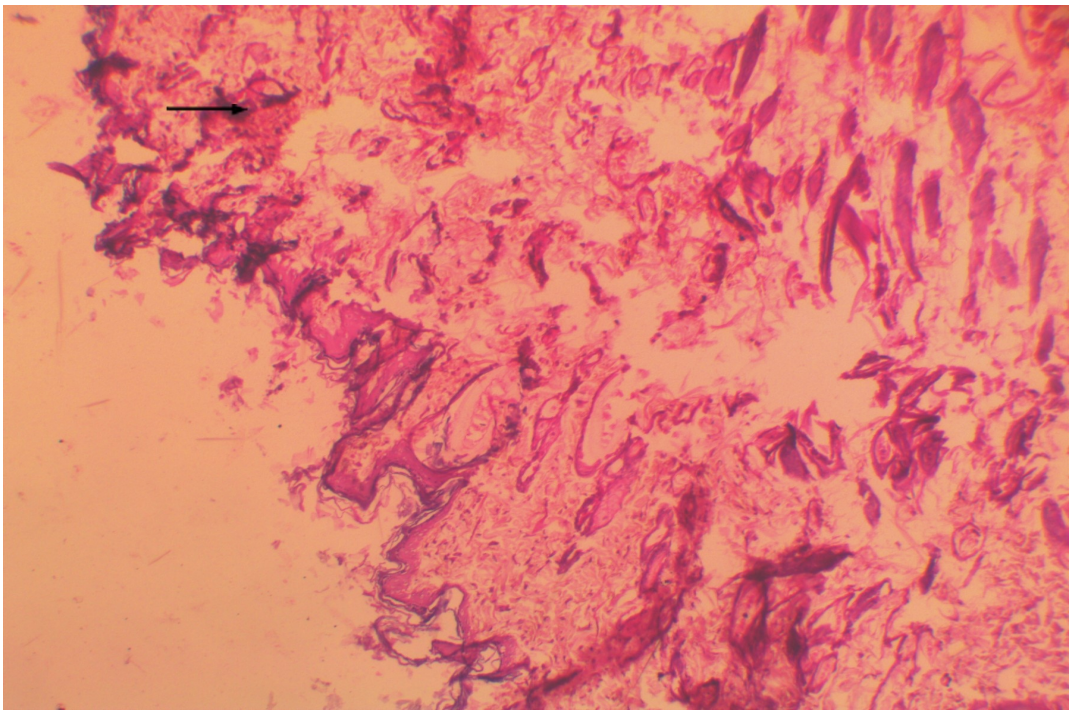


Plate 8. There is dermal congestion and acute inflammatory cell infiltrate. HE x400

No inflammation of the skin and tissue destruction was seen in group 6a (2% plant extract alone) which indicated that the extract was able to prevent the growth of the dermatophyte hyphae. No fungal hyphae were seen. There were moderate hair follicles in the dermis and also moderate amount of sebaceous glands. Same observation was made for group 6b (2% plant extract in emulsion) (Plate 9). This indicated that 2% plant extract alone and 2% plant extract in emulsion are significantly effective as the standard drug.

Table 32 shows the effect of the formulations on the keratin layer of the hair. The untreated control with the value 13.37 ± 0.39 indicated that the hairs were heavily infected with the dermatophyte. The effectiveness of 1% clotrimazole cream (39.86 ± 0.21 µm) in preventing attack by the dermatophyte on the keratin layer of the hair is demonstrated by the high value recorded. However, the group treated with the emulsion alone (15.30 ± 0.22 µm) and the group treated with 0.5% *Kigelia africana* extract emulsion (15.25 ± 0.28 µm) was not too different from the untreated group. 0.5% *Kigelia africana* extract, 1% *Kigelia africana* extract alone and 1% *Kigelia africana* emulsion showed slight efficacy compared to the untreated control. Comparatively, 2% *Kigelia africana* extract alone (30.94 ± 0.38 µm) and 2% *Kigelia africana* extract emulsion (30.53 ± 0.25µm) showed mycological efficacy to other formulations (Fig.10).

Table 90 and Fig.56 (See Appendix) shows that there is significant difference in keratinization within the groups as determined by one way Anova($F_{6,28}$ = 159.174 , $p<0.05$). Tukeys post hoc test shows significant differences when the Control is compared with other groups.

The values for epidermal thickness of the skin biopsies of the various formulations are shown in Table 33. Higher scores indicated significant infection of the dermis with attendance inflammation of the skin and tissue destruction. The untreated group 1 with the value of 92.37 ± 1.76 µm indicated significant infection. Group 2 which is the standard drug (1% clotrimazole cream) showed that the drug was able to reduce the epidermal thickness significantly (36.52 ± 0.54 µm). The values obtained for emulsion alone (89.38 ± 0.56 µm) was not different from the untreated group. However, the efficacy of 0.5% *Kigelia africana* extract, 0.5% *Kigelia africana* extract

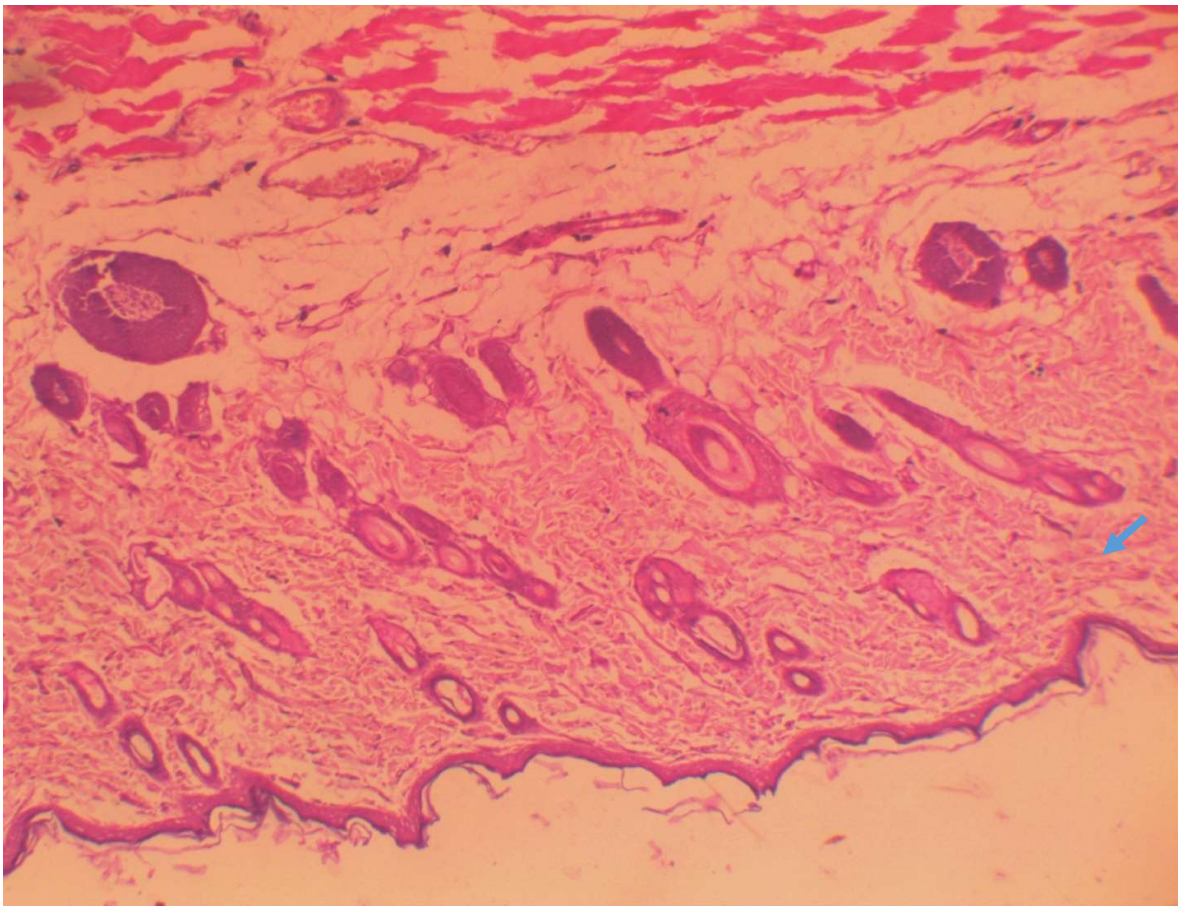


Plate 9 There is no observable lesion(arrow). HE x100

Table 32 Effect of *Kigelia africana* formulations on keratinization (in µm) of skin infected by *Epidermophyton floccosum*

Group 1	14.4	12.48	12.96	14.2	12.8
Group 2	39.36	40.21	40.14	40.24	39.34
Group 3	15.82	15.54	14.61	15.01	15.52
Group 4a	16.55	17.11	16.91	15.20	16.24
Group 4b	15.55	14.29	15.62	15.81	14.96
Group 5a	16.91	17.49	16.38	16.95	16.92
Group 5b	16.32	18.40	17.46	16.86	16.91
Group 6a	30.41	31.29	29.72	31.83	31.43
Group 6b	29.86	30.51	31.10	30.14	31.06

Group 1 – Animals without any treatment – Negative control

Group 2 – Animals treated with cream containing 1% Clotrimazole (Positive Control - a).

Group 3 – Animals treated with placebo (Emulsion base only/Positive control - b)

Group 4a – Animals treated with 0.5% medicinal plant extract alone

Group 4b – Animals treated with emulsion containing 0.5% medicinal plant extract

Group 5a – Animals treated with 1% medicinal plant extract alone

Group 5b – Animals treated with emulsion containing 1% medicinal plant extract

Group 6a- Animals treated with 2% medicinal plant extract alone

Group 6b- Animals treated with emulsion containing 2% medicinal plant extract

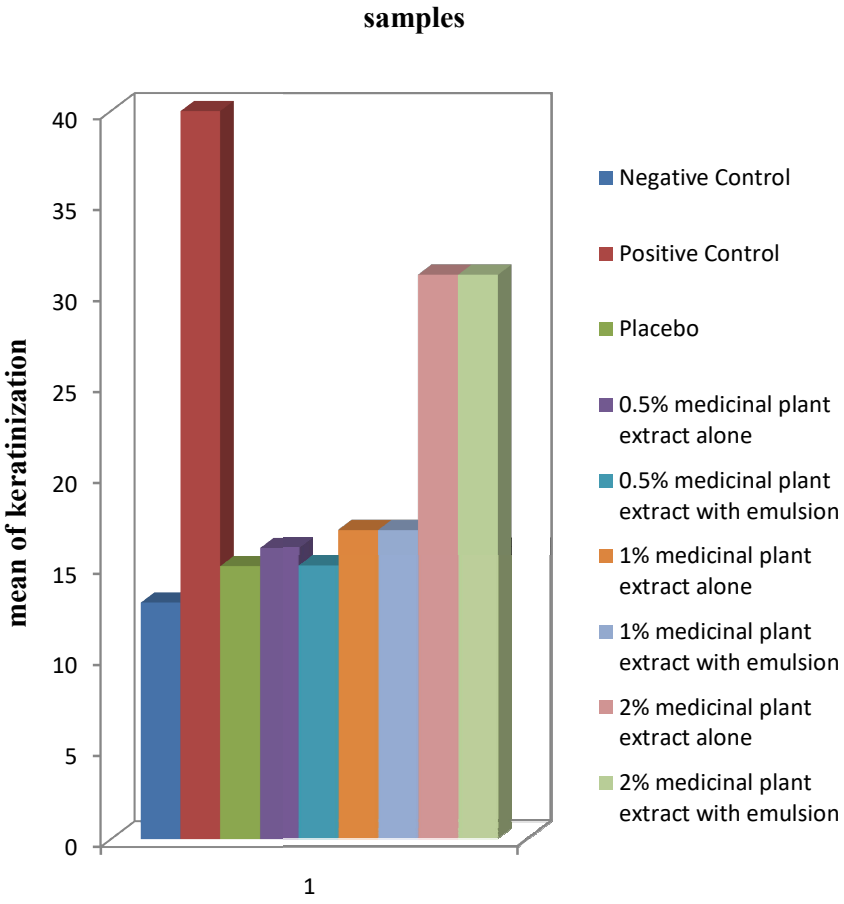


Fig. 10 Effect of *Kigelia africana* extracts on keratinization levels in *Epidermophyton floccosum*infected skin

Table 33 Effect of *Kigelia africana* formulations on Epidermal thickness (in µm) of skin infected by *Epidermophyton floccosum*

Gpr 1	89.76	91.34	90.30	91.76	92.72
Grp 2	41.72	40.87	39.25	40.01	40.18
Grp 3	89.71	90.10	91.44	89.62	90.24
Grp 4a	82.56	80.91	80.52	81.35	80.99
Grp 4b	81.78	82.62	79.45	80.56	81.79
Grp 5a	82.51	82.79	81.19	85.29	81.77
Grp 5b	84.55	81.29	83.79	82.91	83.72
Grp 6a	53.74	50.45	52.99	52.31	53.44
Grp 6b	52.68	54.97	51.72	50.60	49.72

Group 1 – Animals without any treatment – Negative control

Group 2 – Animals treated with cream containing 1% Clotrimazole (Positive Control - a).

Group 3 – Animals treated with placebo (Emulsion base only/Positive control - b)

Group 4a – Animals treated with 0.5% medicinal plant extract alone

Group 4b – Animals treated with emulsion containing 0.5% medicinal plant extract

Group 5a – Animals treated with 1% medicinal plant extract alone

Group 5b – Animals treated with emulsion containing 1% medicinal plant extract

Group 6a- Animals treated with 2% medicinal plant extract alone

Group 6b- Animals treated with emulsion containing 2% medicinal plant extract

emulsion, 1% *Kigelia africana* extract and 1% *Kigelia africana* emulsion in reducing the epidermal thickness is not significant compared with the untreated control. Incontrast, there was significant efficacy of 2% *Kigelia africana* extract and 2% *Kigelia africana* emulsion compared with the values obtained for the standard drug (Fig. 11).
Data showed in Table 91 and Fig.57 (See Appendix) reveals that there is significance difference in epidermal thickness between groups as determined by one way anova ($F_{6,28}$=373.418 , $p<0.05$). A tukey post hoc test revealed that there is significance difference in epidermal thickness between the Control and the test groups.

4.12.1.3 Trichophyton *mentagrophytes*

There was marked inflammation of the skin and foci of discontinuity of the epidermis in group 1 (Table 34). Marked presence of fungal hyphae shows successful infection (Plate 10). There were no hair follicles seen and few sebaceous glands were found. Marked tissue destruction was also observed in the group compared to the other groups which were treated with various formulations.

No inflammation and tissue destruction was observed in group 2 (Table 34). There was absence of fungal hyphae indicating that the drug was able to prevent the infection by the dermatophyte. Numerous hair follicles and sebaceous glands were seen in the dermis.

Group 3 animals treated with emulsion alone had moderate Inflammation and tissue destruction of the skin. There were few presence of fungal hyphae and also few hair follicles. Few sebaceous glands were also seen in the dermis (Plate 10).

There was few presence of fungal hyphae in group 4a. Moderate amount of hair follicles enmeshed within the sparse connective tissue in the dermis were seen.There are few sebaceous glands in the dermis. Same observations made for group 4a were also seen in group 4b.

The inflammation and tissue destruction of the skin were also mild in group 5a (1% plant extract alone) (Table 34). Few amounts of fungal hyphae were seen with moderate amount of hair follicle and sebaceous gland. Same observation were recorded for 5b (1% plant extract in emulsion).

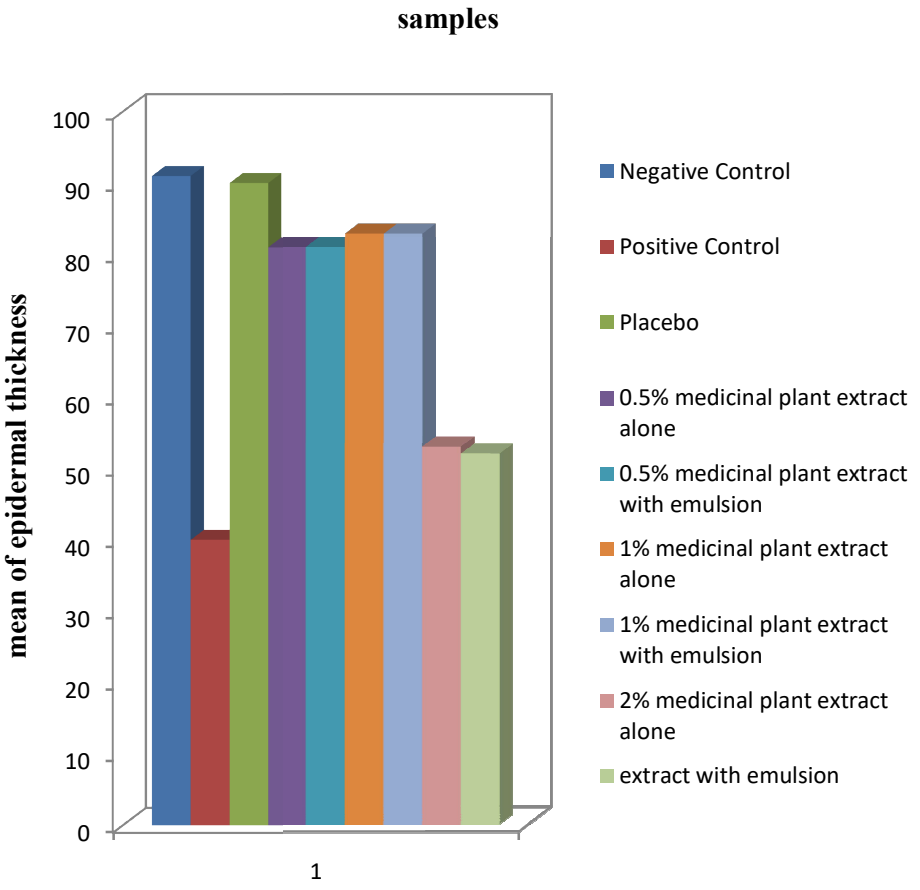


Fig. 11 Effect of *Kigelia africana* extracts on epidermal thickness in *Epidermophyton floccosum* infected skin

Table 34 Mycological efficacy of *Kigelia africana* ethanol formulations against *Trichophyton mentagrophytes*

Group	Fungal hyphae	Hair follicles	Sebaceous gland	Inflammation	Continuity/Tissue destruction
1	+++	-	+	+++	+++
2	-	+++	+++	-	-
3	++	+	+	++	++
4a	+	++	+	++	++
4b	+	++	+	++	++
5a	+	++	++	+	+
5b	+	++	++	+	+
6a	-	+++	++	+	+
6b	-	++	++	+	+

Absent = -, Mild = +, Moderate = ++, Marked = +++

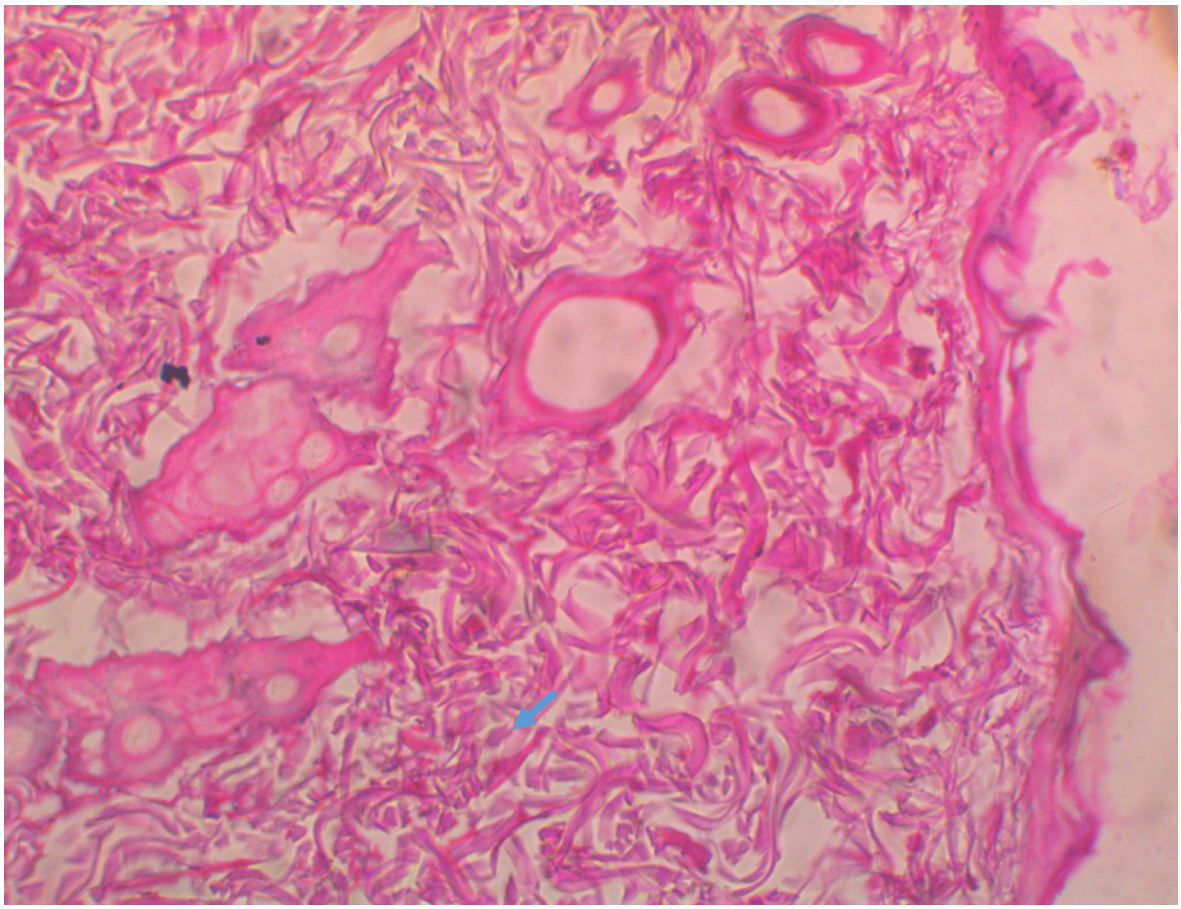


Plate 10 There is diffuse dermal capillary congestion and follicular hyperplasia. HE x400

In group 6a (2% plant extract alone), mild inflammation of the skin and tissue destruction was seen. No fungal hyphae were seen which indicated that the extract was able to prevent the growth of the dermatophyte hyphae. There were numerous hair follicles in the dermis and also moderate amount of sebaceous glands. Same observation was made for group 6b (2% plant extract in emulsion). This shows that 2% plant extract alone and 2% plant extract in emulsion are closely effective as the standard drug (Plate 11). The efficacy of the formulations on the keratin layer of the hair compared with the infected untreated control is shown in Table 35 and Fig. 12. The untreated control group 1, with the value 11.37 ± 0.18 µm indicated that the hairs were heavily infected with the dermatophyte. The standard drug (1% clotrimazole cream) with the value 42.83 ± 0.33 µm showed mycological efficacy in preventing attack by the dermatophyte on the keratin layer of the hair. However, the values obtained for group 3 (18.82 ± 0.22 µm), group 4a (21.50 ± 0.44 µm) and group 4b (20.87 ± 0.60 µm) indicated that their efficacies were not different from one another. This implies that though infected by the dematophytes but not as heavily infected as the untreated control. The values obtained for groups 5a and 5b indicated slight efficacy by the formulations. Comparatively, group 6a (38.84 ± 0.52 µm) and group 6b (37.01 ± 0.39 µm) had close efficacy with the standard drug.

There is significant difference in keratinization between the groups as determined by one way Anover ($F_{6,28}$=316.080 , $p<0.05$). Tukeys post hoc test was used in determining the significant differences when the Control is compared with other groups (Table 92 and Fig.58). See Appendix.

There was significant infection (74.01 ± 0.68 µm) as indicated by the result obtained for the epidermal thickness of untreated group (Table 36 and Fig.13). The values obtained for group 2 (40.41 ± 0.42 µm) indicated high efficacy by reducing the epidermal thickness. However, the values obtained for group 3 (61.81 ± 0.77 µm), group 4a (62.45 ± 0.86 µm), group 4b (62.45 ± 0.60 µm), group 5a (60.72 ± 0.58 µm) and group 5b (60.01 ± 0.42 µm) in reducing the epidermal thickness cannot be said to be significant compared with the untreated control.

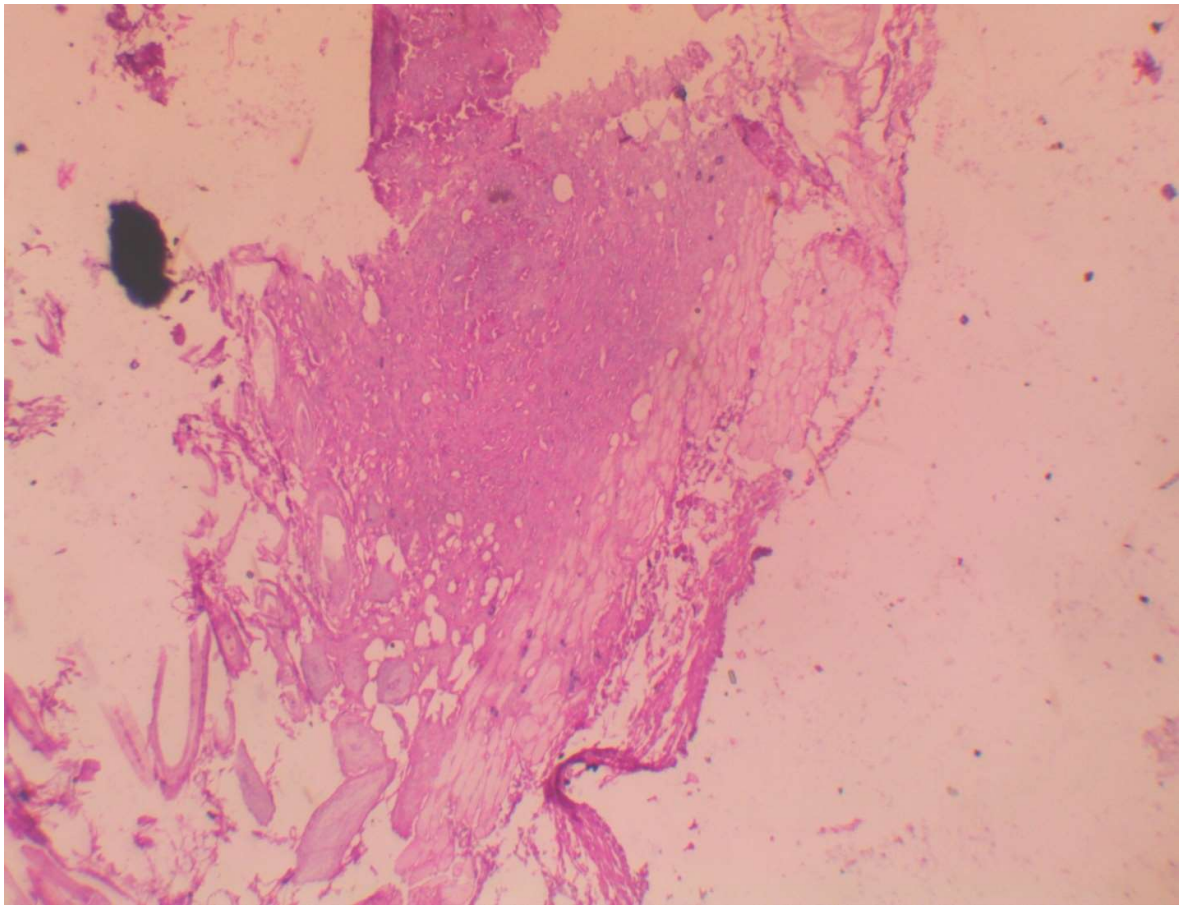


Plate 11 There is cornification and thicknening of the epidermis (HE x100)

Table 35 Effect of *Kigelia africana* formulations on keratinization (in µm) of skin infected by *Trichophyton mentagrophyte*

Group 1	11.82	10.91	11.71	11.00	11.39
Group 2	43.71	43.23	41.72	42.61	42.89
Group 3	18.43	18.51	19.62	18.94	18.62
Group 4a	22.41	20.86	20.69	22.75	20.81
Group 4b	21.91	22.67	19.61	20.42	19.74
Group 5a	24.52	25.91	25.81	24.95	23.34
Group 5b	23.61	24.51	25.01	25.72	21.39
Group 6a	38.91	38.72	40.21	39.32	37.02
Group 6b	37.89	36.51	36.42	38.01	36.24

Group 1 – Animals without any treatment – Negative control

Group 2 – Animals treated with cream containing 1% Clotrimazole (Positive Control - a).

Group 3 – Animals treated with placebo (Emulsion base only/Positive control - b)

Group 4a – Animals treated with 0.5% medicinal plant extract alone

Group 4b – Animals treated with emulsion containing 0.5% medicinal plant extract

Group 5a – Animals treated with 1% medicinal plant extract alone

Group 5b – Animals treated with emulsion containing 1% medicinal plant extract

Group 6a- Animals treated with 2% medicinal plant extract alone

Group 6b- Animals treated with emulsion containing 2% medicinal plant extract

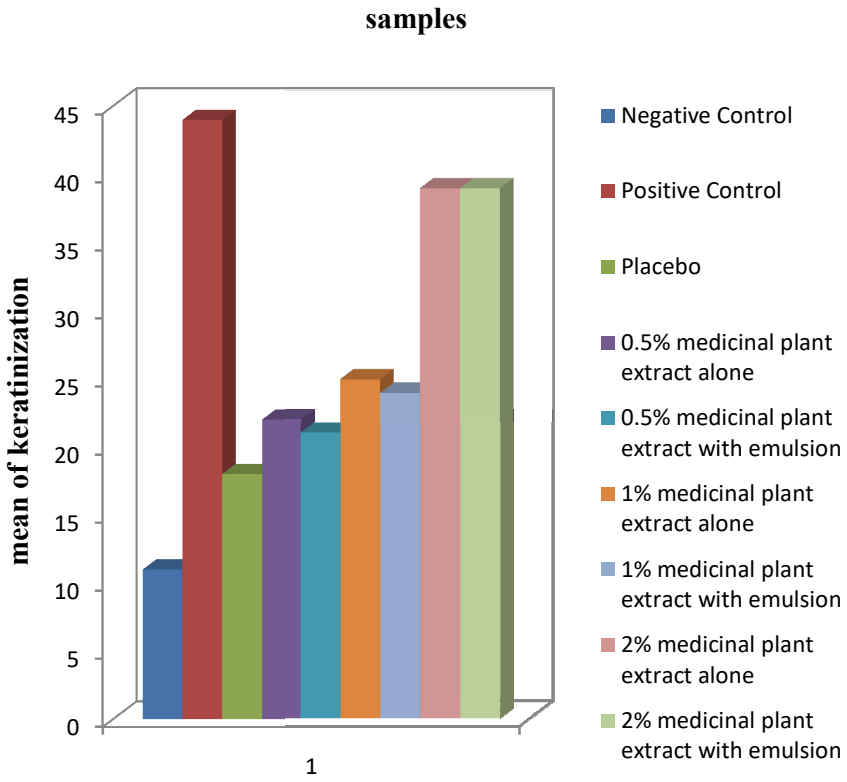


Fig.12 Effect of *Kigelia africana* extracts on keratinization in *Trichophyton mentagrophyte* infected skin

Table 36 Effect of *Kigelia africana* formulations on Epidermal thickness (in µm) of skin infected by *Trichophyton mentagrophyte*

Gpr 1	75.14	74.52	74.65	71.35	74.41
Grp 2	48.24	44.82	45.78	46.16	46.61
Grp 3	63.14	60.81	64.16	60.25	60.71
Grp 4a	65.22	61.51	60.72	63.61	61.11
Grp 4b	62.77	60.56	64.01	63.18	61.72
Grp 5a	58.80	60.14	62.09	61.62	60.93
Grp 5b	60.85	60.11	59.81	62.72	63.16
Grp 6a	40.14	43.55	42.31	40.89	41.91
Grp 6b	41.11	41.38	40.67	42.82	41.85

Group 1 – Animals without any treatment – Negative control

Group 2 – Animals treated with cream containing 1% Clotrimazole (Positive Control - a).

Group 3 – Animals treated with placebo (Emulsion base only/Positive control - b)

Group 4a – Animals treated with 0.5% medicinal plant extract alone

Group 4b – Animals treated with emulsion containing 0.5% medicinal plant extract

Group 5a – Animals treated with 1% medicinal plant extract alone

Group 5b – Animals treated with emulsion containing 1% medicinal plant extract

Group 6a- Animals treated with 2% medicinal plant extract alone

Group 6b- Animals treated with emulsion containing 2% medicinal plant extract

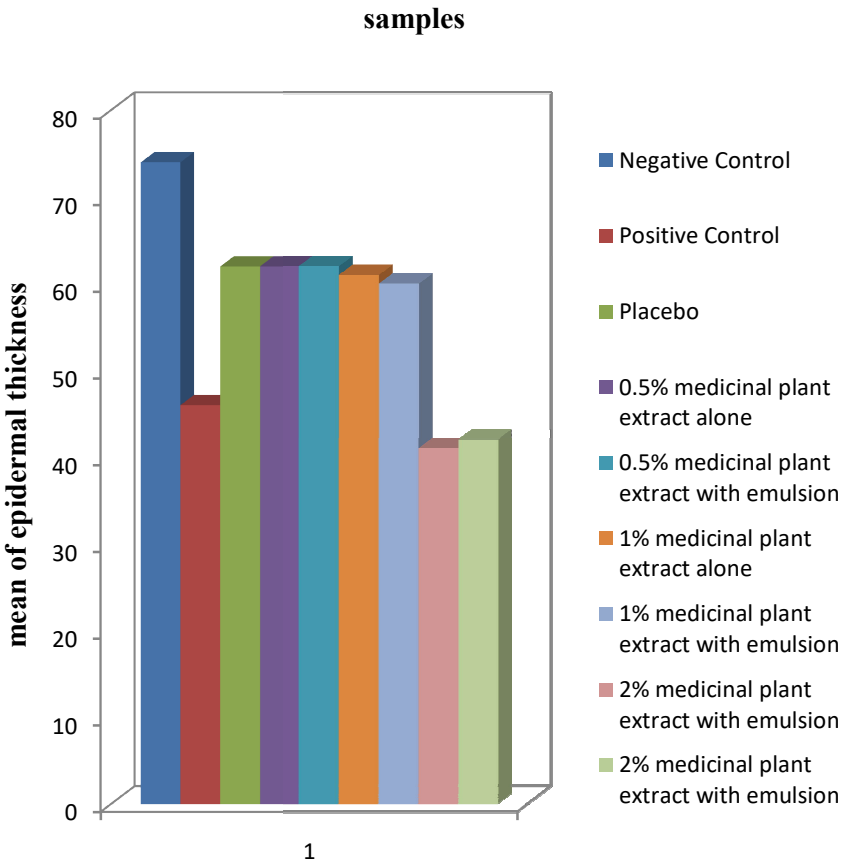


Fig 13 Effect of *Kigelia africana* extracts on epidermal thickness in *Trichophyton mentagrophyte* infected skin

The statistical evaluation as shown in Table 93 shows that there is significance difference in epidermal thickness between groups as determined by one way anova($F_{6,28}$=261.781,p<0.05) . A tukey post hoc test also revealed that there is significance difference in epidermal thickness between the Control and the test groups.

4.12.1.4 *Malassezia furfur*

In the untreated group (group 1), moderate inflammation of the skin and foci of discontinuity of the epidermis were observed (Table 37). There was moderate presence of fungal hyphae which further indicated successful infection. Moderate tissue destruction was also observed in the group.

There were few hair follicles enmeshed within the sparse connective tissue in the dermis which indicated that the dermatophyte is feeding on the follicles. Very few sebaceous glands were seen. No inflammation and tissue destruction was observed in the group treated with 1% clotrimazole (group 2) this is an indication that the drug was able to prevent the growth of the hyphae by the dermatophyte (Plate 12). There were numerous hair follicles in the dermis and also moderate amount sebaceous glands.

Inflammation and tissue destruction of the skin were moderate in the group treated with emulsion alone (group 3). There was moderate presence of fungal hyphae. There were few numbers of hair follicles and sebaceous glands in the dermis (Plate 13).

In group 4a (0.5% plant extract alone) and group 4b (0.5% plant extract in emulsion), inflammation and tissue destruction of the skin were moderate in the group (Table 37). There were few presence of fungal hyphae, hair follicles and sebaceous glands in the dermis. However, in groups 5a (1% plant extract alone) and 5b (1% plant extract in emulsion), few amount of fungal hyphae and hair follicle were seen with moderate amount of sebaceous gland. The inflammation and tissue destruction of the skin were also moderate (Table 37). Same observation were recorded for groups 6a (2% plant extract alone) and 6b (2% plant extract in emulsion). The efficacy of the formulations on the keratin layer of the hair compared with the infected untreated control is shown in Table 38 and Fig.14. The value obtained (26.03 ± 0.30 μm) for the untreated control group 1 indicated that there was moderate infection of the hairs by the dermatophyte.

Table 37 Mycological efficacy of *Kigelia africana* ethanol formulations against *Malassezia furfur*

Group	Fungal hyphae	Hair follicles	Sebaceous gland	Inflammation	Continuity/Tissue destruction
1	++	+	+	++	++
2	-	+++	++	-	-
3	++	+	+	++	++
4a	+	+	+	++	++
4b	+	+	+	++	++
5a	+	+	++	++	++
5b	+	+	++	++	++
6a	+	+	++	++	++
6b	+	+	++	++	++

- = Absent, Mild = +, Moderate = ++, Marked = +++

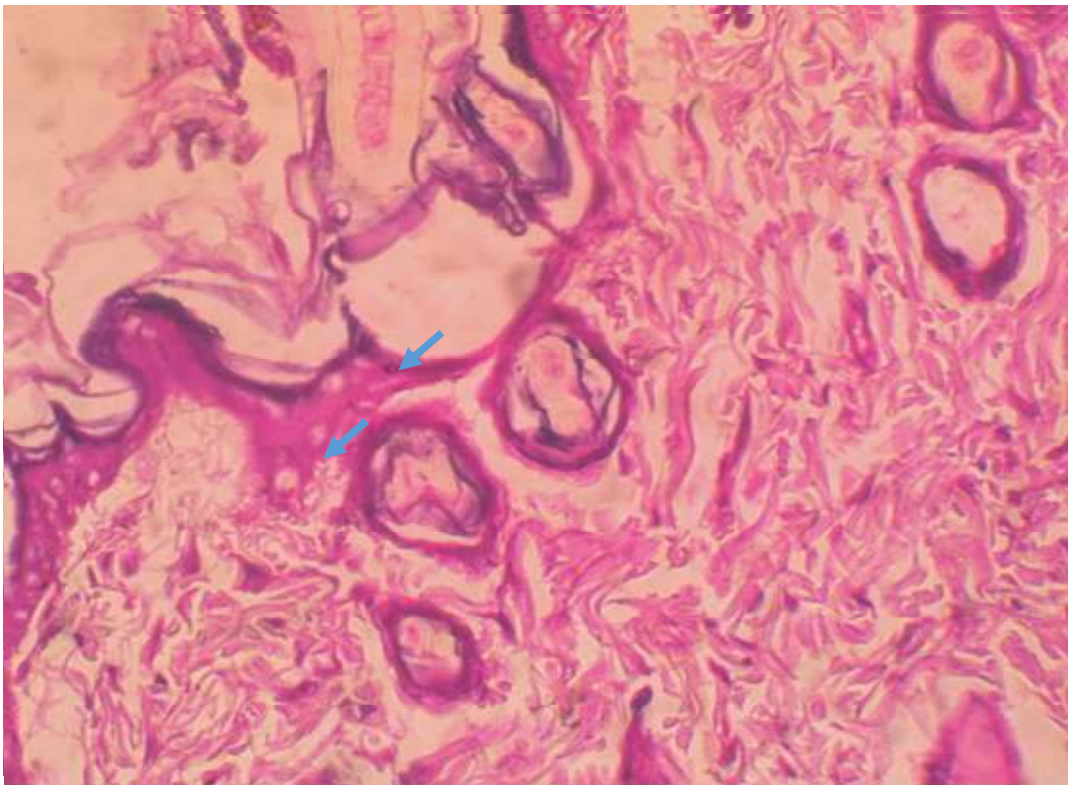


Plate 12 There is mild hydropic degeneration of keratinocytes (arrow). HE x400

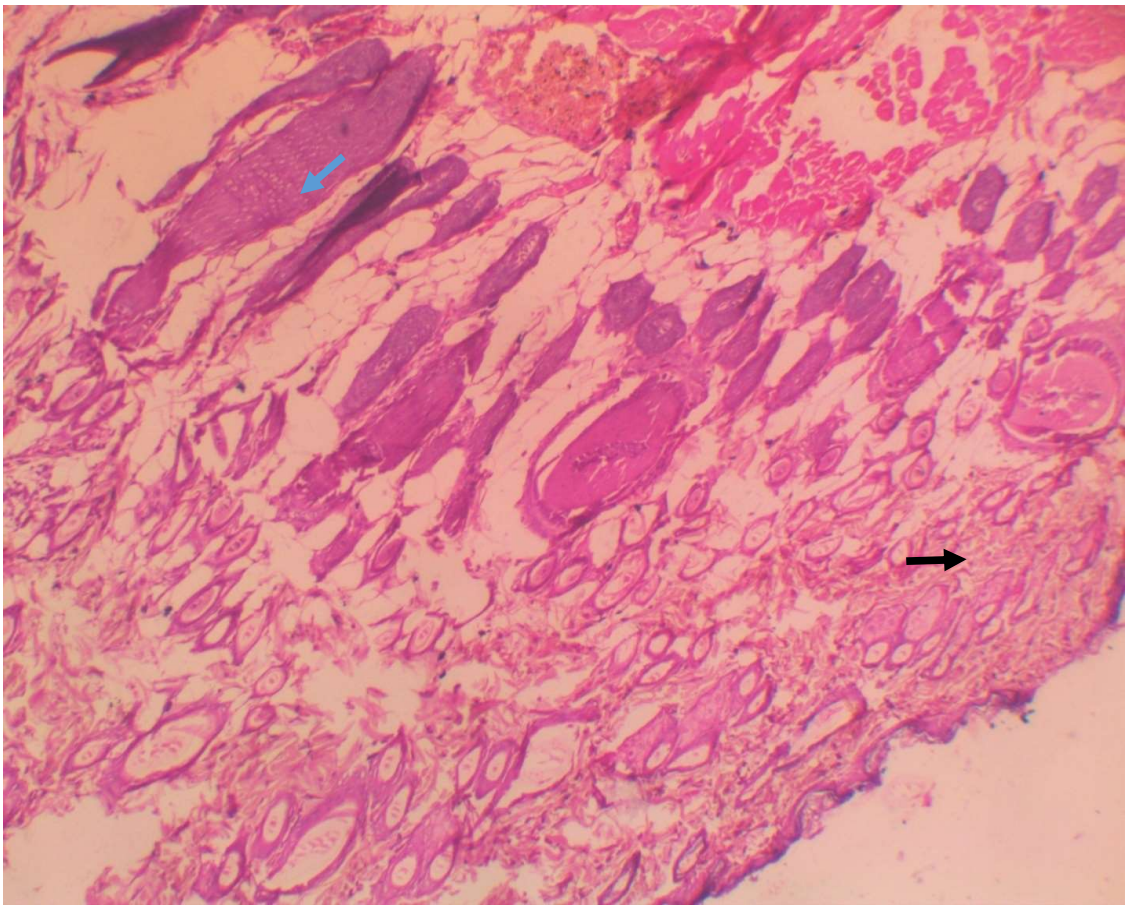


Plate 13 Thicknening of the epidermis(blue arrow) and dermal inflammatory cells Infiltrate(black arrow) (HE x400).

Table 38 Effect of *Kigelia africana* formulations on keratinisation (in µm) of skin infected by *Malassezia furfur*

Group 1	26.92	25.65	25.16	26.24	26.18
Group 2	47.56	48.84	49.31	46.56	48.62
Group 3	29.33	28.61	28.72	27.49	29.54
Group 4a	30.43	30.53	29.92	30.63	29.33
Group 4b	30.02	30.11	29.62	30.73	30.21
Group 5a	30.12	30.38	34.11	29.84	31.65
Group 5b	33.95	31.22	32.16	30.52	30.44
Group 6a	36.44	36.92	36.62	36.35	34.38
Group 6b	35.48	36.71	35.24	36.74	36.95

Group 1 – Animals without any treatment – Negative control

Group 2 – Animals treated with cream containing 1% Clotrimazole (Positive Control - a).

Group 3 – Animals treated with placebo (Emulsion base only/Positive control - b)

Group 4a – Animals treated with 0.5% medicinal plant extract alone

Group 4b – Animals treated with emulsion containing 0.5% medicinal plant extract

Group 5a – Animals treated with 1% medicinal plant extract alone

Group 5b – Animals treated with emulsion containing 1% medicinal plant extract

Group 6a- Animals treated with 2% medicinal plant extract alone

Group 6b- Animals treated with emulsion containing 2% medicinal plant extract

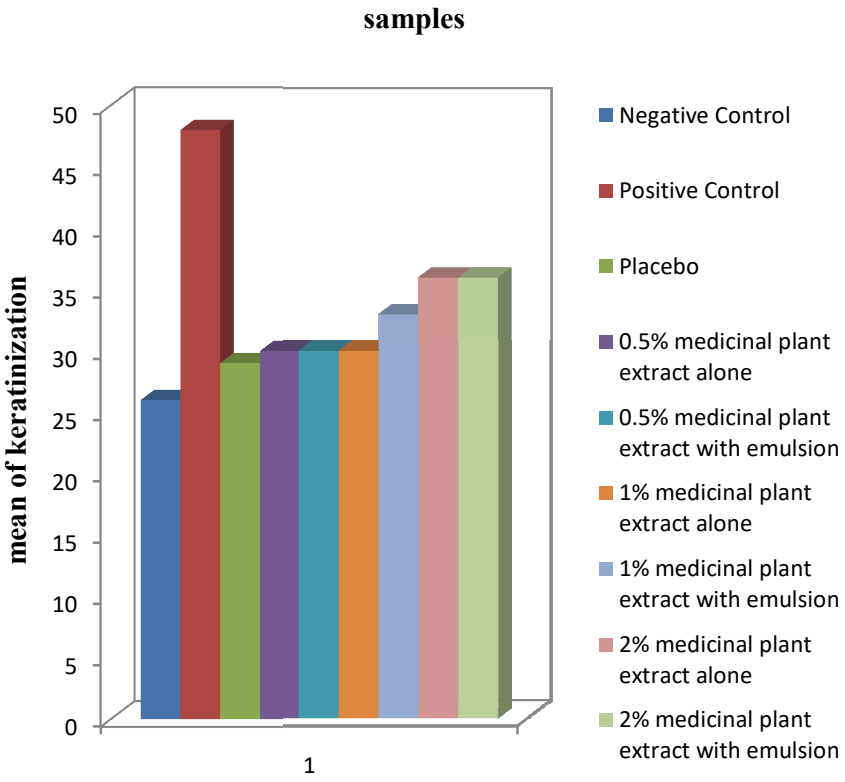


Fig.14 Effect of *Kigelia africana* extracts on keratinization in *Malassezia furfur* infected skin

The value obtained for the standard drug, 1% clotrimazole cream (48.18 ± 0.50 µm) indicated that there is significant prevention of attack by the dermatophyte on the keratin layer of the hair. The observation made for the group treated with the emulsion alone (28.73 ± 0.36 µm) indicated that the infection is gradually moving from moderate to mild. The results obtained for the other groups indicated that the moderate infection has been reduced to mild infection as can be seen in the groups treated with 0.5% *Kigelia africana* extract alone (30.17 ± 0.24 µm), the group treated with 0.5% *Kigelia africana* extract emulsion (30.14 ± 0.14 µm), 1% kigelia africana extract alone (31.22 ± 0.79 µm) and 1% Kigelia africana emulsion (31.66 ± 0.65 µm) which were not different from one another. This implies that there is slight efficacy by the formulations.

However, 2% *kigelia africana* extract alone (36.14 ± 0.45 µm) and 2% *Kigelia africana* extract emulsion (36.22 ± 0.36 µm) showed higher mycological efficacy compared to the other formulations. In conclusion, there is significant difference in keratinization between the groups as determined by one way Anova ($F_{6,28}$=169.737,p<0.05) followed by Turkeys post hoc test when the Control is compared with other groups. (Table 94 and Fig.60) (see Appendix).

The result obtained for the epidermal thickness of the untreated group indicated that there was significant infection (62.21 ± 0.97 µm). However, the values obtained for the standard drug (43.71 ± 0.53 µm) can be said to effectively reduce the epidermal thickness thereby indicating high efficacy against the dermatophyte. The values obtained for emulsion alone (62.14 ± 0.76 µm), 0.5% *Kigelia africana* extract (60.75 ± 0.93 µm), 0.5% *Kigelia africana* extract emulsion (60.37 ± 0.63 µm), 1% *Kigelia africana* extract (59.92±0.34µm), 1% *Kigelia africana* emulsion (58.00±0.82µm), 2% *Kigelia africana* extract alone (57.89±0.76µm) and 2% *Kigelia africana* emulsion with extract (56.91±0.85µm) in reducing the epidermal thickness cannot be said to be significant compared with the untreated control (Table 39 and Fig.15).

The statistical evaluation as shown in (Table 95) and (Fig.61) See Appendix show that there is no significance difference in epidermal thickness between groups as determined by one way anova ($F_{6,28}$=261.781,p>0.05) . A tukey post hoc test also revealed that there is no significance difference in epidermal thickness between the control and the test groups.

Table 39 Effect of *Kigelia africana* formulations on Epidermal thickness (in µm) of skin infected by *Malassezia furfur*

Gpr 1	50.12	51.69	54.38	50.25	54.62
Grp 2	34.76	34.23	31.75	33.53	34.28
Grp 3	54.53	50.32	52.11	52.93	50.81
Grp 4a	55.22	53.35	50.19	53.92	51.05
Grp 4b	52.83	50.88	54.15	53.16	50.85
Grp 5a	55.42	55.63	55.16	54.71	53.69
Grp 5b	50.39	54.34	54.72	51.87	53.71
Grp 6a	53.77	51.16	55.29	51.77	53.94
Grp 6b	54.11	50.63	49.17	49.88	50.76

Group 1 – Animals without any treatment – Negative control

Group 2 – Animals treated with cream containing 1% Clotrimazole (Positive Control - a).

Group 3 – Animals treated with placebo (Emulsion base only/Positive control - b)

Group 4a – Animals treated with 0.5% medicinal plant extract alone

Group 4b – Animals treated with emulsion containing 0.5% medicinal plant extract

Group 5a – Animals treated with 1% medicinal plant extract alone

Group 5b – Animals treated with emulsion containing 1% medicinal plant extract

Group 6a- Animals treated with 2% medicinal plant extract alone

Group 6b- Animals treated with emulsion containing 2% medicinal plant extract

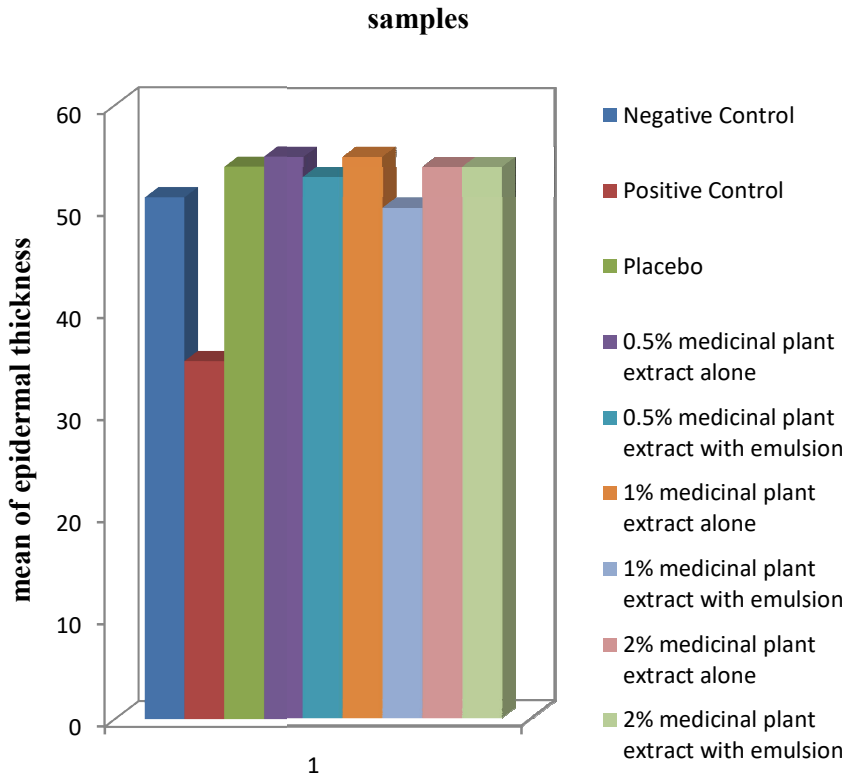


Fig.15 Effect of *Kigelia africana* extracts on epidermal thickness in *Malassezia furfur* infected skin

4.12.2 Effect of *Cassia alata* extracts on dermatopytes

4.12.2.1 *Microsporium aoudini*

There was marked inflammation of the skin and foci of discontinuity of the epidermis observed in group 1. Marked presence of fungal hyphae was also seen in the group. There were no hair follicles seen, but few sebaceous glands were found which further indicated successful infection. Marked tissue destruction was also observed in the group (Table 40).

Plate 16 shows absence of inflammation and tissue destruction in group 2. It was observed that no fungal hyphae were seen in the group 2 indicating that the drug was able to prevent the infection by the dermatophyte. There were numerous hair follicles in the dermis and also numerous amounts of sebaceous glands. Group 3 animals treated with emulsion alone had moderate presence of fungal hyphae. There were few hair follicles and sebaceous glands in the dermis. Skin inflammation and tissue destruction of the skin were marked in the group.

There was moderate presence of fungal hyphae in group 4a. Few amount of hair follicles enmeshed within the sparse connective tissue in the dermis were seen. There are moderate amount of sebaceous glands in the dermis. Inflammation and tissue destruction of the skin were also moderate in the group (Table 40 and Plate 14). Same observations were also seen in group 4b. Few amount of fungal hyphae were seen in group 5a (1% plant extract alone) with moderate amount of hair follicle and sebaceous gland (Plate 15). The inflammation and tissue destruction of the skin were also moderate (Table 40). Same observation were recorded for 5b (1% plant extract in emulsion).

Mild inflammation of the skin and tissue destruction was seen. No fungal hyphae were seen in group 6a (2% plant extract alone) which indicated that the extract was able to prevent the growth of the dermatophyte hyphae. There were moderate hair follicles in the dermis and also moderate amount of sebaceous glands. Same observation was made for group 6b (2% plant extract in emulsion). This indicated that 2% plant extract alone and 2% plant extract in emulsion are closely effective as the standard drug.

Table 40 Mycological efficacy of *Cassia alata* ethanol formulations against *Microsporium aoudini*

Group	Fungal hyphae	Hair follicles	Sebaceous gland	Inflammation	Continuity/Tissue destruction
1	+++	-	+	+++	+++
2	-	+++	+++	-	-
3	++	+	+	+++	+++
4a	++	+	++	++	++
4b	++	+	++	++	++
5a	+	++	++	++	++
5b	+	++	++	++	++
6a	-	++	++	+	+
6b	-	++	++	+	+

- = Absent, Mild = +, Moderate = ++, Marked = +++

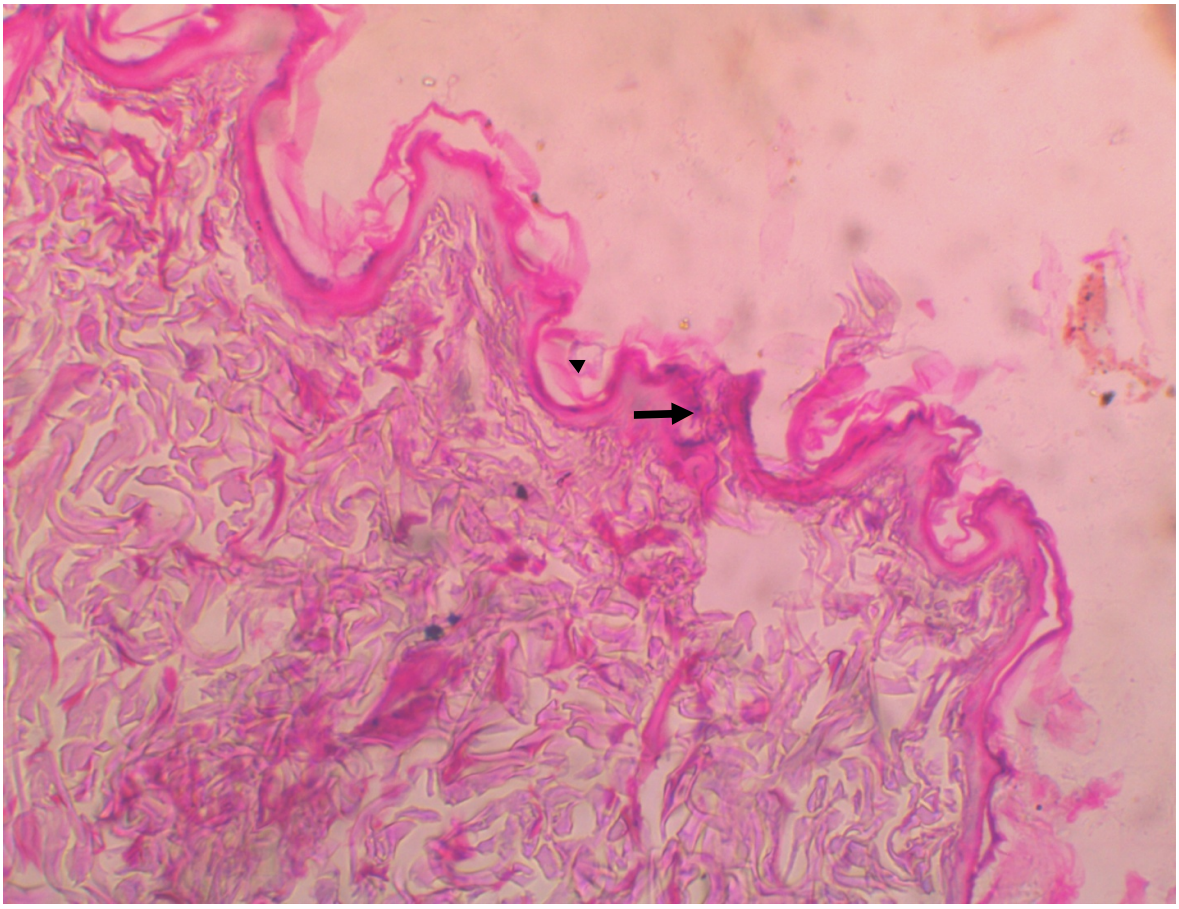


Plate 14 There is thickening of the epidermis due to hyperkeratosis and follicular proliferation (HE x100)

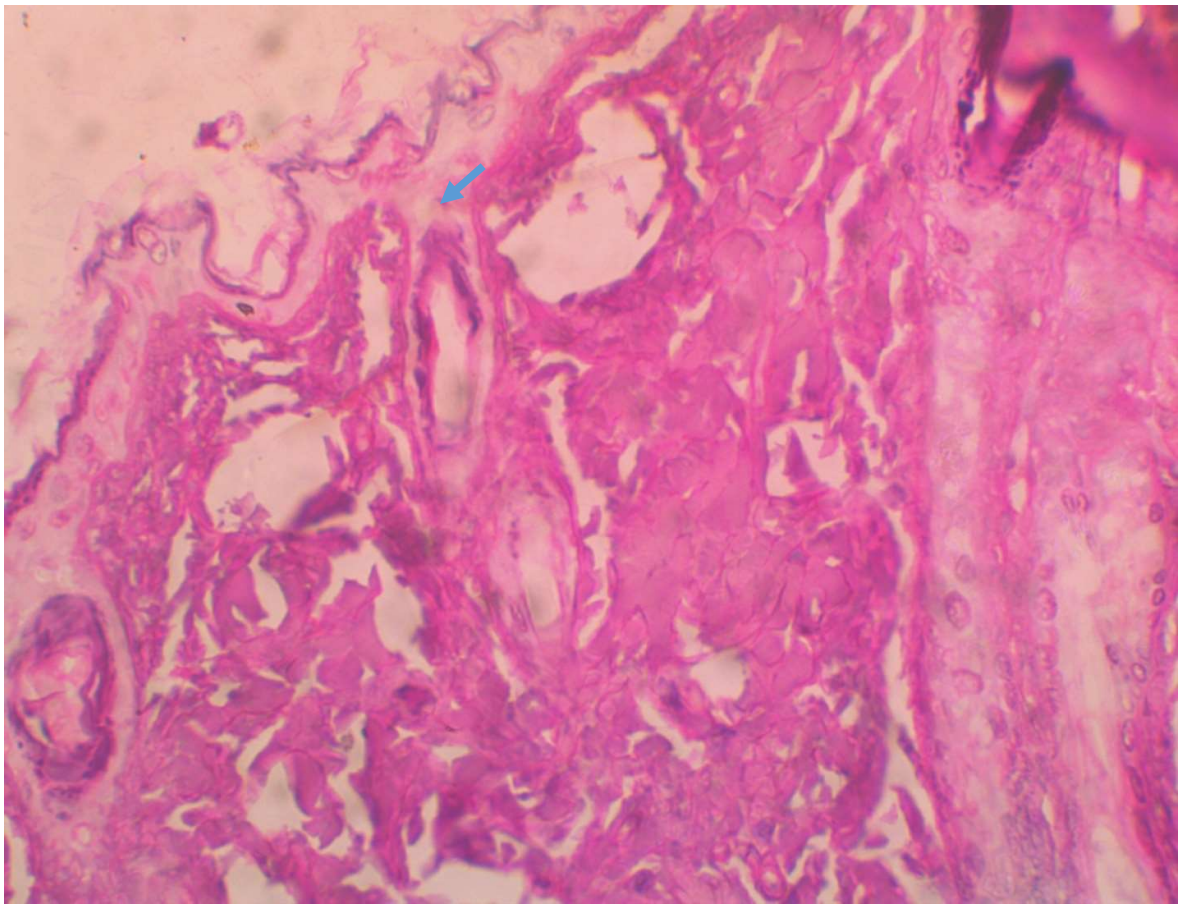


Plate 15 There is conification and thicknening of the epidermis(parakeratosis) HE x100

Table 41 and Fig.16 showed the efficacy of the formulations on the keratin layer of the hair compared with the infected untreated control. The untreated control with the value 26.90 ± 0.53 µm indicated that the hairs were heavily infected with the dermatophyte. The effectiveness of 1% clotrimazole cream (42.57 ± 0.57 µm) in preventing attack by the dermatophyte on the keratin layer of the hair is demonstrated by the high value recorded. However, the group treated with the emulsion alone (27.63 ± 0.75 µm) showed there was no efficacy. Application of 0.5% *Cassia alata* extract emulsion (25.19 ± 0.37 µm) and 0.5% *Cassia alata* extract alone (26.33 ± 0.23 µm) on the infected skin of the animal did not show any significant difference from the untreated group. This implies that they had no effect in curtailing the infection caused by the dermatophyte. The efficacy of the formulations increased as the concentration increases as can been seen in the group treated with 1% *Cassia alata* extract alone(29.23 ± 0.41 µm), 1% *Cassia alata* emulsion (30.06 ± 0.43 µm), 2% *Cassia alata* extract alone (33.23 ± 0.73 µm) and 2% Cassia *alata* extract emulsion (36.17 ± 0.59 µm).

Statistically, there is significant difference in keratinization between the groups as determined by one way Anova ($F_{6,28}$=145.715,$p<0.05$). Using Tukey's post hoc test which confirms the difference. (Table 96 and Fig.62) (See Appendix).

The results obtained for the epidermal thickness of untreated group indicated significant infection (77.97 ± 0.56 µm) compared with the values obtained for the standard drug (45.59 ± 0.05 µm) which showed that it was very effective. The value obtained for emulsion alone (77.75 ± 0.68 µm) indicated that the emulsion had no effect on the epidermal thickness of the animals which was not significant from the untreated group. The efficacy demonstrated by 0.5% *Cassia alata* extract (71.19 ± 0.45 µm), 0.5% *Cassia alata* extract emulsion (73.10 ± 0.67 µm), 1% *Cassia alata* extract (72.75 ± 0.62 µm) and 1% *Cassia alata* emulsion (73.01 ± 0.59 µm) in reducing the epidermal thickness can be said to be slightly significant compared with the untreated control. However, the values obtained for 2% *Cassia alata* extract (62.53 ± 0.56 µm) and 2% *Cassia alata* emulsion (62.27 ± 0.75 µm) showed moderate effectiveness in treating the infection (Table 42) and (Fig.17). The statistical evaluation as shown in Tables 97 and Fig.63 (See Appendix) shows that there is significance difference in epidermal thickness between groups as shown by one way Anova.

Table 41 Effect of *Cassia alata* formulations on keratinisation (in µm) of skin infected by *Microsporium aoudini*

Group 1	26.71	25.32	26.91	28.65	26.92
Group 2	43.08	42.61	44.09	42.45	40.61
Group 3	28.67	26.07	27.59	29.86	25.94
Group 4a	26.55	27.11	26.04	25.75	26.24
Group 4b	25.65	24.06	25.79	25.89	24.58
Group 5a	29.75	27.89	29.83	28.65	30.04
Group 5b	30.71	28.50	30.44	29.73	30.91
Group 6a	33.11	35.49	32.11	31.33	34.09
Group 6b	35.89	37.87	36.09	34.24	36.77

Group 1 – Animals without any treatment – Negative control

Group 2 – Animals treated with cream containing 1% Clotrimazole (Positive Control - a).

Group 3 – Animals treated with placebo (Emulsion base only/Positive control - b)

Group 4a – Animals treated with 0.5% medicinal plant extract alone

Group 4b – Animals treated with emulsion containing 0.5% medicinal plant extract

Group 5a – Animals treated with 1% medicinal plant extract alone

Group 5b – Animals treated with emulsion containing 1% medicinal plant extract

Group 6a- Animals treated with 2% medicinal plant extract alone

Group 6b- Animals treated with emulsion containing 2% medicinal plant extract

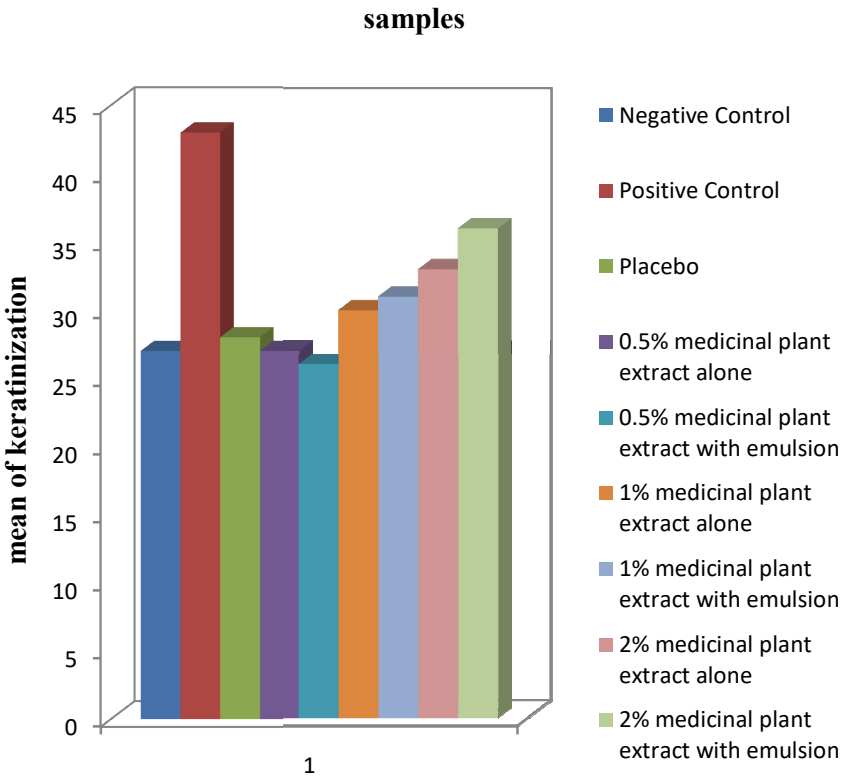


Fig.16 Effect of *Cassia alata* extracts on keratinization in *Microsporium aoudini* infected skin

Table 42 Effect of *Cassia alata* formulations on epidermal thickness (in µm) of skin infected by *Microsporium aoudini*

Gpr 1	79.35	76.43	76.97	78.92	77.96
Grp 2	44.92	48.72	42.39	45.11	46.81
Grp 3	77.80	75.90	76.89	79.92	78.24
Grp 4a	72.68	70.74	70.76	71.64	70.11
Grp 4b	71.81	72.25	79.68	70.56	71.22
Grp 5a	72.11	72.89	71.91	75.09	71.75
Grp 5b	74.55	71.29	73.90	72.11	73.21
Grp 6a	63.44	60.45	62.99	62.31	63.44
Grp 6b	62.53	64.72	61.80	60.07	62.22

Group 1 – Animals without any treatment – Negative control

Group 2 – Animals treated with cream containing 1% Clotrimazole (Positive Control - a).

Group 3 – Animals treated with placebo (Emulsion base only/Positive control - b)

Group 4a – Animals treated with 0.5% medicinal plant extract alone

Group 4b – Animals treated with emulsion containing 0.5% medicinal plant extract

Group 5a – Animals treated with 1% medicinal plant extract alone

Group 5b – Animals treated with emulsion containing 1% medicinal plant extract

Group 6a- Animals treated with 2% medicinal plant extract alone

Group 6b- Animals treated with emulsion containing 2% medicinal plant extract

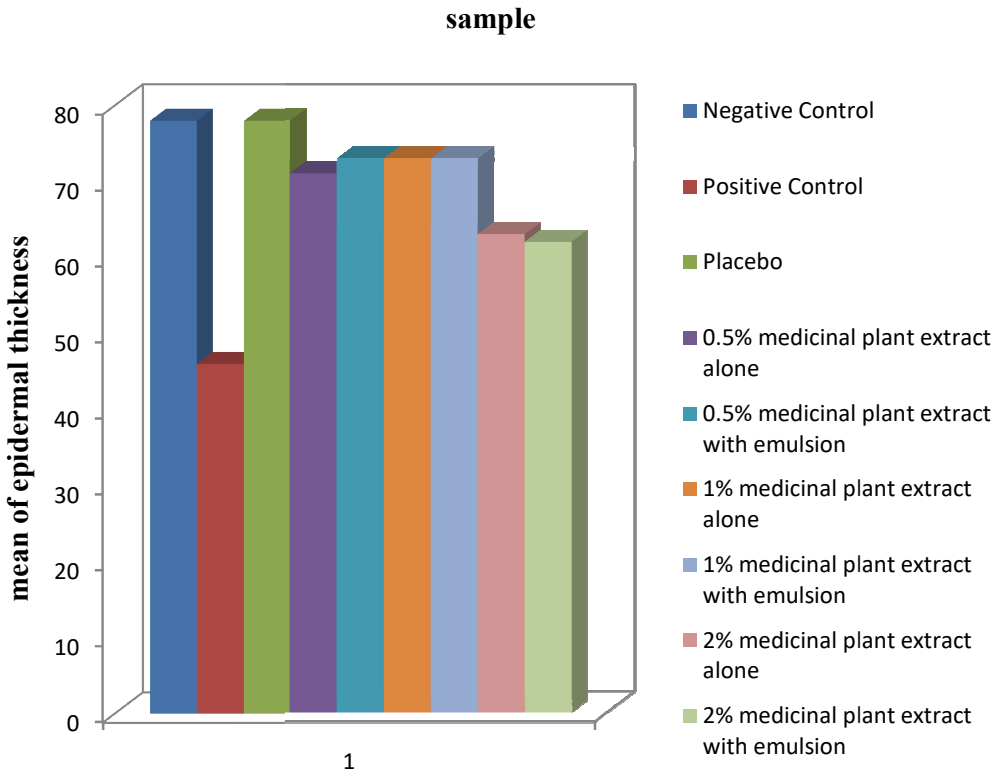


Fig.17 Effect of *Cassia alata* extracts on epidermal thickness in *Microsporium aoudini* infected skin

4.12.2.2 Epidermophyton *floccosum*

There was marked inflammation of the skin and tissue destruction observed in group 1 compared to the other groups which were treated with various formulations. Marked presence of fungal hyphae was seen in the group. Few hair follicles and sebaceous glands were also observed.

No inflammation and tissue destruction was observed in group 2. (Table 43). No fungal hyphae were seen indicating that the drug was able to prevent the infection by the dermatophyte. There were numerous hair follicles in the dermis and also numerous amounts of sebaceous glands. Group 3 animals treated with emulsion alone had moderate skin inflammation, and there was marked tissue destruction of the skin. Moderate presence of fungal hyphae and moderate hair follicles were seen. There were few sebaceous glands in the dermis. (Plate 16).The same observations were made for groups 4a, 4b, 5a and 5b.

There was little presence of fungal hyphae in group 6a. Moderate amount of hair follicles enmeshed within the sparse connective tissue in the dermis were seen. There were also moderate amount of sebaceous glands in the dermis. Inflammation and tissue destruction of the skin were also moderate in the group (Plate 17). Same observations were also seen in group 6b.

Table 44 and Fig.18 shows comparative effectiveness of the formulations on keratinization compared with the infected untreated control. Higher scores indicated improved efficacy compared with the untreated control (14.94 ± 0.38 µm). Significant efficacy was demonstrated by 1% clotrimazole cream (standard drug) with the value 47.05 ± 0.91 µm. 2% *Cassia alata* extract alone (36.88 ± 0.59 µm) and 2% *Cassia alata* extract emulsion (37.49 ± 0.18 µm) can be said to be mycologically effective in prevent the dermotophyte in reducing the keratin layer of the hair. The values obtained for 1% *Cassia alata* extract alone (26.52 ± 0.35 µm), 1% *Cassia alata* extract emulsion (26.71 ± 0.24 µm), 0.5% Cassia alata extract (25.06 ± 0.67 µm) and 0.5% *Cassia alata* extract emulsion (25.66 ± 0.74 µm) showed that the formulations are slightly effective, but not as effective as the 2% formulations. The value obtained for the emulsion alone (22.29 ± 0.44 µm) indicated that it has some antidermatophytic effect compared to the untreated group.

Table 43 Mycological efficacy of *Cassia alata* formulations against *Epidermophyton floccosum*

Group	Fungal hyphae	Hair follicles	Sebaceous gland	Inflammation	Continuity/Tissue destruction
1	+++	+	+	+++	+++
2	-	+++	+++	-	-
3	++	++	+	++	+++
4a	++	++	+	++	+++
4b	++	++	+	++	+++
5a	++	++	+	++	+++
5b	++	++	+	++	+++
6a	+	++	++	++	++
6b	+	++	++	++	++

- = Absent, Mild = +, Moderate = ++, Marked = +++

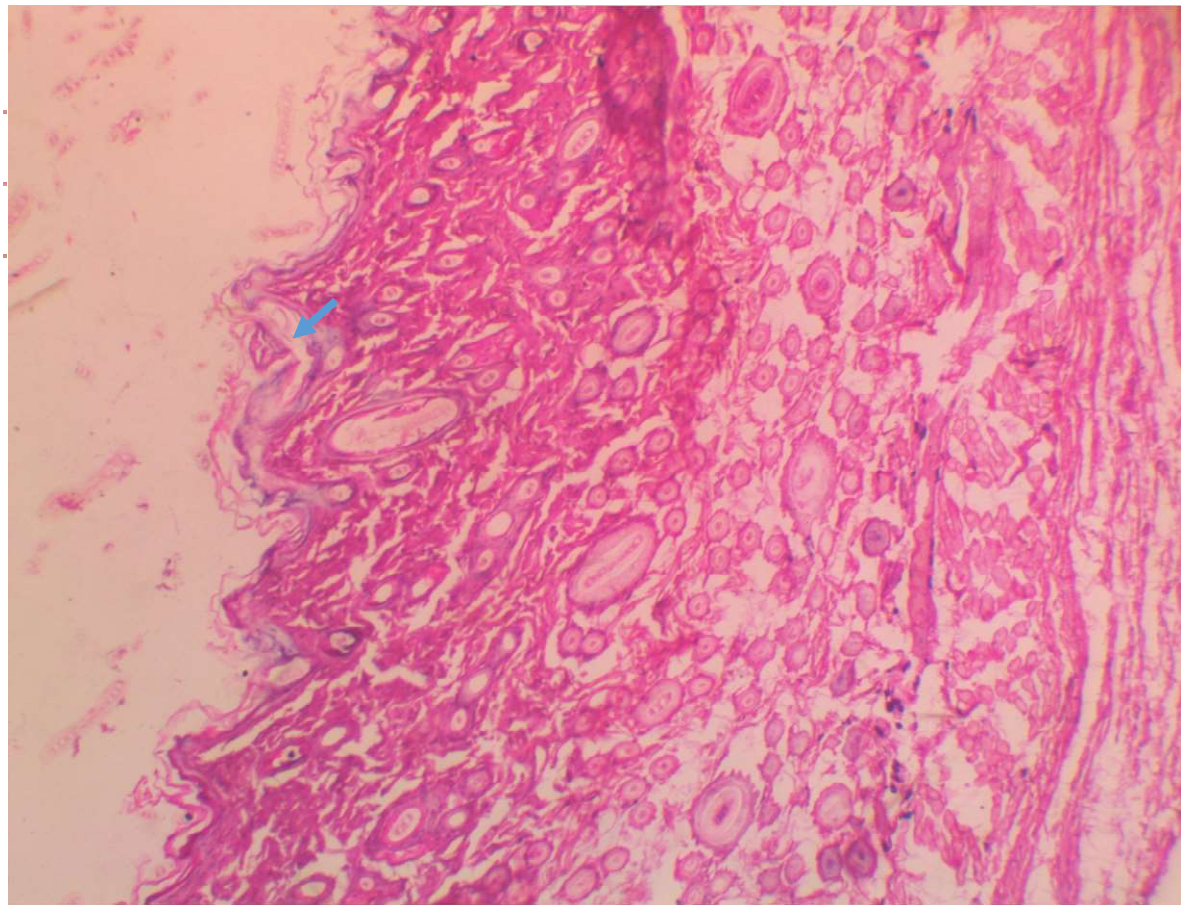


Plate 16. There is moderate thicknening of the epidermis with few acute Inflammatory. HE x100.

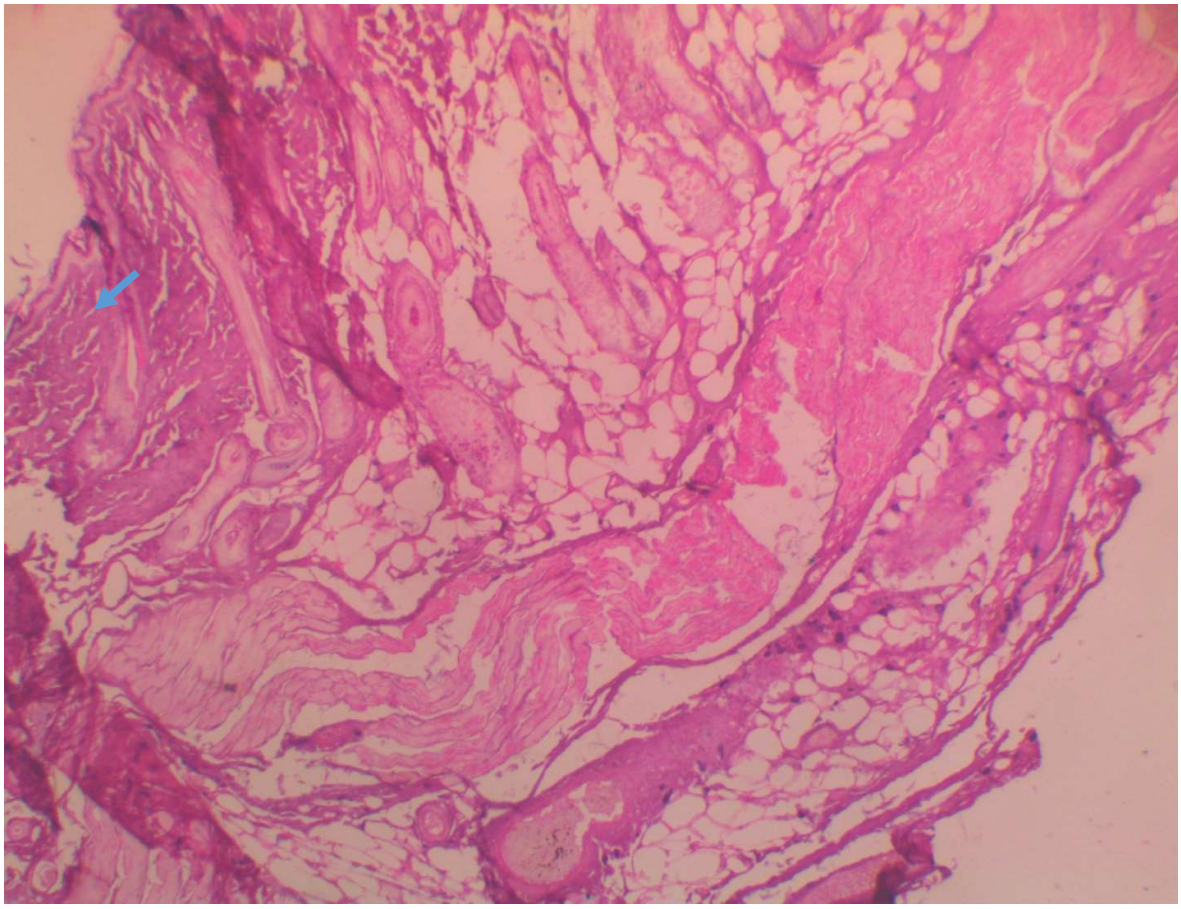


Plate 17 Hyperkeratosis of the epidermis. HE x100.

Table 44 Effect of *Cassia alata* formulations on keratinization (in µm) of skin infected by *Epidermophyton floccosum*

Group 1	14.22	15.67	14.77	14.09	15.96
Group 2	49.68	46.89	48.32	45.90	44.45
Group 3	22.65	22.51	23.43	22.11	20.75
Group 4a	26.35	24.63	25.55	22.67	26.11
Group 4b	26.11	28.89	27.09	26.33	29.87
Group 5a	26.22	26.91	25.37	26.64	27.45
Group 5b	27.09	27.11	26.54	25.84	26.98
Group 6a	36.09	35.22	38.68	37.54	36.89
Group 6b	35.99	37.14	36.44	36.45	36.44

Group 1 – Animals without any treatment – Negative control

Group 2 – Animals treated with cream containing 1% Clotrimazole (Positive Control - a).

Group 3 – Animals treated with placebo (Emulsion base only/Positive control - b)

Group 4a – Animals treated with 0.5% medicinal plant extract alone

Group 4b – Animals treated with emulsion containing 0.5% medicinal plant extract

Group 5a – Animals treated with 1% medicinal plant extract alone

Group 5b – Animals treated with emulsion containing 1% medicinal plant extract

Group 6a- Animals treated with 2% medicinal plant extract alone

Group 6b- Animals treated with emulsion containing 2% medicinal plant extract

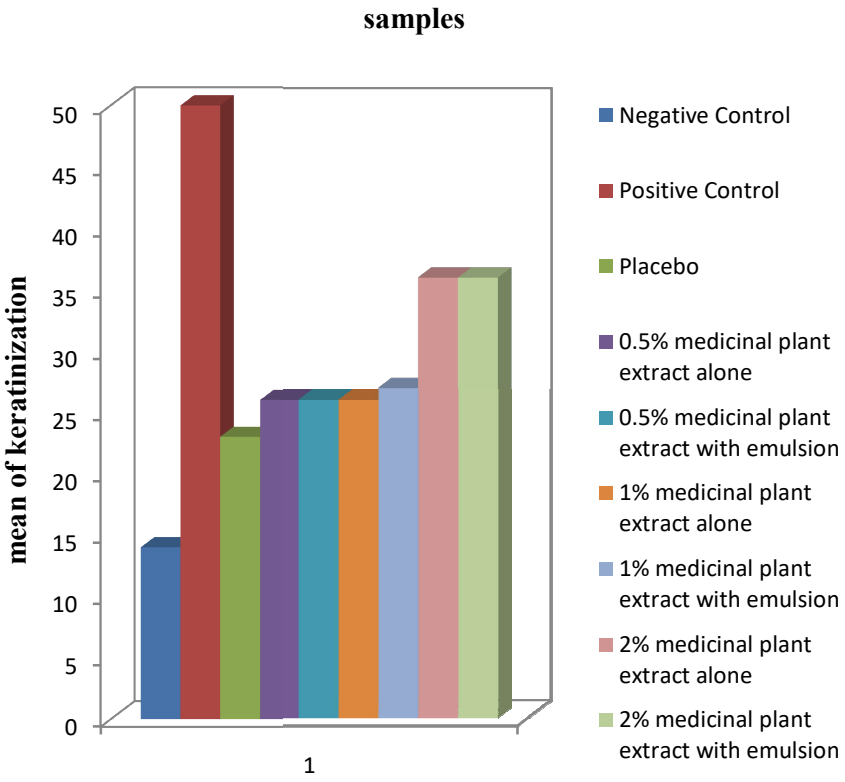


Fig.18 Effect of *Cassia alata* extracts on keratinization in *Epidermophyton floccosum* infected skin

The formulated emulsion alone exhibited some mycological efficacy in the treatment of dermatophytosis. It was also observed that the drug release rate of the formulated emulsion with 2% *Cassia alata* extract is as effective as the standard drug (1% clotrimazole). The efficacy of both formulations could be explained on the basis of the pharmacokinetics of the plant extract on the skin. Similarly, applying 1% clotrimazole topically leads to drug penetration into the upper layers of the stratum corneum of the skin.

Table 98 and Fig.64 (See Appendix), shows that there is significance difference in keratinization between groups as shown by one way Anova ($F_{6,28}$=192.754, $p<0.05$). Using Tukey's post hoc test confirms the difference.

The values for epidermal thickness of the skin biopsies of the various formulations are shown in Table 45. Higher scores indicated significant infection of the dermis with attendance inflammation of the skin and tissue destruction. The untreated group with the value 74.07 ± 0.68 µm indicated significant infection (Fig.19). The values obtained for emulsion alone (63.10 ±0.82µm), 0.5% *Cassia alata* extract (65.36 ± 0.47 µm), 0.5% *Cassia alata* extract emulsion (63.27 ± 0.46 µm), 1% *Cassia alata* extract (64.06 ± 0.55 µm) and 1% *Cassia alata* emulsion (63.39 ± 0.22 µm) indicated that there was slight effect of these formulations on the epidermal thickness of the skin compared with the untreated control. In sharp contrast, there was significant efficacy of 2% *Cassia alata* extract (51.79 ± 0.59 µm) and 2% *Cassia alata* emulsion (51.41 ± 0.35 µm) compared with the values obtained for the standard drug (45.09 ± 0.34 µm). Data showed in Table 99 with Fig.65 (See Appendix) reveals that there is significance difference in epidermal thickness between groups as determined by one way anova ($F_{6,28}$=337.522, $p<0.05$).

A tukey post hoc test revealed that there is significance difference in epidermal thickness between the Control and the test groups.

4.12.2.3 *Trichophyton mentagrophytes*

There was marked inflammation of the skin in group 1. There was also moderate presence of fungal hyphae in the group. Few hair follicles and sebaceous glands were observed. Moderate tissue destruction was also observed in the group. No inflammation and tissue destruction was observed in group 2 (Table 46). No fungal hyphae were seen

Table 45. Effect of *Cassia alata* formulations on epidermal thickness keratinization (in µm) of skin infected by *Epidermophyton floccosum*

Gpr 1	71.54	74.00	74.59	75.55	74.68
Grp 2	44.57	44.22	44.86	45.89	45.89
Grp 3	65.02	63.11	60.16	64.11	63.08
Grp 4a	64.99	66.86	64.07	65.01	65.86
Grp 4b	62.11	64.89	63.45	63.11	62.77
Grp 5a	66.06	63.35	62.86	64.02	63.99
Grp 5b	64.11	63.04	62.85	63.46	63.48
Grp 6a	50.64	53.76	52.46	50.75	51.34
Grp 6b	51.06	51.04	50.54	52.52	51.89

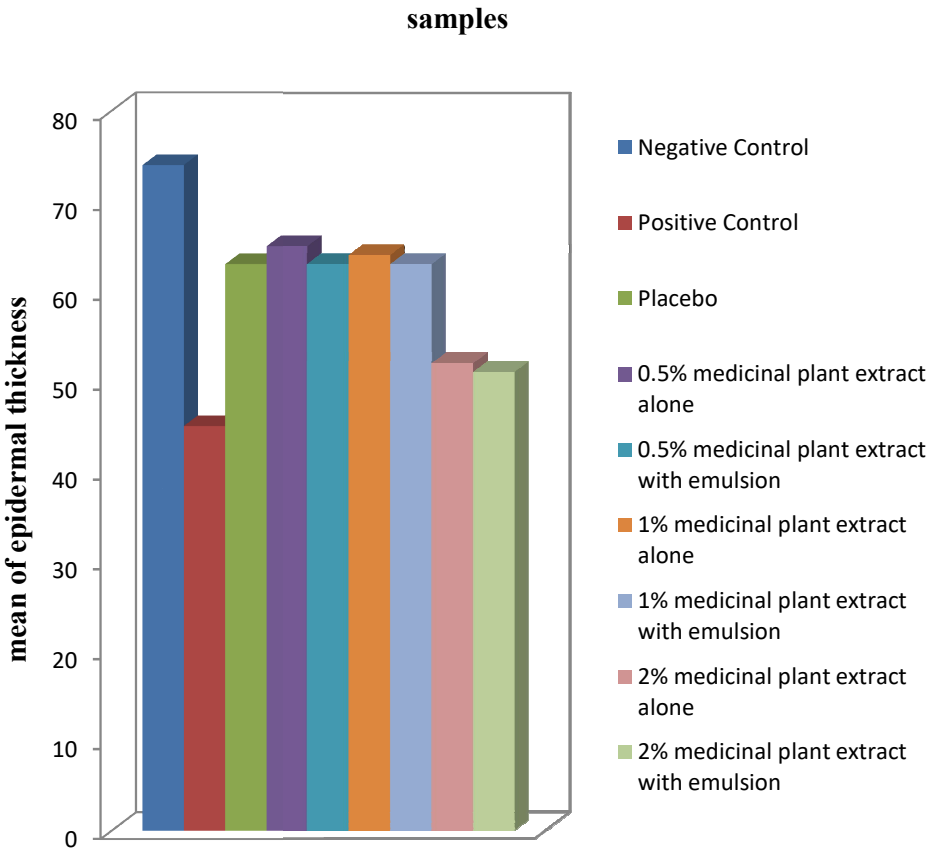


Fig.19 Effect of *Cassia alata* extracts on epidermal thickness in *Epidermophyton floccosum* infected skin

Table 46 Mycological efficacy of *Cassia alata* formulations against *Trichophyton mentagrophytes*

Group	Fungal hyphae	Hair follicles	Sebaceous gland	Inflammation	Continuity/Tissue destruction
1	++	+	+	+++	++
2	-	+++	+++	-	-
3	++	+	+	++	++
4a	++	+	+	++	++
4b	++	+	+	++	++
5a	++	+	+	++	++
5b	++	+	+	++	++
6a	+	++	++	+	+
6b	+	++	++	+	+

- = Absent, Mild = +, Moderate = ++, Marked = +++

in the group indicating that the drug was able to prevent the infection by the dermatophyte. There were numerous hair follicles in the dermis and also numerous amounts of sebaceous glands. Group 3 animals treated with emulsion alone had moderate skin inflammation and moderate tissue destruction of the skin (Plate 18). Moderate presence of fungal hyphae and few hair follicles seen. There were also few sebaceous glands in the dermis. The same observations were made for groups 4a, 4b, 5a and 5b. Inflammation and tissue destruction of the skin were however mild in group 6a (Plate 19). Same observations were also seen in group 6b. There was few presence of fungal hyphae in group 6a. Moderate amount of hair follicles enmeshed within the sparse connective tissue in the dermis were seen. There were also moderate amount of sebaceous glands in the dermis.

The efficacy of the formulations on the keratin layer of the hair compared with the infected untreated control is shown in Table 47. The untreated control with the value 20.11 ± 0.34 µm indicated heavy infection of the hairs by the dermatophyte. The standard drug 1% clotrimazole cream (52.31 ± 0.54 µm) demonstrated high mycological efficacy in preventing attack by the dermatophyte on the keratin layer of the hair (Fig.20) Same goes for the group treated with the emulsion alone (19.39 ± 0.49 µm), the group treated with 0.5% *Kigelia africana* extract alone (19.89 ± 0.43 µm) and the group treated with 0.5% *Kigelia africana* extract emulsion (20.54 ± 0.36 µm) which were not significantly different from one another. The values obtained for 1% *Kigelia africana* extract alone and 1% *Kigelia africana* emulsion indicated slight efficacy by the formulations. Comparatively, 2% *Kigelia africana* extract alone (31.05 ± 0.26 µm) and 2% *Kigelia africana* extract emulsion (30.57 ± 0.41 µm) showed moderate mycological efficacy. Statistically, there is significant difference in keratinization between the groups as determined by one way Anover ($F_{6,28}$=537.588, $p< 0.05$). Tukeys post hoc test was used in determining the significant differences when the Control is compared with other groups. (Table 100 and Fig.66) See Appendix.

There was significant infection (76.76 ± 0.48 µm) as indicated by the result obtained for the epidermal thickness of untreated group. The values obtained for the standard drug (45.59 ± 0.05 µm) indicated that the drug was able to reduce the epidermal thickness considerably. The values obtained for emulsion alone (67.62 ± 0.70

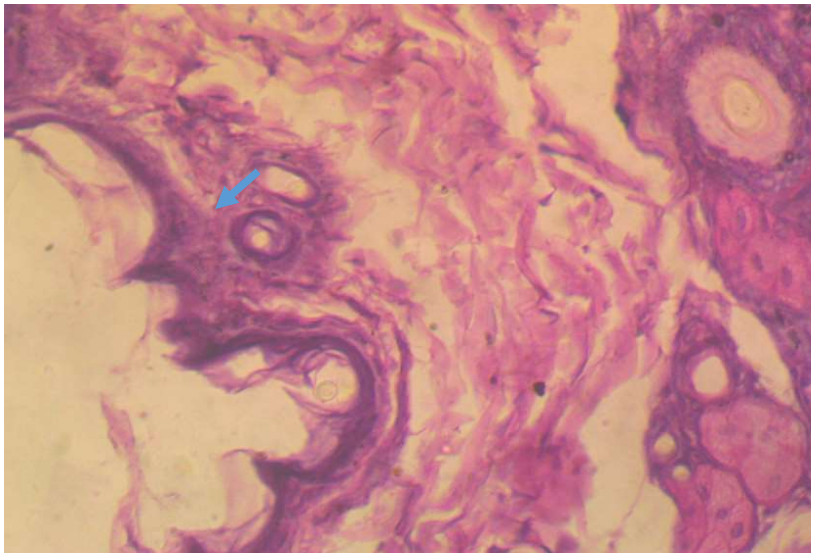


Plate 18. There is no observable lesion. HE x400

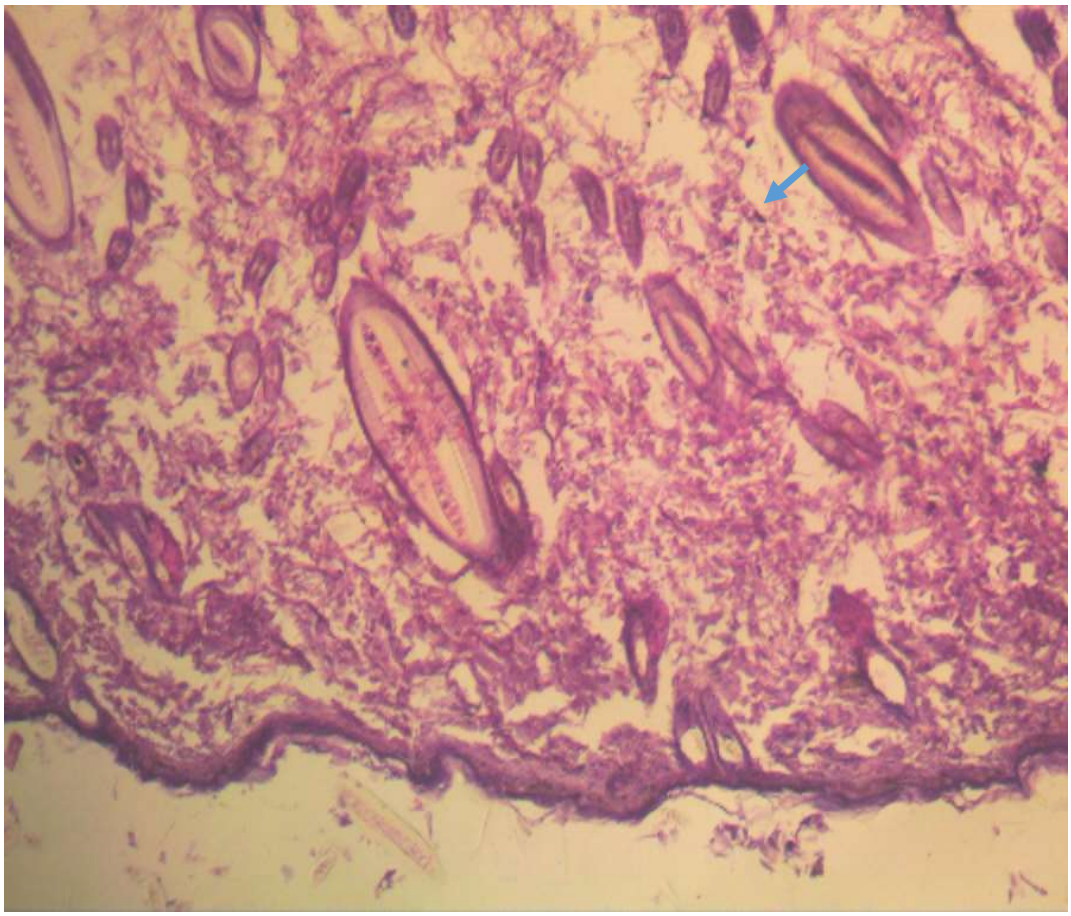


Plate 19. There is no observable lesion. HE x400

Table 47 Effect of *Cassia alata* formulations on keratinization (in µm) of skin infected by *Trichophyton mentagrophyte*

Group 1	19.61	19.43	20.29	21.32	19.92
Group 2	51.82	52.32	54.04	52.64	50.71
Group 3	18.57	20.31	17.89	19.86	20.34
Group 4a	20.93	20.24	19.64	18.36	20.29
Group 4b	19.86	21.75	20.75	20.56	19.78
Group 5a	24.89	25.04	23.96	25.78	23.67
Group 5b	23.24	25.89	24.48	25.64	21.65
Group 6a	31.77	30.69	30.44	31.58	30.75
Group 6b	30.87	31.77	30.24	29.24	30.73

Group 1 – Animals without any treatment – Negative control

Group 2 – Animals treated with cream containing 1% Clotrimazole (Positive Control - a).

Group 3 – Animals treated with placebo (Emulsion base only/Positive control - b)

Group 4a – Animals treated with 0.5% medicinal plant extract alone

Group 4b – Animals treated with emulsion containing 0.5% medicinal plant extract

Group 5a – Animals treated with 1% medicinal plant extract alone

Group 5b – Animals treated with emulsion containing 1% medicinal plant extract

Group 6a- Animals treated with 2% medicinal plant extract alone

Group 6b- Animals treated with emulsion containing 2% medicinal plant extract

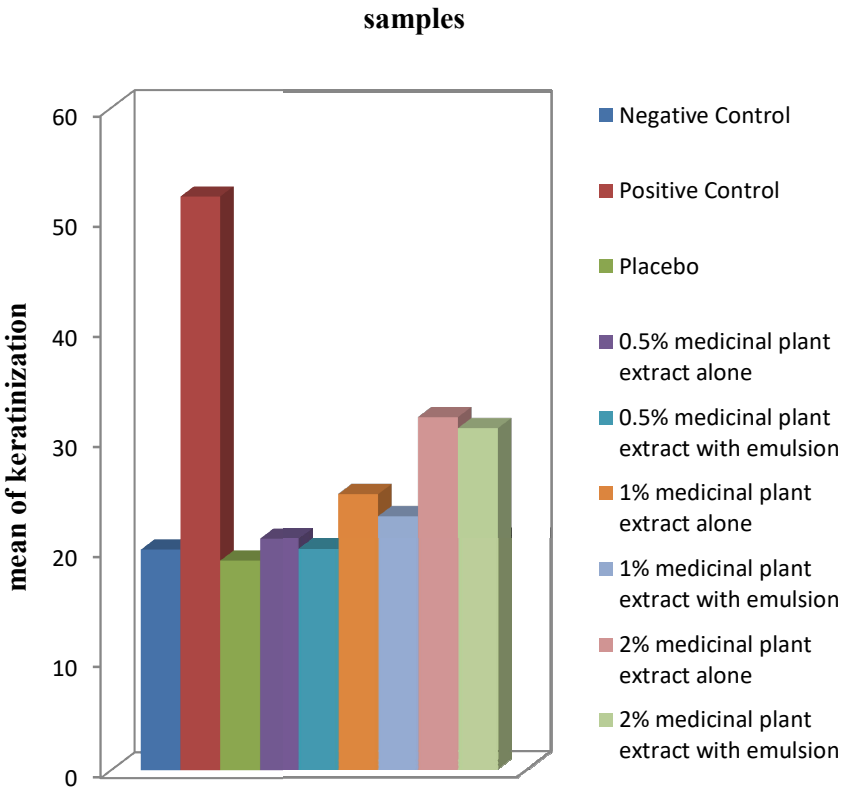


Fig.20 Effect of *Cassia alata* extracts on keratinization in *Trichophyton mentagrophyte* infected skin

μm), 0.5% *Kigelia africana* extract (61.14 ± 0.41 μm), 0.5% *Kigelia africana* extract emulsion (61.10 ± 0.37 μm), 1% *Kigelia africana* extract (62.09 ± 0.35 μm) and 1% *Kigelia africana* emulsion (61.72 ± 0.78 μm) in reducing the epidermal thickness cannot be said to be significant compared with the untreated control.

However, the values obtained for 2% *Kigelia africana* extract alone (57.58 ± 0.75 μm) and 2% *Kigelia africana* emulsion with extract (53.80 ± 0.58 μm) showed indication that there was moderate reduction in the epidermal thickness thereby suggesting that the formulations are mycologically effective against the dermatophyte (Table 48 and Fig.21).

The statistical evaluation as shown in Table 101 and Fig.67 (See Appendix) shows that there is significance difference in epidermal thickness between groups as determined by one way anova ($F_{6,28}$=84.638, $p<0.05$). A tukey post hoc test also revealed that there is significance difference in epidermal thickness between the Control and the test groups.

4.12.2.4 *Malassezia furfur*

Marked inflammation of the skin and foci of discontinuity of the epidermis were observed in group 1 which is the untreated group. This indicated successful infection. Marked tissue destruction was observed in the group compared to the other groups which were treated with various formulations (Plate 20).There was marked presence of fungal hyphae. There were no hair follicles which indicated that the dermatophyte is feeding on the follicles.Very few sebaceous glands were seen. In contrast, no inflammation and tissue destruction was observed in the group treated with 1% clotrimazole (group 2) which indicated that the drug was able to prevent the growth of the hyphae by the dermatophyte (Plate 21).

No fungal hyphae were seen in the group. There were also numerous hair follicles in the dermis and also abundant sebaceous glands (Table 49). In The group treated with emulsion alone (group 3), inflammation and tissue destruction of the skin were moderate in the group (Table 49). There was moderate presence of fungal hyphae which indicated that the cream alone has some medicinal effect on the dermatophyte

Table 48 Effect of *Cassia alata* formulations on epidermal thickness keratinization (in µm) of skin infected by *Trichophyton mentagrophyte*

Gpr 1	75.46	76.43	76.36	78.34	77.23
Grp 2	44.92	48.72	42.39	45.11	46.81
Grp 3	67.87	65.68	66.57	69.75	68.24
Grp 4a	62.38	60.84	60.39	61.78	60.31
Grp 4b	61.31	62.09	60.18	60.31	61.59
Grp 5a	62.90	62.81	61.11	62.09	61.54
Grp 5b	64.51	61.22	60.46	62.18	60.21
Grp 6a	58.37	60.02	57.33	55.72	56.44
Grp 6b	55.23	54.86	52.72	53.89	52.29

Group 1 – Animals without any treatment – Negative control

Group 2 – Animals treated with cream containing 1% Clotrimazole (Positive Control - a).

Group 3 – Animals treated with placebo (Emulsion base only/Positive control - b)

Group 4a – Animals treated with 0.5% medicinal plant extract alone

Group 4b – Animals treated with emulsion containing 0.5% medicinal plant extract

Group 5a – Animals treated with 1% medicinal plant extract alone

Group 5b – Animals treated with emulsion containing 1% medicinal plant extract

Group 6a- Animals treated with 2% medicinal plant extract alone

Group 6b- Animals treated with emulsion containing 2% medicinal plant extract

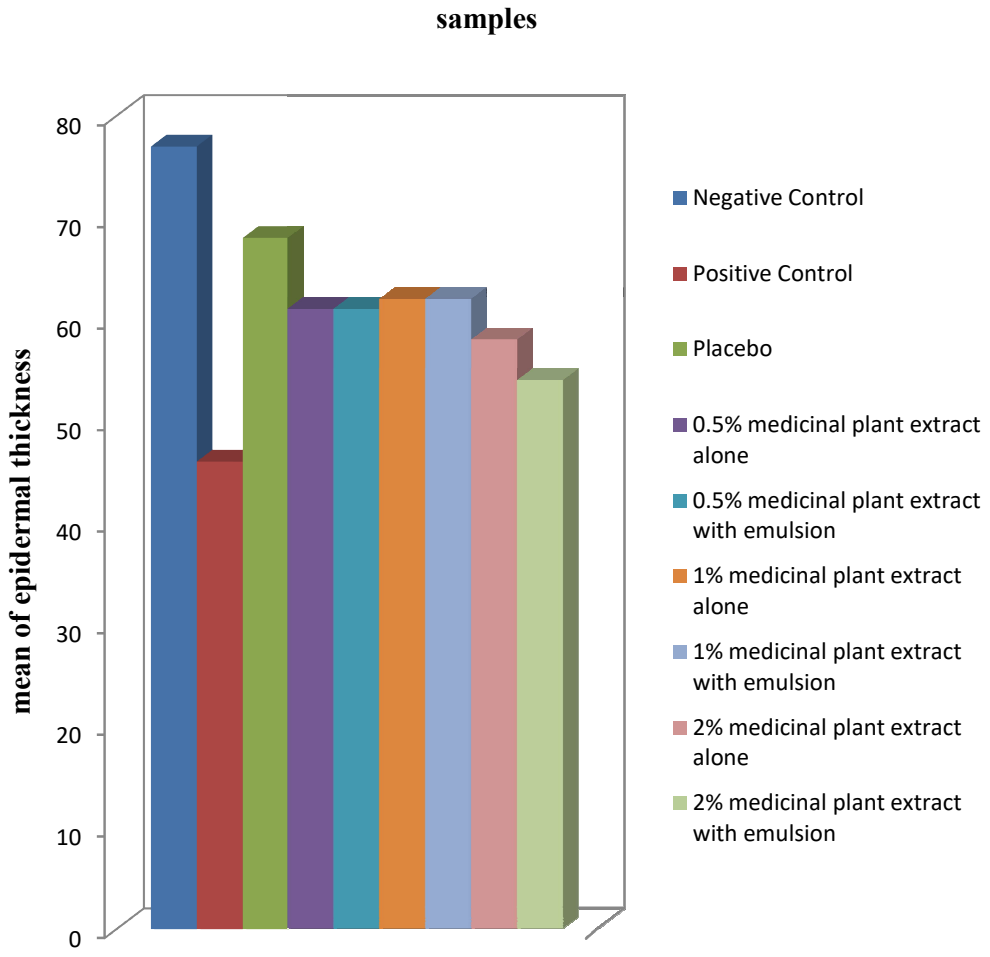


Fig.21 Effect of *Cassia alata* extracts on epidermal thickness in *Trichophyton mentagrophyte* infected skin

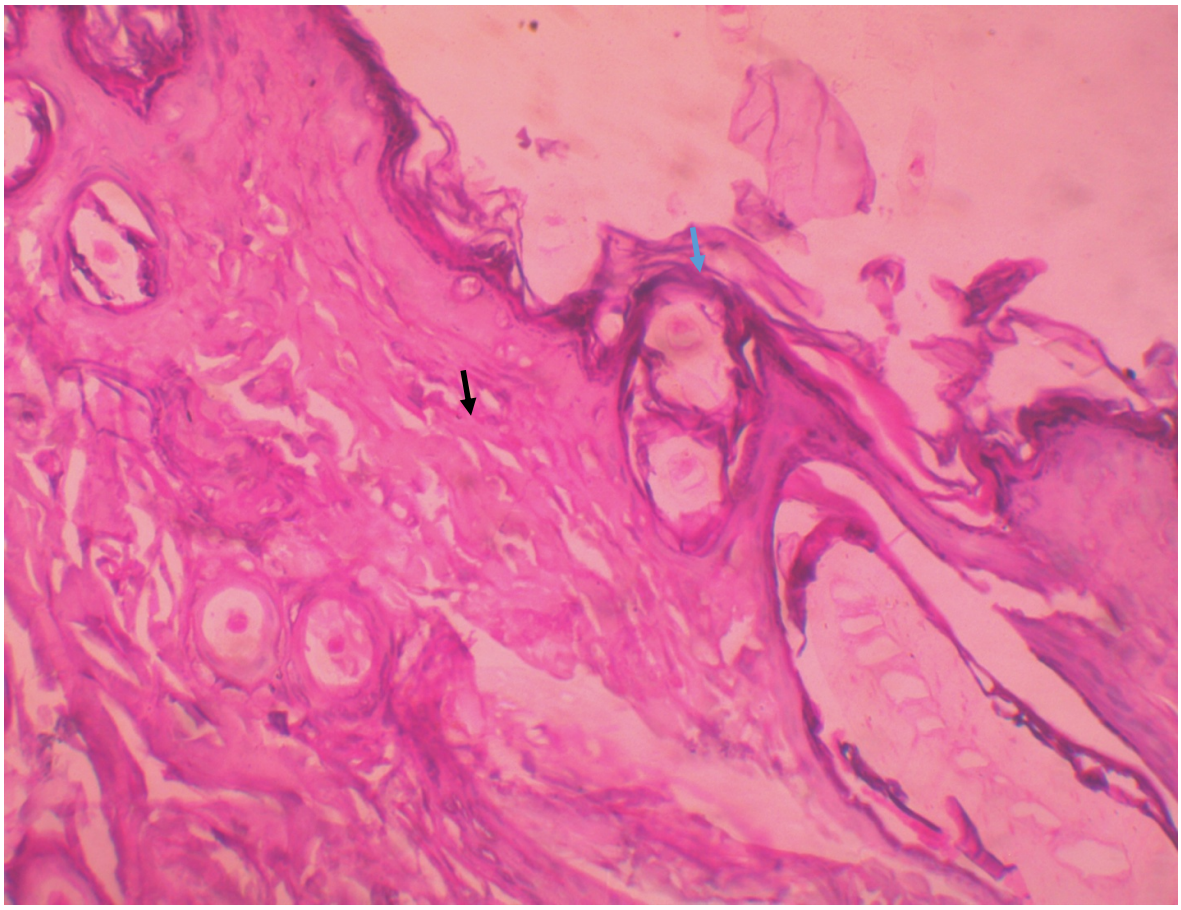


Plate 20. There is increased deposit of connective tissue (black arrow) in the dermis and keratinization of the epidermis (blue arrow). HE x400

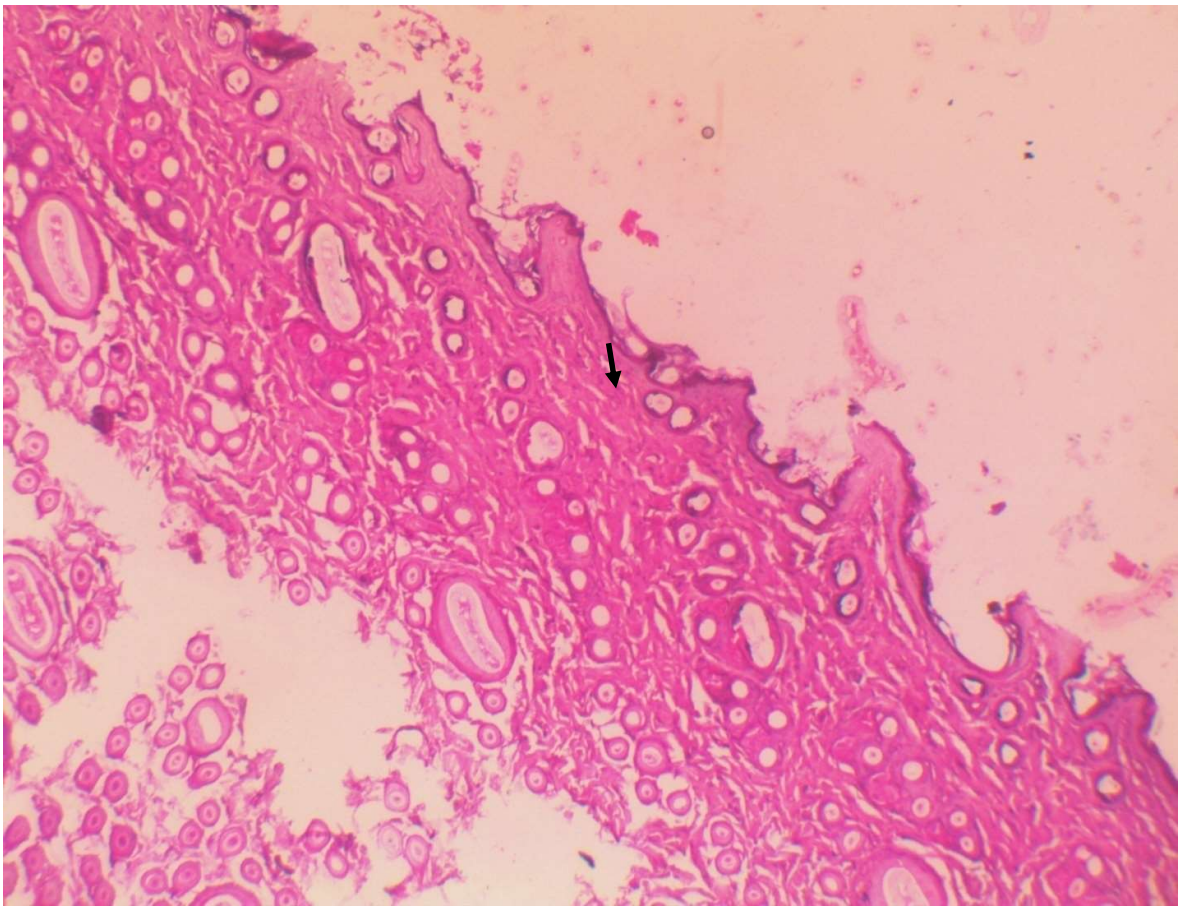


Plate 21. There is congestion of dermal capillaries (arrow). HE x400

Table 49 Mycological efficacy of *Cassia alata* ethanol formulations against *Malassezia furfur*

Group	Fungal hyphae	Hair follicles	Sebaceous gland	Inflammation	Continuity/Tissue destruction
1	+++	-	+	+++	+++
2	-	+++	+++	-	-
3	++	+	+	++	++
4a	++	++	+	++	++
4b	++	++	+	++	++
5a	+	++	++	+	+
5b	+	++	++	+	+
6a	-	++	++	+	+
6b	-	++	++	+	+

- = Absent, Mild = +, Moderate = ++, Marked = +++.

infection and this definitely results in the reduction of numbers of hair follicles as the dermatophye will attack freely. However the group also has few sebaceous glands in the dermis.

Inflammation and tissue destruction of the skin were moderate in group 4a (0.5% plant extract alone). Presence of fungal hyphae was moderate while there are moderate hair follicles enmeshed within the sparse connective tissue in the dermis.There are few sebaceous glands in the dermis.. Same observation was made for animals in group 4b (Table 49).

However, in groups 5a (1% plant extract alone) mild amount of fungal hyphae were seen with moderate amount of hair follicle and sebaceous gland. The inflammation and tissue destruction of the skin were mild (Table 49). Same observation were recorded for 5b (1% plant extract in emulsion).

No fungal hyphae were seen in group 6a (2% plant extract alone) which indicated that the extract was able to prevent the growth of the hyphae by the dermatophyte. There were moderate hair follicles in the dermis and also moderate amount of sebaceous glands. Mild inflammation of the skin and mild tissue destruction was however observed in the group.Same observation was made for group 6b.

Table.50 and Fig.22 show comparative effectiveness of the formulations on keratinization compared with the infected untreated control. Higher scores indicated improved efficacy compared with the untreated control. Significant efficacy was demonstrated by 1% clotrimazole cream (standard drug), 2% *Cassia alata* extract alone and 2% plant extract emulsion. 1% *Cassia alata* extract alone and 1% *Cassia alata* extract emulsion showed slight efficacy, but not as effective as the 2% formulations. Emulsion alone (placebo), 0.5% *Cassia alata* extract and 0.5% *Cassia alata* extract emulsion cannot be said to be mycologically effective compared to the values obtained for the other formulations. Table 102 with Fig.68 (See Appendix) shows that there is statistical difference in keratinization between the groups as determined by one way Anover ($F_{6,28}$ =51.722 , $p<0.05$).

Table 50 Effect of *Cassia alata* formulations on keratinization (in µm) of skin infected by *Malassezia furfur*

Group 1	19.36	19.32	19.82	18.65	16.89
Group 2	38.41	38.11	34.68	35.45	40.35
Group 3	20.33	20.47	18.59	19.46	20.57
Group 4a	23.51	24.05	22.32	20.11	20.88
Group 4b	24.35	24.06	27.64	25.86	24.33
Group 5a	26.41	27.30	24.06	27.65	25.69
Group 5b	28.50	27.62	29.39	28.71	30.11
Group 6a	31.32	31.29	28.76	30.39	29.86
Group 6b	30.73	33.55	32.68	29.89	31.78

Group 1 – Animals without any treatment – Negative control

Group 2 – Animals treated with cream containing 1% Clotrimazole (Positive Control - a).

Group 3 – Animals treated with placebo (Emulsion base only/Positive control - b)

Group 4a – Animals treated with 0.5% medicinal plant extract alone

Group 4b – Animals treated with emulsion containing 0.5% medicinal plant extract

Group 5a – Animals treated with 1% medicinal plant extract alone

Group 5b – Animals treated with emulsion containing 1% medicinal plant extract

Group 6a- Animals treated with 2% medicinal plant extract alone

Group 6b- Animals treated with emulsion containing 2% medicinal plant extract

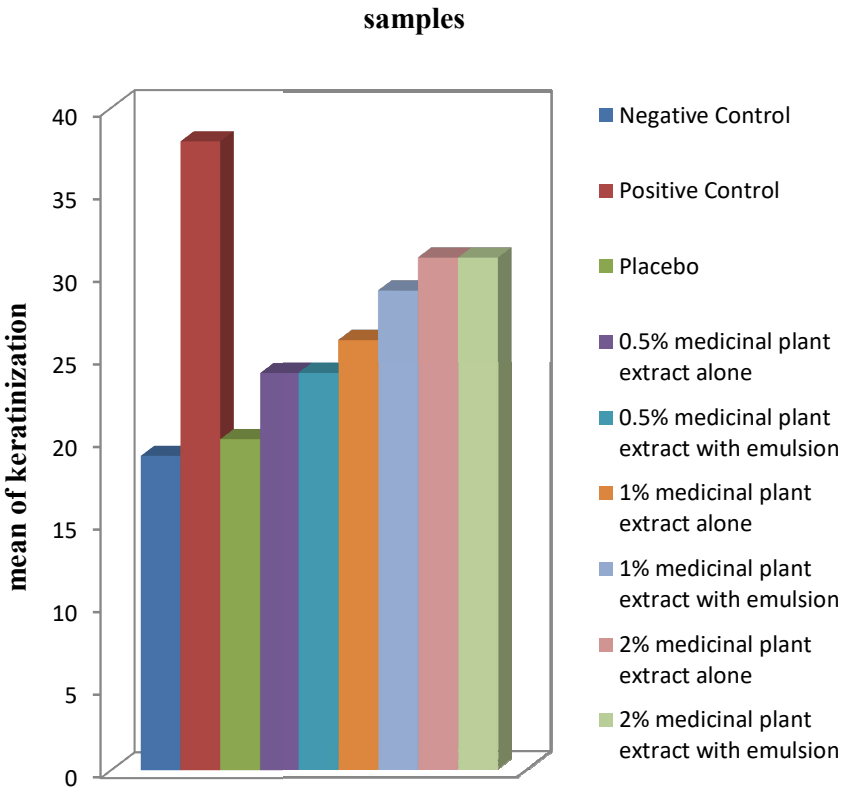


Fig.22 Effect of *Cassia alata* extracts on keratinization in *Malassezia furfur* infected skin

Tukeys post hoc test shows significant differences when the Control is compared with other groups.

The values for epidermal thickness of the skin biopsies of the various formulations are shown in Table 51. Higher scores indicated significant infection of the dermis with attendance inflammation of the skin and tissue destruction. The untreated group with the value 76.76 ± 0.48 μm indicated significant infection. The values obtained for emulsion alone (67.62 ± 0.70 μm) was not statistically different from the untreated group. However, the efficacy of 0.5% *Cassia alata* extract, 0.5% *Cassia alata* extract emulsion, 1% *Cassia alata* extract and 1% Cassia alata emulsion in reducing the epidermal thickness is not significant compared with the untreated control. However, there was moderate efficacy of 2% *Kigelia africana* extract and 2% *Kigelia africana* emulsion compared with the values obtained for the standard drug (Fig.23).

Data showed in Table 103 with Fig.69 (See Appendix) reveals that there is significance difference in epidermal thickness between groups as determined by one way anova ($F_{6,28}$=2.834, $p<0.05$). A tukey post hoc test revealed that there is significance difference in epidermal thickness between the control and the test groups.

The formulated emulsion alone exhibited little or no mycological efficacy in the treatment of dermatophytosis but can only be used as a vehicle to carry the drug to the action site. It was also observed that the drug release rate of the formulated emulsion with 2% *Cassia alata* extract is moderately as effective as the standard drug (1% clotrimazole).

4.12.3 Effect of *Mitracarpus villosus* on dermatophytes

4.12.3.1 *Microsporium aoudini*

There was moderate inflammation of the skin with marked tissue destruction (Table 52) in the untreated group (group 1) indicating that there was infection by the dermatophyte. There was moderate presence of fungal hyphae, and there were no hair follicles. Few sebaceous glands were also seen. No inflammation and tissue destruction was observed in the group treated with 1% clotrimazole (group 2). There was no fungal hyphae were.

There were numerous hair follicles in the dermis and also moderate amount of sebaceous glands (Plate 22).

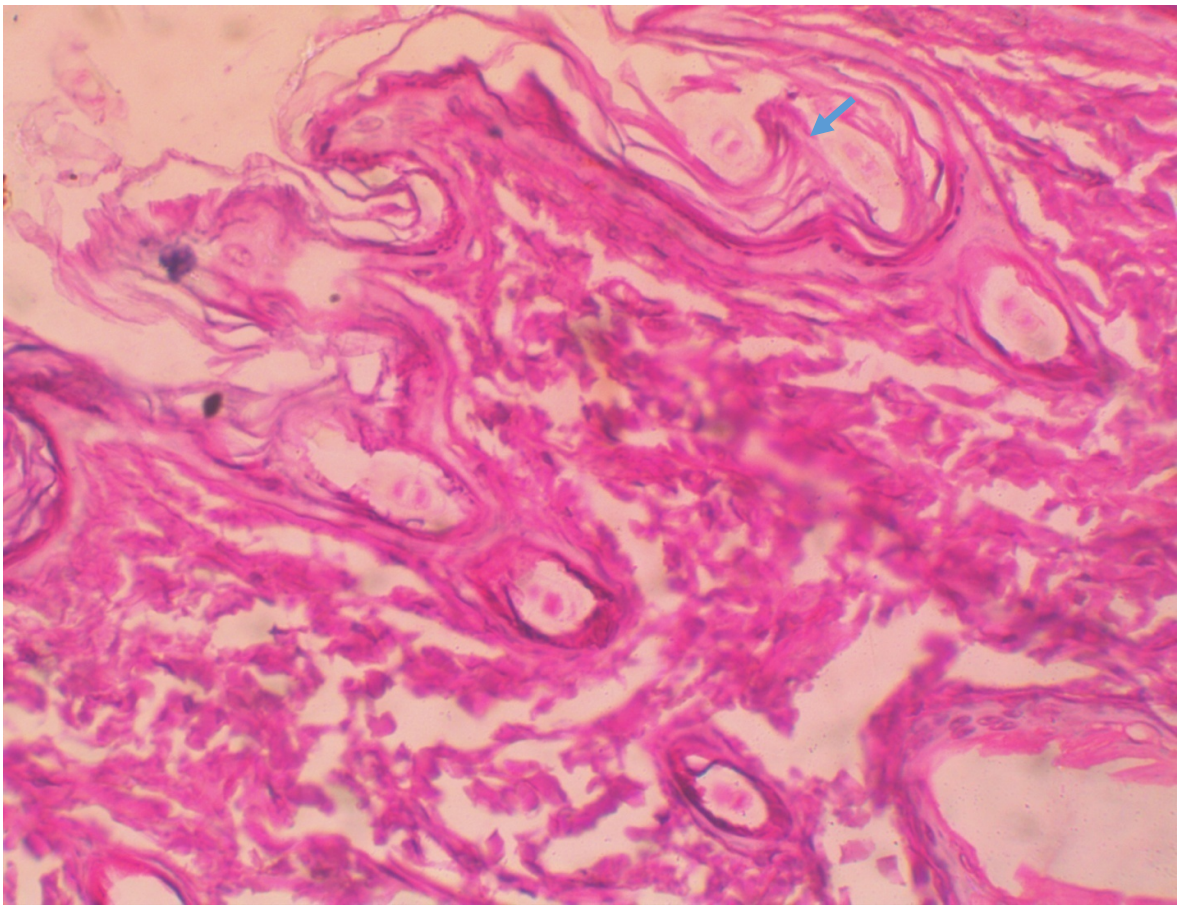


Plate 22 There is severe thickeneing of the epidermis. HE x100.

Table 51 Effect of *Cassia alata* formulations on epidermal thickness (in µm) of skin infected by *Malassezia furfur*

Gpr 1	53.45	56.43	56.83	58.54	57.55
Grp 2	48.32	49.69	50.19	51.67	49.82
Grp 3	54.57	55.46	55.39	58.55	58.89
Grp 4a	53.46	51.44	51.79	51.62	51.51
Grp 4b	50.67	52.29	54.68	51.33	52.62
Grp 5a	51.39	51.41	51.11	50.34	51.35
Grp 5b	52.54	51.19	51.33	51.25	51.35
Grp 6a	52.35	50.39	51.36	50.31	49.45
Grp 6b	51.38	52.72	52.80	56.36	50.42

Group 1 – Animals without any treatment – Negative control

Group 2 – Animals treated with cream containing 1% Clotrimazole (Positive Control - a).

Group 3 – Animals treated with placebo (Emulsion base only/Positive control - b)

Group 4a – Animals treated with 0.5% medicinal plant extract alone

Group 4b – Animals treated with emulsion containing 0.5% medicinal plant extract

Group 5a – Animals treated with 1% medicinal plant extract alone

Group 5b – Animals treated with emulsion containing 1% medicinal plant extract

Group 6a- Animals treated with 2% medicinal plant extract alone

Group 6b- Animals treated with emulsion containing 2% medicinal plant extract

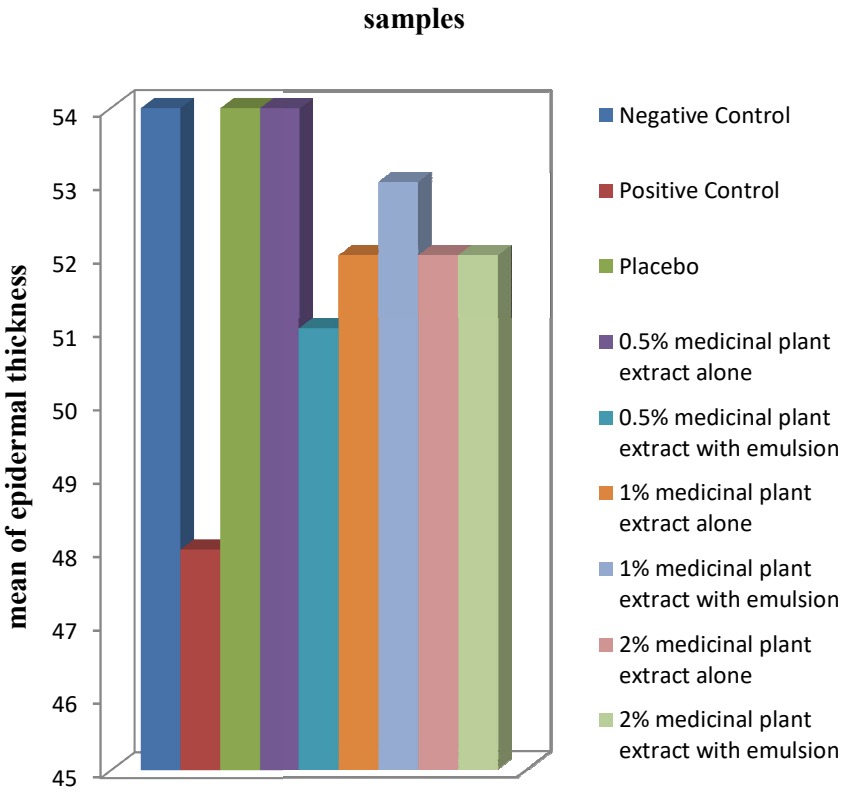


Fig.23 Effect of *Cassia alata* extracts on epidermal thickness in *Malassezia furfur* infected skin

Table 52 Mycological efficacy of *Mitracarpus villosus* ethanol formulations against *Microsporium aoudini*

Group	Fungal hyphae	Hair follicles	Sebaceous gland	Inflammation	Continuity/Tissue destruction
1	++	-	+	++	+++
2	-	+++	++	-	-
3	++	+	+	++	+++
4a	++	+	+	++	++
4b	++	+	+	++	++
5a	+	++	++	++	++
5b	+	++	++	++	++
6a	+	++	++	+	+
6b	-	++	++	+	+

- = Absent, Mild = +, Moderate = ++, Marked = +++

In group 3, there was moderate presence of fungal hyphae with few presence of hair follicle. The group also has few sebaceous glands in the dermis. Inflammation and tissue destruction of the skin were moderate in the group (Table 52).

In group 4a (0.5% plant extract alone), presence of fungal hyphae was moderate while there are moderate hair follicles enmeshed within the sparse connective tissue in the dermis. There are few sebaceous glands in the dermis. Inflammation and tissue destruction of the skin were also moderate in the group. Same observation was made for animals in group 4b (Plate 23).

However, in group 5a (1% plant extract alone) mild amount of fungal hyphae were seen with moderate amount of hair follicle and sebaceous gland. The inflammation and tissue destruction of the skin were moderate (Plate 24). Same observation were recorded for 5b (1% plant extract in emulsion).

Few fungal hyphae were seen in group 6a (2% plant extract alone). There were moderate hair follicles in the dermis and also moderate amount of sebaceous glands. Mild inflammation of the skin and mild tissue destruction was however observed in the group. Same observation was made for group 6b except that there were no hair fungal hyphae in the dermis. The efficacy of the formulations on the keratin layer of the hair compared with the infected untreated control is shown in Table 53 and Fig.24.

The untreated control with the value 16.02 ± 0.26 µm indicated that the hairs were heavily infected with the dermatophyte. The group treated with the emulsion alone (18.77 ± 0.24 µm) showed that there was slight efficacy by the emulsion. The effectiveness of 1% clotrimazole cream (52.47 ± 1.16 µm) in preventing attack by the dermatophyte on the keratin layer of the hair is demonstrated by the high value recorded. Application of 0.5% *Mitracarpus villosus* extract emulsion (26.80 ± 0.77 µm) and 0.5% *Mitracarpus villosus* extract alone (27.93 ± 0.65 µm) on the infected skin of the animal showed difference from the untreated group. This implies that they had moderate effect in curtailing the infection caused by the dermatophyte. The efficacy of the formulations increased as the concentration increases as can been seen in the group treated with 1% ($F_{6,28}$=180.055, $p<0.05$) extract alone (29.76 ± 0.45 µm), 1% *Mitracarpus villosus* emulsion (30.56 ± 0.35 µm), 2% *Mitracarpus villosus* extract

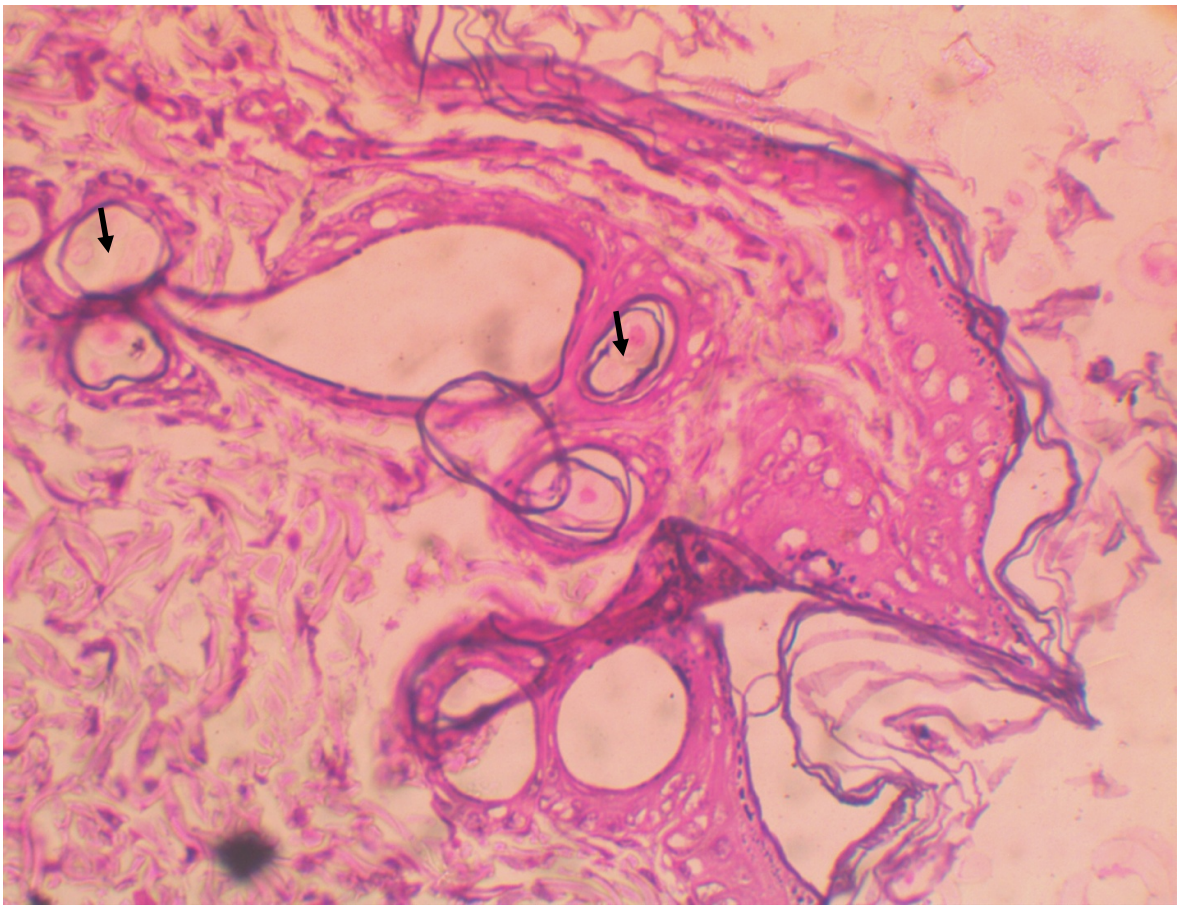


Plate 23 Follicular proliferation(arrows) HE x400)

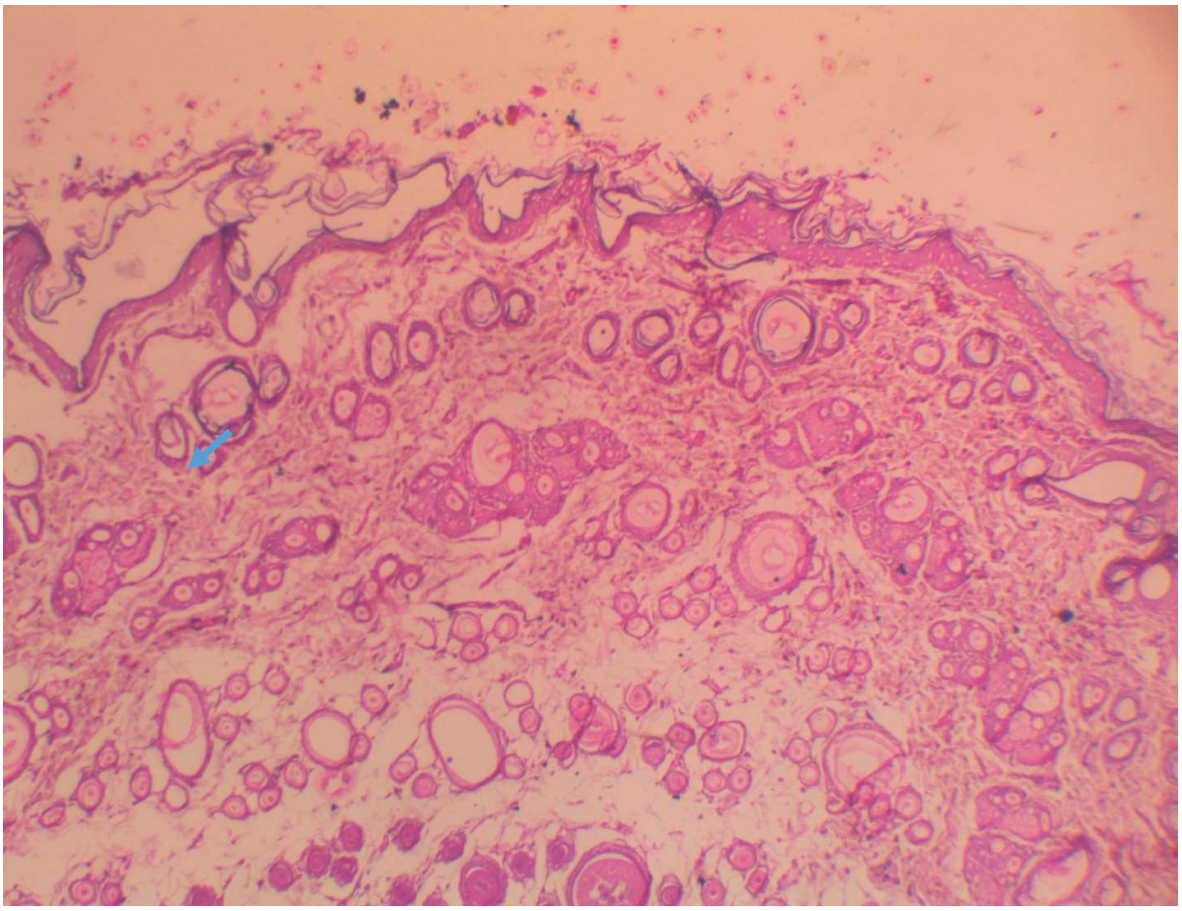


Plate 24 Inflammation of the skin tissues. HE x100.

Table 53 Effect of *Mitracarpus villosus* formulations on keratinization (in µm) of skin infected by *Microsporium aoudini*

Group 1	16.56	15.53	15.26	16.27	16.48
Group 2	51.32	51.14	52.67	56.87	50.35
Group 3	19.08	18.91	18.72	17.89	19.24
Group 4a	25.56	27.75	29.12	28.13	29.08
Group 4b	25.02	25.11	28.22	26.95	28.72
Group 5a	28.12	30.65	30.19	29.49	30.33
Group 5b	30.65	29.46	30.34	30.72	31.65
Group 6a	34.11	35.82	34.82	36.49	35.78
Group 6b	33.97	34.89	35.43	33.79	35.56

Group 1 – Animals without any treatment – Negative control

Group 2 – Animals treated with cream containing 1% Clotrimazole (Positive Control - a).

Group 3 – Animals treated with placebo (Emulsion base only/Positive control - b)

Group 4a – Animals treated with 0.5% medicinal plant extract alone

Group 4b – Animals treated with emulsion containing 0.5% medicinal plant extract

Group 5a – Animals treated with 1% medicinal plant extract alone

Group 5b – Animals treated with emulsion containing 1% medicinal plant extract

Group 6a- Animals treated with 2% medicinal plant extract alone

Group 6b- Animals treated with emulsion containing 2% medicinal plant extract

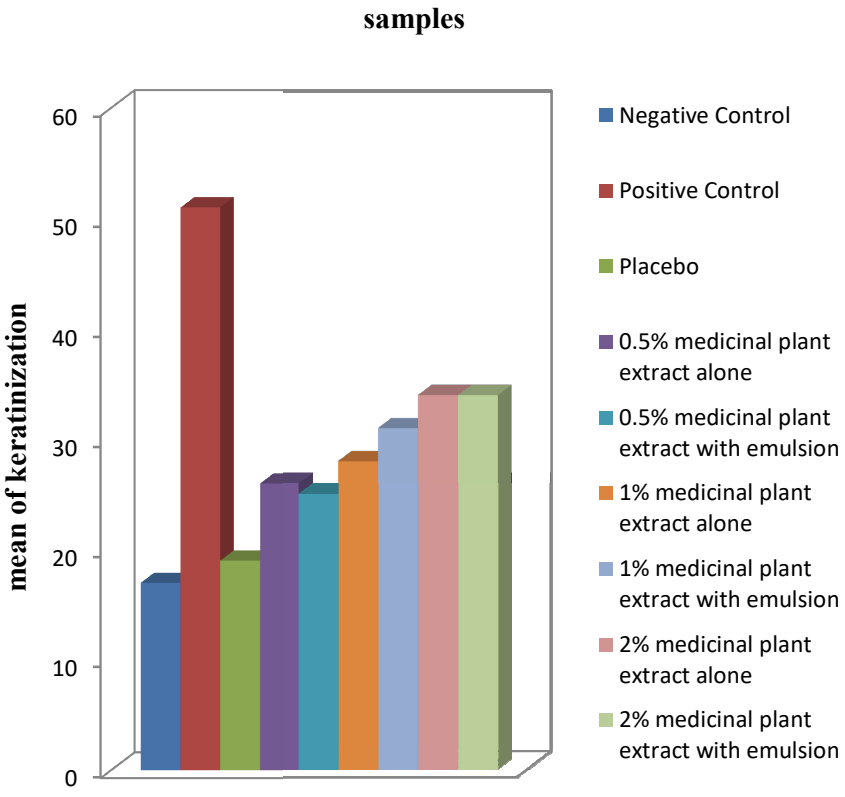


Fig.24 Effect of *Mitracarpus villosus* extracts on keratinization in *Microsporium aoudinin* infected skin

alone (35.40 ± 0.42 µm) and 2% *Mitracarpus villosus* extract emulsion (34.73 ± 0.37 µm).

Statistically, there is significant difference in keratinization between the groups as determined by one way Anova ($F_{6,28}$ =180.055,p<0.05) Using Tukey's post hoc test confirms the difference. (Table 104 and Fig.70) See Appendix.

Table 54 and Fig.25 showed the results and graphical comparison of values obtained for the epidermal thickness of the animals in the groups. The untreated group indicated significant infection (83.19 ± 0.79 µm). The standard drug (38.03 ± 0.67 µm) showed good efficacy by reducing the epidermal thickness considerably compared to the other formulations.

The value obtained for emulsion alone (81.15 ± 0.15 µm) indicated that the emulsion had little effect on the epidermal thickness of the animals which was not significant from the untreated group. The efficacy demonstrated by 0.5% *Mitracarpus villosus* extract (81.82 ± 0.77µm), 0.5% *Mitracarpus villosus* extract emulsion (82.26 ± 0.83µm), 1% *Mitracarpus villosus* (82.59 ± 0.81 µm) and 1% *Mitracarpus villosus* emulsion (82.70 ± 0.82 µm) in reducing the epidermal thickness can be said to be insignificant compared with the untreated control. However, the values obtained for 2% *Mitracarpus villosus* (72.41 ± 0.93µm) and 2% *Mitracarpus villosus* emulsion (69.43 ± 0.44 µm) showed moderate effectiveness in treating the infection.

The statistical evaluation as shown in Tables 105 and Fig.71 shows that there is significance difference in epidermal thickness between groups as shown by one way Anova ($F_{6,28}$=450.625, p<0.05). Using Tukey's post hoc test, there is significant difference between Positive Control and the test groups.

4.12.3.2 E*pidermophyton floccosum*

Marked inflammation of the skin and foci of discontinuity of the epidermis were observed in group 1 which further indicated successful infection (Plate 25). There was marked presence of fungal hyphae in the group. There were no hair follicles seen, and few sebaceous glands were found. Marked tissue destruction was also observed in the group (Table 55).

Table 54 Effect of *Mitracarpus villosus* formulations on epidermal thickness (in µm) of skin infected by *Microsporium aoudini*

Gpr 1	84.54	81.86	84.38	80.75	84.43
Grp 2	38.11	38.13	36.09	37.53	40.28
Grp 3	79.02	80.58	82.41	82.93	80.81
Grp 4a	80.29	83.57	80.19	83.72	81.35
Grp 4b	82.49	80.58	84.85	83.04	80.35
Grp 5a	81.62	85.19	80.76	81.71	83.69
Grp 5b	80.19	84.39	84.26	81.56	83.11
Grp 6a	70.37	71.46	75.73	71.47	73.03
Grp 6b	69.81	67.83	69.35	69.74	70.44

Group 1 – Animals without any treatment – Negative control

Group 2 – Animals treated with cream containing 1% Clotrimazole (Positive Control - a).

Group 3 – Animals treated with placebo (Emulsion base only/Positive control - b)

Group 4a – Animals treated with 0.5% medicinal plant extract alone

Group 4b – Animals treated with emulsion containing 0.5% medicinal plant extract

Group 5a – Animals treated with 1% medicinal plant extract alone

Group 5b – Animals treated with emulsion containing 1% medicinal plant extract

Group 6a- Animals treated with 2% medicinal plant extract alone

Group 6b- Animals treated with emulsion containing 2% medicinal plant extract

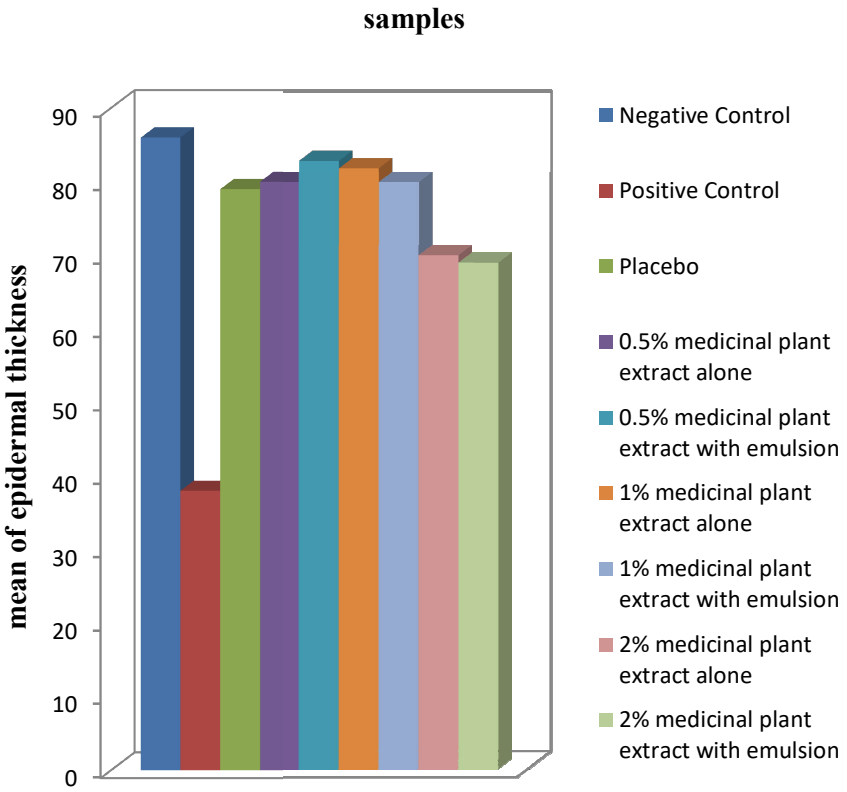


Fig.25 Effect of *Mitracarpus villosus* extracts on epidermal thickness in *Microsporium aoudini* infected skin

Table 55 Mycological efficacy of *Mitracarpus villosus* ethanol formulations against *Epidermophyton floccosum*

Group	Fungal hyphae	Hair follicles	Sebaceous gland	Inflammation	Continuity/Tissue destruction
1	+++	-	+	+++	+++
2	-	+++	+++	-	-
3	+++	+	+	++	+++
4a	++	+	+	++	+++
4b	++	+	+	++	+++
5a	++	++	+	++	+++
5b	+	++	++	++	++
6a	+	++	++	+	+
6b	+	++	++	+	+

- = Absent, Mild = +, Moderate = ++, Marked = +++

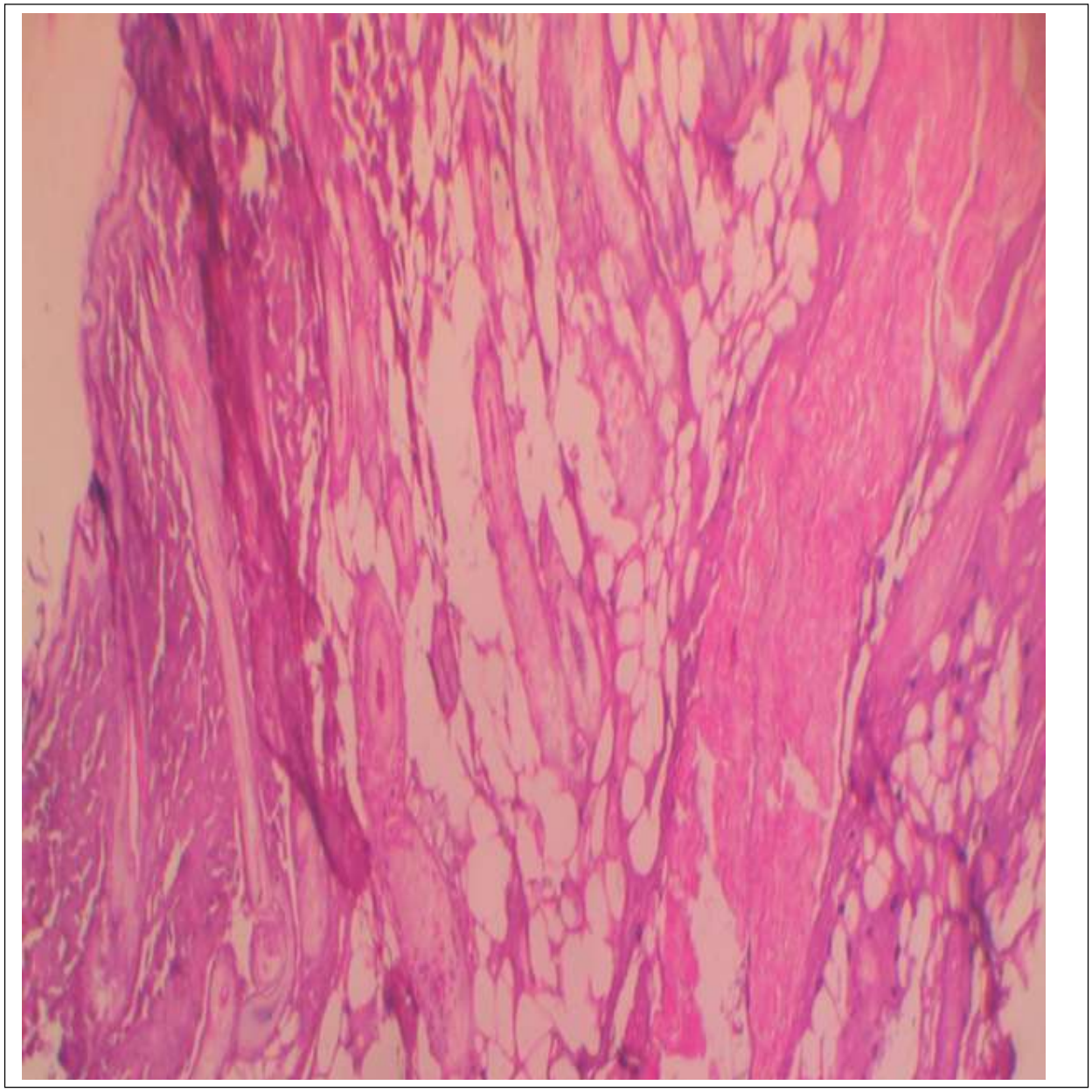


Plate 25. There is observable lesion. HE stain x100

No inflammation and tissue destruction was observed in group 2 (Table 55). There was absence of fungal hyphae indicating that the drug was able to prevent the infection by the dermatophyte. There were numerous hair follicles in the dermis and also numerous amounts of sebaceous glands.

Group 3 animals treated with emulsion alone had marked presence of fungal. There were few hair follicles and sebaceous glands in the dermis. Skin inflammation was moderate while tissue destruction of the skin was marked in the group (Plate 26). There was moderate presence of fungal hyphae in group 4a. Few amount of hair follicles enmeshed within the sparse connective tissue in the dermis were seen. There were also few sebaceous glands in the dermis.

Inflammation of the skin was moderate but tissue destruction of the skin were marked (Table 55). Same observations made for group 4a were also seen in group 4b. Moderate amount of fungal hyphae were seen in group 5a (1% plant extract alone) with moderate amount of hair follicle and few sebaceous glands. Skin inflammation was moderate while tissue destruction of the skin were marked. In group 5b, there were few fungal hyphae with moderate hair follicles and moderate sebaceous gland. Skin inflammation and tissue distruction were moderate.

Few fungal hyphae were seen in group 6a (2% plant extract alone). There were moderate hair follicles in the dermis and also moderate amount of sebaceous glands. However, mild inflammation of the skin and tissue destruction was seen. Same observation was made for group 6b (2% plant extract in emulsion).

The efficacy of the formulations on the keratin layer of the hair compared with the infected untreated control is shown in Table 56 and Fig.26. There was heavy infection of the hair by the dermatophyte as indicated by the value obtained (23.91 ± 0.65 µm). The group treated with the emulsion alone (23.10 ± 1.01 µm) showed that the emulsion had no efficacy whatsoever on the dermatophyte. Application of 0.5% *Mitracarpus villosus* extract emulsion (29.86 ± 0.38 µm) and 0.5% *Mitracarpus villosus* extract alone (29.56 ± 0.46 µm) on the infected skin of the animal showed slight difference from the values obtained for the untreated group. This implies that they had slight effect in curtailing the infection caused by the dermatophyte.

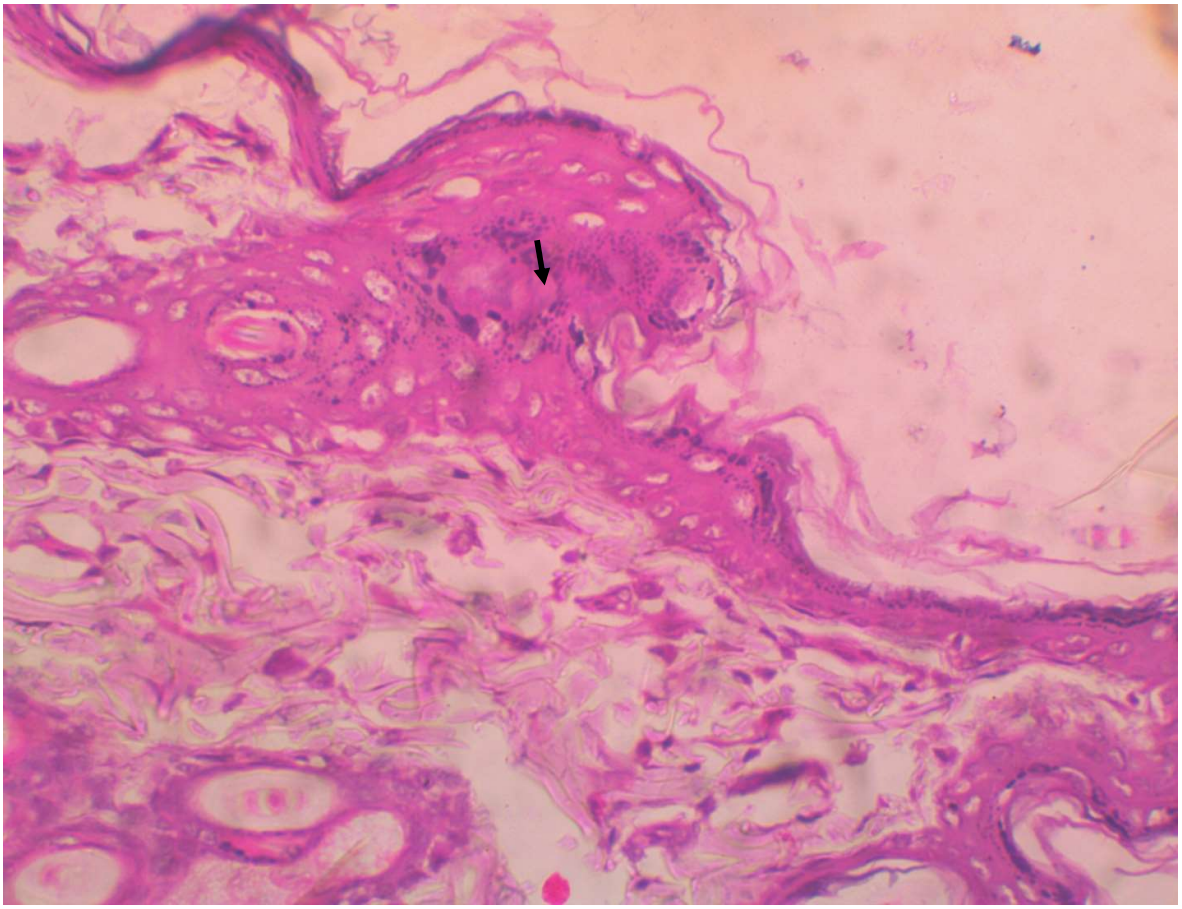


Plate 26 A sustained increase in epidermal thickness(arrow). HE x100

Table 56 Effect of *Mitracarpus villosus* formulations on keratinisation (in µm) of skin infected by *Epidermophyton floccosum*

Group 1	22.32	24.33	22.45	25.53	24.94
Group 2	48.56	50.65	49.76	50.42	49.32
Group 3	21.46	20.86	24.33	22.47	26.40
Group 4a	28.96	28.51	30.75	29.08	29.49
Group 4b	27.87	29.62	29.89	30.66	29.75
Group 5a	30.86	29.46	30.71	31.62	31.75
Group 5b	29.33	30.31	30.40	31.54	31.39
Group 6a	33.68	33.72	31.49	35.11	33.75
Group 6b	34.28	32.42	35.71	33.63	34.46

Group 1 – Animals without any treatment – Negative control

Group 2 – Animals treated with cream containing 1% Clotrimazole (Positive Control - a).

Group 3 – Animals treated with placebo (Emulsion base only/Positive control - b)

Group 4a – Animals treated with 0.5% medicinal plant extract alone

Group 4b – Animals treated with emulsion containing 0.5% medicinal plant extract

Group 5a – Animals treated with 1% medicinal plant extract alone

Group 5b – Animals treated with emulsion containing 1% medicinal plant extract

Group 6a- Animals treated with 2% medicinal plant extract alone

Group 6b- Animals treated with emulsion containing 2% medicinal plant extract

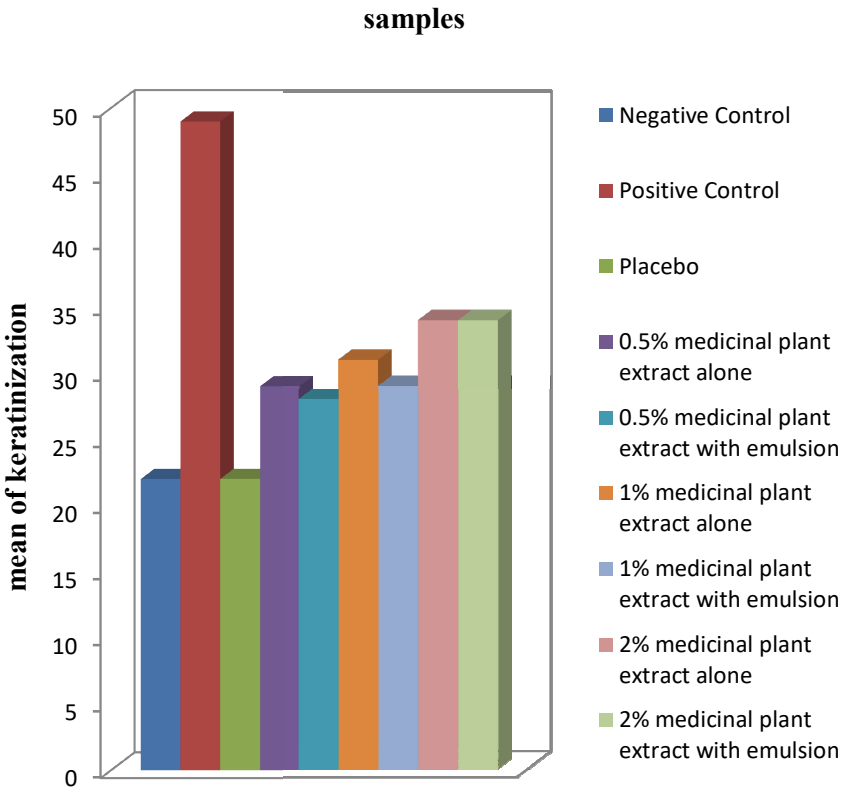


Fig.26 Effect of *Mitracarpus villosus* extracts on keratinization in *Epidermophyton floccosum* infected skin

The trend of activities increasing with concentration increase can been seen in the group treated with 1% extract alone (30.88 ± 0.41 µm), 1% *Mitracarpus villosus* emulsion (30.59 ± 0.40 µm), 2% *Mitracarpus villosus* extract alone (35.55 ± 0.58 µm) and 2% *Mitracarpus villosus* extract emulsion (34.10 ± 0.53 µm). The effectiveness of 1% clotrimazole cream (49.74 ± 0.38 µm) in preventing attack by the dermatophyte on the keratin layer of the hair is demonstrated by the high value recorded.

Statistically, there is significant difference in keratinization between the groups as determined by one way Anova ($F_{6,28}$=248.738, $p<0.05$) Using Tukey's post hoc test confirms the difference (Table 106) and (Fig.72) See Appendix.

The results obtained for the epidermal thickness of untreated group indicated significant infection (82.16 ± 1.04 µm). The value obtained for emulsion alone (73.13 ± 0.41 µm) indicated that the emulsion had slight effect on the epidermal thickness of the animals. The efficacy demonstrated by 0.5% *Mitracarpus villosus* extract (72.76 ± 0.86 µm), 0.5% *Mitracarpus villosus* extract emulsion (72.27 ± 0.75 µm), 1% *Mitracarpus villosus* (71.44 ± 0.91 µm) and 1% *Mitracarpus villosus* emulsion (71.23 ± 0.89 µm) in reducing the epidermal thickness can be said to be slightly significant

compared with the untreated control. However, the values obtained for 2% *Mitracarpus villosus* (62.89 ± 0.65 µm) and 2% *Mitracarpus villosus* emulsion (60.89 ± 0.65 µm) showed moderate effectiveness in treating the infection compared with the values obtained for the standard drug (43.69 ± 0.45 µm) (Table 57 and Fig.27). The statistical evaluation as shown in Table 107 and Fig.73 (See Appendix). shows that there is significance difference in epidermal thickness between groups as shown by one way Anova ($F_{6,28}$=177.781, $p<0.05$). Using Tukey's post hoc test, there is significant difference between Positive Control and the test groups.

4.12.3.3 *Trichophyton mentagrophytes*

There was moderate inflammation of the skin and marked tissue destruction observed in the untreated group 1. There was marked presence of fungal hyphae. There were no hair follicles in the dermis which indicated that the dermatophyte fed on the follicles. Few sebaceous glands were also seen. In contrast, No inflammation and tissue destruction was observed in group 2 treated with 1% clotrimazole. No fungal hyphae were seen

Table 57 Effect of *Mitracarpus villosus* formulations on epidermal thickness (in µm) of skin infected by *Epidermophyton floccosum*

Gpr 1	80.09	79.33	84.53	82.76	84.08
Grp 2	44.19	44.54	41.97	43.63	44.11
Grp 3	74.72	72.97	72.38	72.59	73.01
Grp 4a	75.34	73.43	70.29	73.22	71.54
Grp 4b	72.56	70.37	73.77	73.97	70.69
Grp 5a	70.22	69.47	70.26	74.04	73.19
Grp 5b	70.58	74.67	70.11	71.07	69.71
Grp 6a	63.24	61.76	65.09	61.38	63.00
Grp 6b	64.23	60.63	59.08	59.47	60.53

Group 1 – Animals without any treatment – Negative control

Group 2 – Animals treated with cream containing 1% Clotrimazole (Positive Control - a).

Group 3 – Animals treated with placebo (Emulsion base only/Positive control - b)

Group 4a – Animals treated with 0.5% medicinal plant extract alone

Group 4b – Animals treated with emulsion containing 0.5% medicinal plant extract

Group 5a – Animals treated with 1% medicinal plant extract alone

Group 5b – Animals treated with emulsion containing 1% medicinal plant extract

Group 6a- Animals treated with 2% medicinal plant extract alone

Group 6b- Animals treated with emulsion containing 2% medicinal plant extract

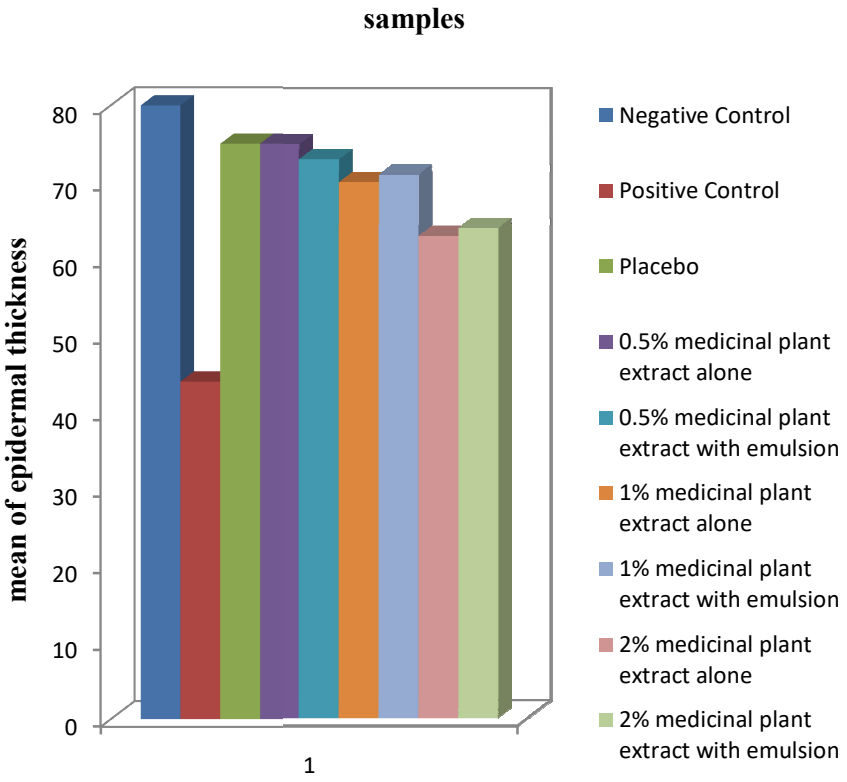


Fig.27 Effect of *Mitracarpus villosus* extracts on epidermal thickness in *Epidermophyton floccosum* infected skin

which indicated that the drug was able to prevent the growth of the hyphae by the dermatophyte. There were moderate hair follicles in the dermis and also abundant sebaceous glands (Plate 27). Skin inflammation was moderate while tissue destruction of the skin was marked in group 3 (Table 58) treated with emulsion alone. There was marked presence of fungal hyphae which indicated that the cream alone has no medicinal effect on the dermatophyte infection and this definitely results in the reduction of numbers of hair follicles as the dermatophye will attack freely. However the group has few sebaceous glands in the dermis. Inflammation and tissue destruction of the skin were moderate in group 4a (0.5% plant extract alone). Presence of fungal hyphae was moderate while there are few hair follicles enmeshed within the sparse connective tissue in the dermis. There are also few sebaceous glands in the dermis (Table 58). The same observation was made for group 4b. However, in groups 5a (1% plant extract alone) moderate amount of fungal hyphae were seen with moderate amount of hair follicle and moderate amount of sebaceous gland. The inflammation and tissue destruction of the skin were mild (Plate 28). Same observations were recorded for groups 5b, 6a and 6b.

The efficacy of the formulations on the keratin layer of the hair compared with the infected untreated control is shown in Table 59 and Fig.28.The untreated control with the value 24.06 ± 0.42 µm indicated heavy infection of the hairs by the dermatophyte. Same goes for the group treated with the emulsion alone (24.21 ± 0.12 µm), the group treated with 0.5% *Mitracarpus villosus* extract alone (24.57 ± 0.80 µm) and the group treated with 0.5% *Mitracarpus villosus* extract emulsions (25.72 ± 0.04 µm) which were not statistically different from one another. The values obtained for 1% *Mitracarpus villosus* extract alone and 1% *Mitracarpus villosus* emulsion indicated slight efficacy by the formulations. Comparatively, 2% *Mitracarpus villosus* extract alone (35.06 ± 0.57 µm) and 2% *Mitracarpus villosus*extract emulsion (36.01 ± 0.26 µm) showed moderate mycological efficacy. The standard drug 1% clotrimazole cream (57.08 ± 0.56 µm) demonstrated high mycological efficacy in preventing attack by the dermatophyte on the keratin layer of the hair. There is significant difference in keratinization between the groups as determined by one way Anova ($F_{6,28}$=318.527,$p<0.05$). Tukeys post hoc test was used in determining the significant

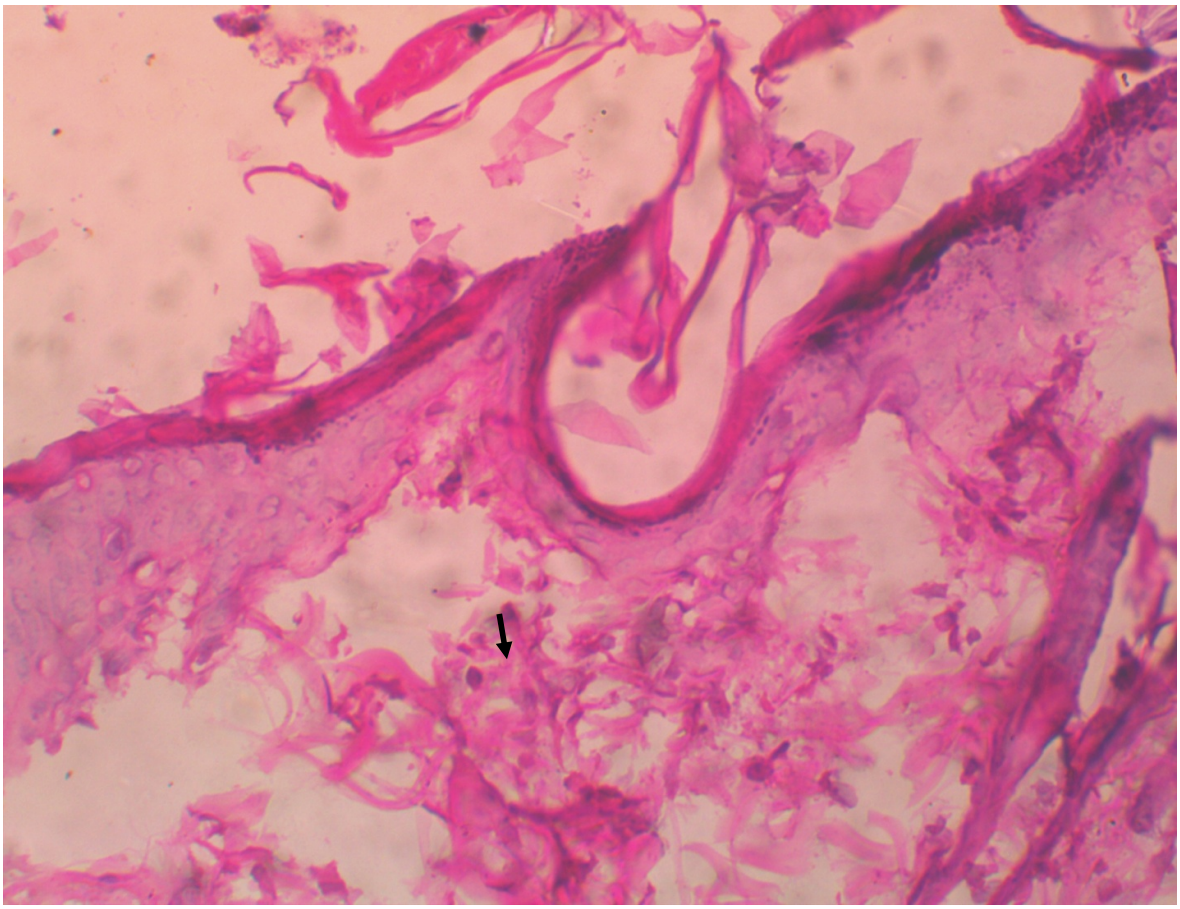


Plate 27 Formation of a mycelia mass in the epidermis. HE x100.

Table 58 Mycological efficacy of *Mitracarpus villosus* formulations against *Trichophyton mentagrophytes*

Group	Fungal hyphae	Hair follicles	Sebaceous gland	Inflammation	Continuity/Tissue destruction
1	+++	-	+	++	+++
2	-	++	+++	-	-
3	+++	+	+	++	+++
4a	++	+	+	++	++
4b	++	+	+	++	++
5a	++	++	++	+	+
5b	++	++	++	+	+
6a	++	++	++	+	+
6b	++	++	++	+	+

- = Absent, Mild = +, Moderate = ++, Marked = +++

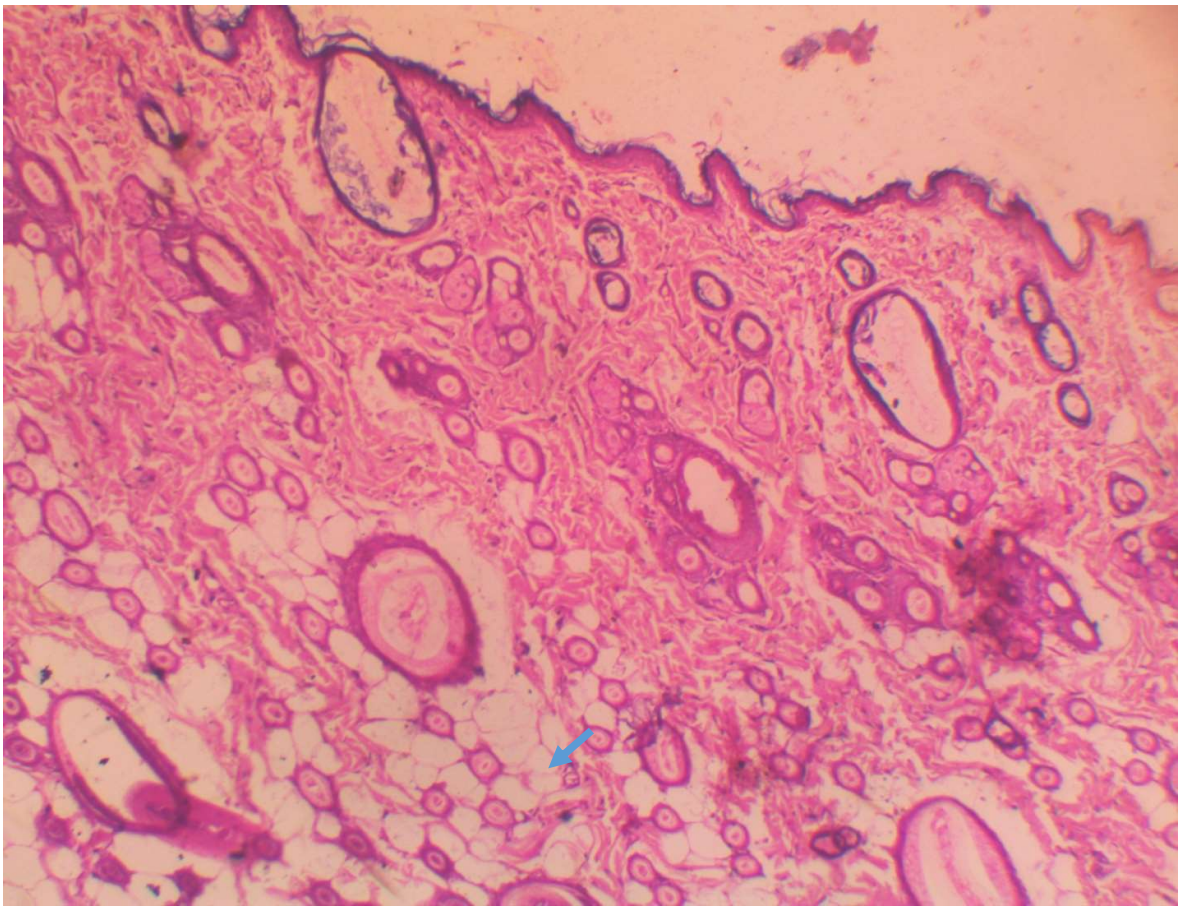


Plate 28 There is diffuse dermal capillary congestion and follicular hyperplasia. HE x400

Table 59 Effect of *Mitracarpus villosus* formulations on keratinization (in µm) of skin infected by *Trichophyton mentagrophytes*

Group 1	22.65	24.32	25.25	23.92	24.18
Group 2	55.28	58.11	57.04	56.56	58.39
Group 3	22.63	23.38	21.39	26.39	27.25
Group 4a	24.73	24.11	21.92	26.84	25.23
Group 4b	23.72	25.64	23.28	27.03	28.93
Group 5a	28.33	30.43	29.03	29.45	30.29
Group 5b	30.15	30.22	29.71	31.33	29.72
Group 6a	34.24	33.72	35.86	34.69	36.78
Group 6b	35.33	35.91	36.69	36.49	35.64

Group 1 – Animals without any treatment – Negative control

Group 2 – Animals treated with cream containing 1% Clotrimazole (Positive Control - a).

Group 3 – Animals treated with placebo (Emulsion base only/Positive control - b)

Group 4a – Animals treated with 0.5% medicinal plant extract alone

Group 4b – Animals treated with emulsion containing 0.5% medicinal plant extract

Group 5a – Animals treated with 1% medicinal plant extract alone

Group 5b – Animals treated with emulsion containing 1% medicinal plant extract

Group 6a- Animals treated with 2% medicinal plant extract alone

Group 6b- Animals treated with emulsion containing 2% medicinal plant extract

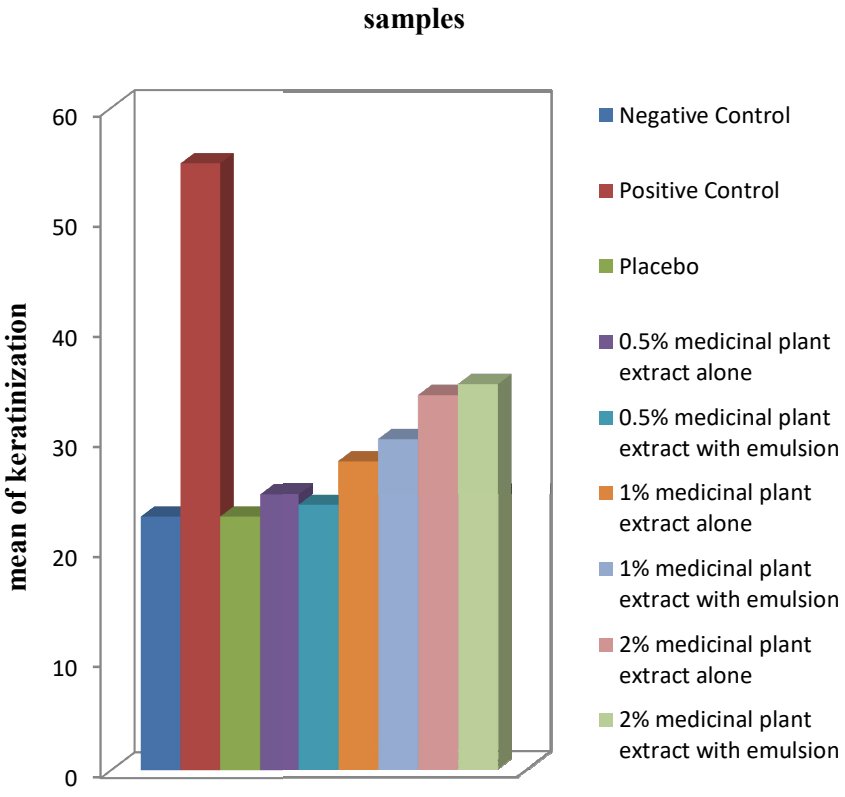


Fig.28 Effect of *Mitracarpus villosus* extracts on keratinization in *Trichophyton mentagrophytes* infected skin

differences when the Control is compared with other groups (Table 108 and Fig.74).See Appendix.

There was significant infection (85.69 ± 0.52 µm) as indicated by the result obtained for the epidermal thickness of untreated group. The values obtained for emulsion alone (72.14 ± 0.76 µm),0.5% *Mitracarpus villosus* extract (72.55 ± 0.81 µm),0.5% *Mitracarpus villosus* extract emulsion (72.37 ± 0.65 µm) indicated slight reduction in the epidermal thickness. However, 1% *Mitracarpus villosus* (64.92 ± 0.34 µm) and 1% *Mitracarpus villosus* emulsion (63.81 ± 0.51 µm) in reducing the epidermal thickness can be said to be mild compared with the untreated control. The values obtained for 2% *Mitracarpus villosus* extract alone (53.59 ± 0.82 µm) and 2% *Mitracarpus villosus* emulsion with extract (50.9 1± 0.39 µm) showed indication that there was moderate reduction in the epidermal thickness thereby suggesting that the formulations are mycologically effective against the dermatophyte compared with the values obtained for the standard drug (43.71 ± 0.53 µm) (Table 60 and Fig.29).

The statistical evaluation as shown in Table 109 and Fig.75 (See Appendix) show that there is significance difference in epidermal thickness between groups as determined by one way anova ($F_{6,28}$=314.002,p<0.05). A tukey post hoc test also revealed that there is significance difference in epidermal thickness between the Control and the test groups.

4.12.3.4 *Malassezia furfur*

There were marked inflammation of the skin and marked tissue destruction was also observed in group 1. There was marked presence of fungal hyphae in the group. Few hair follicles and sebaceous glands were also observed.

No inflammation and tissue destruction was observed in group 2 (Table 61). No fungal hyphae were seen in the group. There were numerous hair follicles in the dermis and also numerous amounts of sebaceous glands. There was marked skin inflammation and marked tissue destruction of the skin in group 3 (Plate 29) animals treated with emulsion alone. There was marked presence of fungal hyphae and few hair follicles. There were also moderate amount of sebaceous glands in the dermis.

Table 60 Effect of *Mitracarpus villosus* formulations on epidermal thickness (in µm) of skin infected by *Trichophyton mentagrophytes*

Gpr 1	86.53	87.11	84.78	85.71	84.32
Grp 2	44.76	44.23	41.75	43.53	44.28
Grp 3	74.53	70.32	72.11	72.93	70.81
Grp 4a	74.22	73.35	70.19	73.92	71.05
Grp 4b	72.83	70.88	74.15	73.16	70.85
Grp 5a	65.42	65.63	65.16	64.71	63.69
Grp 5b	64.39	64.34	64.72	61.87	63.71
Grp 6a	55.77	51.16	55.29	51.77	53.94
Grp 6b	51.11	50.63	52.17	50.88	49.76

Group 1 – Animals without any treatment – Negative control

Group 2 – Animals treated with cream containing 1% Clotrimazole (Positive Control - a).

Group 3 – Animals treated with placebo (Emulsion base only/Positive control - b)

Group 4a – Animals treated with 0.5% medicinal plant extract alone

Group 4b – Animals treated with emulsion containing 0.5% medicinal plant extract

Group 5a – Animals treated with 1% medicinal plant extract alone

Group 5b – Animals treated with emulsion containing 1% medicinal plant extract

Group 6a- Animals treated with 2% medicinal plant extract alone

Group 6b- Animals treated with emulsion containing 2% medicinal plant extract

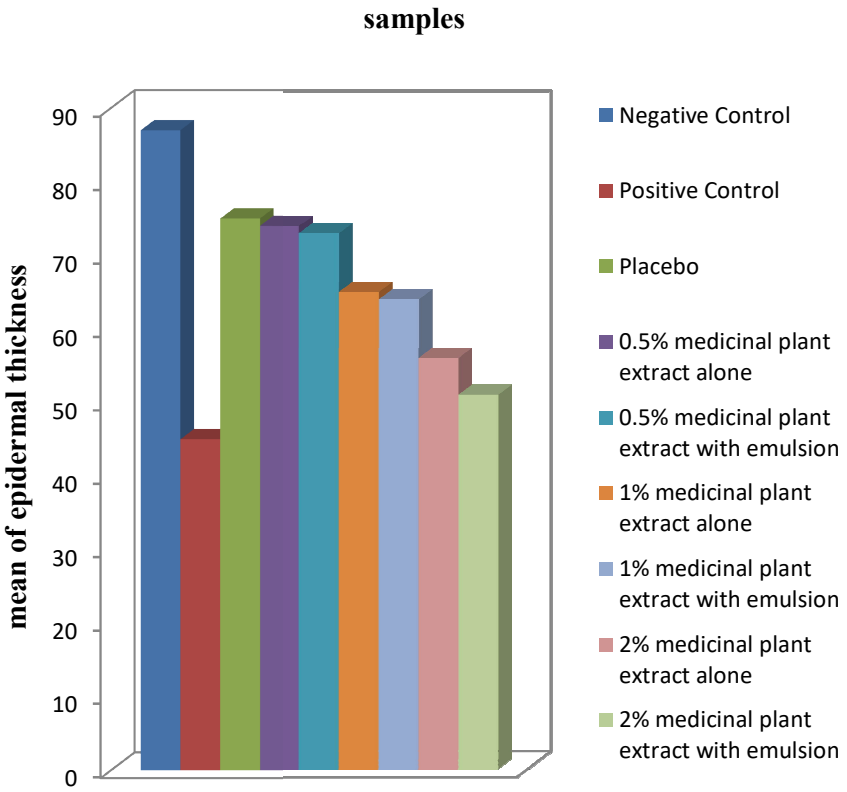


Fig.29 Effect of *Mitracarpus villosus* extracts on epidermal thickness in *Trichophyton mentagrophytes* infected skin

Table 61 Mycological efficacy of *Mitracarpus villosus* ethanol formulations against *Malassezia furfur*

Group	Fungal hyphae	Hair follicles	Sebaceous gland	Inflammation	Continuity/Tissue destruction
1	+++	+	+	+++	+++
2	-	+++	+++	-	-
3	+++	+	++	+++	+++
4a	++	+	++	++	++
4b	++	+	++	++	++
5a	++	++	++	++	++
5b	++	++	++	++	++
6a	+	++	++	+	+
6b	+	++	++	+	+

- = Absent, Mild = +, Moderate = ++, Marked = +++

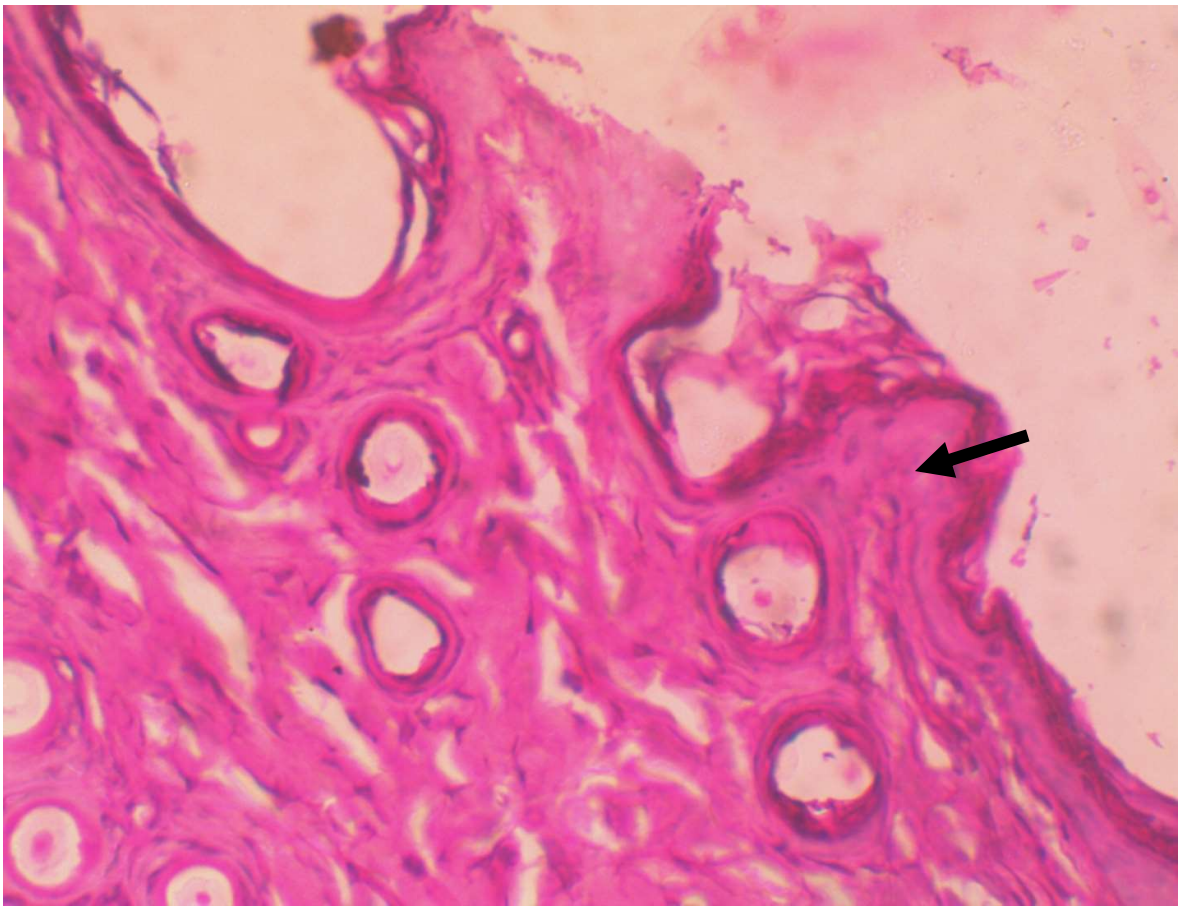


Plate 29 There is severe thicknening of the epidermis. HE x100

Inflammation and tissue destruction of the skin were however moderate in the group (Plate 30). Same observations were also seen in group 5b.
In group 6a, there was few presence of fungal hyphae in the group. Moderate amount of hair follicles enmeshed within the sparse connective tissue in the dermis were seen. There were also moderate amount of sebaceous glands in the dermis. Inflammation and tissue destruction of the skin were however mild in the group (Table 61).

Same observations were also seen in group 6b. Table 62 and Fig.30 show comparative effectiveness of the formulations on keratinization compared with the infected untreated control. Higher scores indicated improved efficacy compared with the untreated control. Significant efficacy was demonstrated by 1% clotrimazole cream (standard drug), 2% *Mitracarpus villosus* extract alone and 2% *Mitracarpus villosus* extract emulsion. 1% *Mitracarpus villosus* extract alone and 1% *Mitracarpus villosus* extract emulsion showed slight efficacy, but not as effective as the 2% formulations. Emulsion alone (placebo), 0.5% *Mitracarpus villosus* extract and 0.5% *Mitracarpus villosus* extract emulsion cannot be said to be mycologically effective compared to the values obtained for the other formulations.

Table 110 with Fig.76 (See Appendix) shows that there is significant difference in keratinization between the groups as determined by one way Anover. ($F_{6,28}$=345.757, $p<0.05$). Tukeys post hoc test shows asignificant differences when the Control is compared with other groups.

The values for epidermal thickness of the skin biopsies of the various formulations are shown in Table 63. Higher scores indicated significant infection of the dermis with attendance inflammation of the skin and tissue destruction. The values obtained for the untreated group, the groups treated with emulsion alone, 0.5% *Mitracarpus villosus* extract, and 0.5% *Mitracarpus villosus* extract emulsion was not statistically different. However, the efficacy of 1% *Mitracarpus villosus* extract and 1% *Mitracarpus villosus* emulsion in reducing the epidermal thickness is slightly significant compared with the untreated control. In contrast, there was significant efficacy of 2% *Mitracarpus villosus* extract and 2% *Mitracarpus villosus* emulsion compared with the values obtained for the standard drug (Fig.31).

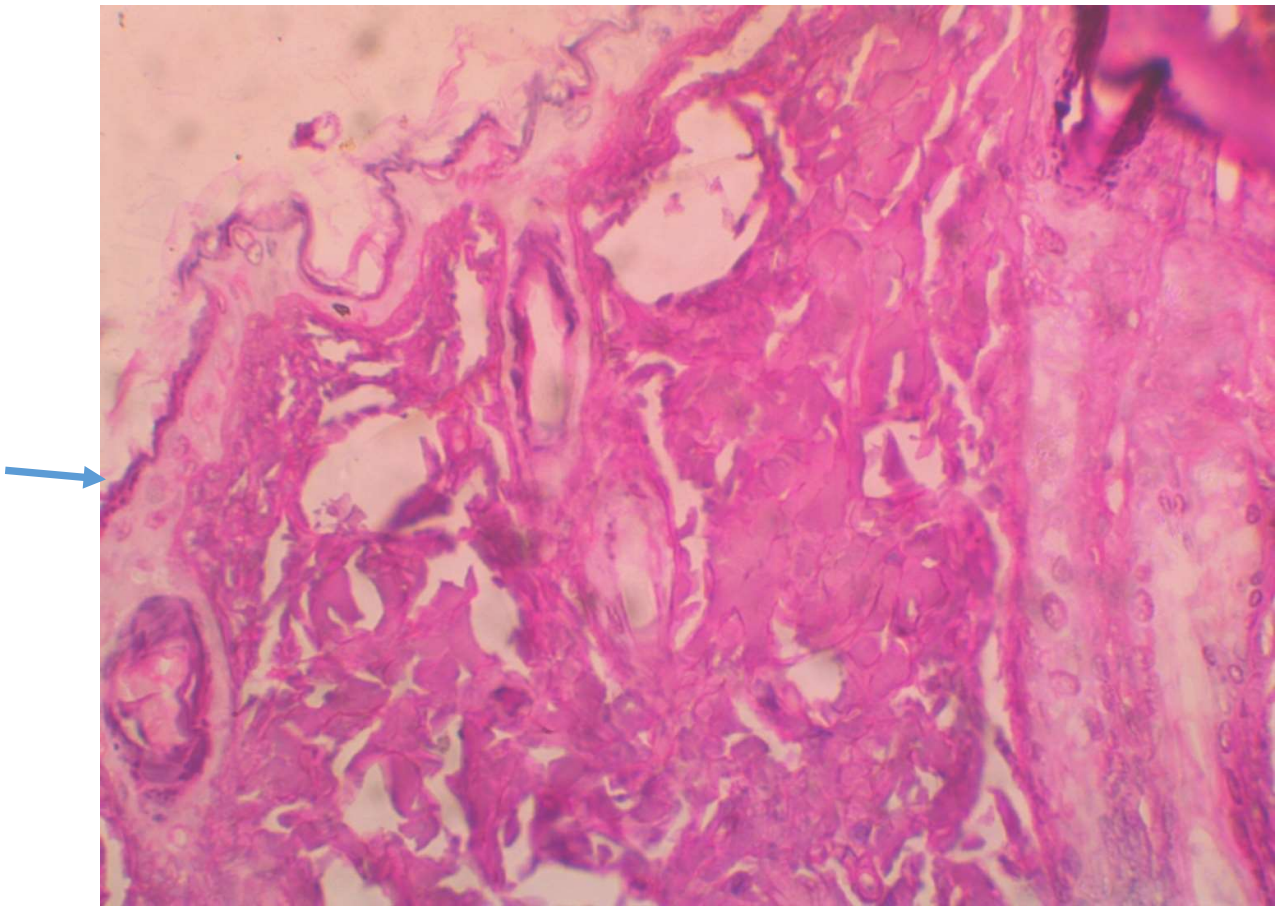


Plate 30 There is diffuse dermal capillary congestion and follicular hyperplasia. HE x400

Table 62 Effect of *Mitracarpus villosus* formulations on keratinization (µm) of skin infected by *Malassezia furfur*

Group 1	28.71	28.11	25.38	27.56	26.72
Group 2	56.28	58.22	56.41	56.19	58.34
Group 3	30.26	29.28	29.72	30.09	29.72
Group 4a	31.33	32.29	30.12	31.62	29.29
Group 4b	30.56	31.98	34.46	31.96	31.69
Group 5a	31.45	31.47	33.57	32.92	32.43
Group 5b	34.05	35.48	33.76	36.42	31.69
Group 6a	45.72	46.92	46.09	46.38	44.36
Group 6b	45.08	46.33	45.69	46.47	46.39

Group 1 – Animals without any treatment – Negative control

Group 2 – Animals treated with cream containing 1% Clotrimazole (Positive Control - a).

Group 3 – Animals treated with placebo (Emulsion base only/Positive control - b)

Group 4a – Animals treated with 0.5% medicinal plant extract alone

Group 4b – Animals treated with emulsion containing 0.5% medicinal plant extract

Group 5a – Animals treated with 1% medicinal plant extract alone

Group 5b – Animals treated with emulsion containing 1% medicinal plant extract

Group 6a- Animals treated with 2% medicinal plant extract alone

Group 6b- Animals treated with emulsion containing 2% medicinal plant extract

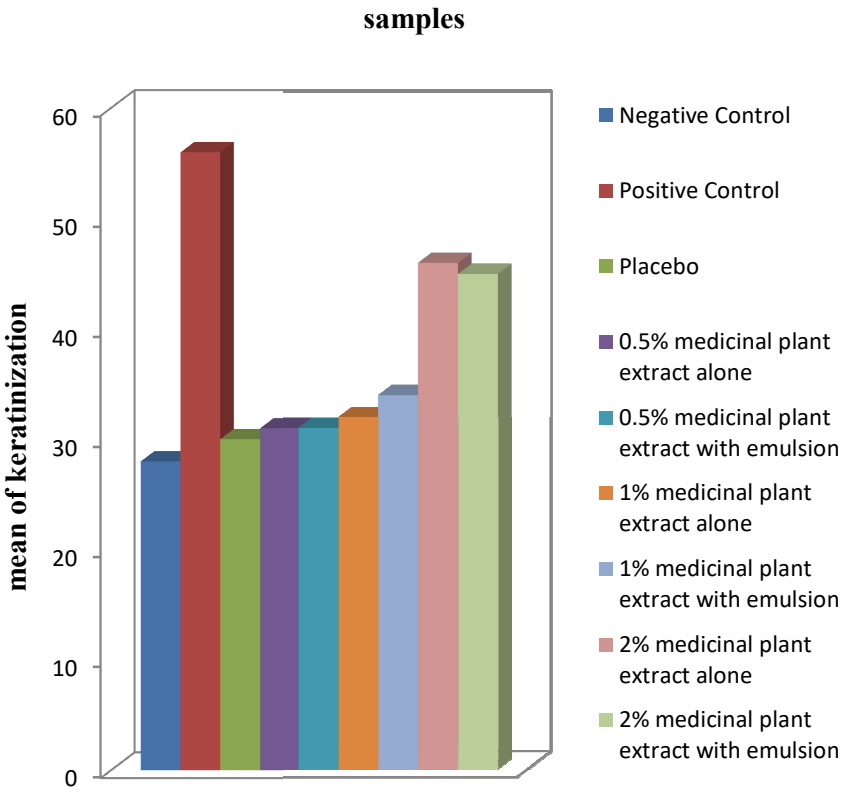


Fig. 30 Effect of *Mitracarpus villosus* extracts on keratinization in *Malassezia furfur* infected skin

Table 63 Effect of *Mitracarpus villosus* formulations on epidermal thickness (µm) of skin infected by *Malassezia furfur*

Gpr 1	52.93	54.05	52.22	51.77	53.39
Grp 2	31.25	31.24	31.05	32.83	32.46
Grp 3	52.63	51.32	50.47	52.93	53.76
Grp 4a	49.27	48.89	50.47	49.92	51.67
Grp 4b	50.33	49.86	51.61	52.76	51.72
Grp 5a	42.39	45.03	45.85	44.24	43.73
Grp 5b	40.39	44.34	44.47	41.95	43.04
Grp 6a	36.75	38.28	35.11	36.74	33.74
Grp 6b	34.68	30.43	36.47	33.43	30.48

Group 1 – Animals without any treatment – Negative control

Group 2 – Animals treated with cream containing 1% Clotrimazole (Positive Control - a).

Group 3 – Animals treated with placebo (Emulsion base only/Positive control - b)

Group 4a – Animals treated with 0.5% medicinal plant extract alone

Group 4b – Animals treated with emulsion containing 0.5% medicinal plant extract

Group 5a – Animals treated with 1% medicinal plant extract alone

Group 5b – Animals treated with emulsion containing 1% medicinal plant extract

Group 6a- Animals treated with 2% medicinal plant extract alone

Group 6b- Animals treated with emulsion containing 2% medicinal plant extract

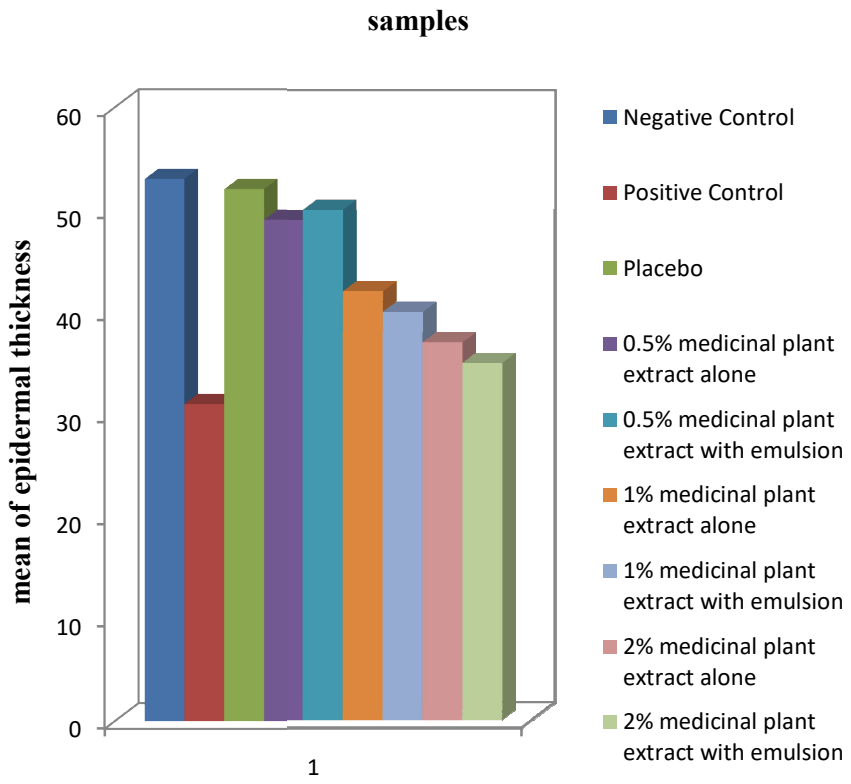


Fig.31 Effect of *Mitracarpus villosus* extracts on epidermal thickness in *Malassezia furfur* infected skin

Data showed in Table 111 and Fig.77 (See Appendix) reveals staistically that there is significance difference in epidermal thickness between groups as determined by one way anova ($F_{6,28}$=121.134,p<0.05). A tukey post hoc test revealed that there is significance difference in epidermal thickness between the control and the test groups. The formulated emulsion alone exhibited little or no mycological efficacy in the treatment of dermatophytosis.

4.12.4 Effect of *Cassia occidentalis* on dermatophytes

4.12.4.1 *Microsporium aoudinin*

There were marked inflammation of the skin and marked tissue destruction observed in group 1. There was also marked presence of fungal hyphae in the group (Plate 31). Few hair follicles and sebaceous glands were seen. No inflammation and tissue destruction was observed in group 2 (Table 64). No fungal hyphae were seen in this group. There were numerous hair follicles in the dermis and also numerous amounts of sebaceous glands. Group 3 animals treated with emulsion alone had marked skin inflammation and marked tissue destruction of the skin. Moderate presence of fungal hyphae and few hair follicles were seen.

There were also few amounts of sebaceous glands in the dermis. It was observed that skin inflammation and tissue destruction were moderate in group 4a. There was also moderate presence of fungal hyphae and few hair follicles. There were also few amounts of sebaceous glands. The same observations were made for group 4b.

In group 5a, Inflammation and tissue destruction of the skin were moderate (Plate 32). There was moderate presence of fungal hyphae in the group. Moderate amount of hair follicles enmeshed within the sparse connective tissue in the dermis were seen. There were also moderate amount of sebaceous glands in the dermis. Same observations were also seen in group 5b. In group 6a, Inflammation and tissue destruction of the skin were mild (Table 64). There were few presence of fungal hyphae in the group. Moderate amount of hair follicles enmeshed within the sparse connective tissue in the dermis were seen. There were also moderate amount of sebaceous glands in the dermis. Same observations were also seen in group 6b. The efficacy of the formulations on the keratin layer of the hair compared with the infected untreated control is shown in Table 65 and

Table 64 Mycological efficacy of *Cassia occidentalis* formulations against *Microsporium aoudini*

Group	Fungal hyphae	Hair follicles	Sebaceous gland	Inflammation	Continuity/Tissue destruction
1	+++	+	+	+++	+++
2	-	+++	+++	-	-
3	++	+	+	+++	+++
4a	++	+	+	++	++
4b	++	+	+	++	++
5a	++	++	++	++	++
5b	++	++	++	++	++
6a	+	++	++	+	+
6b	+	++	++	+	+

- = Absent, Mild = +, Moderate = ++, Marked = +++

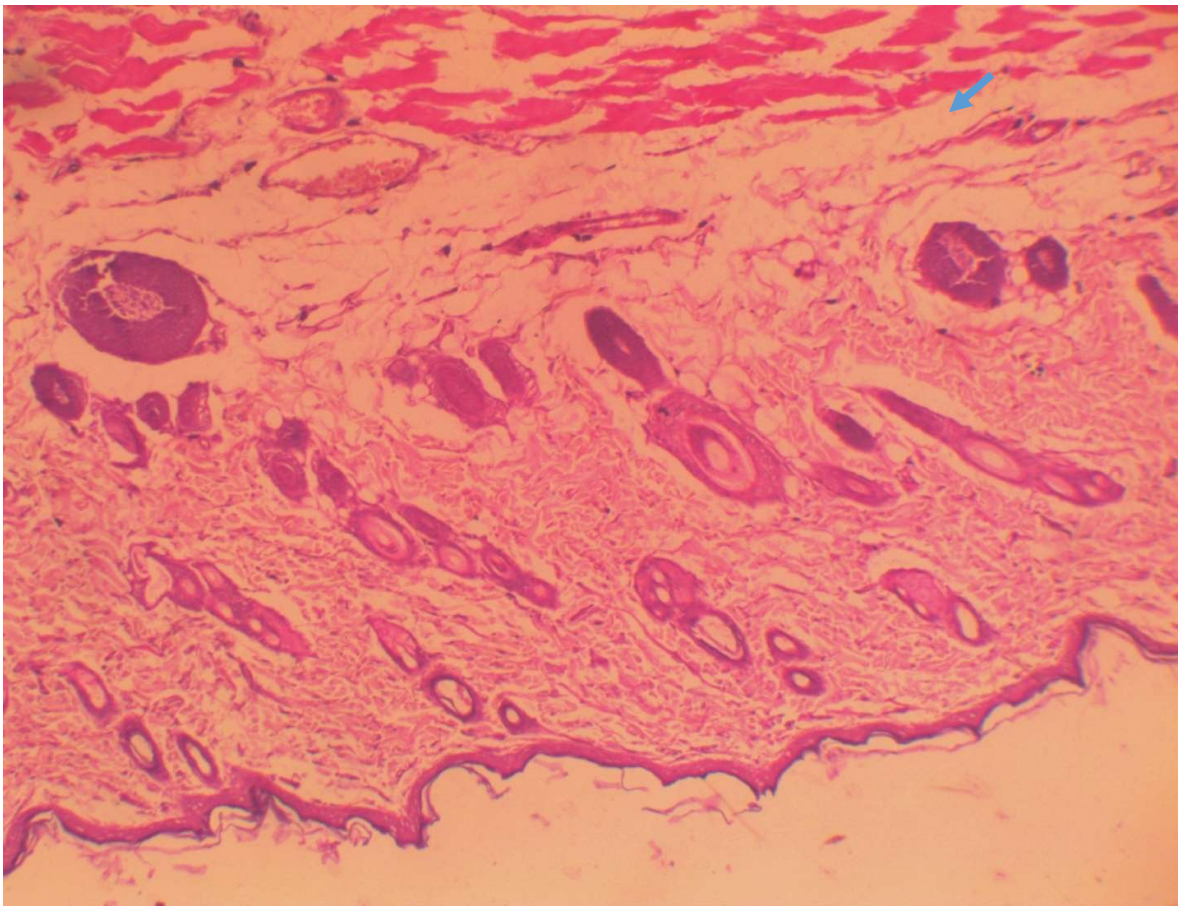


Plate 31 There is no observable lesion. HE x100

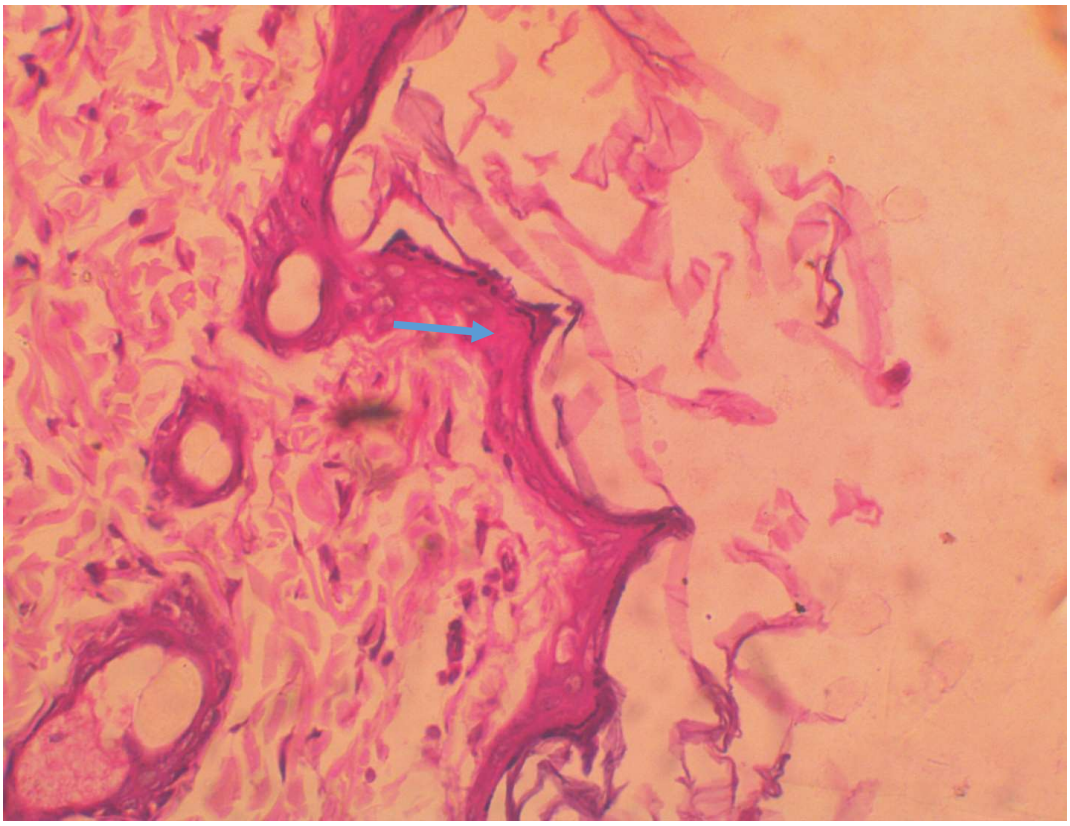


Plate 32. There is cornification and thicknening of the epidermis HE x100

Table 65 Effect of *Cassia occidentalis* formulations on keratinization (µm) of skin infected by *Microsporium aoudini*

Group 1	23.65	24.11	23.54	24.57	26.38
Group 2	46.64	48.09	44.65	45.25	43.46
Group 3	28.35	26.11	28.60	29.17	27.52
Group 4a	28.24	29.35	24.68	26.11	27.65
Group 4b	27.65	26.33	27.04	28.32	26.89
Group 5a	26.82	26.24	26.57	27.33	26.09
Group 5b	25.31	26.22	24.57	26.63	25.09
Group 6a	29.38	28.65	28.86	28.60	29.84
Group 6b	28.64	30.57	29.91	29.02	30.35

Group 1 – Animals without any treatment – Negative control

Group 2 – Animals treated with cream containing 1% Clotrimazole (Positive Control - a).

Group 3 – Animals treated with placebo (Emulsion base only/Positive control - b)

Group 4a – Animals treated with 0.5% medicinal plant extract alone

Group 4b – Animals treated with emulsion containing 0.5% medicinal plant extract

Group 5a – Animals treated with 1% medicinal plant extract alone

Group 5b – Animals treated with emulsion containing 1% medicinal plant extract

Group 6a- Animals treated with 2% medicinal plant extract alone

Group 6b- Animals treated with emulsion containing 2% medicinal plant extract

Fig.32. The untreated control with the value 24.45 ± 0.52 µm indicated that the hairs were heavily infected with the dermatophyte. However, the group treated with the emulsion alone (27.95 ± 0.53 µm) showed slight efficacy.

Application of 0.5% *Cassia occidentalis* extract emulsion (27.25 ± 0.34 µm), 0.5% *Cassia occidentalis* extract alone (27.21 ± 0.82 µm), 1% *Cassia occidentalis* extract alone (27.61 ± 0.22 µm) and 1% *Cassia occidentalis* emulsion (27.56 ± 0.38 µm) on the infected skin of the animal also showed slight difference from the untreated group. This implies that they had some effect in curtailing the infection caused by the dermatophyte. There is moderate efficacy exhibited by 2% *Cassia occidentalis* extract alone (29.07 ± 0.24 µm) and 2% *Cassia occidentalis* extract emulsion (29.70 ± 0.37 µm).

The effectiveness of 1% clotrimazole cream (45.62 ± 0.80 µm) in preventing attack by the dermatophyte on the keratin layer of the hair is demonstrated by the high value recorded. There is significant difference in keratinization between the groups as determined by one way Anova ($F_{6,28}$=186.682,$p<0.05$). Using Turkey's post hoc test confirms the difference (Table 112 and Fig.78) see Appendix.

The results obtained for the epidermal thickness of untreated group indicated significant infection (86.51 ± 0.87 µm) Table 66 and Fig.33. The value obtained for emulsion alone (76.43 ± 0.87 µm) indicated that the emulsion had little effect on the epidermal thickness of the animals which was not significant from the untreated group. The efficacy demonstrated by 0.5% *Cassia occidentalis* extract (71.90 ± 0.37 µm), 0.5% *Cassia occidentalis* extract emulsion (71.22 ± 0.75 µm), 1% *Cassia occidentalis* extract (71.02 ± 0.20 µm), 1% *Cassia occidentalis* emulsion (71.27 ± 0.32 µm), 2% *Cassia occidentalis* extract (71.15 ± 0.51 µm) and 2% *Cassia occidentalis* emulsion. The statistical evaluation as shown in Tables 113 and Fig.79 (See Appendix) shows that there is significance difference in epidermal thickness between groups as shown by one way Anova ($F_{6,28}$=550.218,$p<0.05$). Using Tukey's post hoc test there is significant difference between positive control and the test groups.

4.12.4.2 *Epidermophyton floccosum*

There were moderate inflammation of the skin and marked tissue destruction observed in group 1. There was marked presence of fungal hyphae and few hair follicles and

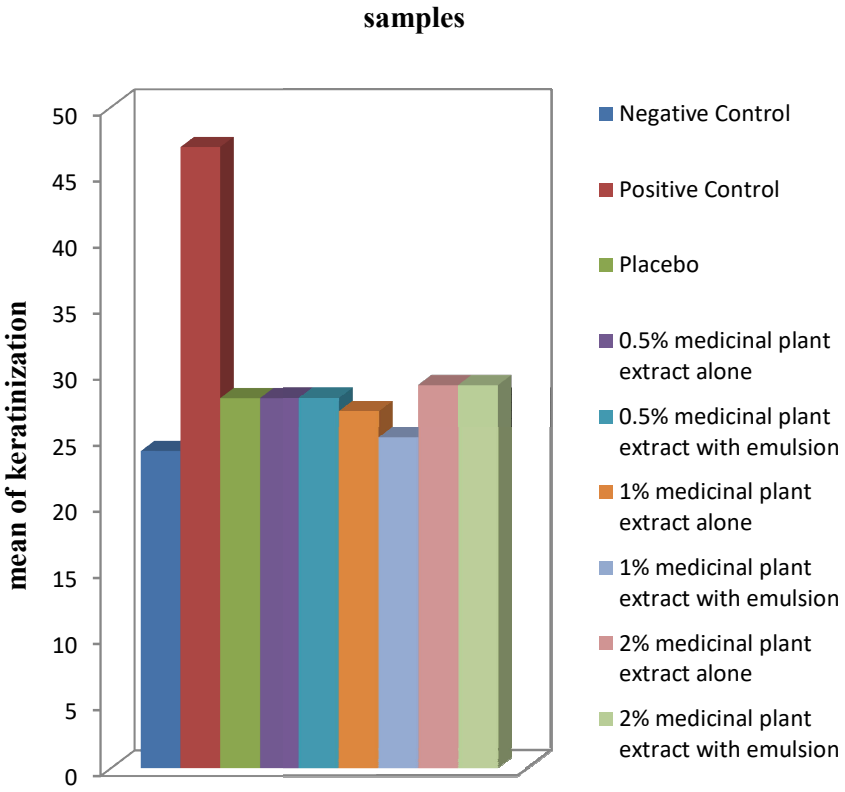


Fig.32 Effect of *Cassia occidentalis* extracts on keratinisation in *Microsporium aoudinin* infected skin

Table 66 Effect of *Cassia occidentalis* formulations on epidermal thickness (µm) of skin infected by *Microsporium aoudini*

Gpr 1	83.28	86.64	86.73	88.38	87.54
Grp 2	38.64	39.62	40.24	41.62	39.47
Grp 3	74.48	75.33	75.30	78.11	78.91
Grp 4a	73.22	71.48	71.89	71.22	71.67
Grp 4b	70.17	72.29	74.68	71.33	72.64
Grp 5a	71.45	71.11	71.19	70.26	71.08
Grp 5b	72.04	70.87	71.53	70.25	71.65
Grp 6a	72.85	70.56	71.66	70.69	69.97
Grp 6b	71.38	72.72	72.80	74.96	70.82

Group 1 – Animals without any treatment – Negative control

Group 2 – Animals treated with cream containing 1% Clotrimazole (Positive Control - a).

Group 3 – Animals treated with placebo (Emulsion base only/Positive control - b)

Group 4a – Animals treated with 0.5% medicinal plant extract alone

Group 4b – Animals treated with emulsion containing 0.5% medicinal plant extract

Group 5a – Animals treated with 1% medicinal plant extract alone

Group 5b – Animals treated with emulsion containing 1% medicinal plant extract

Group 6a- Animals treated with 2% medicinal plant extract alone

Group 6b- Animals treated with emulsion containing 2% medicinal plant extract

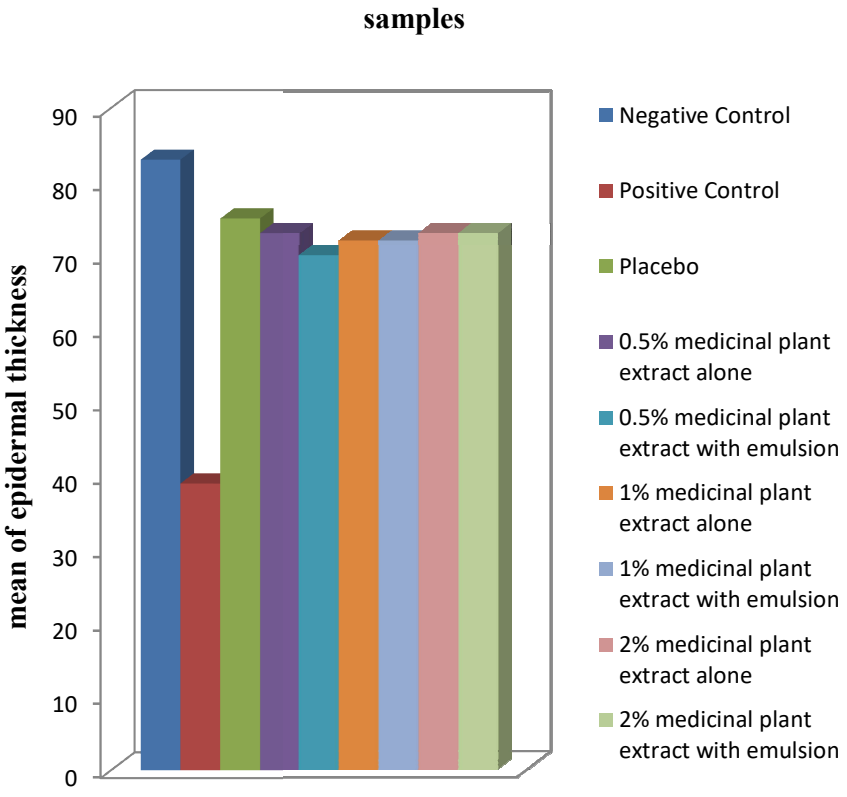


Fig.33 Effect of *Cassia occidentalis* extracts on epidermal thickness in *Microsporium aoudinin* infected skin

sebaceous glands were observed. In group 2, No inflammation and tissue destruction was observed (Table 67).

No fungal hyphae were seen. There were numerous hair follicles in the dermis and moderate amounts of sebaceous glands. Group 3 animals treated with emulsion alone had moderate presence of fungal hyphae and few hair follicles. There were also few amounts of sebaceous glands in the dermis. There was moderate skin inflammation and moderate tissue destruction of the skin in the group (Plate 33). This same observation was recorded for 4a, 4b, 5a and 5b. In group 6a, Inflammation and tissue destruction of the skin were moderate (Plate 34). There was moderate presence of fungal hyphae in the group. Few amount of hair follicles enmeshed within the sparse connective tissue in the dermis were seen. There were also moderate amount of sebaceous glands in the dermis. Same observations were also seen in group 6b.

The efficacy of the formulations on the keratin layer of the hair compared with the infected untreated control is shown in Table 68 and Fig.34. The value obtained (21.41 ± 0.58 µm) for the untreated control group 1 indicated that there was moderate infection of the hairs by the dermatophyte. The same conclusion can be made for the group treated with the emulsion alone (21.47 ± 0.64 µm). The results obtained for the other groups 0.5% *Cassia occidentalis* extract alone (20.70 ± 0.36 µm), 0.5% *Cassia occidentalis* extract emulsion (20.90±0.56µm), 1% *Cassia occidentalis* extract alone (22.03 ± 0. 85 µm), 1% *Cassia occidentalis* emulsion (22.82 ± 0.36 µm), 2% *Cassiaoccidentalis* extract alone (23.51 ± 0.58 µm) and 2% *Cassia occidentalis* extract emulsion (23.69 ± 0.42 µm) showed indication that there was no mycological efficacy compared to the other untreated group.

The value obtained for the standard drug 1% clotrimazole cream (47.37 ± 0.63 µm) is statistically significant in preventing attack by the dermatophyte on the keratin layer of the hair.

In conclusion, Table 114 and Fig.80 (See Appendix) reveals that there is a significance difference in keratinization in at least between two groups as revealed by one way Anova($F_{6,28}$= 298.278,$p<0.05$). Tukey's post hoc test was used in determining the significant differences when the Control is compared with other groups.

Table 67 Mycological efficacy of *Cassia occidentalis* formulations against *Epidermophyton floccosum*

Group	Fungal hyphae	Hair follicles	Sebaceous gland	Inflammation	Continuity/Tissue destruction
1	+++	+	+	++	+++
2	-	+++	++	-	-
3	++	+	+	++	++
4a	++	+	+	++	++
4b	++	+	+	++	++
5a	++	+	+	++	++
5b	++	+	+	++	++
6a	++	+	++	++	++
6b	++	+	++	++	++

- = Absent, Mild = +, Moderate = ++, Marked = +++

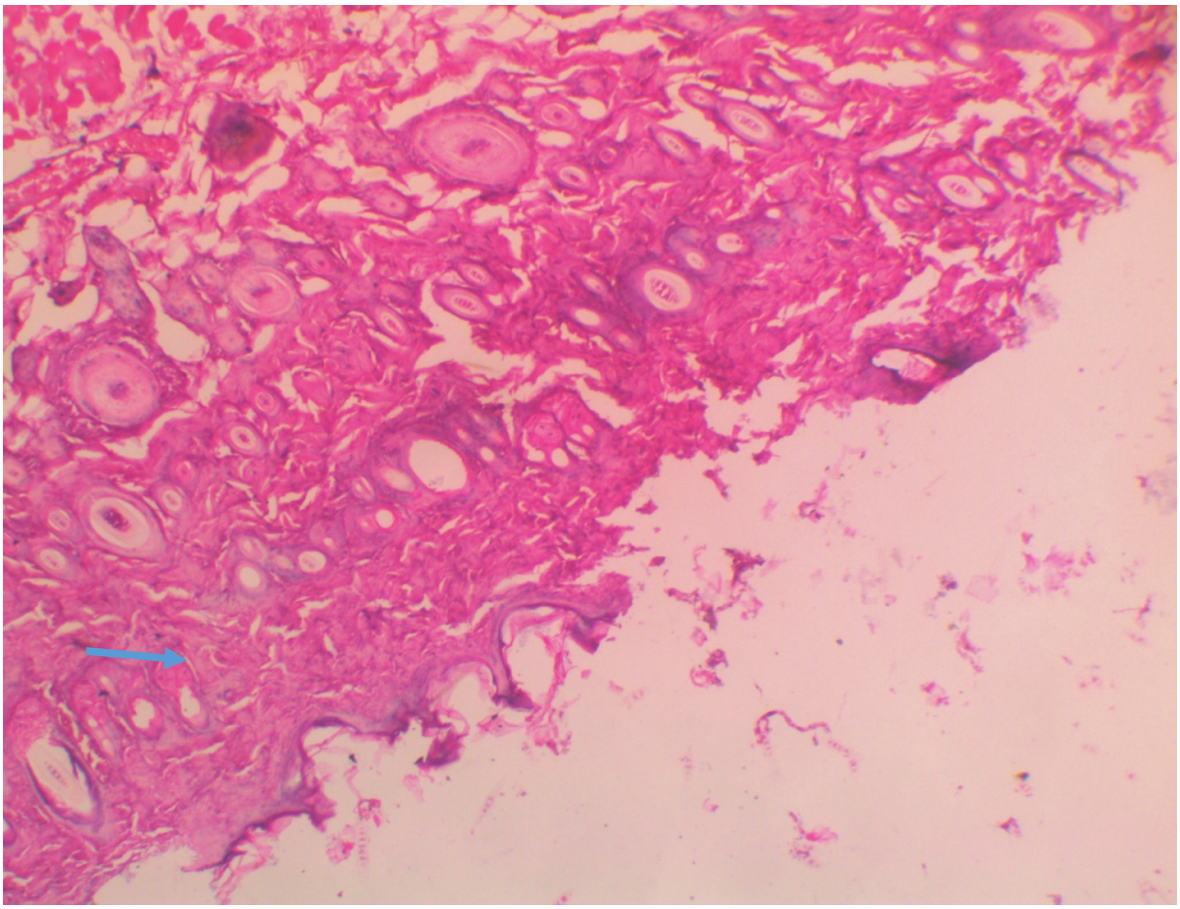


Plate 33. There is thicknening of the epidermis due to hyperkeratosis. HE x100.

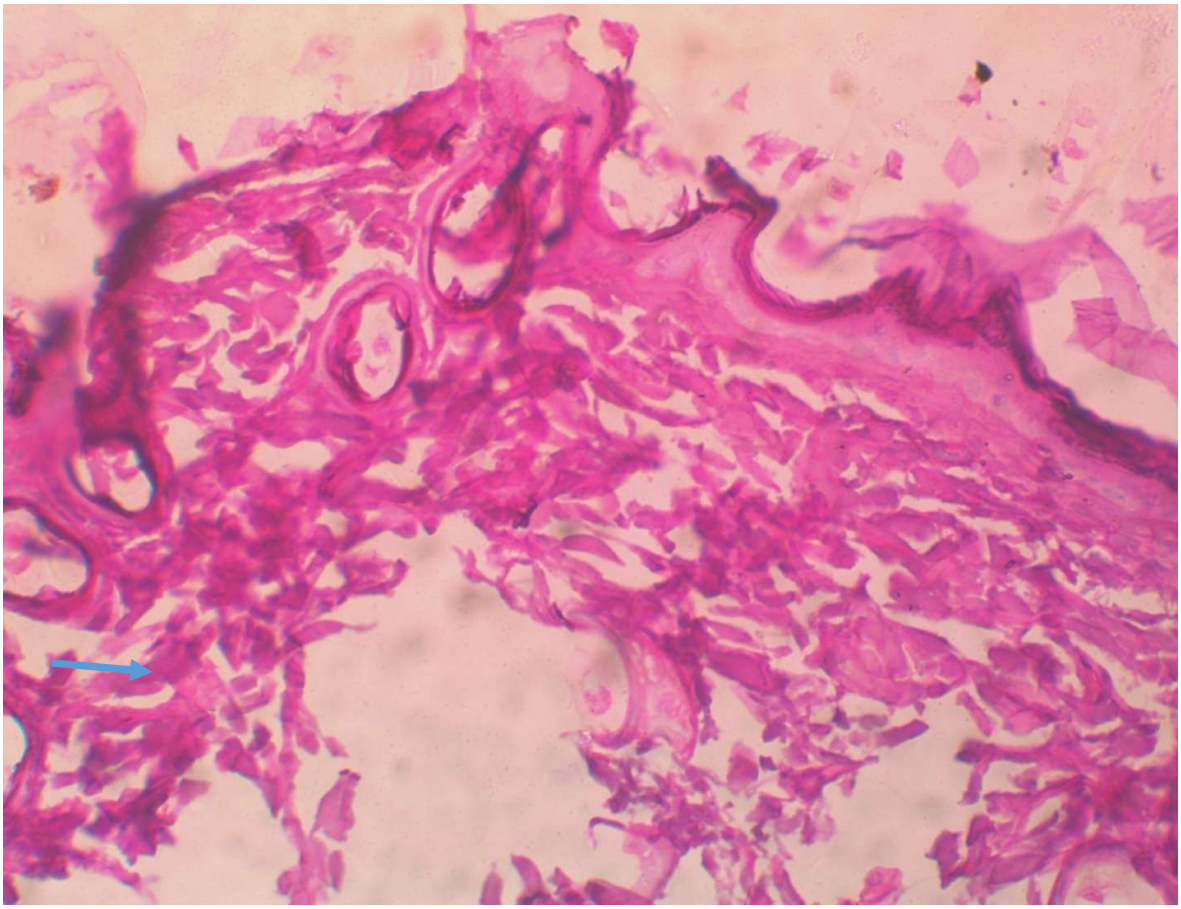


Plate 34 Discontinuity of skin tissues. HE x100.

Table 68 Effect of *Cassia occidentalis* formulations on keratinization (µm) of skin infected by *Epidermophyton floccosum*

Group 1	20.54	20.72	21.39	20.75	23.64
Group 2	46.26	49.79	47.26	46.65	46.89
Group 3	20.48	21.54	21.07	23.89	20.37
Group 4a	20.24	19.74	20.47	21.37	21.69
Group 4b	19.45	22.31	22.14	20.38	20.22
Group 5a	20.24	21.64	20.29	24.38	23.61
Group 5b	21.23	20.22	20.54	22.01	20.11
Group 6a	24.82	21.92	23.84	22.39	24.59
Group 6b	24.53	23.78	22.69	24.68	22.76

Group 1 – Animals without any treatment – Negative control

Group 2 – Animals treated with cream containing 1% Clotrimazole (Positive Control - a).

Group 3 – Animals treated with placebo (Emulsion base only/Positive control - b)

Group 4a – Animals treated with 0.5% medicinal plant extract alone

Group 4b – Animals treated with emulsion containing 0.5% medicinal plant extract

Group 5a – Animals treated with 1% medicinal plant extract alone

Group 5b – Animals treated with emulsion containing 1% medicinal plant extract

Group 6a- Animals treated with 2% medicinal plant extract alone

Group 6b- Animals treated with emulsion containing 2% medicinal plant extract

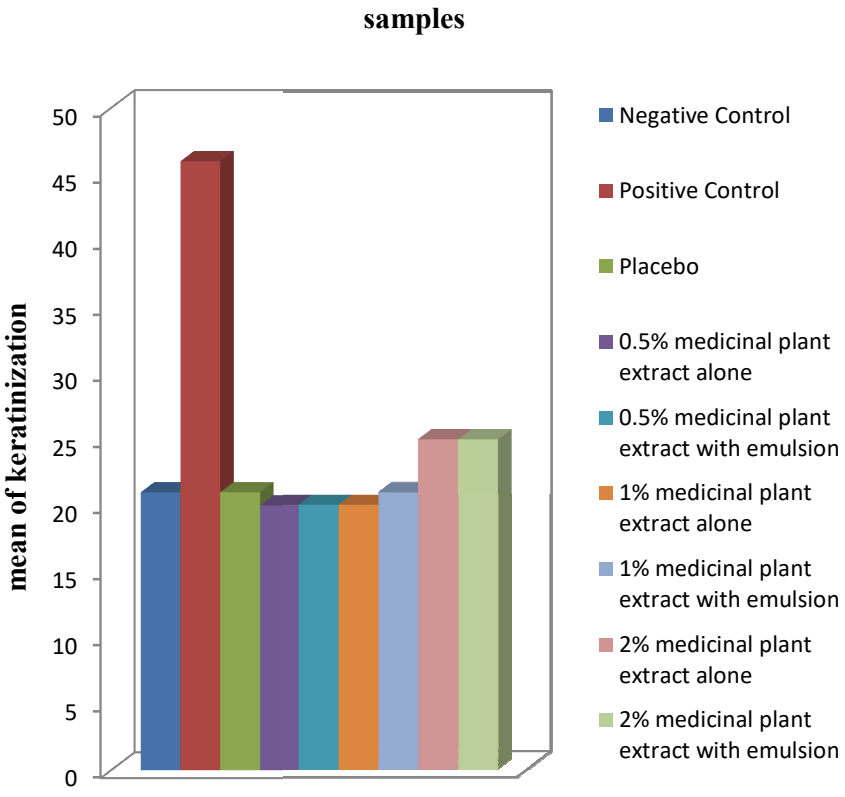


Fig.34 Effect of *Cassia occidentalis* extracts on keratinization in *Epidermophyton floccosum* infected skin

The result obtained for the epidermal thickness of the untreated group indicated that there was significant infection (86.64 ± 0.82 µm). The values obtained for emulsion alone (82.97 ± 0.82 µm), 0.5% *Cassia occidentalis* extract (82.05 ± 0.38 µm) and 0.5% *Cassia occidentalis* extract emulsion (81.61 ± 0.34 µm) in reducing the epidermal thickness cannot be said to be significant compared with the untreated control. 1% *Cassia occidentalis* extracts (77.75 ± 0.05 µm) and 1% *Cassia occidentalis* (77.19 ± 0.42 µm) showed slight reduction in the epidermal thickness.

The values obtained for 2% *Cassia occidentalis* extract alone (62.77 ± 0.71 µm) and 2% *Cassia occidentalis* emulsion with extract (62.88 ± 0.01 µm) indicated significant reduction in epidermal thickness compared with the untreated control. However, the values obtained for the standard drug (42.83 ± 0.87 µm) can be said to effectively reduce the epidermal thickness thereby indicating high efficacy against the dermatophyte (Table 69) and (Fig. 35).

The statistical evaluation as shown in Table 115 and Fig.81 (See Appendix) shows that there is no significance difference in epidermal thickness between groups as determined by one way Anova ($F_{6,28}$= 230.172,$p<0.05$). A turkey post hoc test also revealed that there is no significance difference in epidermal thickness between the Control and the test groups.

4.12.4.3 *Trichophyton mentagrophytes*

There were marked inflammation of the skin and marked tissue destruction observed in group 1. There was marked presence of fungal hyphae. There were no hair follicles and no sebaceous glands observed (Plate 35).

No inflammation and tissue destruction was however observed in the group 2 (Table 70). No fungal hyphae were seen in the group. There were numerous hair follicles in the dermis and also numerous amounts of sebaceous glands.

Group 3 animals treated with emulsion alone had moderate inflammation and tissue destruction of the skin (Plate 36). Moderate presence of fungal hyphae and few hair follicles were seen. There were also few amounts of sebaceous glands in the dermis. There was moderate presence of fungal hyphae and few hair follicles in group 4a. There

Table 69 Effect of *Cassia occidentalis* formulations on epidermal thickness (µm) of skin infected by *Epidermophyton floccosum*

Gpr 1	83.76	86.63	86.43	88.64	87.75
Grp 2	42.69	44.83	40.29	41.67	44.68
Grp 3	80.87	85.66	83.75	82.65	81.92
Grp 4a	83.54	81.79	81.74	81.85	81.34
Grp 4b	81.27	82.33	80.68	81.33	82.46
Grp 5a	79.41	79.89	74.18	78.64	76.64
Grp 5b	78.44	76.26	77.33	76.28	77.65
Grp 6a	62.35	60.39	61.36	60.31	69.45
Grp 6b	61.38	62.72	62.80	66.63	60.89

Group 1 – Animals without any treatment – Negative control

Group 2 – Animals treated with cream containing 1% Clotrimazole (Positive Control - a).

Group 3 – Animals treated with placebo (Emulsion base only/Positive control - b)

Group 4a – Animals treated with 0.5% medicinal plant extract alone

Group 4b – Animals treated with emulsion containing 0.5% medicinal plant extract

Group 5a – Animals treated with 1% medicinal plant extract alone

Group 5b – Animals treated with emulsion containing 1% medicinal plant extract

Group 6a- Animals treated with 2% medicinal plant extract alone

Group 6b- Animals treated with emulsion containing 2% medicinal plant extract

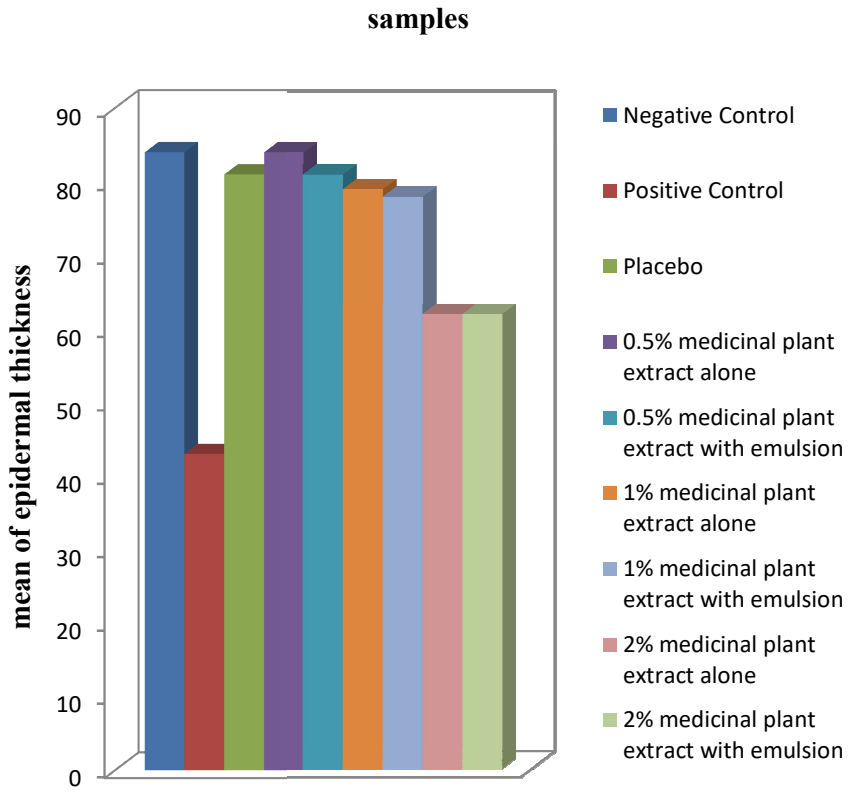


Fig.35 Effect of *Cassia occidentalis* extracts on epidermal thickness in *Epidermophyton floccosum* infected skin

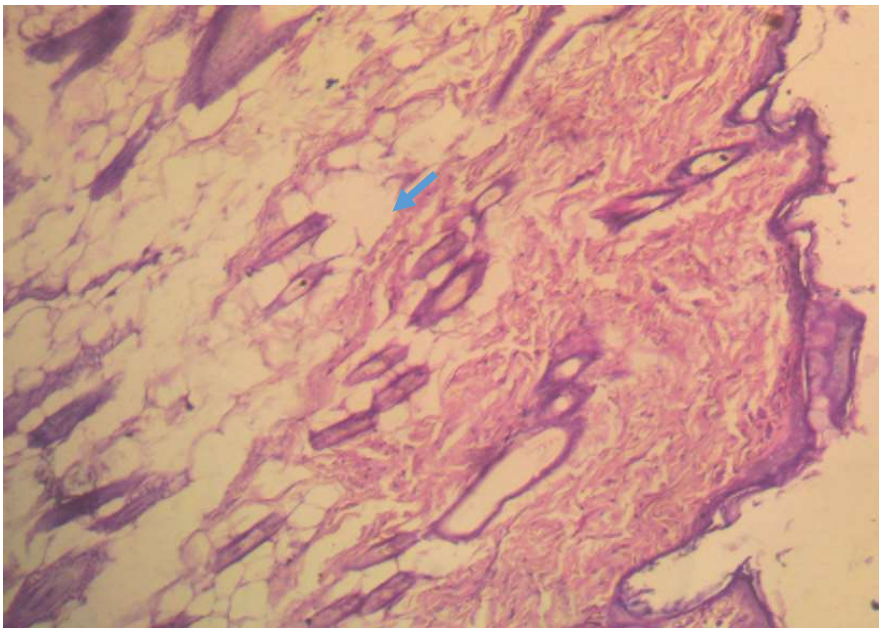


Plate 35 Epidermis chronically infected. HE x100.

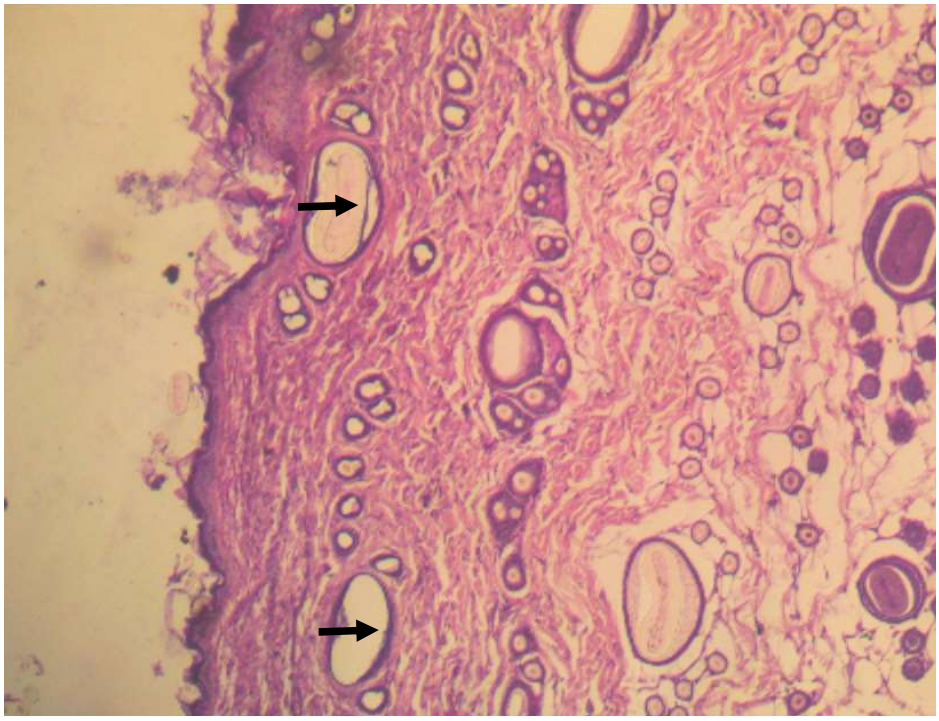


Plate 36 Folicular proliferation (arrow). HE x100.

Table 70 Mycological efficacy of *Cassia occidentalis* formulations against *Trichophyton mentagrophytes*

Group	Fungal hyphae	Hair follicles	Sebaceous gland	Inflammation	Continuity/Tissue destruction
1	+++	-	-	+++	+++
2	-	+++	+++	-	-
3	++	+	+	++	++
4a	++	+	+	++	++
4b	++	+	+	++	++
5a	++	++	++	+	++
5b	++	++	++	+	++
6a	+	++	++	+	+
6b	+	++	++	+	+

- = Absent, Mild = +, Moderate = ++, Marked = +++

were also few amounts of sebaceous glands. It was observed that skin inflammation and tissue destruction were moderate. The same observations were made for group 4b. In group 5a, there was moderate presence of fungal hyphae in the group. Moderate amount of hair follicles enmeshed within the sparse connective tissue in the dermis were seen. There were also moderate amount of sebaceous glands in the dermis. Inflammation of the skin was mild and tissue destruction of the skin were however moderate in the group (Table 70). Same observations were also seen in group 5b. In group 6a, there was few presence of fungal hyphae in the group. Moderate amount of hair follicles enmeshed within the sparse connective tissue in the dermis were seen. There were also moderate amount of sebaceous glands in the dermis. Inflammation and tissue destruction of the skin were however mild in the group. Same observations were also seen in group 6b.

The efficacy of the formulations on the keratin layer of the hair compared with the infected untreated control is shown in Table 71 and Fig.36. The untreated control with the value 18.66±0.26μm indicated heavy infection of the hairs by the dermatophyte. Same goes for the group treated with the emulsion alone (18.27 ± 0.79 μm), the group treated with 0.5% *Cassia occidentalis* extract alone (20.72 ± 0.35 μm) and the group treated with 0.5% *Cassia occidentalis* extract emulsion (19.63 ± 0.71 μm) which were not statistically different from one another. The values obtained for 1% *Cassia occidentalis* extract alone and 1% *Cassia occidentalis* emulsion indicated slight efficacy by the formulations. Comparatively, 2% *Cassia occidentalis* extract alone (28.64 ± 0.53 μm) and 2% a *Cassia occidentalis* extract emulsion (28.34 ± 0.52 μm) showed moderate mycological efficacy. The standard drug 1% clotrimazole cream (44.02 ± 0.79 μm) demonstrated high mycological efficacy in preventing attack by the dermatophyte on the keratin layer of the hair. Statistically, there is significant difference in keratinization between the groups as determined by one way Anova ($F_{6,28}$=211.553,p<0.05). Tukeys post hoc test was used in determining the significant differences when the Control is compared with other groups. (Table 116) and (Fig.82) (See Appendix).

There was significant infection (77.32 ± 0.38 μm) as indicated by the result obtained for the epidermal thickness of untreated group. The values obtained for emulsion alone (67.18 ± 0.71 μm), 0.5% *Cassia occidentalis* extract (61.96 ± 0.38 μm),

Table 71 Effect of *Cassia occidentalis* formulations on keratinization (µm) of skin infected by *Trichophyton mentagrophytes*

Group 1	18.42	19.46	18.82	18.72	17.89
Group 2	45.61	42.72	44.66	45.48	41.65
Group 3	17.37	17.67	18.67	18.06	19.57
Group 4a	20.63	20.39	19.82	20.78	21.98
Group 4b	19.15	21.45	17.35	20.81	19.39
Group 5a	23.69	23.38	21.34	22.05	22.70
Group 5b	23.83	21.72	24.84	23.78	24.71
Group 6a	28.62	29.24	27.76	27.39	30.38
Group 6b	27.47	29.57	27.33	29.61	27.70

Group 1 – Animals without any treatment – Negative control

Group 2 – Animals treated with cream containing 1% Clotrimazole (Positive Control - a).

Group 3 – Animals treated with placebo (Emulsion base only/Positive control - b)

Group 4a – Animals treated with 0.5% medicinal plant extract alone

Group 4b – Animals treated with emulsion containing 0.5% medicinal plant extract

Group 5a – Animals treated with 1% medicinal plant extract alone

Group 5b – Animals treated with emulsion containing 1% medicinal plant extract

Group 6a- Animals treated with 2% medicinal plant extract alone

Group 6b- Animals treated with emulsion containing 2% medicinal plant extract

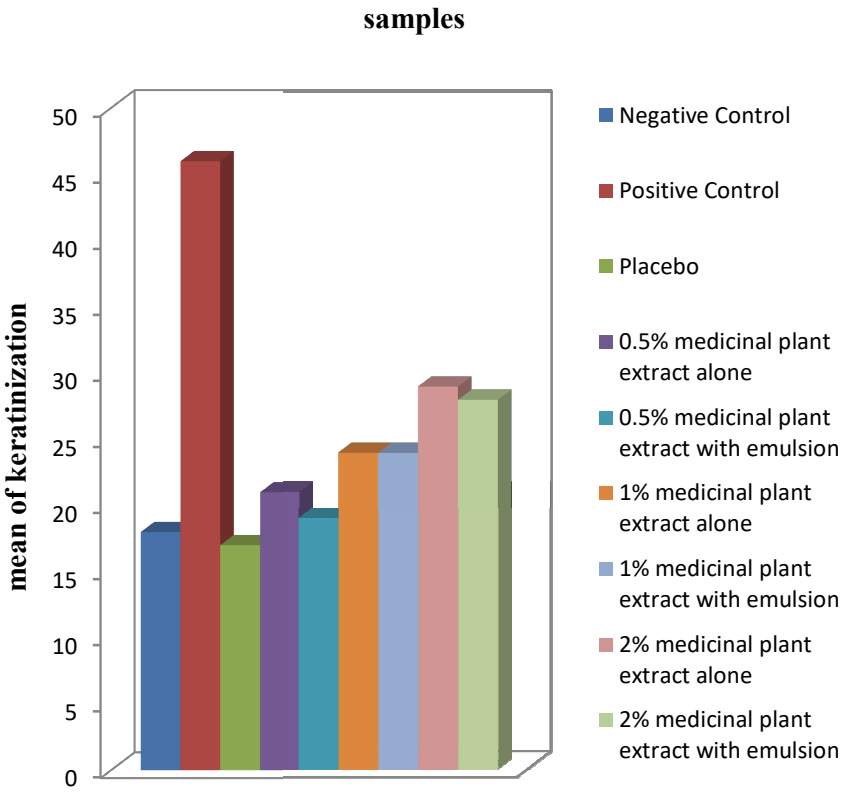


Fig.36 Effect of *Cassia occidentalis* extracts on keratinization in *Trichophyton mentagrophytes* infected skin

0.5% *Cassia occidentalis* extracts emulsion (61.52 ± 0.90 µm), 1% *Cassia occidentalis* extract (57.32 ± 0.47 µm) and 1% *Cassia occidentalis* emulsion (56.84 ± 0.61 µm) in reducing the epidermal thickness can be said to be slightly significant compared with the untreated control. However, the values obtained for 2% *Cassia occidentalis* extract alone(51.88±0.44µm) and 2% *Cassia occidentalis* emulsion with extract (51.71±0.65µm) showed indication that there was moderate reduction in the epidermal thickness thereby suggesting that the formulations are mycologically effective against the dermatophyte compared with the values obtained for the standard drug (39.24±0.93µm) (Table 72) and (Fig.37).

The statistical evaluation as shown in Table 117 and Fig.83 (See Appendix) shows that there is significance difference in epidermal thickness between groups as determined by one way Anova ($F_{6,28}$=140.269,p<0.05). A turkey post hoc test also revealed that there is significance difference in epidermal thickness between the Control and the test groups.

4.12.4.4 Malassezia*Furfur*

There was marked presence of fungal hyphae in group 1. There were no hair follicles and no sebaceous glands were observed. There were marked inflammation of the skin and marked tissue destruction was also observed in the group (Table 73). No fungal hyphae were seen in group 2. There were numerous hair follicles in the dermis and also numerous amounts of sebaceous glands. No inflammation and tissue destruction was however observed in the group (Table 73).

Group 3 animals treated with emulsion alone had moderate presence of fungal hyphae and few hair follicles. There were also few amounts of sebaceous glands in the dermis. There was moderate skin inflammation and moderate tissue destruction of the skin in the group (Plate 37).

There was moderate presence of fungal hyphae and few hair follicles in group 4a. There were also few amounts of sebaceous glands. It was observed that skin inflammation and tissue destruction were moderate. The same observations were made for group 4b (Plate 38). In group 5a, there was moderate presence of fungal hyphae in the group. Moderate

Table 72 Effect of *Cassia occidentalis* formulations on epidermal thickness (µm) of skin infected by *Trichophyton mentagrophytes*

Gpr 1	76.85	76.44	76.93	78.54	77.86
Grp 2	35.69	39.72	40.28	41.08	39.42
Grp 3	68.07	65.43	65.49	68.35	68.58
Grp 4a	63.46	61.44	61.79	61.62	61.51
Grp 4b	60.67	62.29	64.68	60.33	59.62
Grp 5a	56.39	57.41	56.11	58.34	58.35
Grp 5b	56.54	56.19	57.33	55.25	58.89
Grp 6a	50.89	53.39	51.36	52.31	51.45
Grp 6b	49.75	51.76	50.90	53.46	52.68

Group 1 – Animals without any treatment – Negative control

Group 2 – Animals treated with cream containing 1% Clotrimazole (Positive Control - a).

Group 3 – Animals treated with placebo (Emulsion base only/Positive control - b)

Group 4a – Animals treated with 0.5% medicinal plant extract alone

Group 4b – Animals treated with emulsion containing 0.5% medicinal plant extract

Group 5a – Animals treated with 1% medicinal plant extract alone

Group 5b – Animals treated with emulsion containing 1% medicinal plant extract

Group 6a- Animals treated with 2% medicinal plant extract alone

Group 6b- Animals treated with emulsion containing 2% medicinal plant extract

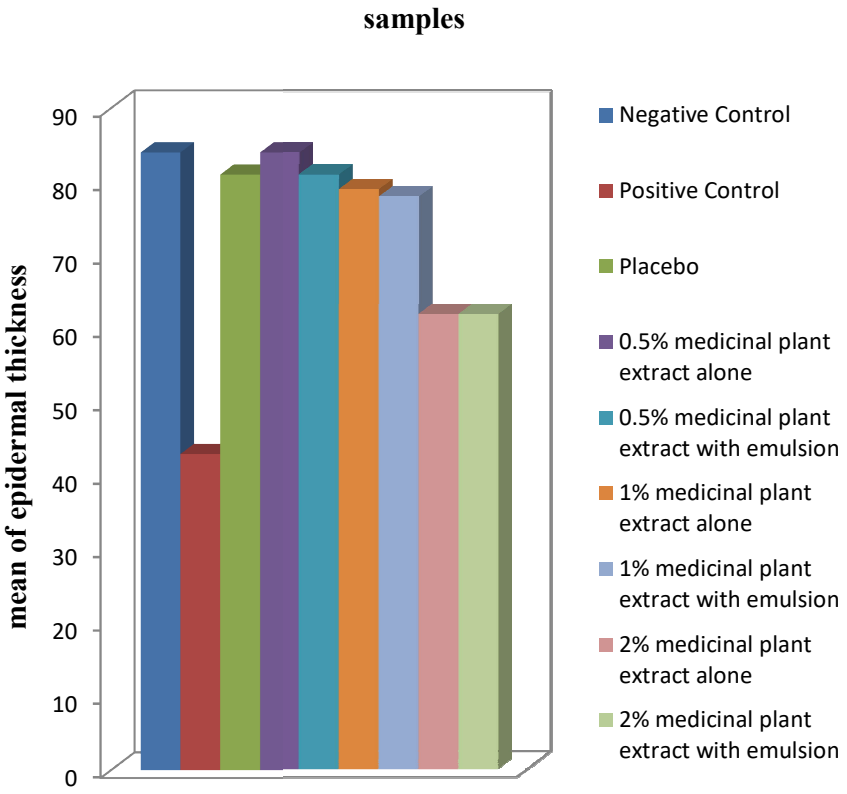


Fig.37 Effect of *Cassia occidentalis* extracts on epidermal thickness in *Trichophyton mentagrophytes* infected skin

Table 73 Mycological efficacy of *Cassia occidentalis* formulations against *Malassezia furfur*

Group	Fungal hyphae	Hair follicles	Sebaceous gland	Inflammation	Continuity/Tissue destruction
1	++	+	+	+++	+++
2	-	+++	++	+	-
3	++	+	+	++	++
4a	++	+	+	++	++
4b	++	+	+	++	++
5a	++	+	+	++	++
5b	++	+	+	++	++
6a	++	++	++	+	+
6b	++	++	++	+	+

- = Absent, Mild = +, Moderate = ++, Marked = +++

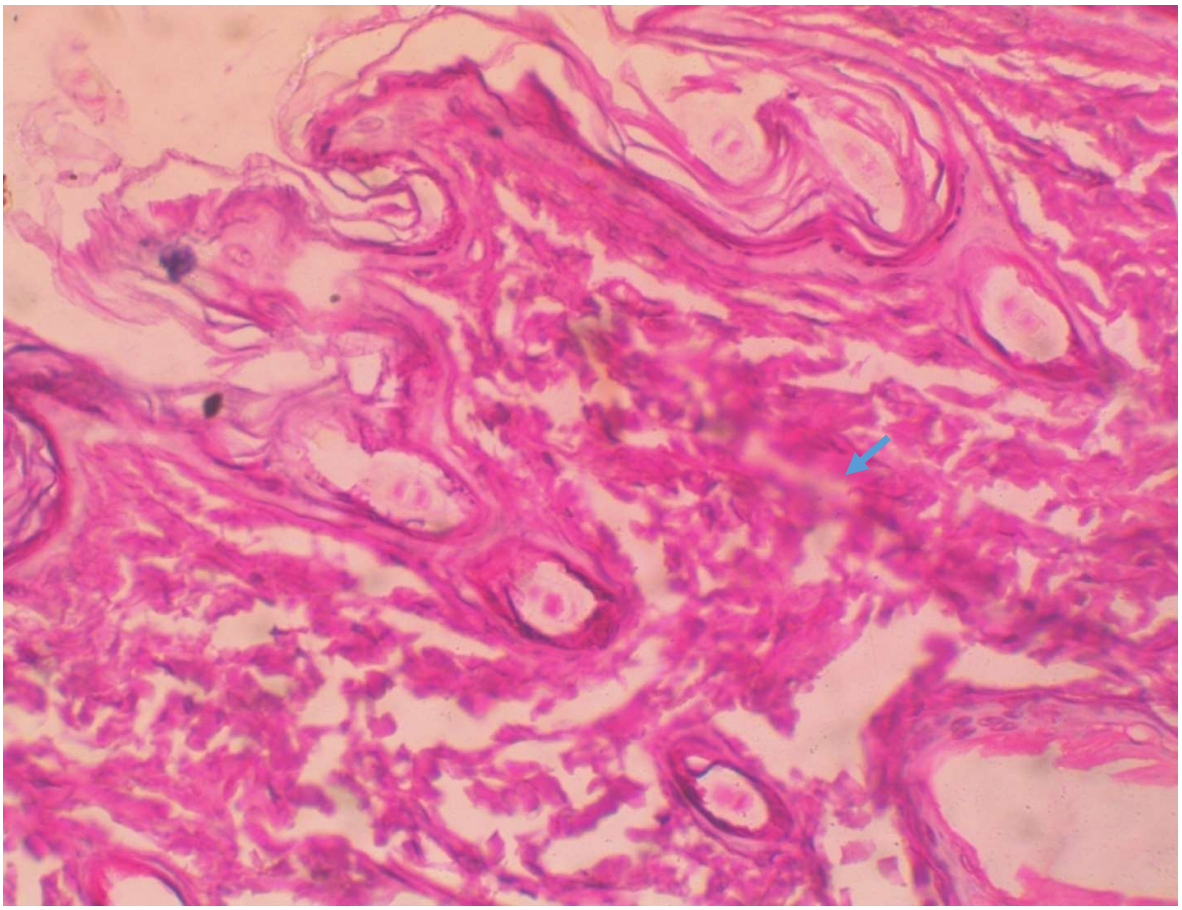


Plate 37 There is absence of sebaceous gland. HE x100

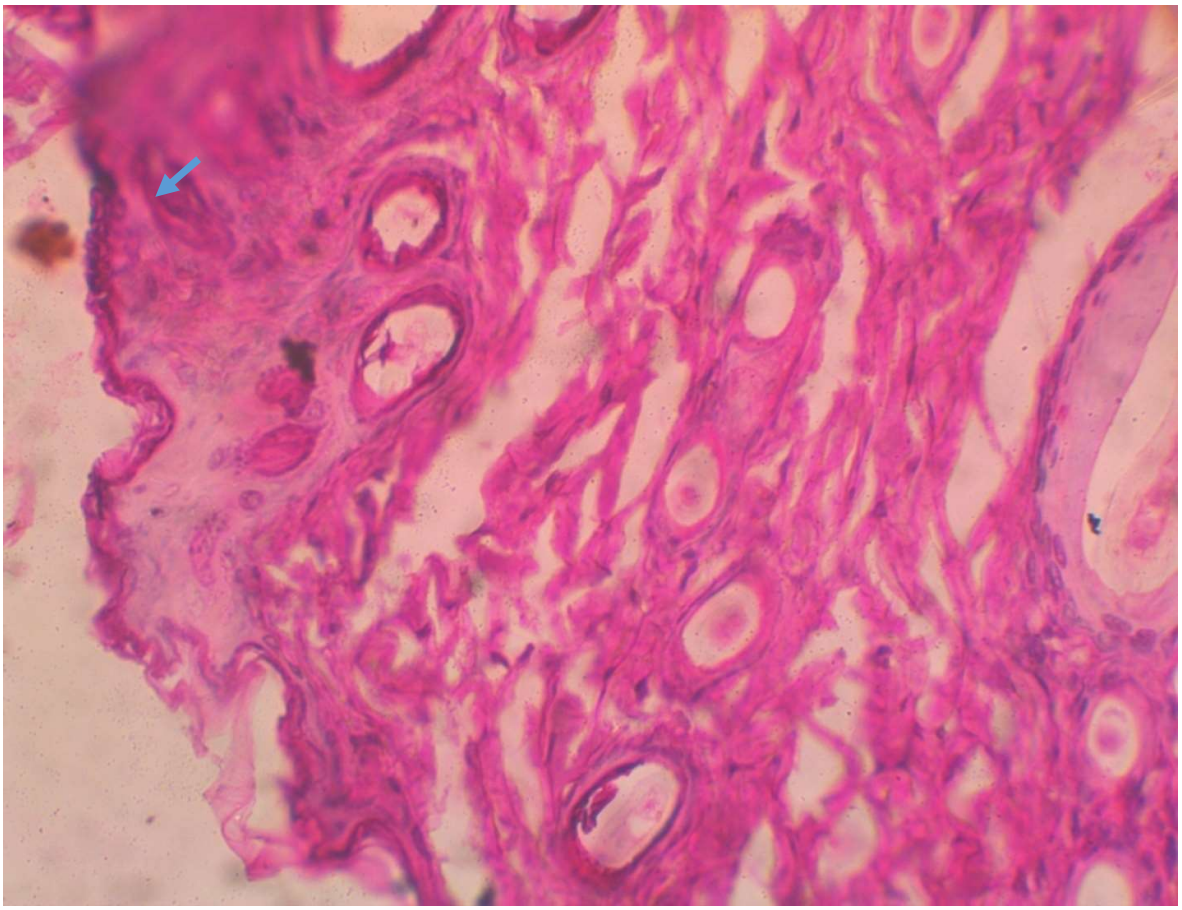


Plate 38 Inflamation of the epidermal(parakeratosis). HE x100)

amount of hair follicles enmeshed within the sparse connective tissue in the dermis were seen.

There were also moderate amount of sebaceous glands in the dermis. Inflammation of the skin was mild and tissue destruction of the skin were however moderate in the group. Same observations were also seen in group 5b. In group 6a, there was few presence of fungal hyphae in the group. Moderate amount of hair follicles enmeshed within the sparse connective tissue in the dermis were seen. There were also moderate amount of sebaceous glands in the dermis. Inflammation and tissue destruction of the skin were however mild in the group. Same observations were also seen in group 6b.

The efficacy of the formulations on the keratin layer of the hair compared with the infected untreated control is shown in Table 74 and Fig.38. The value obtained (23.41±0.63µm) for the untreated control group 1 indicated that there was moderate infection of the hairs by the dermatophyte. There was mild infection by the dermatophyte for the group treated with the emulsion alone (27.28 ± 1.00 µm), 0.5% *Cassia occidentalis* extract alone (27.17 ± 0.97 µm), the group treated with 0.5% *Cassia occidentalis* extract emulsion (27.91 ± 0.92 µm), 1% *Cassia occidentalis* extract alone (28.13 ± 0.86 µm) and 1% *Cassia occidentalis* emulsion (28.16 ± 0.18 µm) which were not statistically different from one another. This implies that there is slight efficacy by the formulations. However, 2% *Cassia occidentalis* extract alone (30.95 ± 0.75 µm) and 2% *Cassia occidentalis* extract emulsion (31.87 ± 0.78 µm) showed higher mycological efficacy compared to the other formulations. The value obtained for the standard drug 1% clotrimazole cream (55.80 ± 0.67 µm) is statistically significant in preventing attack by the dermatophyte on the keratin layer of the hair.

In conclusion, there is significant difference in keratinization between the groups as determined by one way Anova ($F_{6,28}$=182.154,p<0.05). Tukeys post hoc test was used in determining the significant differences when the Control is compared with other groups. (Table 118 and Fig.84) See Appendix.

The result obtained for the epidermal thickness of the untreated group indicated that there was significant infection (47.56 ± 0.42 µm). The values obtained for emulsion

Table 74 Effect of *Cassia occidentalis* formulations on keratinization (µm) of skin infected by *Malassezia furfur*

Group 1	24.36	21.32	22.82	23.65	24.89
Group 2	54.41	58.11	54.68	55.45	56.35
Group 3	24.33	25.47	28.59	29.46	28.57
Group 4a	26.51	26.05	25.32	27.11	30.88
Group 4b	23.65	25.06	24.64	27.86	28.33
Group 5a	28.81	28.35	24.77	29.05	29.65
Group 5b	28.68	27.97	28.42	27.61	28.11
Group 6a	33.32	30.29	28.78	31.68	30.66
Group 6b	31.43	31.55	30.68	34.89	30.78

Group 1 – Animals without any treatment – Negative control

Group 2 – Animals treated with cream containing 1% Clotrimazole (Positive Control - a).

Group 3 – Animals treated with placebo (Emulsion base only/Positive control - b)

Group 4a – Animals treated with 0.5% medicinal plant extract alone

Group 4b – Animals treated with emulsion containing 0.5% medicinal plant extract

Group 5a – Animals treated with 1% medicinal plant extract alone

Group 5b – Animals treated with emulsion containing 1% medicinal plant extract

Group 6a- Animals treated with 2% medicinal plant extract alone

Group 6b- Animals treated with emulsion containing 2% medicinal plant extract

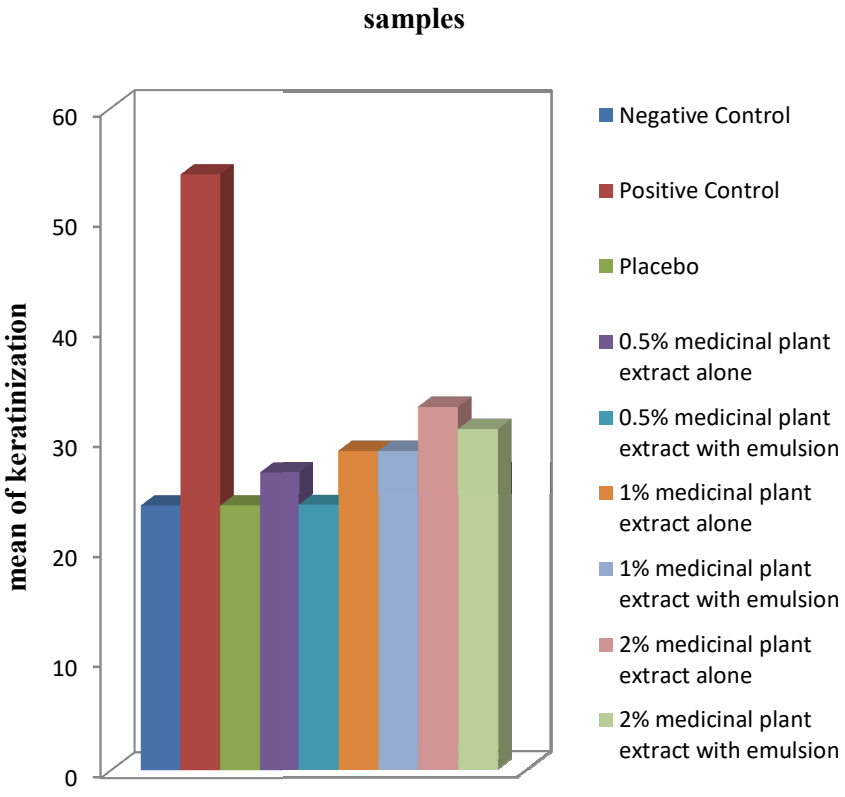


Fig.38 Effect of *Cassia occidentalis* extracts on keratinization in *Malassezia furfur* infected skin

alone (47.37 ± 0.65 µm), 0.5% *Cassia occidentalis* (41.96 ± 0.38 µm) and 0.5% *Cassia occidentalis* extract emulsion (42.32±0.65µm) indicated that there was no significant difference from the untreated group. The values obtained for 1% *Cassia occidentalis* (39.32 ± 0.74 µm), 1% *Cassia occidentalis* (39.93 ± 0.69 µm), 2% *Cassia occidentalis* extract alone (38.17 ± 0.65 µm) and 2% *Cassia occidentalis* emulsion with extract (37.74 ± 0.71 µm) indicated slight efficacy in reducing the epidermal thickness which is significant compared with the untreated control.

However, the values obtained for the standard drug (30.54 ± 0.32 µm) can be said to effectively reduce the epidermal thickness thereby indicating high efficacy against the dermatophyte (Table 75) and (Fig.39). The statistical evaluation as shown in Table 119 and Fig.85 (See Appendix) shows that there is no significance difference in epidermal thickness between groups as determined by one way Anova ($F_{6,28}$=0.736,p<0.05). A tukey post hoc test also revealed that there is no significance difference in epidermal thickness between the control and the test groups.

4.12.5 Effect of *Acalypha wilkesiana* extracts on dermatophytes

4.12.5.1 *Microsporium aoudini*

There were marked inflammation of the skin and moderate tissue destruction observed in group 1. There was moderate presence of fungal hyphae. There were also few hair follicles and few sebaceous glands were.

No inflammation and tissue destruction were seen in group 2 (Table 76). No fungal hyphae were seen in the group. There were numerous hair follicles in the dermis and also numerous amounts of sebaceous glands. Group 3 animals treated with emulsion alone had moderate skin inflammation and moderate tissue destruction of the skin. Moderate presence of fungal hyphae and few hair follicles were observed. There were also few amounts of sebaceous glands in the dermis. The same observations were recorded for groups 4a, 4b, 5a and 5b (Plate 39) In group 6a, Inflammation and tissue destruction of the skin were however mild. There was few presence of fungal hyphae in the group. Moderate amount of hair follicles enmeshed within the sparse connective tissue in the dermis were seen. There were also moderate amount of sebaceous glands in the dermis. Same observations were also seen in group 6b (Plate 40). The efficacy of

Table 75 Effect of *Cassia occidentalis* formulations on epidermal thickness (µm) of skin infected by *Malassezia furfur*

Gpr 1	48.45	46.43	46.83	48.54	47.55
Grp 2	30.32	30.69	30.19	31.67	29.82
Grp 3	47.57	45.46	46.39	48.55	48.89
Grp 4a	43.46	41.44	41.79	41.62	41.51
Grp 4b	40.67	42.29	44.68	41.33	42.62
Grp 5a	38.39	39.41	37.11	40.34	41.35
Grp 5b	37.54	41.19	41.33	40.25	39.35
Grp 6a	37.35	36.39	38.36	40.31	38.45
Grp 6b	37.38	37.72	36.80	36.36	40.42

Group 1 – Animals without any treatment – Negative control

Group 2 – Animals treated with cream containing 1% Clotrimazole (Positive Control - a).

Group 3 – Animals treated with placebo (Emulsion base only/Positive control - b)

Group 4a – Animals treated with 0.5% medicinal plant extract alone

Group 4b – Animals treated with emulsion containing 0.5% medicinal plant extract

Group 5a – Animals treated with 1% medicinal plant extract alone

Group 5b – Animals treated with emulsion containing 1% medicinal plant extract

Group 6a- Animals treated with 2% medicinal plant extract alone

Group 6b- Animals treated with emulsion containing 2% medicinal plant extract

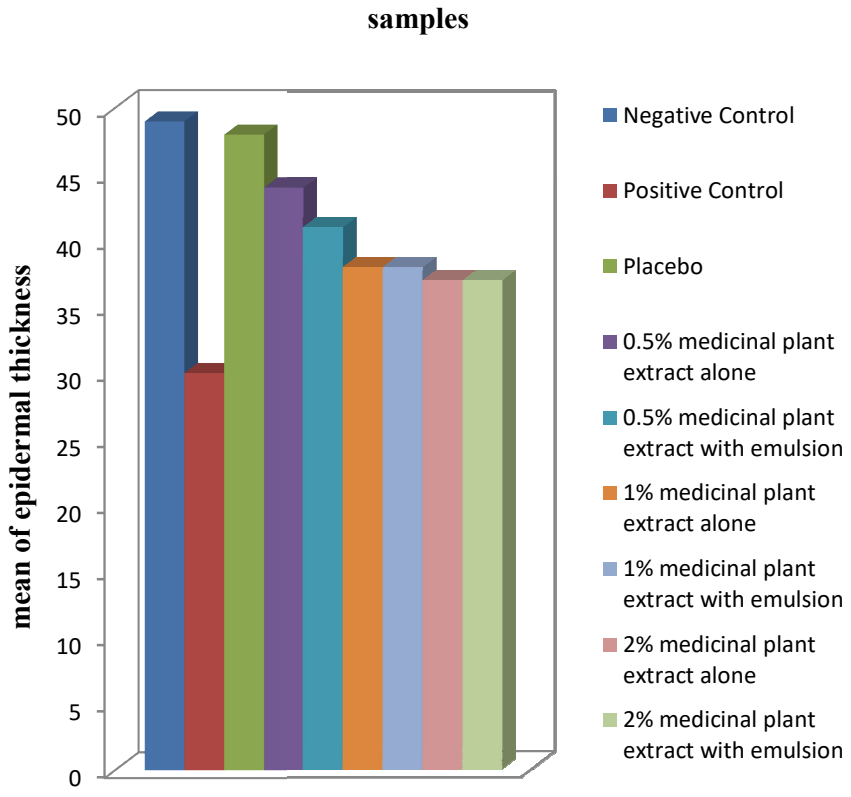


Fig.39 Effect of *Cassia occidentalis* extracts on epidermal thickness in *Malassezia furfur* infected skin

Table 76 Mycological efficacy of *Acalypha wilkesiana* formulations against *Microsporium aoudini*

Group	Fungal hyphae	Hair follicles	Sebaceous gland	Inflammation	Continuity/Tissue destruction
1	++	+	+	+++	++
2	-	+++	+++	-	-
3	++	+	+	++	++
4a	++	+	+	++	++
4b	++	+	+	++	++
5a	++	+	+	++	++
5b	++	+	+	++	++
6a	+	++	++	+	+
6b	+	++	++	+	+

- = Absent, Mild = +, Moderate = ++, Marked = +++

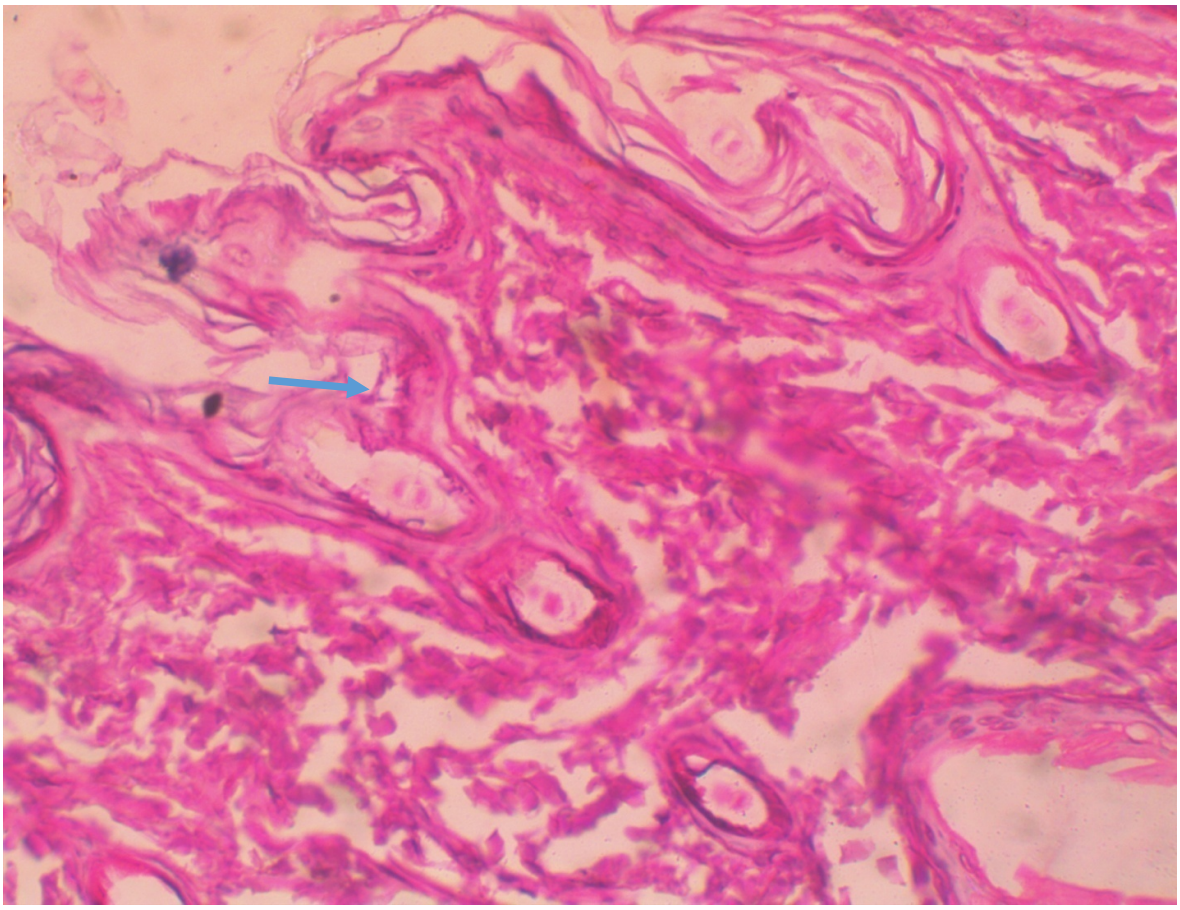


Plate 39 There is diffuse dermal capillary congestion and follicular hyperplasia. HE x400

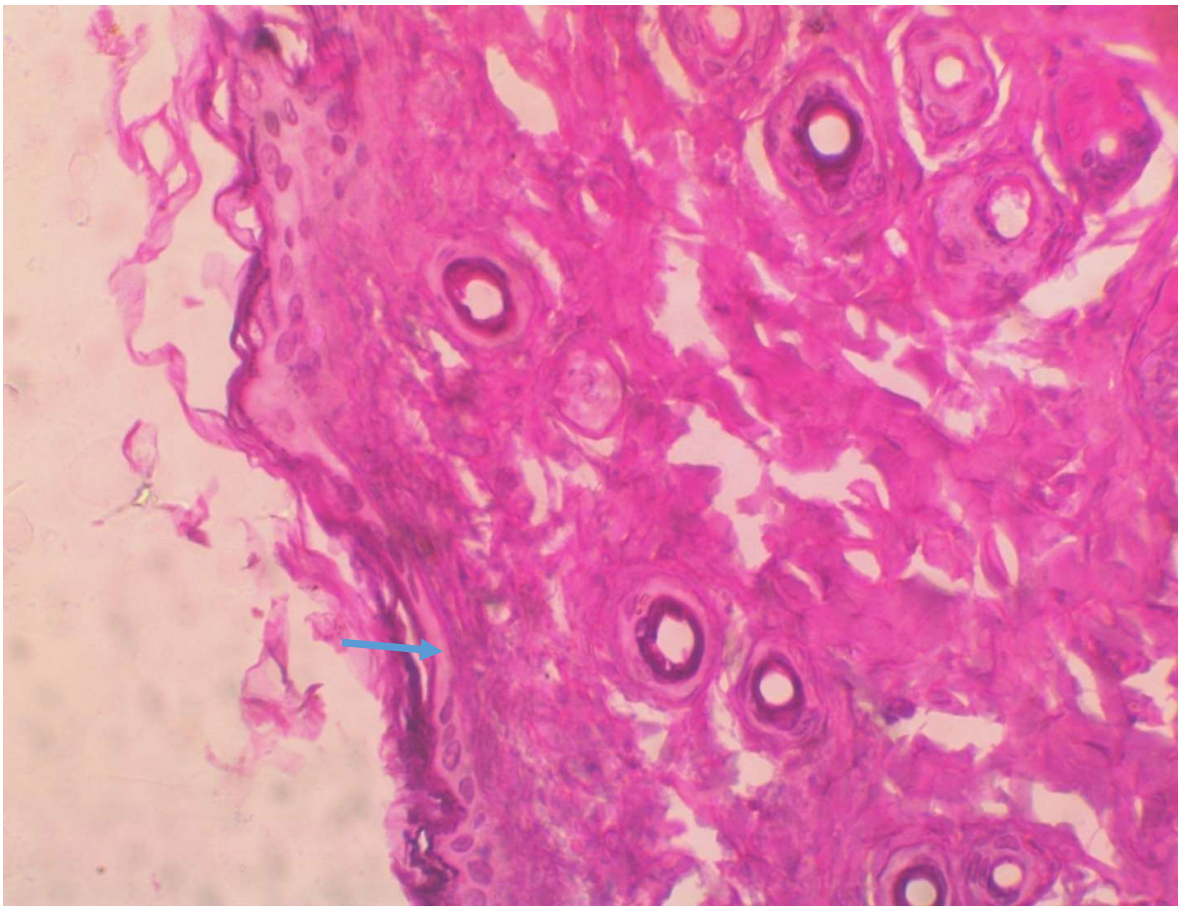


Plate 40 Thicknening of the epidermis due to hyperkeratosis. HE x100.

the formulations on the keratin layer of the hair compared with the infected untreated control is shown in Table 77 and Fig.40.

The untreated control with the value 21.18 ± 0.24 µm indicated heavy infection of the hairs by the dermatophyte. Same goes for the group treated with the emulsion alone (20.49 ± 0.50 µm), the group treated with 0.5% *Acalypha wilkesiana* extract alone (20.79 ± 0.42 µm) and the group treated with 0.5% *Acalypha wilkesiana* extract emulsion (20.58 ± 0.39 µm) which were not statistically different from one another. The values obtained for 1% *Acalypha wilkesiana* extract alone and 1% *Acalypha wilkesiana* emulsion indicated slight efficacy by the formulations. Comparatively, 2% *Acalypha wilkesiana* extract alone (31.11 ± 0.37 µm) and 2% *Acalypha wilkesiana* extract emulsion (31.01 ± 0.62 µm) showed moderate mycological efficacy.

The standard drug 1% clotrimazole cream (51.41 ± 0.56 µm) demonstrated high mycological efficacy in preventing attack by the dermatophyte on the keratin layer of the hair.

There is significant difference in keratinization between the groups as determined by one way Anova ($F_{6,28}$=537.588, $p< 0.05$). Tukeys post hoc test was used in determining the significant differences when the Control is compared with other groups. (Table 120 and Fig.86) (See Appendix). There was significant infection (75.47 ± 0.61 µm) as indicated by the result obtained for the epidermal thickness of untreated group. The values obtained for emulsion alone (68.93 ± 0.34 µm), 0.5% *Acalypha wilkesiana* extract (61.32 ± 0.62 µm), 0.5% *Acalypha wilkesiana* extract emulsion (60.96 ± 0.42 µm), 1% *Acalypha wilkesiana* extract (63.11 ± 0.53 µm) and 1% *Acalypha wilkesiana* emulsion (62.68 ± 0.72 µm) in reducing the epidermal thickness cannot be said to be significant compared with the untreated control.

However, the values obtained for 2% *Acalypha wilkesiana* extract alone (58.36 ± 0.42 µm) and 2% *Acalypha wilkesiana* emulsion with extract (54.96 ± 0.69 µm) showed indication that there was moderate reduction in the epidermal thickness

thereby suggesting that the formulations are mycologically effective against the dermatophyte compared with the values obtained for the standard drug (44.62 ± 0.18 µm) (Table 78) and (Fig.41). The statistical evaluation as shown in Table 121 and Fig.87 (See Appendix) shows that there is significance difference in epidermal thickness between groups as determined by one way anova ($F_{6,28}$=84.638,$p<0.05$).

Table 77 Effect of *Acalypha wilkesiana* formulations on keratinization (µm) of skin infected by *Microsporium aoudini*

Group 1	20.36	21.30	21.12	20.65	21.29
Group 2	51.31	50.89	51.75	51.45	50.35
Group 3	20.18	20.54	20.63	20.46	20.62
Group 4a	20.41	20.45	20.55	20.34	20.88
Group 4b	20.59	20.38	20.76	20.69	20.73
Group 5a	21.69	21.54	21.97	21.74	21.82
Group 5b	20.90	21.38	20.91	21.05	21.19
Group 6a	30.69	31.54	30.97	31.24	31.22
Group 6b	30.30	30.98	31.11	31.05	31.19

Group 1 – Animals without any treatment – Negative control

Group 2 – Animals treated with cream containing 1% Clotrimazole (Positive Control - a).

Group 3 – Animals treated with placebo (Emulsion base only/Positive control - b)

Group 4a – Animals treated with 0.5% medicinal plant extract alone

Group 4b – Animals treated with emulsion containing 0.5% medicinal plant extract

Group 5a – Animals treated with 1% medicinal plant extract alone

Group 5b – Animals treated with emulsion containing 1% medicinal plant extract

Group 6a- Animals treated with 2% medicinal plant extract alone

Group 6b- Animals treated with emulsion containing 2% medicinal plant extract

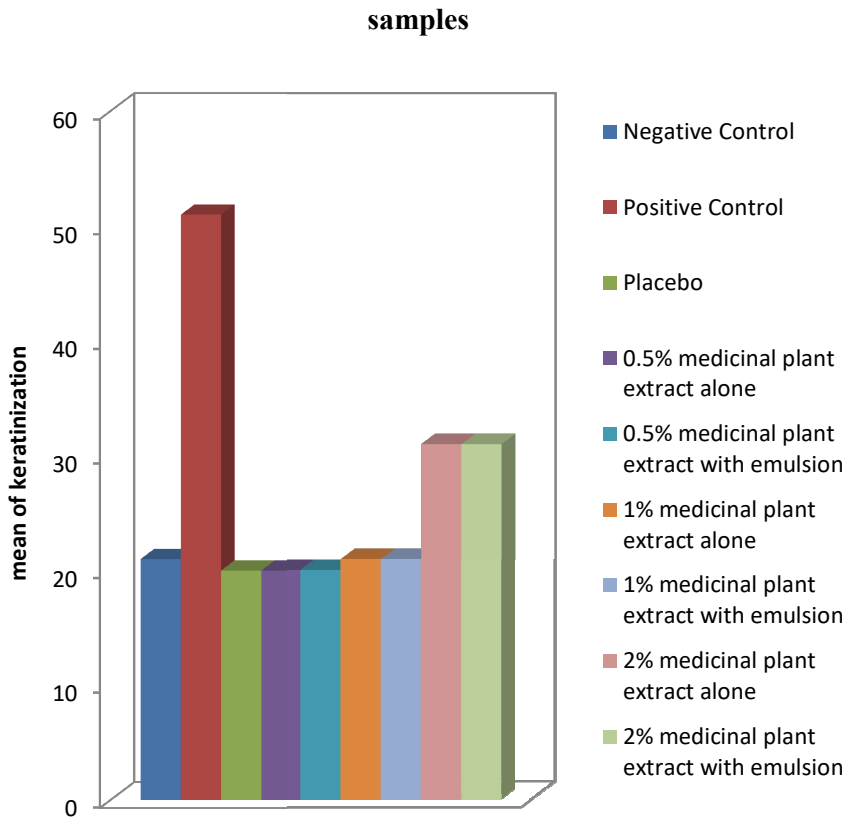


Fig.40 Effect of *Acalypha wilkesiana* extracts on keratinization in *Microsporium aoudini* infected skin

Table 78 Effect of *Acalypha wilkesiana* formulations on epidermal thickness (µm) of skin infected by *Microsporium aoudini*

Gpr 1	75.45	75.43	75.23	74.94	75.55
Grp 2	43.86	44.77	43.96	44.79	43.21
Grp 3	67.54	68.62	67.45	68.75	68.85
Grp 4a	61.26	61.38	61.55	61.22	61.31
Grp 4b	60.67	59.89	61.08	60.93	60.62
Grp 5a	63.39	62.87	63.21	62.96	62.85
Grp 5b	61.54	61.19	61.78	61.25	62.42
Grp 6a	58.25	59.15	58.29	57.86	58.25
Grp 6b	54.38	53.92	55.10	54.55	54.39

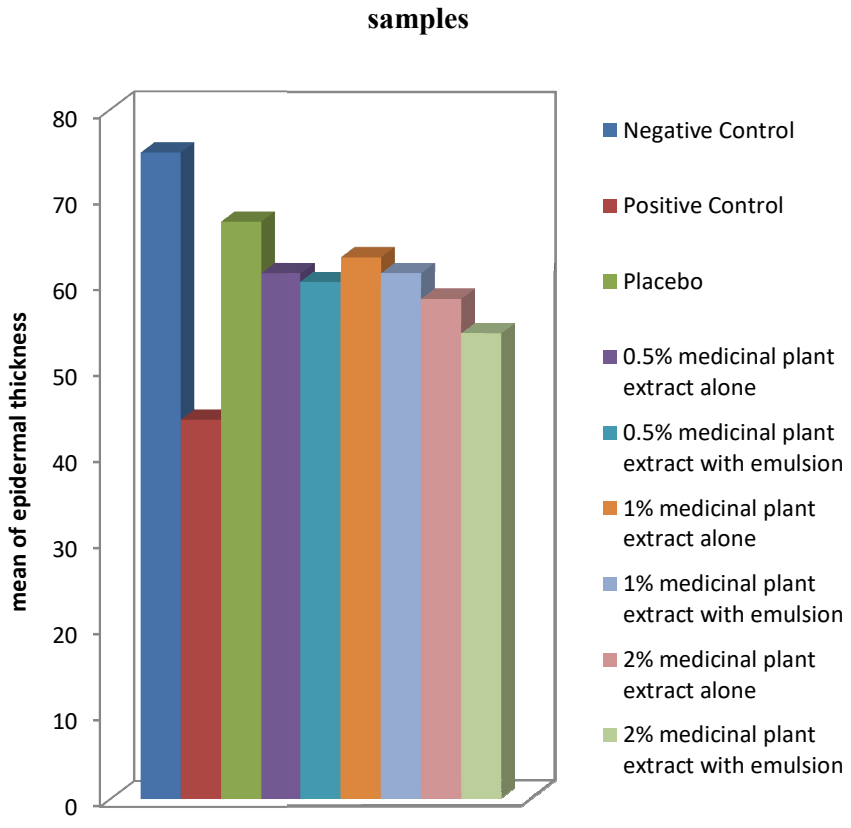


Fig.41 Effect of *Acalypha wilkesiana* extracts on epidermal thickness in *Microsporium aoudini* infected skin

A tukey post hoc test also revealed that there is significance difference in epidermal thickness between the Control and the test groups.

4.12.5.2 *Epidermophyton floccosum*

There was marked presence of fungal hyphae in group 1. No hair follicle was seen and there was few sebaceous glands observed. There were marked inflammation of the skin and marked tissue destruction was also observed in the group. No fungal hyphae were seen in group 2.There were numerous hair follicles in the dermis and also numerous amounts of sebaceous glands. No inflammation and tissue destruction was however observed in the group (Table 79).

Group 3 animals treated with emulsion alone had marked presence of fungal hyphae and few hair follicles. There were also few amounts of sebaceous glands in the dermis. There was moderate skin inflammation and marked tissue destruction of the skin in the group (Plate 41). There was moderate presence of fungal hyphae and few hair follicles in group 4a. There were also few amounts of sebaceous glands. It was observed that skin inflammation was moderate while tissue destruction was marked. The same observations were made for group 4b (Plate 42).

In group 5a, there was moderate presence of fungal hyphae in the group. Moderate amount of hair follicles enmeshed within the sparse connective tissue in the dermis were seen. There were few amounts of sebaceous glands in the dermis. Skin inflammation was moderate while tissue destruction of the skin was however marked. (Plate 44). In group 5b, there was mild presence of fungal hyphae in the group. Moderate amount of hair follicles enmeshed within the sparse connective tissue in the dermis were seen. There were moderate amounts of sebaceous glands in the dermis. Skin inflammation was moderate while tissue destruction of the skin was however marked.

In group 6a, there was few presence of fungal hyphae in the group. Moderate amount of hair follicles enmeshed within the sparse connective tissue in the dermis were seen. There were also moderate amount of sebaceous glands in the dermis. Inflammation and tissue destruction of the skin were however mild in the group (Table 79). Same observations were also seen in group 6b

Table 79 Mycological efficacy of *Acalypha wilkesiana* formulations against *Epidermophyton floccosum*

Group	Fungal hyphae	Hair follicles	Sebaceous gland	Inflammation	Continuity/Tissue destruction
1	+++	-	+	+++	+++
2	-	+++	+++	-	-
3	+++	+	+	++	+++
4a	++	+	+	++	+++
4b	++	+	+	++	+++
5a	++	++	+	++	+++
5b	+	++	++	++	++
6a	+	++	++	+	+
6b	+	++	++	+	+

- = Absent Mild = +, Moderate = ++, Marked = +++

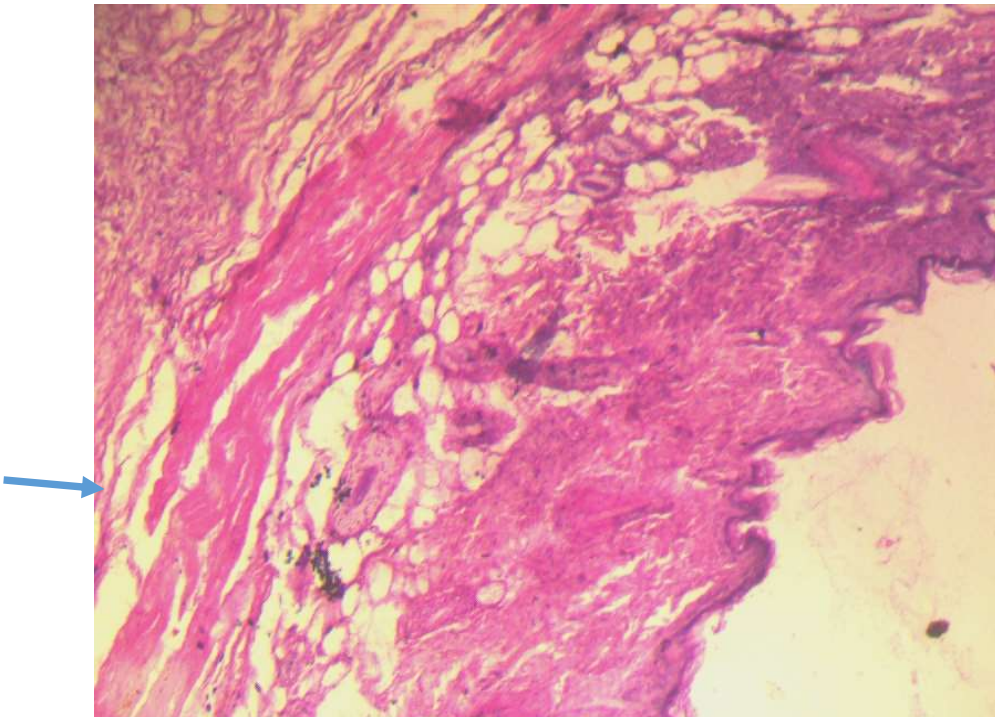


Plate 41. Cornification and inflammation of the epidermis.HE x100.

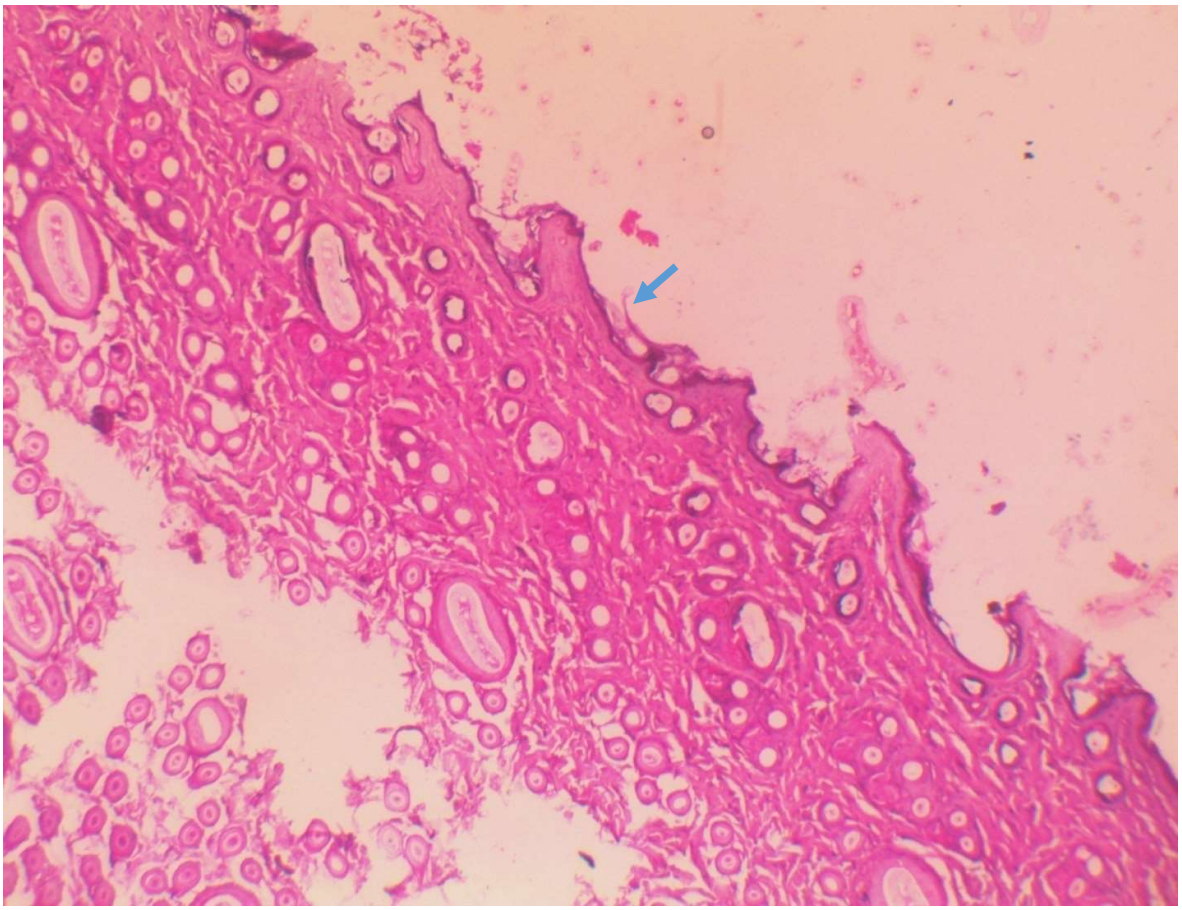


Plate 42 There is dermal congestion and acute inflammatory cell infiltrate. HE x400

The efficacy of the formulations on the keratin layer of the hair compared with the infected untreated control is shown in Table 80 and Fig.42. There was heavy infection of the hair by the dermatophyte as indicated by the value obtained (22.61 ± 0.45 µm).

The group treated with the emulsion alone (24.21 ± 0.72 µm) showed that the emulsion had no efficacy whatsoever on the dermatophyte. Application of 0.5% *Acalypha wilkesiana* extract emulsion (28.11 ± 0.51 µm) and 0.5% *Acalypha wilkesiana* extract alone (28.42 ± 0.24 µm) on the infected skin of the animal showed slight difference from the values obtained for the untreated group. This implies that they had slight effect in curtailing the infection caused by the dermatophyte. The trend of activities increasing with concentration increase can been seen in the group treated with 1% extract alone (31.26 ± 0.18 µm), 1% *Acalypha wilkesiana* emulsion (31.29 ± 0.63 µm), 2% *Acalypha wilkesiana* extract alone (34.22 ± 0.08 µm) and 2% *Acalypha wilkesiana* extract emulsion (33.65 ± 0.74 µm). The effectiveness of 1% clotrimazole cream (49.74 ± 0.38 µm) in preventing attack by the dermatophyte on the keratin layer of the hair is demonstrated by the high value recorded. Statistically, there is significant difference in keratinization between the groups as determined by one way Anova ($F_{6,28}$=248.738,p<0.05) Using Tukey's post hoc test confirms the difference. (Table 122 and Fig.88) See appendix.

The results obtained for the epidermal thickness of untreated group indicated significant infection (81.26 ± 0.35 µm). The value obtained for emulsion alone (75.26 ± 0.32 µm) indicated that the emulsion had slight effect on the epidermal thickness of the animals. The efficacy demonstrated by 0.5% *Acalypha wilkesiana* extract (73.37 ± 0.53 µm), 0.5% *Acalypha wilkesiana* extract emulsion (73.18 ± 0.14 µm), 1% *Acalypha wilkesiana* (72.33 ± 0.61 µm) and 1% *Acalypha wilkesiana* emulsion (71.52 ± 0.72 µm) in reducing the epidermal thickness can be said to be slightly significant compared with the untreated control. However, the values obtained for 2% *Acalypha wilkesiana* (61.25 ± 0.33 µm) and 2% *Acalypha wilkesiana* emulsion (61.18±0.15µm) showed moderate effectiveness in treating the infection compared with the values obtained for the standard drug (43.69 ± 0.45 µm) (Table 81) and (Fig.43). The statistical evaluation as shown in Tables 123 and Fig.89 (See appendix) shows that there is significance difference in epidermal thickness between the groups.

Table 80 Effect of *Acalypha wilkesiana* formulations on keratinization (µm) of skin infected by *Epidermophyton floccosum*

Group 1	22.36	22.45	21.92	22.65	22.35
Group 2	50.42	50.34	51.01	50.25	50.32
Group 3	24.11	24.34	24.25	24.24	23.98
Group 4a	28.29	27.89	28.45	28.51	27.88
Group 4b	28.09	28.21	28.06	27.97	28.03
Group 5a	31.21	31.16	31.07	31.24	31.33
Group 5b	31.10	31.42	30.91	31.15	31.22
Group 6a	34.26	34.14	34.18	33.79	34.02
Group 6b	34.05	32.98	33.61	34.15	34.19

Group 1 – Animals without any treatment – Negative control

Group 2 – Animals treated with cream containing 1% Clotrimazole (Positive Control - a).

Group 3 – Animals treated with placebo (Emulsion base only/Positive control - b)

Group 4a – Animals treated with 0.5% medicinal plant extract alone

Group 4b – Animals treated with emulsion containing 0.5% medicinal plant extract

Group 5a – Animals treated with 1% medicinal plant extract alone

Group 5b – Animals treated with emulsion containing 1% medicinal plant extract

Group 6a- Animals treated with 2% medicinal plant extract alone

Group 6b- Animals treated with emulsion containing 2% medicinal plant extract

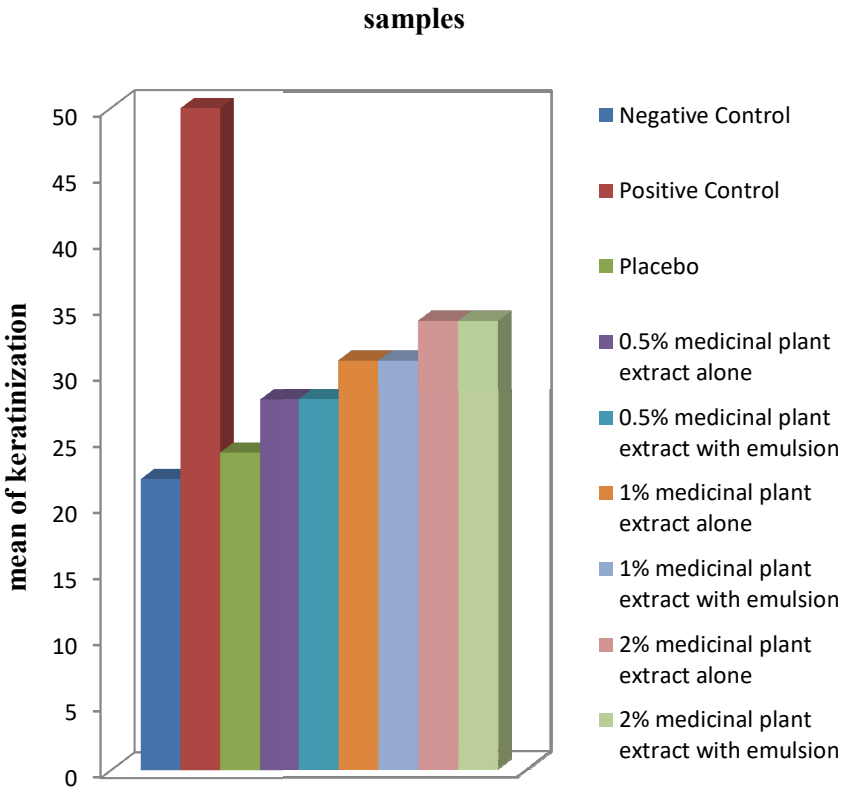


Fig.42 Effect of *Acalypha wilkesiana* extracts on keratinization in *Epidermophyton floccosum* infected skin

Table 81 Effect of *Acalypha wilkesiana* formulations on epidermal thickness (µm) of skin infected by *Epidermophyton floccosum*

Gpr 1	81.15	80.94	81.23	81.14	81.09
Grp 2	43.91	44.58	44.23	44.19	44.21
Grp 3	75.06	74.92	75.15	75.33	74.95
Grp 4a	73.26	73.25	73.42	73.28	73.31
Grp 4b	73.08	72.89	73.21	72.96	73.12
Grp 5a	73.39	72.97	73.21	73.27	72.85
Grp 5b	71.54	71.31	71.35	71.41	72.02
Grp 6a	60.93	61.15	61.32	61.26	61.29
Grp 6b	61.18	61.08	61.25	61.15	61.22

Group 1 – Animals without any treatment – Negative control

Group 2 – Animals treated with cream containing 1% Clotrimazole (Positive Control - a).

Group 3 – Animals treated with placebo (Emulsion base only/Positive control - b)

Group 4a – Animals treated with 0.5% medicinal plant extract alone

Group 4b – Animals treated with emulsion containing 0.5% medicinal plant extract

Group 5a – Animals treated with 1% medicinal plant extract alone

Group 5b – Animals treated with emulsion containing 1% medicinal plant extract

Group 6a- Animals treated with 2% medicinal plant extract alone

Group 6b- Animals treated with emulsion containing 2% medicinal plant extract

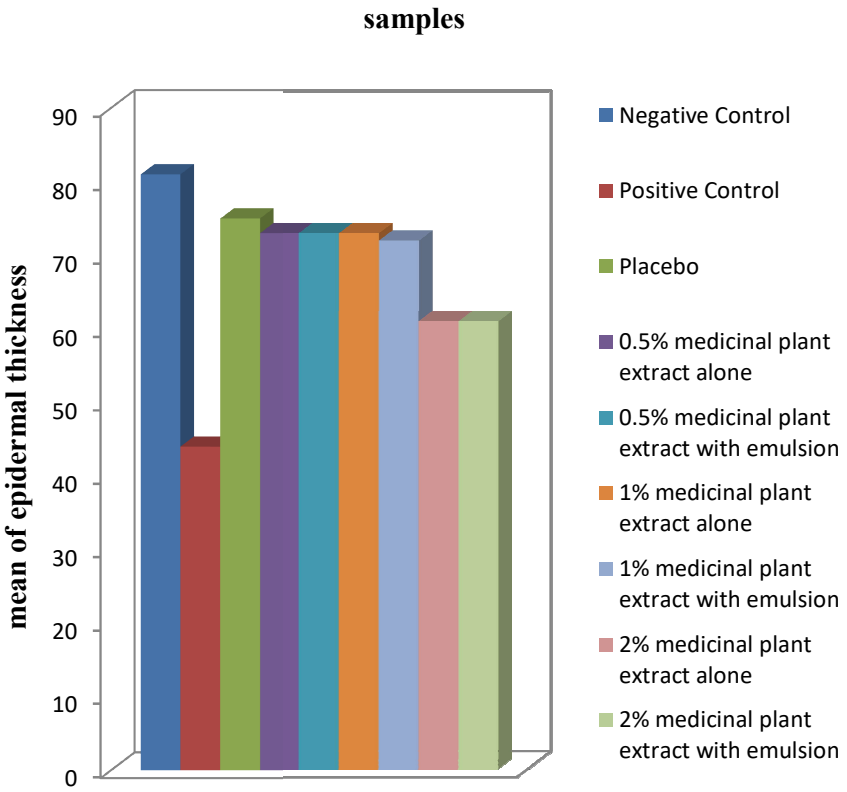


Fig.43 Effect of *Acalypha wilkesiana* extracts on epidermal thickness in *Epidermophyton floccosum* infected skin

4.12.5.3 *Trichophyton mentagrophytes*

There was moderate inflammation of the skin and tissue destruction. There was moderate presence of fungal hyphae in group 1. Few hair follicles and few sebaceous glands were observed.

No inflammation and tissue destruction was however observed in the group (Table 82). No fungal hyphae were seen in group 2. There were numerous hair follicles in the dermis and moderate amounts of sebaceous glands.

There was moderate skin inflammation and tissue destruction of the skin in the group 3 animals treated with emulsion alone. There was moderate presence of fungal hyphae and few hair follicles. There were also few amounts of sebaceous glands in the dermis(Plate 43)

There was few presence of fungal hyphae and few hair follicles in group 4a. There were also few amounts of sebaceous glands. It was observed that skin inflammation and tissue destruction was moderate. The same observations were made for group 4b.

In group 5a, there was few presence of fungal hyphae in the group. Few amount of hair follicles enmeshed within the sparse connective tissue in the dermis were seen. There were moderate amounts of sebaceous glands in the dermis. Skin inflammation and tissue destruction were moderate (Plate 44). The same observations were seen for groups 5b, 6a and 6b. The efficacy of the formulations on the keratin layer of the hair compared with the infected untreated control is shown in Table 83 and Fig.44. The value obtained (27.24 ± 0.52 μm) for the untreated control group 1 indicated that there was moderate infection of the hairs by the dermatophyte. The same conclusion can be made for the group treated with the emulsion alone (27.25 ± 0.44 μm).

The results obtained for the other groups indicated that the moderate infection has been reduced to mild infection as can be seen in the groups treated with 0.5% *Acalypha wilkesiana* extract alone (31.21 ± 0.33 μm), the group treated with 0.5% *Acalypha wilkesiana* extract emulsion (30.14 ± 0.18 μm), 1% *Acalypha wilkesian a*extract alone (32.41 ± 0.56 μm) and 1% *Acalypha wilkesiana* emulsion (31.72 ± 0.46 μm) which were not statistically different from one another. This implies that there is slight efficacy by the formulations. However, 2% *Acalypha wilkesiana* extract alone (37.52 ±0.37 μm)

Table 82 Mycological efficacy of *Acalypha wilkesiana* formulations against *Trichophyton mentagrophytes*

Group	Fungal hyphae	Hair follicles	Sebaceous gland	Inflammation	Continuity/Tissue destruction
1	++	+	+	++	++
2	-	+++	++	-	-
3	++	+	+	++	++
4a	+	+	+	++	++
4b	+	+	+	++	++
5a	+	+	++	++	++
5b	+	+	++	++	++
6a	+	+	++	++	++
6b	+	+	++	++	++

- = Absent, Mild = +, Moderate = ++, Marked = +++

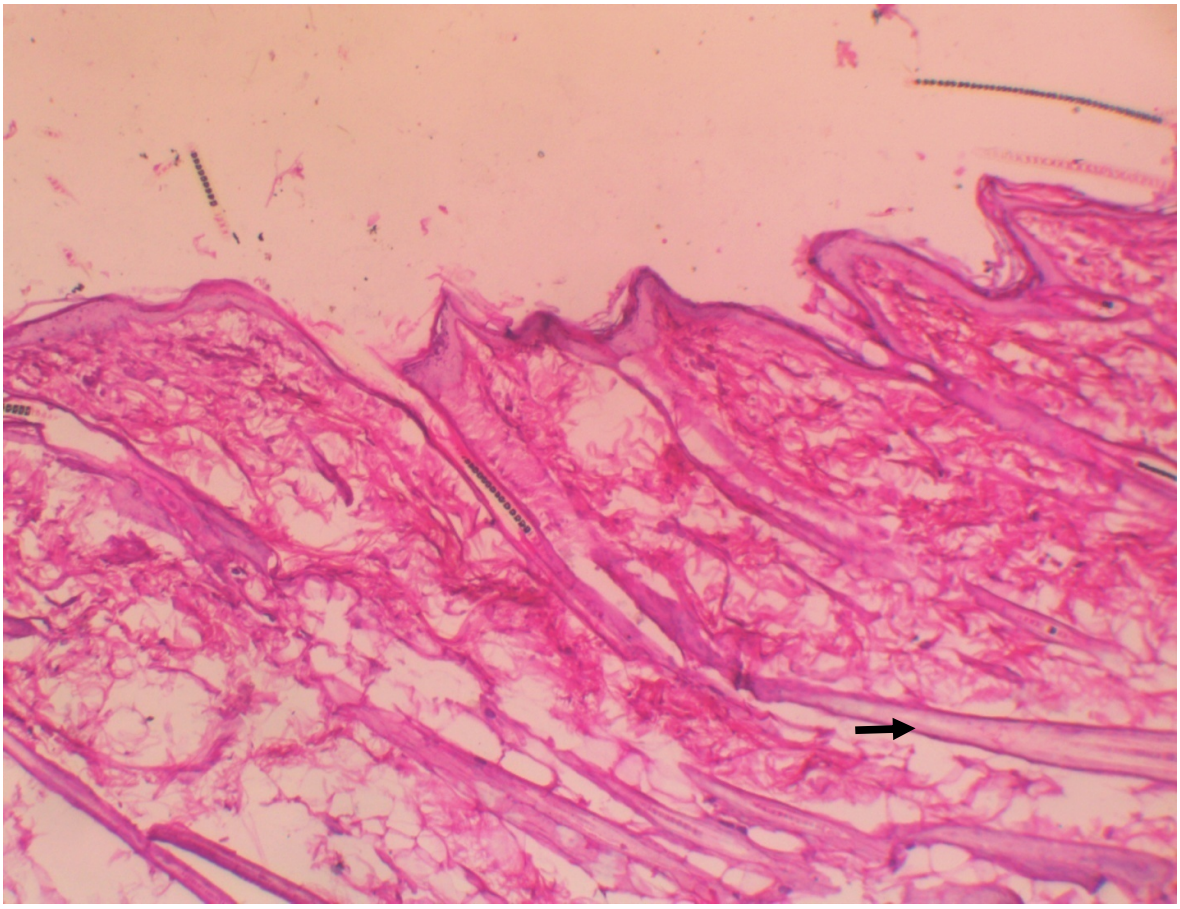


Plate 43 There is hair follicular hyperplasia (arrow). HE x400

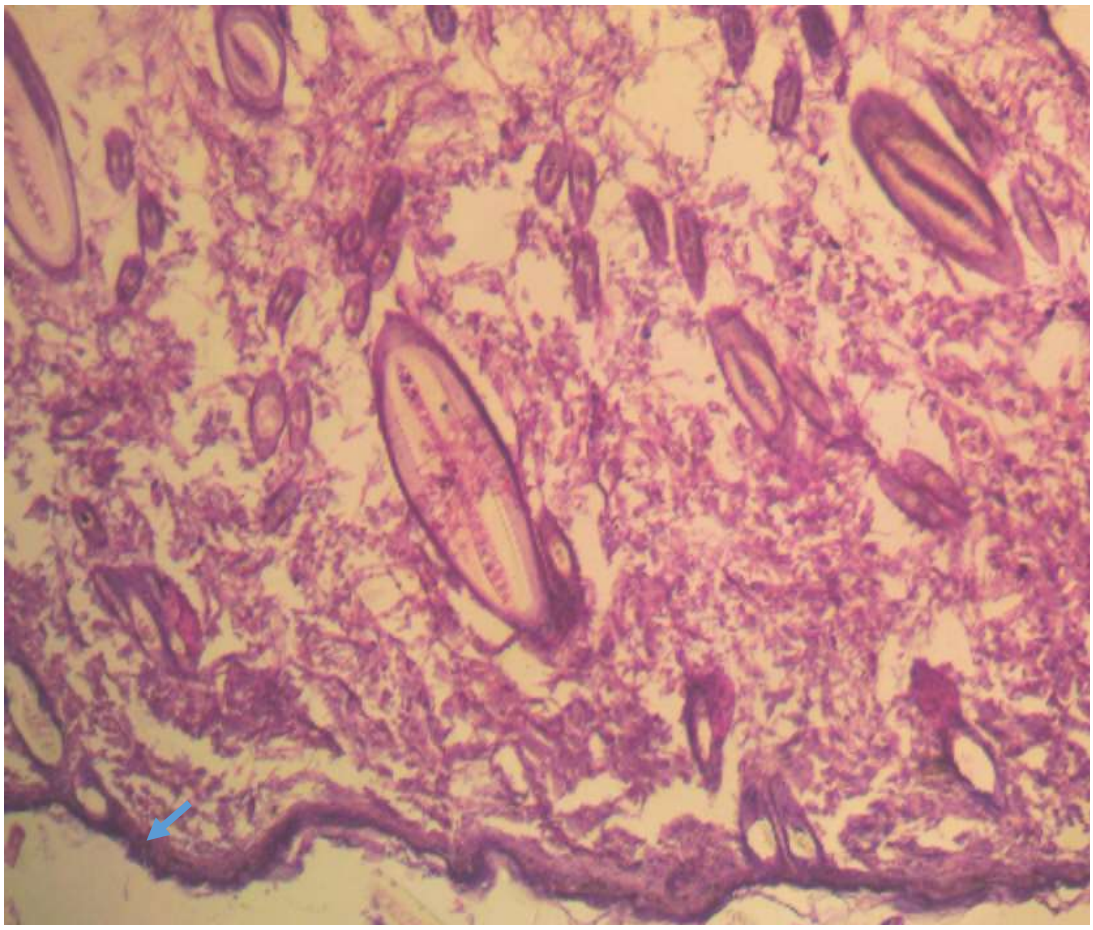


Plate 44 Moderate thicknening of the epidermis. HE x100.

Table 83 Effect of *Acalypha wilkesiana* formulations on keratinization (µm) of skin infected by *Trichophyton mentagrophytes*

Group 1	27.25	27.30	27.19	27.15	27.21
Group 2	47.21	46.91	47.24	47.09	47.28
Group 3	27.22	27.43	27.28	27.19	27.35
Group 4a	31.23	31.15	31.42	31.27	31.24
Group 4b	30.19	30.08	30.16	30.23	30.13
Group 5a	32.40	31.54	32.38	32.14	32.42
Group 5b	31.76	31.54	30.98	31.25	31.29
Group 6a	37.54	37.36	37.65	36.84	37.43
Group 6b	37.21	37.18	37.19	37.05	37.22

Group 1 – Animals without any treatment – Negative control

Group 2 – Animals treated with cream containing 1% Clotrimazole (Positive Control - a).

Group 3 – Animals treated with placebo (Emulsion base only/Positive control - b)

Group 4a – Animals treated with 0.5% medicinal plant extract alone

Group 4b – Animals treated with emulsion containing 0.5% medicinal plant extract

Group 5a – Animals treated with 1% medicinal plant extract alone

Group 5b – Animals treated with emulsion containing 1% medicinal plant extract

Group 6a- Animals treated with 2% medicinal plant extract alone

Group 6b- Animals treated with emulsion containing 2% medicinal plant extract

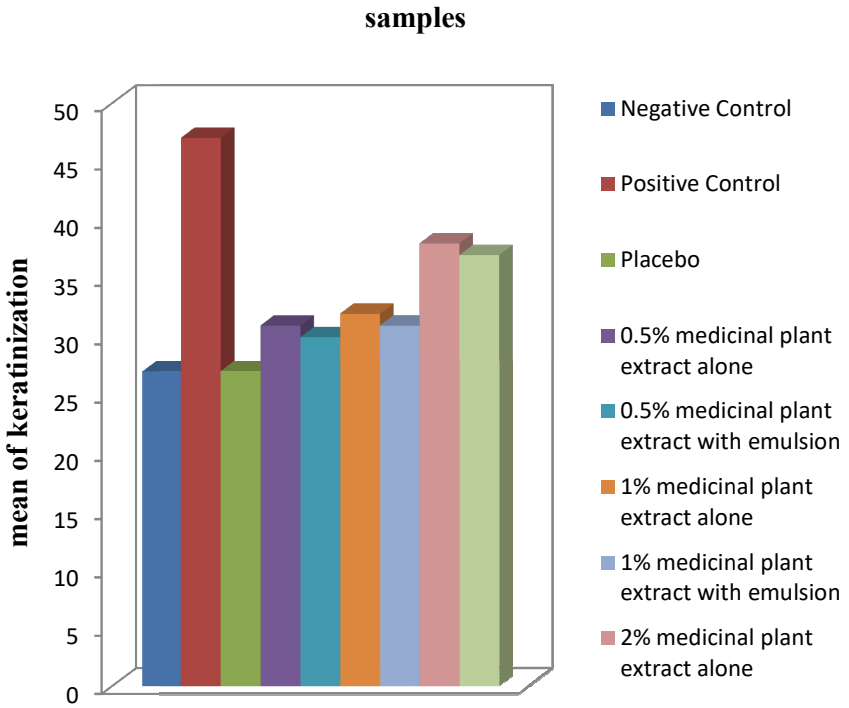


Fig.44 Effect of *Acalypha wilkesiana* extracts on keratinization in *Trichophyton mentagrophyte* infected skin

and 2% *Acalypha wilkesiana* extract emulsion (37.15 ± 0.21 μm) showed higher mycological efficacy compared to the other formulations. The value obtained for the standard drug 1% clotrimazole cream (47.22 ± 0.41 μm) is statistically significant in preventing attack by the dermatophyte on the keratin layer of the hair.

In conclusion, there is significant difference in keratinization between the groups as determined by one way Anova ($F_{6,28}$=169.737,$p<0.05$). Tukeys post hoc test was used in determining the significant differences when the Control is compared with other groups (Table 124 and Fig.90). See Appendix.

The result obtained for the epidermal thickness of the untreated group indicated that there was significant infection (61.21 ± 0.97 μm). The values obtained for emulsion alone (63.14 ± 0.76 μm), 0.5% *Acalypha wilkesiana* extract (61.75 ± 0.93 μm), 0.5% *Acalypha wilkesiana* extract emulsion (61.37 ± 0.63 μm), 1% *Acalypha wilkesiana* extract (58.92 ± 0.34 μm), 1% *Acalypha wilkesiana* emulsion (58.31 ± 0.82 μm), 2% *Acalypha wilkesiana* extract alone (59.89 ± 0.76 μm) and 2% *Acalypha wilkesiana* emulsion with extract (57.91 ± 0.85 μm) in reducing the epidermal thickness cannot be said to be significant compared with the untreated control. However, the values obtained for the standard drug (43.71 ± 0.53 μm) can be said to effectively reduce the epidermal thickness thereby indicating high efficacy against the dermatophyte (Table 84) and (Fig.45). The statistical evaluation as shown in Table 125 and Fig.91 (See Appendix) shows that there is no significance difference in epidermal thickness between groups as determined by one way anova ($F_{6,28}$=261.781,$p>0.05$). A Tukey post hoc test also revealed that there is no significance difference in epidermal thickness between the Control and the test groups.

4.12.5.4 *Malassezia furfur*

There was marked presence of fungal hyphae in group 1. No hair follicles were seen and few sebaceous glands were observed. There was moderate inflammation of the skin and marked tissue destruction (Table 85). No fungal hyphae were seen in group 2. There were moderate hair follicles in the dermis and numerous amounts of sebaceous glands. No inflammation and tissue destruction was however observed in the group (Plate 45). Group 3 animals treated with emulsion alone had marked presence of fungal

hyphae and few hair follicles. There were also few amounts of sebaceous glands in the dermis

Table 84 Effect of *Acalypha wilkesiana* formulations on epidermal thickness (µm) of skin infected by *Trichophyton mentagrophytes*

Gpr 1	61.25	61.35	61.42	61.24	61.19
Grp 2	44.69	44.49	44.39	44.61	43.96
Grp 3	63.24	63.12	63.31	63.15	63.22
Grp 4a	61.73	61.69	61.75	61.38	61.61
Grp 4b	61.27	61.33	61.18	61.21	61.32
Grp 5a	59.86	59.91	59.74	59.96	59.65
Grp 5b	59.43	59.29	59.31	59.53	59.22
Grp 6a	58.25	59.15	58.29	57.86	58.25
Grp 6b	57.28	57.89	57.33	57.24	57.43

Group 1 – Animals without any treatment – Negative control

Group 2 – Animals treated with cream containing 1% Clotrimazole (Positive Control - a).

Group 3 – Animals treated with placebo (Emulsion base only/Positive control - b)

Group 4a – Animals treated with 0.5% medicinal plant extract alone

Group 4b – Animals treated with emulsion containing 0.5% medicinal plant extract

Group 5a – Animals treated with 1% medicinal plant extract alone

Group 5b – Animals treated with emulsion containing 1% medicinal plant extract

Group 6a- Animals treated with 2% medicinal plant extract alone

Group 6b- Animals treated with emulsion containing 2% medicinal plant extract

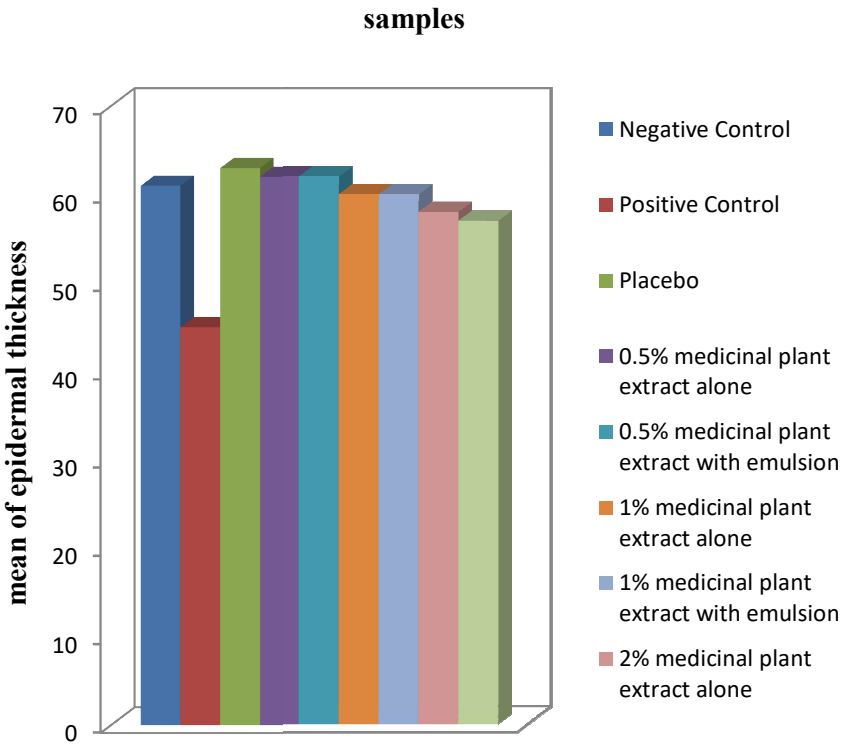


Fig.45 Effect of *Acalypha wilkesiana* extracts on epidermal thickness in *Trichophyton mentagrophyte* infected skin

Table 85 Mycological efficacy of *Acalypha wilkesiana* formulations against *Malassezia furfur*

Group	Fungal hyphae	Hair follicles	Sebaceous gland	Inflammation	Continuity/Tissue destruction
1	+++	-	+	++	+++
2	-	++	+++	-	-
3	+++	+	+	++	+++
4a	++	+	+	++	++
4b	++	+	+	++	++
5a	++	++	++	+	+
5b	++	++	++	+	+
6a	++	++	++	+	+
6b	++	++	++	+	+

- = Absent, Mild = +, Moderate = ++, Marked = +++

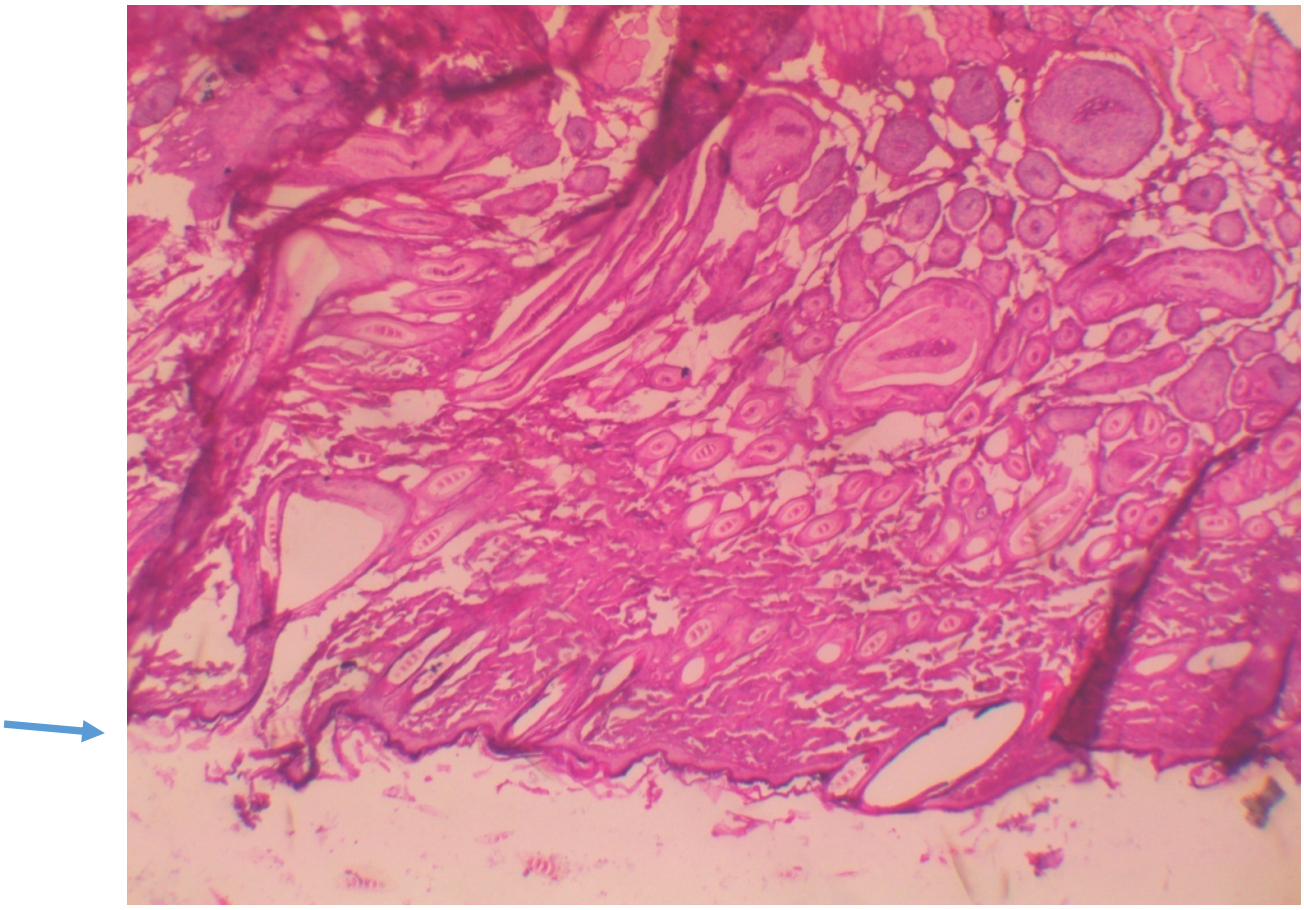


Plate 45 Hyperkeratosis of the epidermis and and congestion of dermal capillaries.HE x400.

There was moderate skin inflammation and marked tissue destruction of the skin in the group (Plate 46).

There was moderate presence of fungal hyphae and few hair follicles in group 4a. There were also few amounts of sebaceous glands. It was observed that skin inflammation and tissue destruction was moderate. The same observations were made for group 4b.

In group 5a, there was moderate presence of fungal hyphae in the group. Moderate amount of hair follicles enmeshed within the sparse connective tissue in the dermis were seen. There were moderate amounts of sebaceous glands in the dermis. Skin inflammation and tissue destruction were however mild (Table 85). The same observations were seen for groups 5b, 6a and 6b.

The efficacy of the formulations on the keratin layer of the hair compared with the infected untreated control is shown in Table 86 and Fig.46. The untreated control with the value 25.22 ± 0.35 µm indicated heavy infection of the hairs by the dermatophyte. Same goes for the group treated with the emulsion alone (25.01 ± 0.14 µm), the group treated with 0.5% *Acalypha wilkesiana* extract alone (25.37 ± 0.37 µm) and the group treated with 0.5% *Acalypha wilkesiana* extract emulsion (25.12 ± 0.11 µm) which was not statistically different from one another. The values obtained for 1% *Acalypha wilkesiana* extract alone and 1% *Acalypha wilkesiana* emulsion indicated slight efficacy by the formulations. Comparatively, 2% *Acalypha wilkesiana* extract alone (35.83 ± 0.57 µm) and 2% *Acalypha wilkesiana* extract emulsion (35.11 ± 0.44 µm) showed moderate mycological efficacy. The standard drug 1% clotrimazole cream (58.18 ± 0.82 µm) demonstrated high mycological efficacy in preventing attack by the dermatophyte on the keratin layer of the hair.

There is significant difference in keratinization between the groups as determined by one way Anova ($F_{6,28}$=320.527,$p<0.05$). Tukeys post hoc test was used in determining the significant differences when the Control is compared with other groups (Table 126 and Fig.92). See Appendix.

There was significant infection (88.39 ± 0.43 µm) as indicated by the result obtained for the epidermal thickness of untreated group. The values obtained for emulsion alone (71.69 ± 0.82 µm), 0.5% *Acalypha wilkesiana* extract(73.55 ± 0.81 µm),

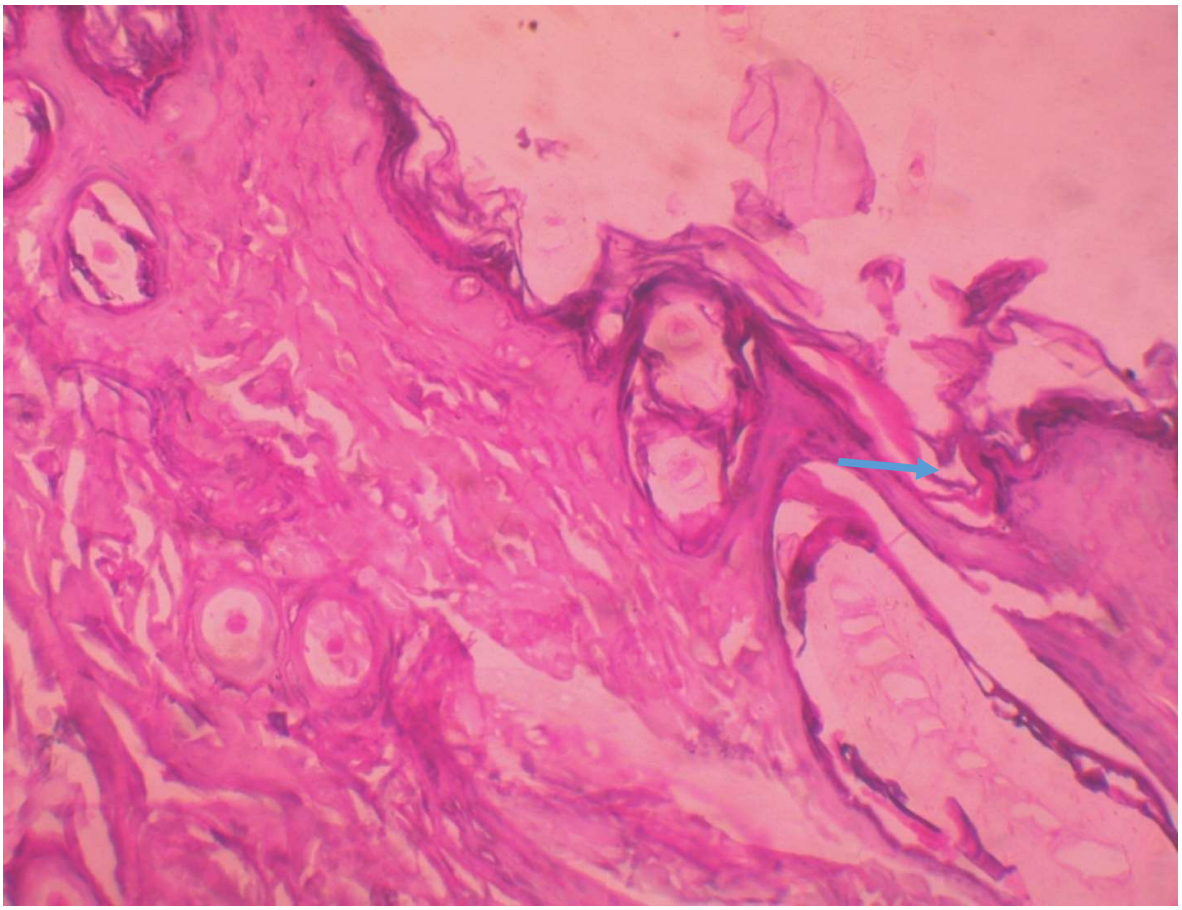


Plate 46 Tissue destruction and discontinuity of the epidermis. HE stain x100)

Table 86 Effect of *Acalypha wilkesiana* formulations on keratinization (µm) of skin infected by *Malassezia furfur*

Group 1	25.28	25.30	22.22	25.40	25.35
Group 2	58.22	58.19	58.40	58.35	58.25
Group 3	25.21	25.32	25.36	25.16	25.22
Group 4a	25.37	25.45	25.39	25.42	25.43
Group 4b	25.19	25.28	25.16	25.29	25.15
Group 5a	27.39	27.29	27.53	27.42	27.28
Group 5b	27.10	27.06	27.11	27.05	27.20
Group 6a	35.96	36.06	35.63	35.79	35.88
Group 6b	35.28	35.32	35.19	35.24	35.41

Group 1 – Animals without any treatment – Negative control

Group 2 – Animals treated with cream containing 1% Clotrimazole (Positive Control - a).

Group 3 – Animals treated with placebo (Emulsion base only/Positive control - b)

Group 4a – Animals treated with 0.5% medicinal plant extract alone

Group 4b – Animals treated with emulsion containing 0.5% medicinal plant extract

Group 5a – Animals treated with 1% medicinal plant extract alone

Group 5b – Animals treated with emulsion containing 1% medicinal plant extract

Group 6a- Animals treated with 2% medicinal plant extract alone

Group 6b- Animals treated with emulsion containing 2% medicinal plant extract

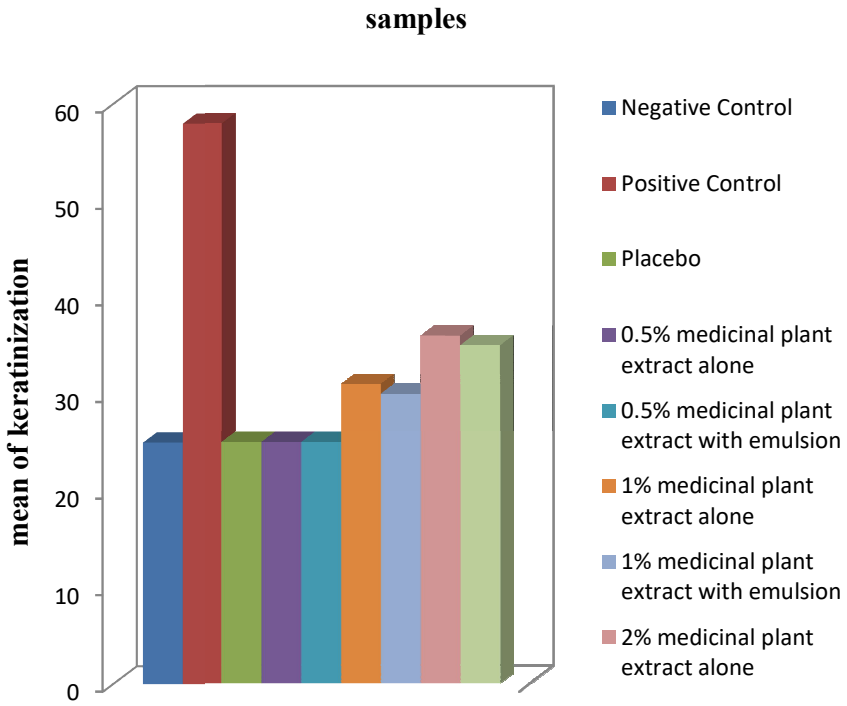


Fig.46 Effect of *Acalypha wilkesiana* extracts on keratinization in *Malassezia furfur* infected skin

0.5% *Acalypha wilkesiana* extract emulsion (73.37 ± 0.65 µm) indicated slight reduction in the epidermal thickness. However, 1% *Acalypha wilkesiana* (65.92 ± 0.34 µm) and 1% *Acalypha wilkesiana* emulsion (64.81 ± 0.51 µm) in reducing the epidermal thickness can be said to be mild compared with the untreated control. The values obtained for 2% *Acalypha wilkesiana* extract alone (54.59 ± 0.82 µm) and 2% *Acalypha wilkesiana* emulsion with extract (52.91 ± 0.39 µm) showed indication that there was moderate reduction in the epidermal thickness thereby suggesting that the formulations are mycologically effective against the dermatophyte compared with the values obtained for the standard drug (43.71 ± 0.53 µm) (Table 87) and (Fig.47).

The statistical evaluation as shown in Table 127 and Fig.93 (See Appendix) shows that there is significance difference in epidermal thickness between groups as determined by one way anova ($F_{6,28}$=316.002,p<0.05). A Tukey post hoc test also revealed that there is significance difference in epidermal thickness between the Control and the test groups.

4.13 Efficacy of emulsion preparations against test microorganisms

4.13.1 Efficacy of emulsion preparations against *Trychopyhton mentagrophyte*

Evaluation of the formulations tested indicated that their efficacy against the dermatophyte is concentration dependent. Emulsions containing 2% of all the medicinal plants extracts possess significant mycological efficacy against dermatophytic infection caused by *T.mentagrophyte*. The high efficacy could be explained on the basis of pharmacokinetics of the extracts on the skin. Data obtained for keratinization indicated that efficacy of 2% *Kigelia africana* and 2% *Acalypha wilkesiana* were statistically significant (P<0.05) with 37.01 ± 0.39 µm and 37.15 ± 0.21 µm respectively. The result obtained agrees with the works carried out on *Kigelia africana* by Jeyachandran and Mahesh (2007) and Akinyemi *et al* (2005) on *Acalypha wilkesiana*. Similarly, previous data obtained during the in-vitro antifungal tests we carried out (*Kigelia africana*, 15mm and *Acalypha wilkesiana*, 14mm) further support the efficacy of the formulations. The activity of 2% *Mitracarpus villosus* was also significant. This also agrees with our data for the medicinal plants extract during the in-vitro antifungal test.

Also our results is in agreement with the findings of Odima *et al.,* 2014 and Aboh *et al.,* 2014 who demonstrated the effectiveness of ethanolic extracts of *Mitracarpus villosus* against *T.mentagrophye*.

Table 87 Effect of *Acalypha wilkesiana* formulations on epidermal thickness (µm) of skin infected by *Malassezia furfur*

Gpr 1	88.61	88.55	88.32	88.34	88.64
Grp 2	42.64	43.17	42.51	42.38	42.29
Grp 3	71.28	71.54	71.29	71.35	71.41
Grp 4a	73.69	73.64	73.65	73.48	73.61
Grp 4b	73.37	73.29	73.41	73.34	73.42
Grp 5a	65.92	65.85	65.69	65.85	65.85
Grp 5b	64.81	64.76	64.84	64.69	64.58
Grp 6a	54.59	54.65	54.49	54.50	54.61
Grp 6b	52.91	53.06	52.88	52.93	53.79

Group 1 – Animals without any treatment – Negative control

Group 2 – Animals treated with cream containing 1% Clotrimazole (Positive Control - a).

Group 3 – Animals treated with placebo (Emulsion base only/Positive control - b)

Group 4a – Animals treated with 0.5% medicinal plant extract alone

Group 4b – Animals treated with emulsion containing 0.5% medicinal plant extract

Group 5a – Animals treated with 1% medicinal plant extract alone

Group 5b – Animals treated with emulsion containing 1% medicinal plant extract

Group 6a- Animals treated with 2% medicinal plant extract alone

Group 6b- Animals treated with emulsion containing 2% medicinal plant extract

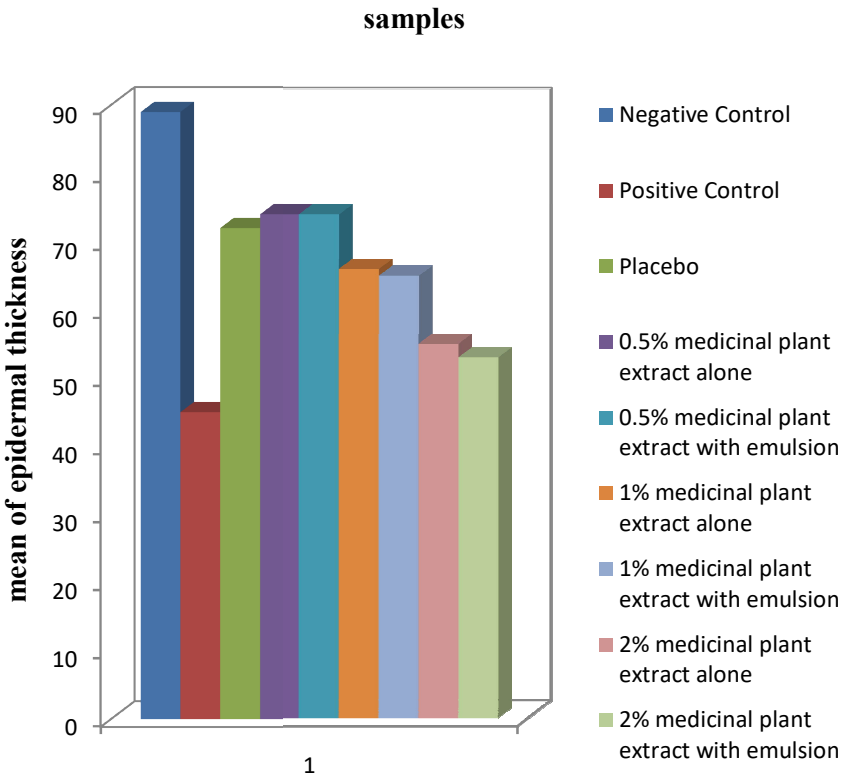


Fig.47 Effect of *Acalypha wilkesiana* extracts on epidermal thickness in *Malassezia furfur* infected skin

Though 2% *Cassia alata* (30.57 ± 0.41 µm) and 2% *Cassia occidentalis* (28.34 ± 0.52 µm) formulations also showed significant activity (P<0.05) but not as significant as the other medicinal plants formulations. The results obtained suggest that the formulations containing ethanolic extract of *K. africana* and *Acalypha wilkesiana* has the best efficacy compared to the other medicinal plants extracts formulations. This further lend support to the observation recorded during the susceptibility test carried out by Owolabi *et al.,* (2007) who reported a better activity of the ethanolic extract against C. albicans with zones of inhibition measuring 20.75 ± 4.6 mm and an MIC of 7.92 ± 1.52 mg/mL. Oyelami *et al.,* (2003) in their work evaluated the efficacy of *Acalypha wilkesiana* ointment in the treatment of superficial fungal skin diseases (Tinea corporis), the results obtained by them is comparable with what was obtained for the formulated emulsion with *Acalypha wilkesiana* in this study. Makinde *et al., (*2007) demonstrated the effects of *Cassia alata* on *T.mentagrophytes*, this study however confirms the effectiveness in treating skin disease caused by *Trychopyhton mentagrophyte.* Comparing data obtained for epidermal thickness indicated that *Kigelia africana* emulsion was the most effective (41.72 ± 0.76 µm) >*Mitracarpus villosus* emulsion (50.91 ± 0.39 µm) >*Cassia occidentalis* emulsion (51.71 ± 0.65 µm) >*Cassia alata* emulsion (53.80 ± 0.58 µm) >*Acalypha wilkesiana* emulsion (57.91 ± 0.85 µm).

4.13.2 Efficacy of emulsion preparations against *Microsporum aoudini*

2% formulations of *Cassia alata* (36.17 ± 0.59 µm) and *Mitracarpus villosus* (34.73 ± 0.37 µm) have close activity significantly (P < 0.05) compared to the reference drug. The activity of 2% *Acalypha wilkesiana* (31.01 ± 0.62 µm) and *Kigelia Africana* (31.37 ± 0.53 µm) is also of note. However, slight activity is noted in the *Cassia occidentalis* emulsion (29.70 ± 0.37 µm). Previous in-vitro studies showed that *C.alata* have considerable activities against human pathogens (Owolabi *et al*., 2007, Jeyachandran and Mahesh, 2007, John Britto, 2001, Singh and Singh, 2000, Okafor *et al*., 2001 and Makinde *et al*.,2007) which possibly makes the emulsions prepared with the extracts to be effective against the pathogen. Emulsion formulated with 2% *Mitracarpus villosus* also showed activity by reducing the epidermal thickness significantly (69.43 ± 0.44 µm). Our data however indicated that emulsion formulated with 2% *Kigelia africana*

was able to reduce the epidermal thickness significantly (44.11 ± 0.03 µm) ($P<0.05$). Comparing data obtained for epidermal thickness indicated that *Kigelia africana* emulsion was the most effective (44.11 ± 0.03 µm) >*Acalypha wilkesiana* emulsion (54.96 ± 0.69 µm) >*Cassia alata* emulsion (62.27 ± 0.75 µm) >*Mitracarpus villosus* emulsion (69.43 ± 0.44 µm) >*Cassia occidentalis* emulsion (72.54 ± 0.72 µm).

4.13.3 Efficacy of emulsion preparations against *Epidermophyton floccosum*

The high efficacy of 2% *Cassia alata* formulation against *E.floccosum* is very significant ($P<0.05$). Data obtained showed that the formulation was able to reduce the dekeratinisation (37.49 ± 0.18 µm) of the hair follicle by the dermatophyte and also reduce the epidermal thickness of the stratum cornuem (51.41 ± 0.35 µm). The keratinization by 2% *Mitracarpus villosus* and 2% *Acalypha wilkesiana* formulations exhibited noticeable activity against the dermatophytes, 34.10 ± 0.53 µm and 33.65 ± 0.74 µm respectively, which is significant ($P<0.05$). The efficacy of *Kigelia africana* is also significant (30.53 ± 0.25 µm). However, the formulation with 2% *Cassia occidentalis* (23.69 ± 0.42 µm) was not significantly active ($P>0.05$) against the dermatophyte. Comparing data obtained for epidermal thickness, it was observed that *Cassia alata* emulsion 51.41 ± 0.35 µm) was the most effective >*Mitracarpus villosus* emulsion (60.79 ± 0.91 µm) >*Acalypha wilkesiana* emulsion (61.18 ± 0.15 µm) >*Kigelia africana* emulsion (30.53 ± 0.25 µm) >*Cassia occidentalis* emulsion (23.69 ± 0.42 µm). The antifungal studies by various researchers earlier reported in 4.14.2 corroborate the observed results.

4.13.4 Efficacy of emulsion preparations against *Malassezia furfur*

Data obtained for keratinization indicated that 2% *Mitracarpus villosus* has the highest efficacy (45.99 ± 0.27 µm) which is statistically significant ($P<0.05$) against *Malassier furfur*. The activities of 2% *Kigelia africana* and 2% *Acalypha wilkesiana* can be said to be close (36.22 ± 0.36 µm) and (35.83 ± 0.57 µm) respectively. However, data obtained for both *Cassia occidentalis* and *Cassia alata* indicated that the formulations has close activities (31.87 ± 0.78 µm) and (31.73 ± 0.66 µm) which is very significant compared to that recorded for reference drug. Comparing data obtained for epidermal thickness, it was observed that *Mitracarpus villosus* emulsion (33.10 ± 0.18 µm) was the most effective >*Cassia occidentalis* emulsion (37.74 ± 0.71 µm) >*Cassia alata* emulsion

(52.74 ± 0.01 µm) >*Acalypha wilkesiana* emulsion (52.91 ± 0.39 µm) >*Kigelia africana* emulsion (56.91 ± 0.85 µm). Various scientific studies indicated the usefulness of developing drugs from *M.villosus* extract for treating skin ailments (Onawunmi *et al*., 2012, Arti and Kanika 2014, Odima *et al*., 2014, Aboh *et al.*, 2014 and Abere *et al.,* 2007).

CHAPTER FIVE

CONCLUSION AND RECOMMENDATION

5.1 **Conclusion**

1. This study showed that ethanol solvent produced more extracts of medicinal plants than water and petroleum ether.

2. All extracts were active against some of the pathogens in a concentration dependent manner. However, ethanol extracts were more active against more of the pathogens.

3. Formulated dermal creams were all stable with the exception of *Cassia occidentalis* containing cream which was unstable to centrifugation at 4000 rpm for 30 minutes.

4. Effectiveness of the dermal creams against the pathogens were in the order *Kigelia africana Mitracarpus villosus >Cassia alata >Cassia occidentalis >Acalypha wilkesiana.*

5. The formulated creams had very low skin irritation potential and comparable efficacy to positive control.

6. The formulated dermal creams are likely to be within the reach of average family and also environmentally friendly to the skin.

5.2 Contribution to knowledge

Scientific production of dermal creams from local medicinal plants which are more likely to be acceptable and available to low income people. Also the incidence of resistance and toxicity associated with long-term treatment with conventional antifungal drugs will be reduced if not completely eliminated.

5.3 Recommendation

Effective control and management of skin infections caused by dermatophytes can be achieved by regular use of previously developed herbal medicated soap with the newly developed herbal creams. Investment in the industrial production of the dermal creams on a large scale will yield great financial returns. It will also support the Government

efforts in providing jobs for our teeming youth in achieving the Millenium Development Goals program of the Government. When clinical trials must have been carried out on the dermal creams, it is recommended that characterization of the identity of the anti-dermatophytic components found within these plants be determined. It is also recommended that full spectrum of efficacy of the products on the dermatophytes be carried out and appropriate low–cost technology for the industrial production of the herbal body cream be developed.

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APPENDIX

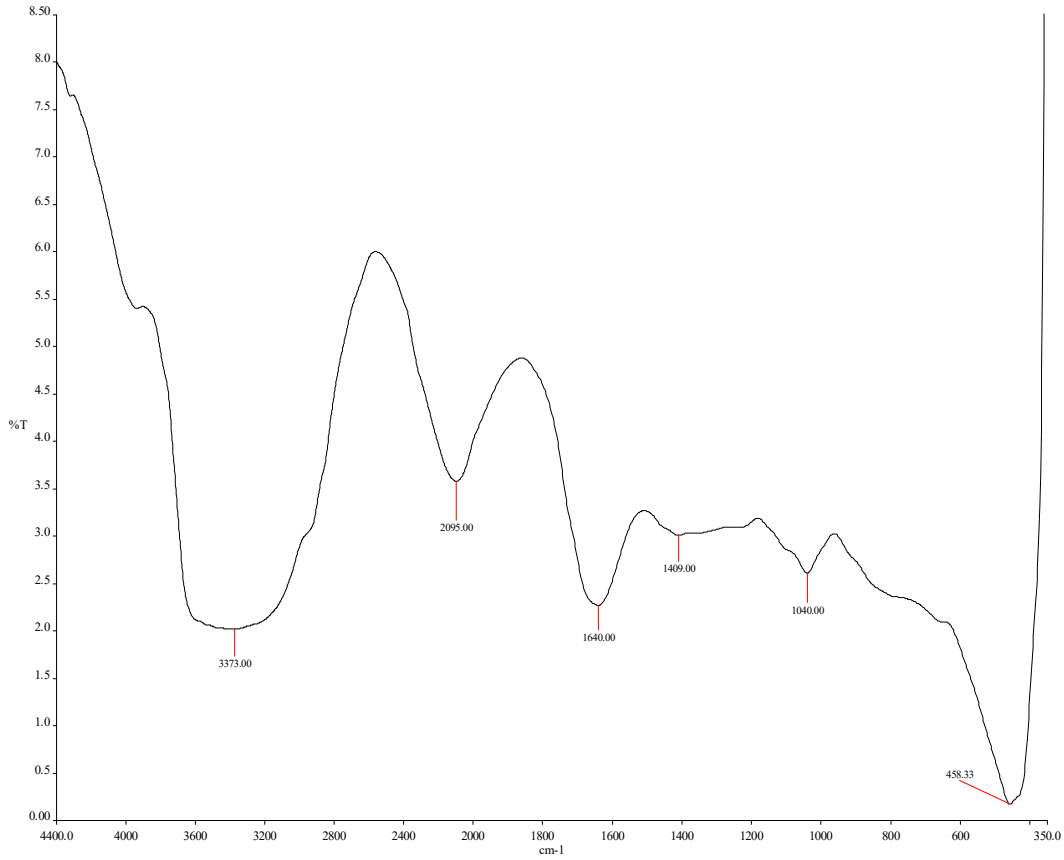

Figure 48. FTIR Spectrum of Emulsion alone (Placebo)

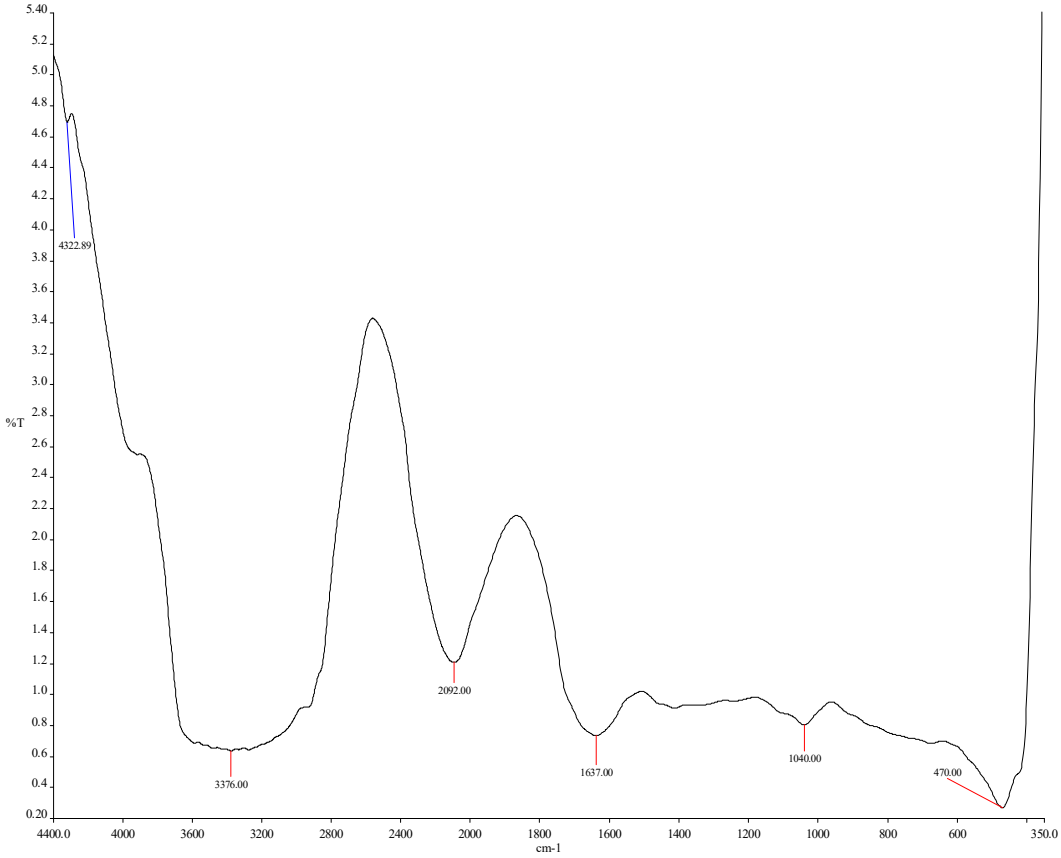

Figure 49.FTIR Spectrum of *Cassia alata* cosmeceutical

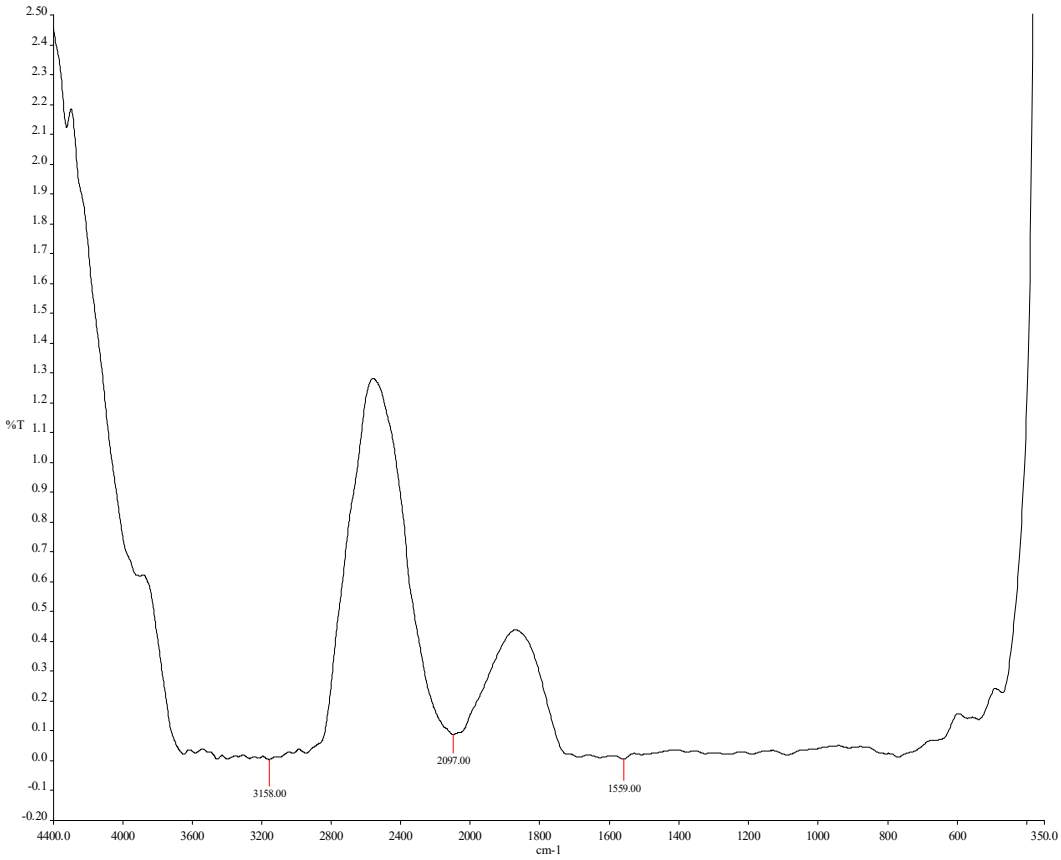

Figure 50 FTIR Spectrum of *Cassia occidentalis* cosmeceutical

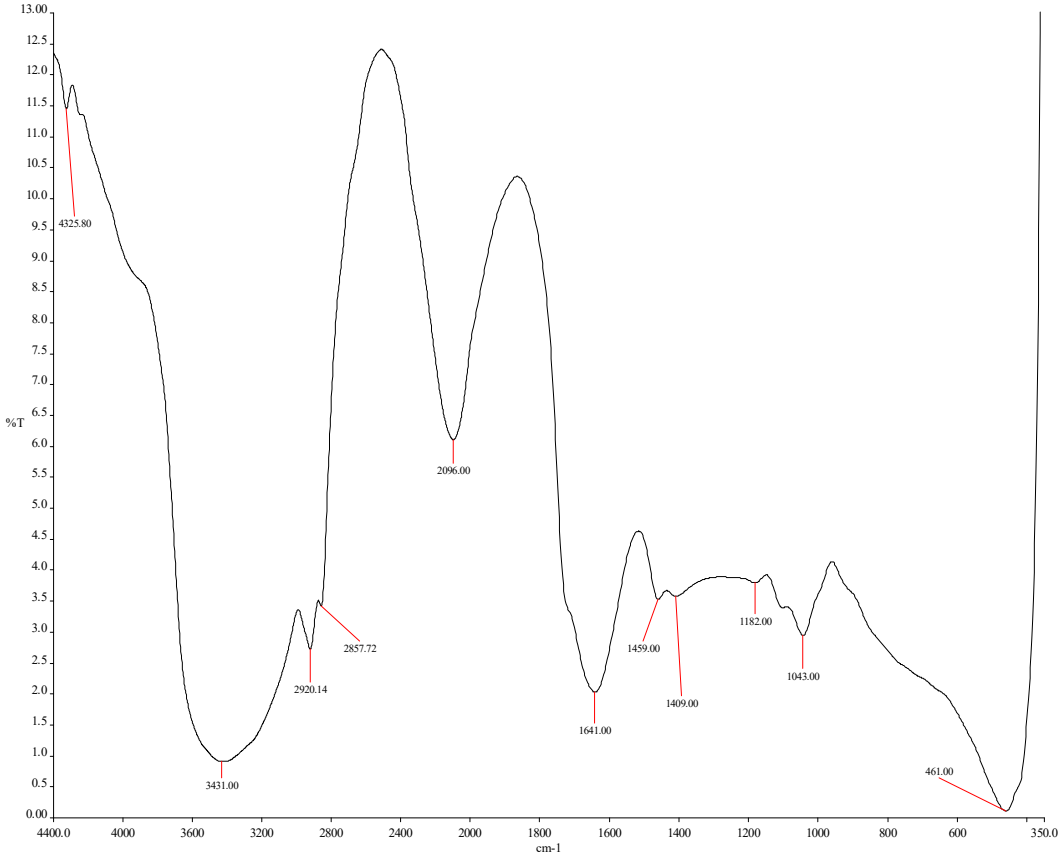

Figure 51. FTIR Spectrum of *Mitracarpus villosus* cosmeutical

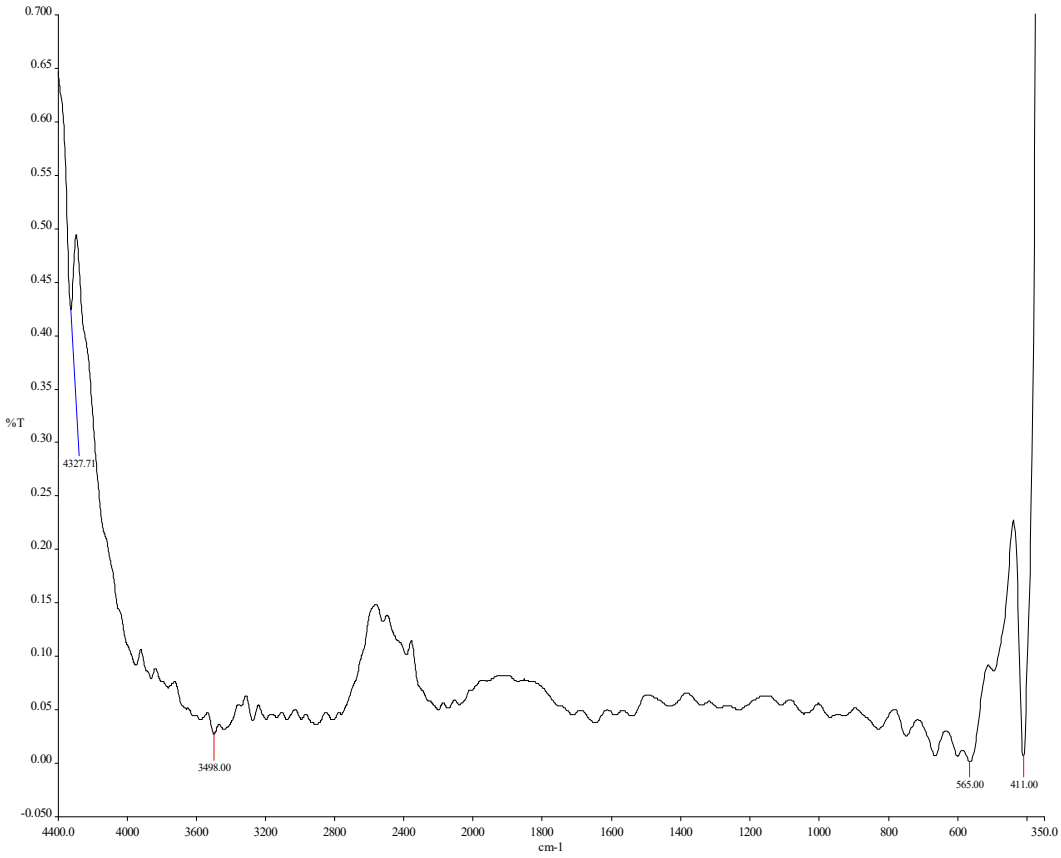

Figure 52. FTIR Spectrum of *Acalypha wilkesiana* cosmeutical

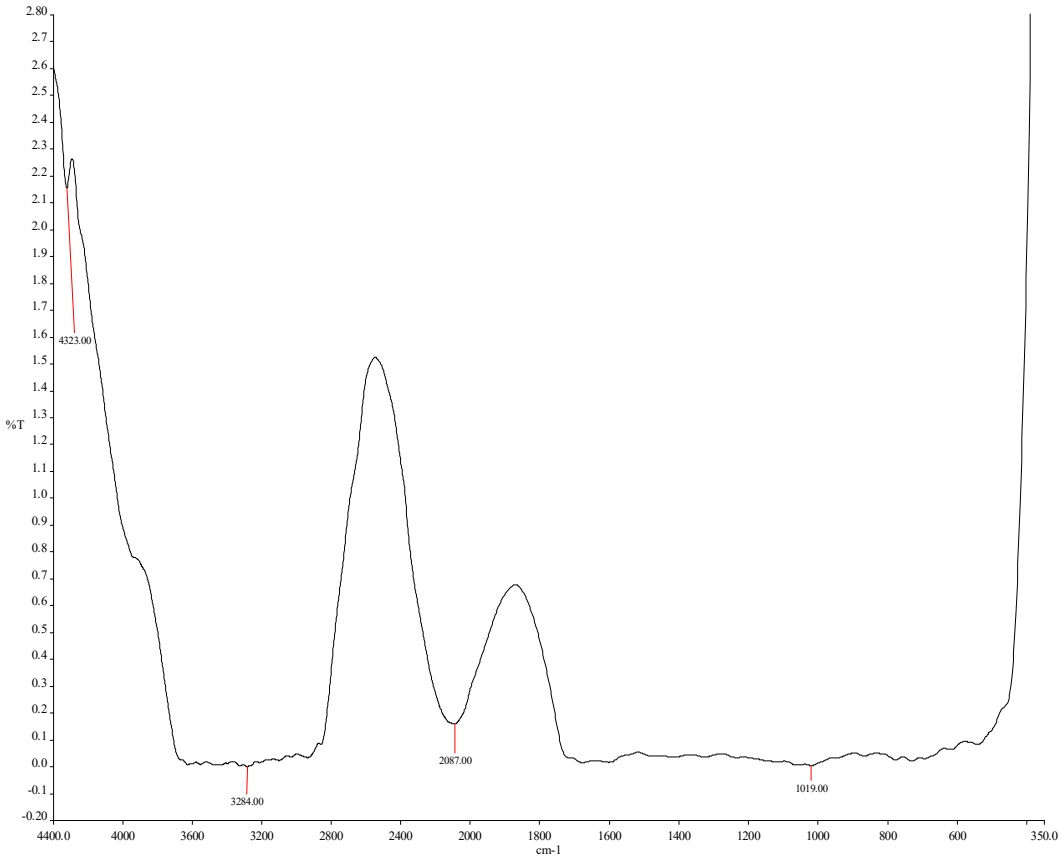

Figure 53. FTIR Spectrum of *Kigelia africana* cosmeceutical

Fig. 54. Keratinization comparison of *Kigelia africana* extracts with the positive control against *Microsporium aoudinin*

a= Positive Control vs 0.5% medicinal plant extract alone

b=Positive Control vs 0.5% medicinal plant extract with emulsion

c=Positive Control vs 1% medicinal plant extract alone

d=Positive Control vs 1% medicinal plant extract with emulsion

e=Positive Control vs 2% medicinal plant extract alone

f=Positive Control vs 2% medicinal plant extract with emulsion

Table 88 Analysis of variance showing the difference in the keratinization within the groups of *Kigelia africana* ethanol extracts against *Microsporium aoudinin*

	Sum of Squares	Df	Mean Square	F	P	N	Remark
Between Groups	1617.479	6	269.580	159.174	0.000	35	Significant
Within Groups	47.421	28	1.694				
Total	1664.900	34					

p value is computed 3 decimal places

p value is computed at 95% confidence interval

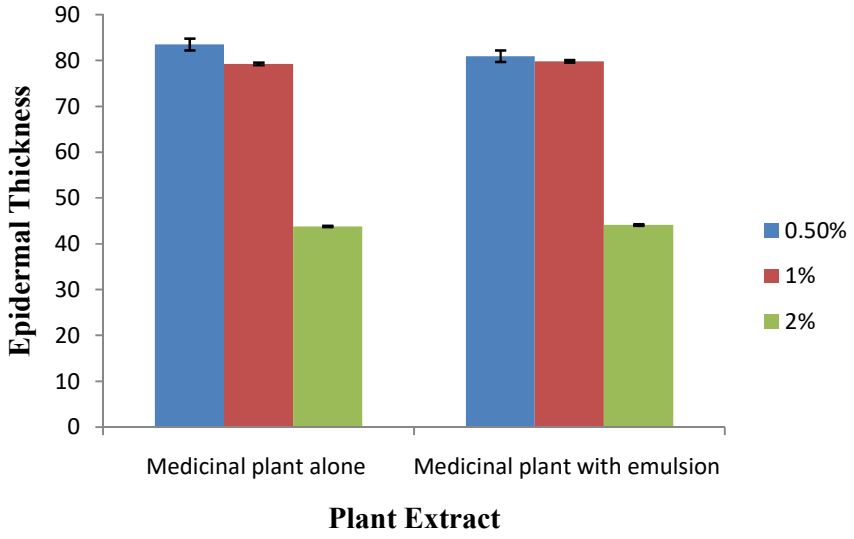

Fig. 55 Epidermal thickness comparison of *Kigelia* extracts with the positive control against *Microsporium aoudini*

a= Positive Control vs 0.5% medicinal plant extract alone

b=Positive Control vs 0.5% medicinal plant extract with emulsion

c=Positive Control vs 1% medicinal plant extract alone

d=Positive Control vs 1% medicinal plant extract with emulsion

e=Positive Control vs 2% medicinal plant extract alone

f=Positive Control vs 2% medicinal plant extract with emulsion

Table 89 Analysis of variance showing the difference in the epidermal thickness Within the groups of *Kigelia africana* ethanol extracts against *Microsporium aoudini*

	Sum of Squares	Df	Mean Square	F	P	N	Remark
Between Groups	13538.070	6	2256.345				
Within Groups	169.188	28	6.042	373.418	0.000	35	Significant
Total	13707.258	34					

P value is computed at 3 decimal places

P value is computed at 95% confidence interval

.

Fig.56. Keratinization comparison of *Kigelia africana* extracts with the positive control against *Epidermophyton floccosum*.

a= Positive Control vs 0.5% medicinal plant extract alone

b=Positive Control vs 0.5% medicinal plant extract with emulsion

c=Positive Control vs 1% medicinal plant extract alone

d=Positive Control vs 1% medicinal plant extract with emulsion

e=Positive Control vs 2% medicinal plant extract alone

f=Positive Control vs 2% medicinal plant extract with emulsion

Table 90 Analysis of variance showing the difference in the keratinization within the groups of *Kigelia africana* ethanol extracts against *Epidermophyton floccosum*

	Sum of Squares	df	Mean Square	F	P	N	Remark
Between Groups	2864.419	6	477.403				
Within Groups	11.893	28	.425	1123.932	0.000	35	Significant
Total	2876.312	34					

P value is computed at 3 decimal places

P value is computed at 95% confidence interval

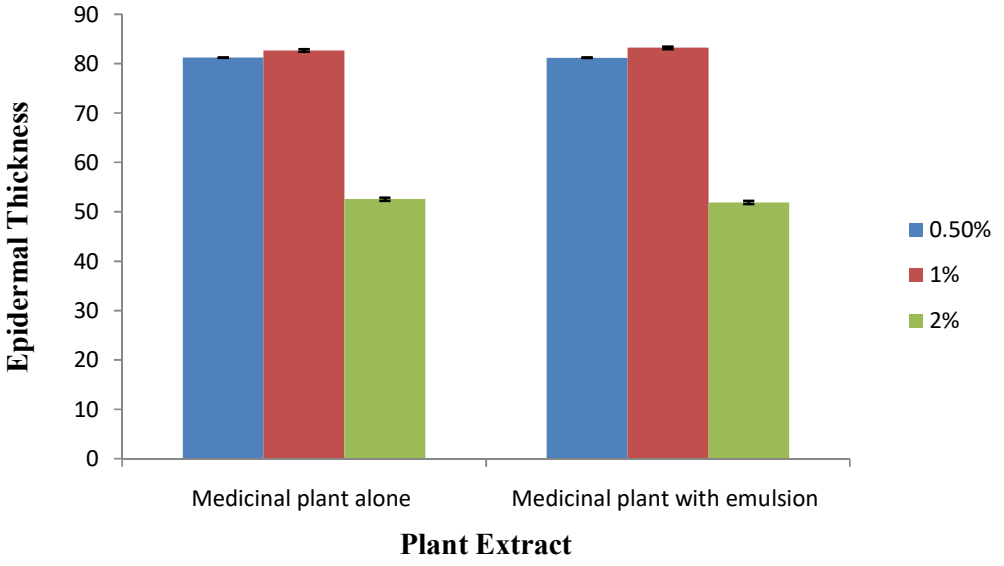

Fig.57 Epidermal thickness comparison of *Kigelia africana* ethanol extracts with the positive control against *Epidermophyton floccosum*.

a= Positive Control vs 0.5% medicinal plant extract alone

b=Positive Control vs 0.5% medicinal plant extract with emulsion

c=Positive Control vs 1% medicinal plant extract alone

d=Positive Control vs 1% medicinal plant extract with emulsion

e=Positive Control vs 2% medicinal plant extract alone

f=Positive Control vs 2% medicinal plant extract with emulsion

Table 91 Analysis of variance showing the difference in the epidermal thickness between 6 different groups of *Kigelia africana* ethanol extracts against *Epidermophyton floccosum*

	Sum of Squares	Df	Mean Square	F	P	N	Remark
Between Groups	10281.664	6	1713.611	931.449	0.000	35	Significant
Within Groups	51.512	28	1.840				
Total	10333.176	34					

P value is computed at 3 decimal places

P value is computed at 95% confidence interval

Fig.58 Keratinization comparison of *Kigelia africana* extracts with the positive control against *Trichophyton mentagrophyte*

a= Positive Control vs 0.5% medicinal plant extract alone

b=Positive Control vs 0.5% medicinal plant extract with emulsion

c=Positive Control vs 1% medicinal plant extract alone

d=Positive Control vs 1% medicinal plant extract with emulsion

e=Positive Control vs 2% medicinal plant extract alone

f=Positive Control vs 2% medicinal plant extract with emulsion

Table 92 Analysis of variance showing the difference in the keratinization between 6 different groups of *Kigelia africana* ethanol extracts against *Trichophyton mentagrophytes*

	Sum of Squares	Df	Mean Square	F	P	N	Remark
Between Groups	2544.227	6	424.038	316.080	.000	35	Significant
Within Groups	37.563	28	1.342				
Total	2581.790	34					

P value is computed at 3 decimal places

P value is computed at 95% confidence interval

.

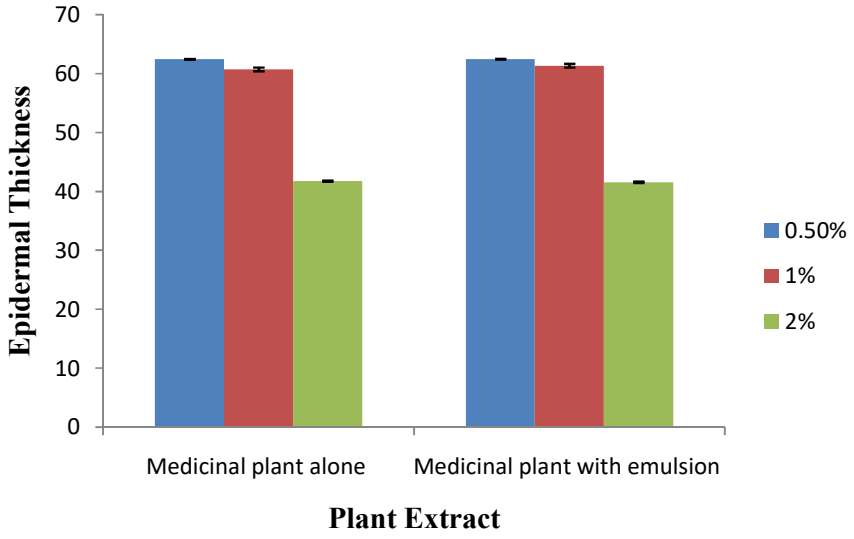

Fig.59 Epidermal thickness comparison of *Kigelia africana* extracts with the positive control against *Trichophyton mentagrophytes*

a= Positive Control vs 0.5% medicinal plant extract alone

b=Positive Control vs 0.5% medicinal plant extract with emulsion

c=Positive Control vs 1% medicinal plant extract alone

d=Positive Control vs 1% medicinal plant extract with emulsion

e=Positive Control vs 2% medicinal plant extract alone

f=Positive Control vs 2% medicinal plant extract with emulsion

Table 93 Analysis of variance showing the difference in epidermal thickness between different groups of *Kigelia africana* formulations against *Trichophyton mentagrophytes*

	Sum of Squares	df	Mean Square	F	N	P	Remark
Between Groups	1973.364	6	328.894	192.754			
Within Groups	47.776	28	1.706		35	0.000	Significant
Total	2021.140	34					

p value is computed 3 decimal places

p value is computed at 95% confidence interval

Fig.60 Keratinization comparison of *Kigelia africana* extracts with the positive control against *Malassezia furfur*

a= Positive Control vs 0.5% medicinal plant extract alone

b=Positive Control vs 0.5% medicinal plant extract with emulsion

c=Positive Control vs 1% medicinal plant extract alone

d=Positive Control vs 1% medicinal plant extract with emulsion

e=Positive Control vs 2% medicinal plant extract alone

f=Positive Control vs 2% medicinal plant extract with emulsion

Table 94 Analysis of variance showing the difference in the keratinization between 6 different groups of *Kigelia africana* ethanol extracts formulations against *Malassezia furfur*

	Sum of Squares	df	Mean Square	F	Sig.	N	Remark
Between Groups	1243.378	6	207.230				
Within Groups	34.185	28	1.221	169.737	0.000	35	Significant
Total	1277.562	34					

p value is computed at 3 decimal places

p value is computed at 95% confidence interval

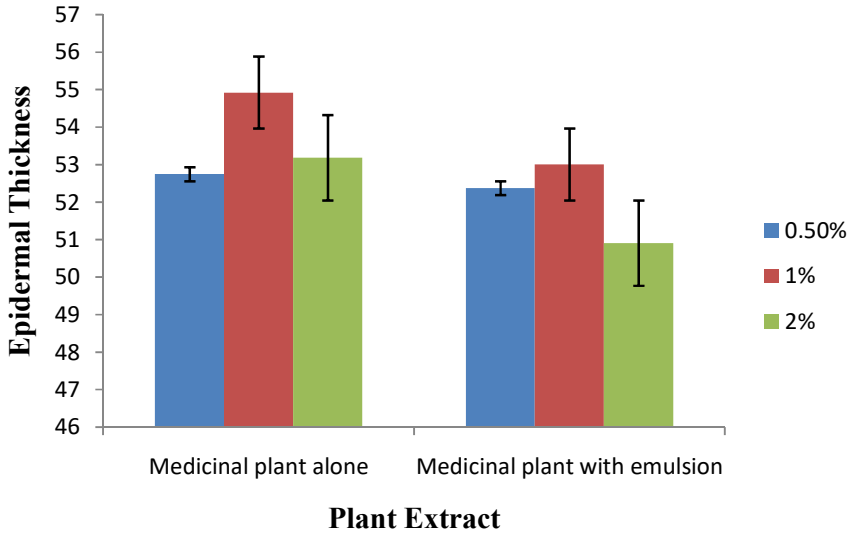

Fig.61 Epidermal thickness comparison of *Kigelia africana* extracts with the positive control against *Malassezia furfur*

a= Positive Control vs 0.5% medicinal plant extract alone

b=Positive Control vs 0.5% medicinal plant extract with emulsion

c=Positive Control vs 1% medicinal plant extract alone

d=Positive Control vs 1% medicinal plant extract with emulsion

e=Positive Control vs 2% medicinal plant extract alone

f=Positive Control vs 2% medicinal plant extract with emulsion

Table 95 Analysis of variance showing the difference in the epidermal thickness between 6 different groups of *Kigelia africana* extracts formulations against *Malassezia furfur*

	Sum of Squares	Df	Mean Square	F	P	N	Remark
Between Groups	1613.386	6	268.898				
Within Groups	72.971	28	2.606	103.180	0.000	35	Significant
Total	1686.356	34					

P value is computed at 3 decimal places

P value is computed at 95% confidence interval

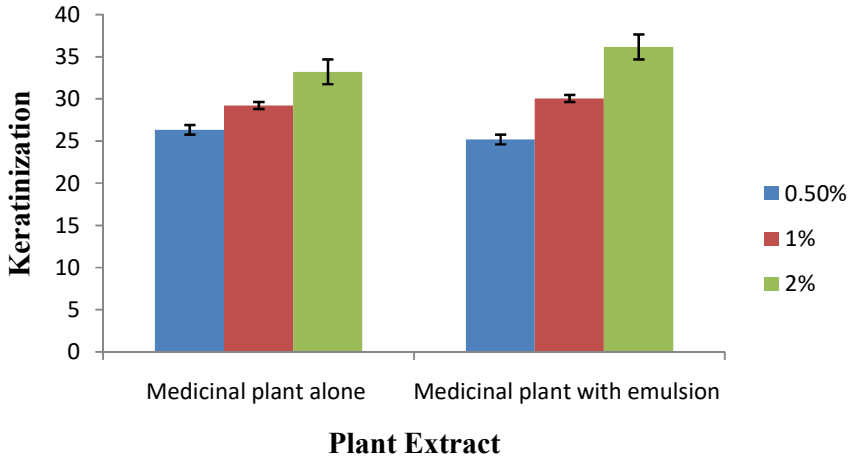

Fig.62 Keratinization comparison of *Cassia alata* extracts with the positive control against *Microsporium aoudinin.*

a= Positive Control vs 0.5% medicinal plant extract alone

b=Positive Control vs 0.5% medicinal plant extract with emulsion

c=Positive Control vs 1% medicinal plant extract alone

d=Positive Control vs 1% medicinal plant extract with emulsion

e=Positive Control vs 2% medicinal plant extract alone

f=Positive Control vs 2% medicinal plant extract with emulsion

Table 96 Analysis of variance showing the difference in the keratinization between 6 different groups of *Cassia alata* formulations against *Microsporium aoudinin*

	Sum of Squares	Df	Mean Square	F	N	P	Remark
Between Groups	1100.973	6	183.495	145.715	35	0.000	Significant
Within Groups	35.260	28	1.259				
Total	1136.232	34					

p value is computed 3 decimal places

p value is computed at 95% confidence interval

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Fig.63 Epidermal thickness comparison of *Cassia alata* extracts with the positive control against *Microsporium aoudinin.*

a= Positive Control vs 0.5% medicinal plant extract alone

b=Positive Control vs 0.5% medicinal plant extract with emulsion

c=Positive Control vs 1% medicinal plant extract alone

d=Positive Control vs 1% medicinal plant extract with emulsion

e=Positive Control vs 2% medicinal plant extract alone

f=Positive Control vs 2% medicinal plant extract with emulsion

Table 97 Analysis of variance showing the difference in the epidermal thickness between 6 different groups of *Cassia alata* ethanol formulations against *Microsporium aoudinin*

	Sum of Squares	df	Mean Square	F	N.	P	Remark
Between Groups	3555.592	6	592.599	148.430			
Within Groups	111.788	28	3.992		35	0.000	Significant
Total	3667.380	34					

p value is computed 3 decimal places

p value is computed at 95% confidence interval

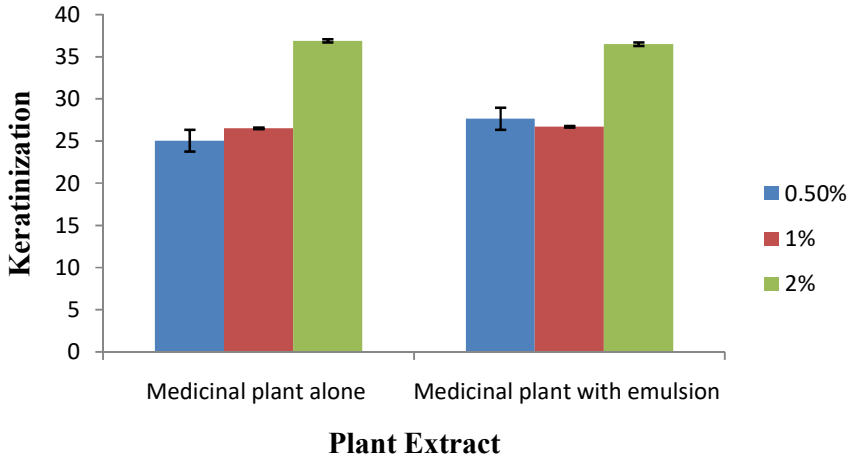

Fig.64 Keratinization comparison of *Cassia alata* extracts with the positive control against *Epidermophyton floccosum*

a= Positive Control vs 0.5% medicinal plant extract alone

b=Positive Control vs 0.5% medicinal plant extract with emulsion

c=Positive Control vs 1% medicinal plant extract alone

d=Positive Control vs 1% medicinal plant extract with emulsion

e=Positive Control vs 2% medicinal plant extract alone

f=Positive Control vs 2% medicinal plant extract with emulsion

Table 98 Analysis of variance showing the difference in keratinization between different groups of *Cassia alata* formulations against *Epidermophyton floccosum*

	Sum of Squares	df	Mean Square	F	N	P	Remark
Between Groups	1973.364	6	328.894	192.754			
Within Groups	47.776	28	1.706		35	0.000	Significant
Total	2021.140	34					

p value is computed 3 decimal places

p value is computed at 95% confidence interval

Fig.65 Epidermal thickness comparison of *Cassia alata* extracts with the positive control against *Epidermophyton floccosum*

a= Positive Control vs 0.5% medicinal plant extract alone

b=Positive Control vs 0.5% medicinal plant extract with emulsion

c=Positive Control vs 1% medicinal plant extract alone

d=Positive Control vs 1% medicinal plant extract with emulsion

e=Positive Control vs 2% medicinal plant extract alone

f=Positive Control vs 2% medicinal plant extract with emulsion

Table 99 Analysis of variance showing the difference in the epidermal thickness between 6 different groups of *Cassia alata* formulations against *Epidermophyton floccosum*

	Sum of Squares	df	Mean Square	F	N	P	Remark
Between Groups	1979.766	6	329.961	337.522	35	0.000	Significant
Within Groups	27.373	28	978				
Total	2007.138	34					

p value is computed 3 decimal places

p value is computed at 95% confidence interval

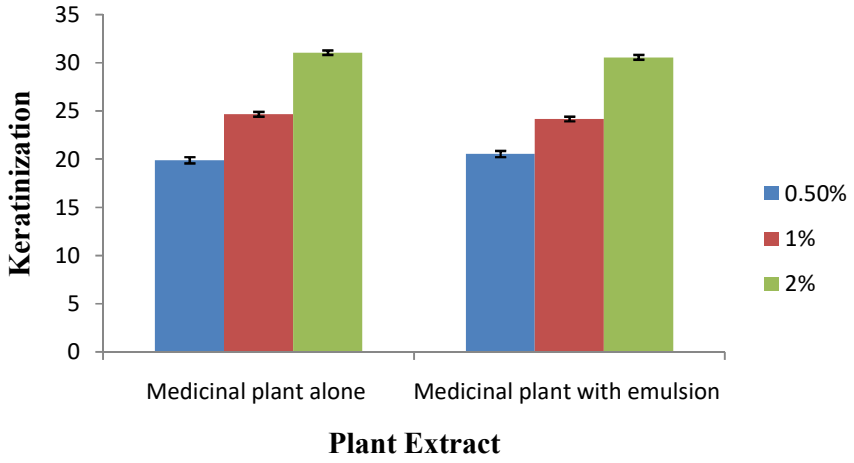

Fig.66 Keratinization comparison of *Cassia alata* extracts with the positive control against *Trichophyton mentagrophytes*

a= Positive Control vs 0.5% medicinal plant extract alone

b=Positive Control vs 0.5% medicinal plant extract with emulsion

c=Positive Control vs 1% medicinal plant extract alone

d=Positive Control vs 1% medicinal plant extract with emulsion

e=Positive Control vs 2% medicinal plant extract alone

f=Positive Control vs 2% medicinal plant extract with emulsion

Table 100 Analysis of variance showing the difference in the keratinization between 6 different groups of *Cassia alata* formulations against *Trichophyton mentagrophytes*

	Sum of Squares	Df	Mean Square	F	N	P	Remark
Between Groups	3731.704	6	621.951				
Within Groups	32.394	28	1.157	537.588	35	0.000	Significant
Total	3764.098	34					

p value is computed 3 decimal places

p value is computed at 95% confidence interval

Fig.67 Epidermal thickness comparison of *Cassia alata* ethanol extracts with the positive control against *Trichophyton mentagrophytes*

a= Positive Control vs 0.5% medicinal plant extract alone

b=Positive Control vs 0.5% medicinal plant extract with emulsion

c=Positive Control vs 1% medicinal plant extract alone

d=Positive Control vs 1% medicinal plant extract with emulsion

e=Positive Control vs 2% medicinal plant extract alone

f=Positive Control vs 2% medicinal plant extract with emulsion

Table 101 Analysis of variance showing the difference in the epidermal thickness between 6 different groups of *Cassia alata* formulations against *Trichophyton mentagrophytes*

	Sum of Squares	df	Mean Square	F	N	P	Remark
Between Groups	1102.728	6	183.788				
Within Groups	60.801	28	2.171	84.638	35	0.000	Significant
Total	1163.529	34					

p value is computed 3 decimal places

p value is computed at 95% confidence interval

Fig.68 Keratinisation comparison of *Cassia alata* extracts with the positive control against *Malassezia furfur*

a= Positive Control vs 0.5% medicinal plant extract alone

b=Positive Control vs 0.5% medicinal plant extract with emulsion

c=Positive Control vs 1% medicinal plant extract alone

d=Positive Control vs 1% medicinal plant extract with emulsion

e=Positive Control vs 2% medicinal plant extract alone

f=Positive Control vs 2% medicinal plant extract with emulsion

Table 102 Analysis of variance showing the difference in the keratinization between 6 different groups of *Cassia alata* formulations against *Malassezia furfur*

	Sum of Squares	df	Mean Square	F	N	P	Remark
Between Groups	739.983	6	123.330				
Within Groups	66.765	28	2.384	51.722	35	0.000	Significant
Total	806.748	34					

p value is computed 3 decimal places

p value is computed at 95% confidence interval

Fig.69. Epidermal thickness comparison of *Cassia alata* extracts with the positive control against *Malassezia furfur*

a= Positive Control vs 0.5% medicinal plant extract alone

b=Positive Control vs 0.5% medicinal plant extract with emulsion

c=Positive Control vs 1% medicinal plant extract alone

d=Positive Control vs 1% medicinal plant extract with emulsion

e=Positive Control vs 2% medicinal plant extract alone

f=Positive Control vs 2% medicinal plant extract with emulsion

Table 103 Analysis of variance showing the difference in the epidermal thickness between 6 different groups of *Cassia alata* formulations against *Malassezia furfur*

	Sum of Squares	df	Mean Square	F	N	P	Remark
Between Groups	27.627	6	4.604	2.841	35	0.027	Significant
Within Groups	45.375	28	1.621				
Total	73.002	34					

p value is computed 3 decimal places

p value is computed at 95% confidence interval

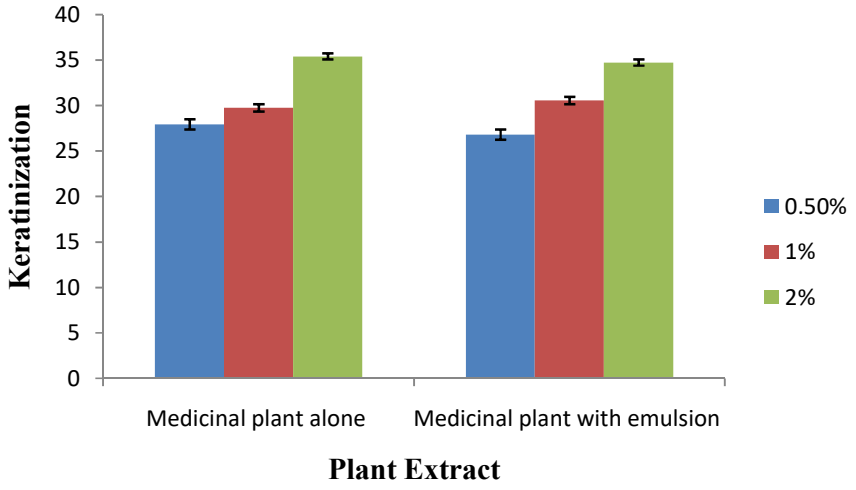

Fig.70 Keratinisation comparison of *Mitracarpus villosus* extracts with the positive control against *Microsporium aoudinin*

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a= Positive Control vs 0.5% medicinal plant extract alone
b=Positive Control vs 0.5% medicinal plant extract with emulsion
c=Positive Control vs 1% medicinal plant extract alone
d=Positive Control vs 1% medicinal plant extract with emulsion
e=Positive Control vs 2% medicinal plant extract alone
f=Positive Control vs 2% medicinal plant extract with emulsion

Table 104 Analysis of variance showing the difference in the keratinization between 6 different groups of *Mitracarpus villosus* ethanol formulations against *Microsporium aoudinin*

	Sum of Squares	df	Mean Square	F	Sig.	P	Remark
Between Groups	2310.471	6	385.079	180.055	.000		
Within Groups	59.883	28	2.139				
Total	2370.354	34					

p value is computed 3 decimal places

p value is computed at 95% confidence interval

Fig.71 Epidermal thickness comparison of *Mitracarpus villosus* extracts with the positive control against *Microsporium aoudinin*

a= Positive Control vs 0.5% medicinal plant extract alone

b=Positive Control vs 0.5% medicinal plant extract with emulsion

c=Positive Control vs 1% medicinal plant extract alone

d=Positive Control vs 1% medicinal plant extract with emulsion

e=Positive Control vs 2% medicinal plant extract alone

f=Positive Control vs 2% medicinal plant extract with emulsion

Table 105 Analysis of variance showing the difference in the epidermal thickness between 6 different groups of *Mitracarpus villosus* formulations against *Microsporium aoudinin*

	Sum of Squares	df	Mean Square	F	N	P	Remark
Between Groups	7927.448	6	1321.241				
Within Groups	82.096	28	2.932	450.625	35	0.000	Significant
Total	8009.545	34					

p value is computed 3 decimal places

p value is computed at 95% confidence interval

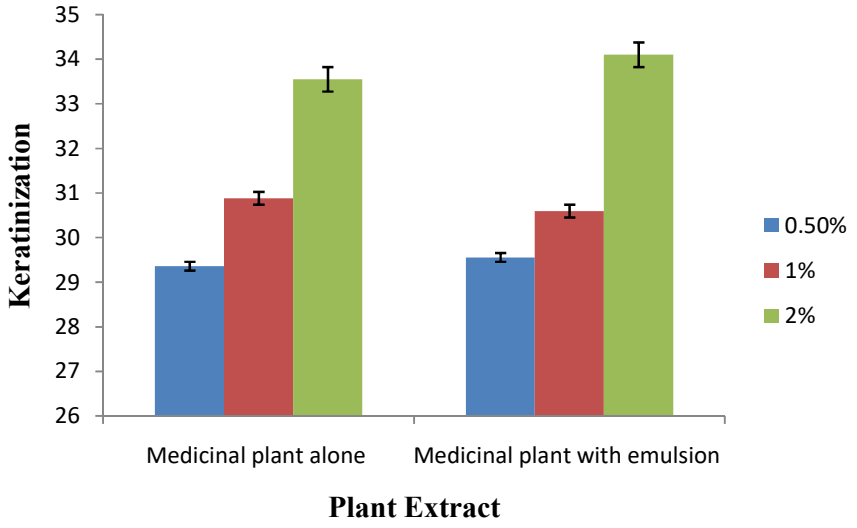

Fig.72 Keratinisation comparison of *Mitracarpus villosus* extracts with the positive control against *Epidermophyton floccosum.*

a= Positive Control vs 0.5% medicinal plant extract alone

b=Positive Control vs 0.5% medicinal plant extract with emulsion

c=Positive Control vs 1% medicinal plant extract alone

d=Positive Control vs 1% medicinal plant extract with emulsion

e=Positive Control vs 2% medicinal plant extract alone

f=Positive Control vs 2% medicinal plant extract with emulsion

Table 106 Analysis of variance showing the difference in the keratinization between 6 different groups of *Mitracarpus villosus* ethanol formulations against *Epidermophyton floccosum*

	Sum of Squares	df	Mean Square	F	N	P	Remark
Between Groups	1553.155	6	258.859	248.738	35	0.000	Significant
Within Groups	29.139	28	1.041				
Total	1582.295	34					

p value is computed 3 decimal places

p value is computed at 95% confidence interval

Fig.73 Epidermal thickness comparison of *Mitracarpus villosus* extracts with the positive control against *Epidermophyton floccosum*

a= Positive Control vs 0.5% medicinal plant extract alone

b=Positive Control vs 0.5% medicinal plant extract with emulsion

c=Positive Control vs 1% medicinal plant extract alone

d=Positive Control vs 1% medicinal plant extract with emulsion

e=Positive Control vs 2% medicinal plant extract alone

f=Positive Control vs 2% medicinal plant extract with emulsion

Table 107 Analysis of variance showing the difference in the epidermal thickness between 6 different groups of *Mitracarpus villosus* ethanol formulations against *Epidermophyton floccosum*

	Sum of Squares	Df	Mean Square	F	N	P	Remark
Between Groups	3348.743	6	558.124				
Within Groups	87.903	28	3.139	177.781	35	0.000	Significant
Total	3436.646	34					

p value is computed 3 decimal places

p value is computed at 95% confidence interval

Fig.74 Keratinisation comparison of *Mitracarpus villosus* extracts with the positive control against *Trichophyton mentagrophytes*

a= Positive Control vs 0.5% medicinal plant extract alone

b=Positive Control vs 0.5% medicinal plant extract with emulsion

c=Positive Control vs 1% medicinal plant extract alone

d=Positive Control vs 1% medicinal plant extract with emulsion

e=Positive Control vs 2% medicinal plant extract alone

f=Positive Control vs 2% medicinal plant extract with emulsion

Table 108 Analysis of variance showing the difference in the keratinization between 6 different groups of *Mitracarpus villosus* formulations against *Trichophyton mentagrophytes*

	Sum of Squares	df	Mean Square	F	N	P	Remark
Between Groups	3648.317	6	608.053	318.527	35	0.000	Significant
Within Groups	53.451	28	1.909				
Total	3701.767	34					

p value is computed 3 decimal places

p value is computed at 95% confidence interval

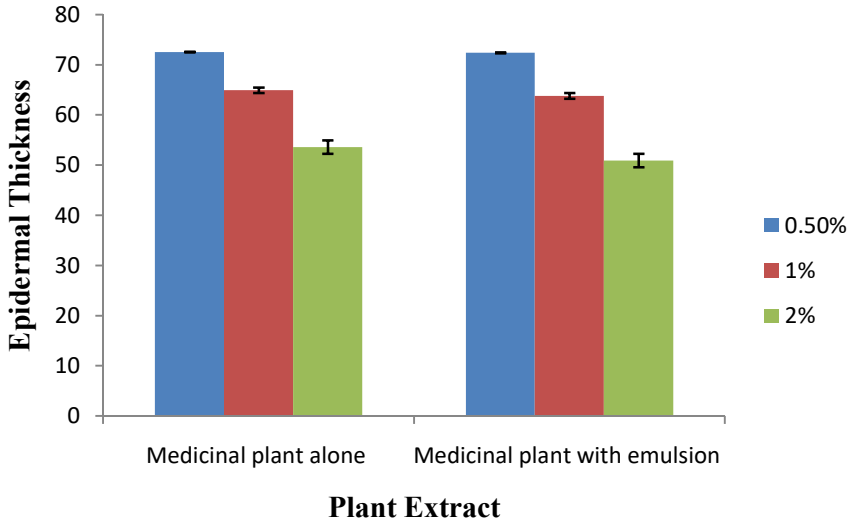

Fig.75 Epidermal thickness comparison of *Mitracarpus villosus* extracts with the positive control against *Trichophyton mentagrophytes*.

a= Positive Control vs 0.5% medicinal plant extract alone

b=Positive Control vs 0.5% medicinal plant extract with emulsion

c=Positive Control vs 1% medicinal plant extract alone

d=Positive Control vs 1% medicinal plant extract with emulsion

e=Positive Control vs 2% medicinal plant extract alone

f=Positive Control vs 2% medicinal plant extract with emulsion

Table 109 Analysis of variance showing the difference in the epidermal thickness between 6 different groups of *Mitracarpus villosus* formulations against *Trichophyton mentagrophytes*

	Sum of Squares	df	Mean Square	F	N	P	Remark
Between Groups	3689.351	6	614.892				
Within Groups	54.831	28	1.958	314.002	35	0.000	Significant
Total	3744.182	34					

p value is computed 3 decimal places

p value is computed at 95% confidence interval

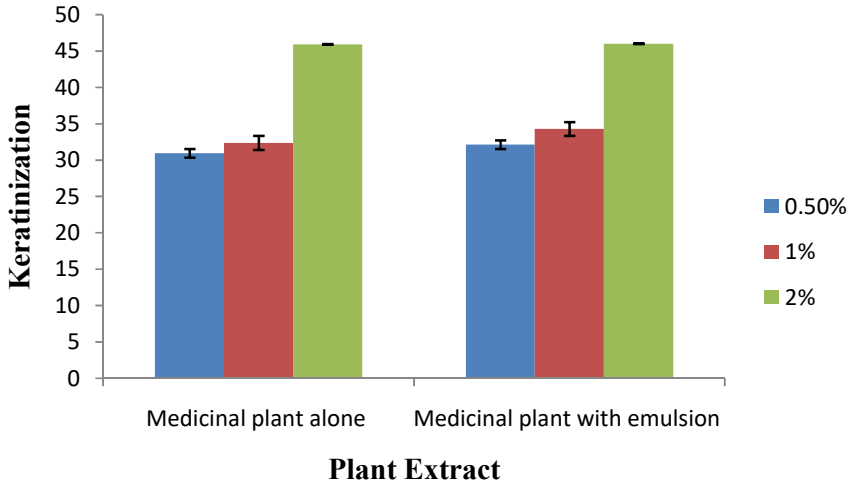

Fig.76 Keratinisation comparison of *Mitracarpus villosus* extracts with the positive control against *Malassezia furfur*

*=means difference is significant at p=0.05

a= Positive Control vs 0.5% medicinal plant extract alone

b=Positive Control vs 0.5% medicinal plant extract with emulsion

c=Positive Control vs 1% medicinal plant extract alone

d=Positive Control vs 1% medicinal plant extract with emulsion

e=Positive Control vs 2% medicinal plant extract alone

f=Positive Control vs 2% medicinal plant extract with emulsion

Table 110 Analysis of variance showing the difference in the keratinization between 6 different groups of *Mitracarpus villosus* formulations against *Malassezia furfur*

	Sum of Squares	df	Mean Square	F	N	P	Remark
Between Groups	2987.813	6	497.969				
Within Groups	40.326	28	1.440	345.757	35	0.000	Significant
Total	3028.139	34					

p value is computed 3 decimal places

p value is computed at 95% confidence interval

Fig.77 Epidermal thickness comparison of *Mitracarpus villosus* extracts with the positive control against *Malassezia furfur*.

a= Positive Control vs 0.5% medicinal plant extract alone

b=Positive Control vs 0.5% medicinal plant extract with emulsion

c=Positive Control vs 1% medicinal plant extract alone

d=Positive Control vs 1% medicinal plant extract with emulsion

e=Positive Control vs 2% medicinal plant extract alone

f=Positive Control vs 2% medicinal plant extract with emulsion

Table 111 Analysis of variance showing the difference in the epidermal thickness between 6 different groups of *Mitracarpus villosus* formulations against *Malassezia furfur*

	Sum of Squares	Df	Mean Square	F	N	P	Remark
Between Groups	1857.929	6	309.655				
Within Groups	71.577	28	2.556	121.134	35	0.000	Significant
Total	1929.506	34					

p value is computed 3 decimal places

p value is computed at 95% confidence interval

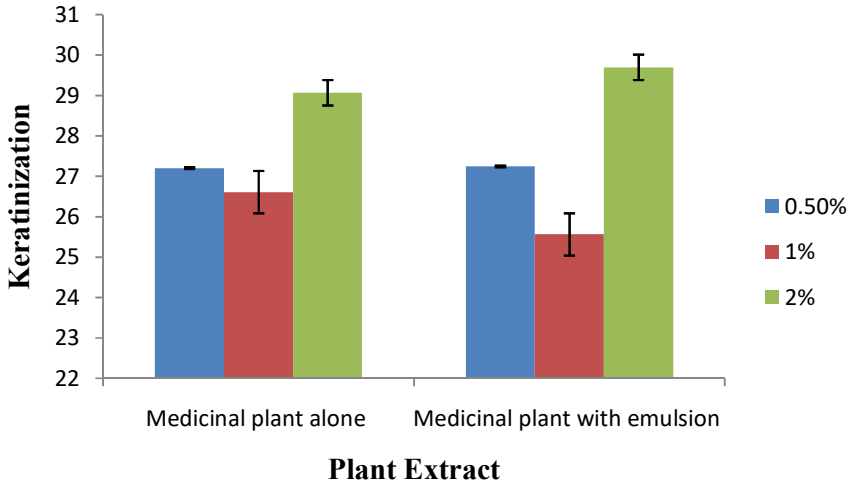

Fig.78 Keratinisation comparison of *Cassia occidentalis* extracts with the positive control against *Microsporium aoudinin*.

a= Positive Control vs 0.5% medicinal plant extract alone

b=Positive Control vs 0.5% medicinal plant extract with emulsion

c=Positive Control vs 1% medicinal plant extract alone

d=Positive Control vs 1% medicinal plant extract with emulsion

e=Positive Control vs 2% medicinal plant extract alone

f=Positive Control vs 2% medicinal plant extract with emulsion

Tabel 112 Analysis of variance showing the difference in the keratinization between 6 different groups of *Cassia occidentalis* formulations against *Microsporium aoudinin*

	Sum of Squares	Df	Mean Square	F	N	P	Remark
Between Groups	1456.507	6	242.751	186.682	35	0.000	Significant
Within Groups	36.410	28	1.300				
Total	1492.917	34					

p value is computed 3 decimal places
p value is computed at 95% confidence interval

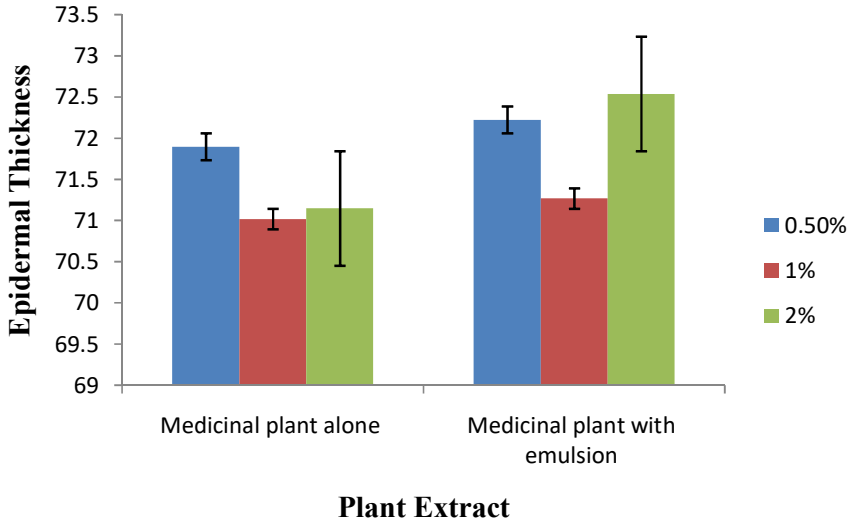

Fig.79 Epidermal thickness comparison of *Cassia occidentalis* extracts with the positive control against *Microsporium aoudinin*

a= Positive Control vs 0.5% medicinal plant extract alone

b=Positive Control vs 0.5% medicinal plant extract with emulsion

c=Positive Control vs 1% medicinal plant extract alone

d=Positive Control vs 1% medicinal plant extract with emulsion

e=Positive Control vs 2% medicinal plant extract alone

f=Positive Control vs 2% medicinal plant extract with emulsion

Table 113 Analysis of variance showing the difference in the epidermal thickness between 6 different groups of *Cassia occidentalis* formulations against *Microsporium aoudinin*.

	Sum of Squares	df	Mean Square	F	N	P	Remark
Between Groups	4333.638	6	722.273				
Within Groups	36.756	28	1.313	550.218	35	0.000	Significant
Total	4370.394	34					

p value is computed 3 decimal places
p value is computed at 95% confidence interval

Fig.80 Keratinization comparison of *Cassia occidentalis* extracts with the positive control against *Epidermophyton floccosum*.

a= Positive Control vs 0.5% medicinal plant extract alone

b=Positive Control vs 0.5% medicinal plant extract with emulsion

c=Positive Control vs 1% medicinal plant extract alone

d=Positive Control vs 1% medicinal plant extract with emulsion

e=Positive Control vs 2% medicinal plant extract alone

f=Positive Control vs 2% medicinal plant extract with emulsion

Table 114 Analysis of variance showing the difference in the keratinization between 6 different groups of *Cassia occidentalis* formulations against *Epidermophyton floccosum*

	Sum of Squares	df	Mean Square	F	N	P	Remark
Between Groups	2817.922	6	469.654				
Within Groups	44.087	28	1.575	298.278	35	0.000	Significant
Total	2862.009	34					

p value is computed 3 decimal places
p value is computed at 95% confidence interval

Fig.81 Epidermal thickness comparison of *Cassia occidentalis* extracts with the positive control against *Epidermophyton floccosum*.

a= Positive Control vs 0.5% medicinal plant extract alone

b=Positive Control vs 0.5% medicinal plant extract with emulsion

c=Positive Control vs 1% medicinal plant extract alone

d=Positive Control vs 1% medicinal plant extract with emulsion

e=Positive Control vs 2% medicinal plant extract alone

f=Positive Control vs 2% medicinal plant extract with emulsion

Table 115 Analysis of variance showing the difference in the epidermal thickness between 6 different groups of *Cassia occidentalis* formulations against *Epidermophyton floccosum*

	Sum of Squares	df	Mean Square	F	N	P	Remark
Between Groups	6158.663	6	1026.444				
Within Groups	124.865	28	4.459	230.172	35	0.0000	Significant
Total	6283.528	34					

p value is computed 3 decimal places
p value is computed at 95% confidence interval

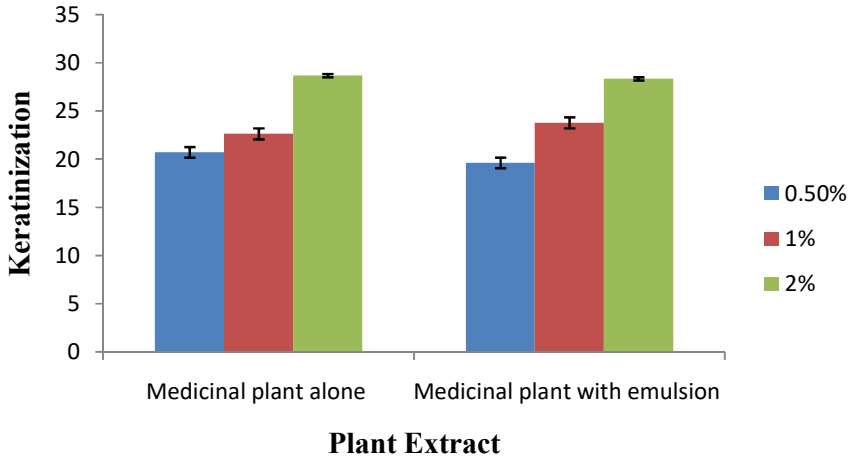

Fig.82 Keratinisation comparison of *Cassia occidentalis* extracts with the positive control against *Trichophyton mentagrophytes*.

a= Positive Control vs 0.5% medicinal plant extract alone

b=Positive Control vs 0.5% medicinal plant extract with emulsion

c=Positive Control vs 1% medicinal plant extract alone

d=Positive Control vs 1% medicinal plant extract with emulsion

e=Positive Control vs 2% medicinal plant extract alone

f=Positive Control vs 2% medicinal plant extract with emulsion

Table 116 Analysis of variance showing the difference in the keratinization between 6 different groups of *Cassia occidentalis* formulations against *Trichophyton mentagrophytes*

	Sum of Squares	df	Mean Square	F	N	P	Remark
Between Groups	2087.195	6	347.866				
Within Groups	46.042	28	1.644	211.553	35	0.000	Significant
Total	2133.236	34					

p value is computed 3 decimal places
p value is computed at 95% confidence interval

Fig.83 Epidermal thickness comparison of *Cassia occidentalis* extracts with the positive control against *Trichophyton mentagrophytes*

a= Positive Control vs 0.5% medicinal plant extract alone

b=Positive Control vs 0.5% medicinal plant extract with emulsion

c=Positive Control vs 1% medicinal plant extract alone

d=Positive Control vs 1% medicinal plant extract with emulsion

e=Positive Control vs 2% medicinal plant extract alone

f=Positive Control vs 2% medicinal plant extract with emulsion

Table 117 Analysis of variance showing the difference in the epidermal thickness between 6 different groups of *Cassia occidentalis* formulations against *Trichophyton mentagrophytes*

	Sum of Squares	df	Mean Square	F	N	P	Remark
Between Groups	1829.086	6	304.848				
Within Groups	60.852	28	2.173	140.269	35	0.000	Significant
Total	1889.939	34					

p value is computed 3 decimal places
p value is computed at 95% confidence interval

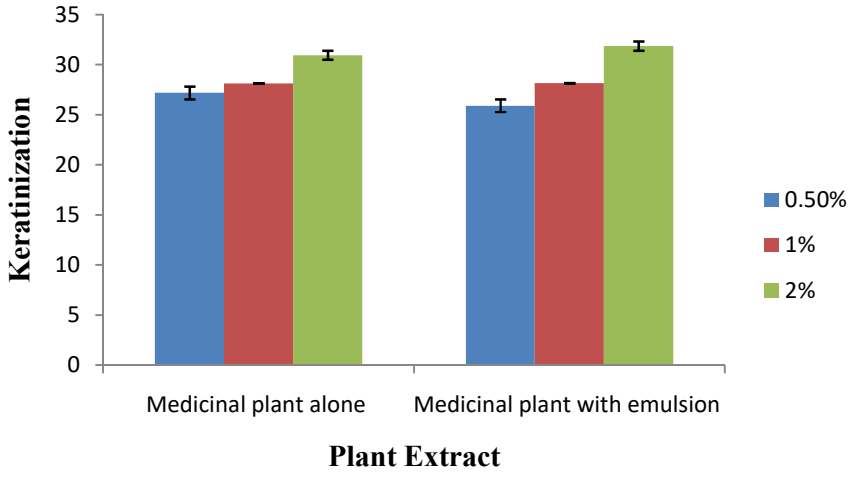

Fig.84 Keratinisation comparison of *Cassia occidentalis* extracts with the positive control against *Malassezia furfur*.

a= Positive Control vs 0.5% medicinal plant extract alone

b=Positive Control vs 0.5% medicinal plant extract with emulsion

c=Positive Control vs 1% medicinal plant extract alone

d=Positive Control vs 1% medicinal plant extract with emulsion

e=Positive Control vs 2% medicinal plant extract alone

f=Positive Control vs 2% medicinal plant extract with emulsion

Table 118 Analysis of variance showing the difference in the keratinization between 6 different groups of *Cassia occidentalis* formulations against *Malassezia furfur*

	Sum of Squares	df	Mean Square	F	N	P	Remark
Between Groups	3277.399	6	546.233	182.154	35	0.000	Significant
Within Groups	83.965	28	2.999				
Total	3361.364	34					

p value is computed 3 decimal places
p value is computed at 95% confidence interval

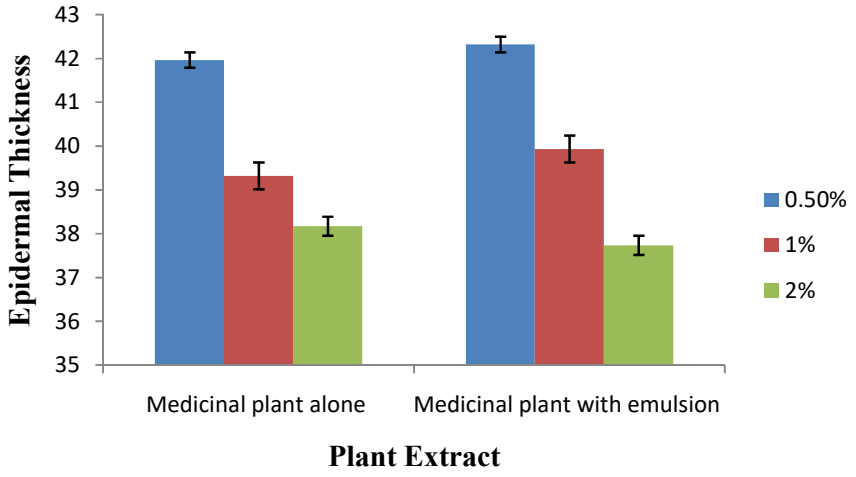

Fig.85 Epidermal thickness comparison of *Cassia occidentalis* extracts with the positive control against *Malassezia furfur*

a= Positive Control vs 0.5% medicinal plant extract alone

b=Positive Control vs 0.5% medicinal plant extract with emulsion

c=Positive Control vs 1% medicinal plant extract alone

d=Positive Control vs 1% medicinal plant extract with emulsion

e=Positive Control vs 2% medicinal plant extract alone

f=Positive Control vs 2% medicinal plant extract with emulsion

Table 119 Analysis of variance showing the difference in the epidermal thickness between 6 different groups of *Cassia occidentalis* formulations against *Malassezia furfur*

	Sum of Squares	df	Mean Square	F	N	P	Remark
Between Groups	466.756	6	77.793				
Within Groups	53.471	28	1.910	40.736	35	0.000	Significant
Total	520.227	34					

p value is computed 3 decimal places

p value is computed at 95% confidence interval

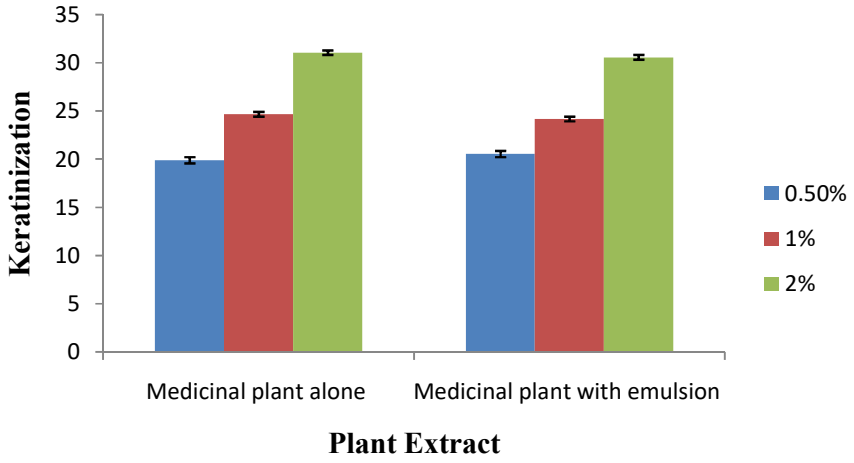

Fig.86 Keratinisation comparison of *Acalypha wilkesiana* extracts with the positive control against *Microsporium aoudinin*

a= Positive Control vs 0.5% medicinal plant extract alone

b=Positive Control vs 0.5% medicinal plant extract with emulsion

c=Positive Control vs 1% medicinal plant extract alone

d=Positive Control vs 1% medicinal plant extract with emulsion

e=Positive Control vs 2% medicinal plant extract alone

f=Positive Control vs 2% medicinal plant extract with emulsion

Table 120 Analysis of variance showing the difference in the keratinization between 6 different groups of *Acalypha wilkesiana* formulations against *Microsporium aoudinin*

	Sum of Squares	df	Mean Square	F	N	P	Remark
Between Groups	3731.704	6	621.951				
Within Groups	32.394	28	1.157	537.588	35	0.000	Significant
Total	3764.098	34					

p value is computed 3 decimal places
p value is computed at 95% confidence interval

Fig.87 Epidermal thickness comparison of *Acalypha wilkesiana* extracts with the positive control against *Microsporium aoudini.*

a= Positive Control vs 0.5% medicinal plant extract alone

b=Positive Control vs 0.5% medicinal plant extract with emulsion

c=Positive Control vs 1% medicinal plant extract alone

d=Positive Control vs 1% medicinal plant extract with emulsion

e=Positive Control vs 2% medicinal plant extract alone

f=Positive Control vs 2% medicinal plant extract with emulsion

Table 121 Analysis of variance showing the difference in the epidermal thickness between 6 different groups of *Acalypha wilkesiana* formulations against *Microsporium aoudinin*

	Sum of Squares	df	Mean Square	F	N	P	Remark
Between Groups	1102.728	6	183.788				
Within Groups	60.801	28	2.171	84.638	35	0.000	Significant
Total	1163.529	34					

p value is computed 3 decimal places
p value is computed at 95% confidence interval

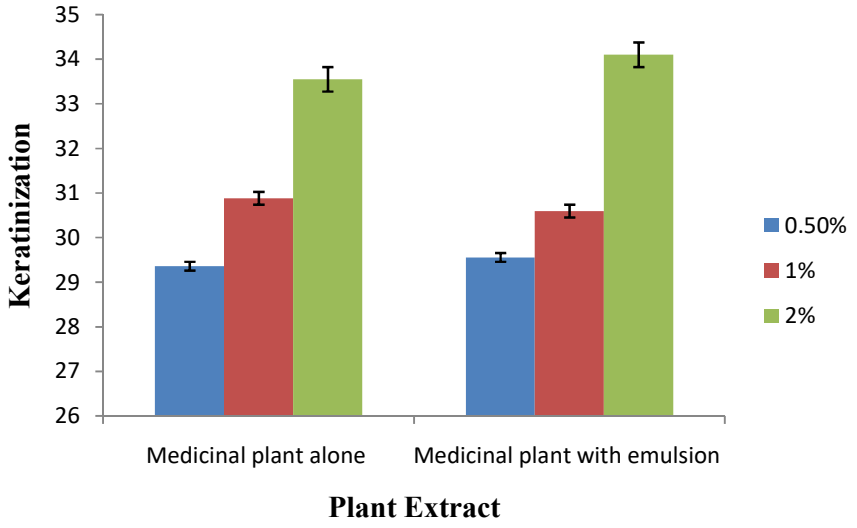

Fig.88 Keratinisation comparison of *Acalypha wilkesiana* extracts with the positive control gainst *Epidermophyton floccosum*

a= Positive Control vs 0.5% medicinal plant extract alone

b=Positive Control vs 0.5% medicinal plant extract with emulsion

c=Positive Control vs 1% medicinal plant extract alone

d=Positive Control vs 1% medicinal plant extract with emulsion

e=Positive Control vs 2% medicinal plant extract alone

f=Positive Control vs 2% medicinal plant extract with emulsion

Table 122 Analysis of variance showing the difference in the keratinization between 6 different groups of *Acalypha wilkesiana* formulations against *Epidermophyton floccosum*

	Sum of Squares	df	Mean Square	F	N	P	Remark
Between Groups	1553.155	6	258.859				
Within Groups	29.139	28	1.041	248.738	35	0.000	Significant
Total	1582.295	34					

p value is computed 3 decimal places
p value is computed at 95% confidence interval

Fig.89 Epidermal thickness comparison of *Acalypha wilkesiana* extracts with the positive control against *Epidermophyton floccosum*

a= Positive Control vs 0.5% medicinal plant extract alone

b=Positive Control vs 0.5% medicinal plant extract with emulsion

c=Positive Control vs 1% medicinal plant extract alone

d=Positive Control vs 1% medicinal plant extract with emulsion

e=Positive Control vs 2% medicinal plant extract alone

f=Positive Control vs 2% medicinal plant extract with emulsion

Table 123 Analysis of variance showing the difference in the epidermal thickness between 6 different groups of *Acalypha wilkesiana* formulations against *Epidermophyton floccosum*

	Sum of Squares	df	Mean Square	F	N	P	Remark
Between Groups	3348.743	6	558.124				
Within Groups	87.903	28	3.139	177.781	35	0.000	Significant
Total	3436.646	34					

p value is computed 3 decimal places
p value is computed at 95% confidence interval

Fig.90 Keratinisation comparison of *Acalypha wilkesiana* ethanol extracts with the positive control against *Trichophyton mentagrophytes*

a= Positive Control vs 0.5% medicinal plant extract alone

b=Positive Control vs 0.5% medicinal plant extract with emulsion

c=Positive Control vs 1% medicinal plant extract alone

d=Positive Control vs 1% medicinal plant extract with emulsion

e=Positive Control vs 2% medicinal plant extract alone

f=Positive Control vs 2% medicinal plant extract with emulsion

Table 124 Analysis of variance showing the difference in the keratinization between 6 different groups of *Acalypha wilkesiana* formulations against *Trichophyton mentagrophytes*

	Sum of Squares	df	Mean Square	F	Sig.	N	Remark
Between Groups	1243.378	6	207.230				
Within Groups	34.185	28	1.221	169.737	0.000	35	Significant
Total	1277.562	34					

p value is computed at 3 decimal places

p value is computed at 95% confidence interval

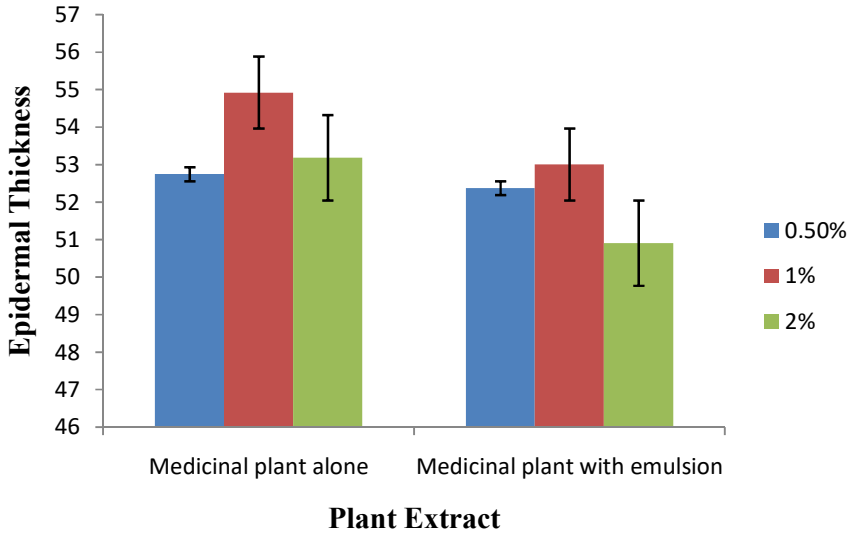

Fig.91 Epidermal thickness comparison of *Acalypha wilkesiana* extracts with the positive control against *Trichophyton mentagrophytes*

a= Positive Control vs 0.5% medicinal plant extract alone

b=Positive Control vs 0.5% medicinal plant extract with emulsion

c=Positive Control vs 1% medicinal plant extract alone

d=Positive Control vs 1% medicinal plant extract with emulsion

e=Positive Control vs 2% medicinal plant extract alone

f=Positive Control vs 2% medicinal plant extract with emulsion

Table 125 Analysis of variance showing the difference in the epidermal thickness between 6 different groups of *Acalypha wilkesiana* formulations against *Trichophyton mentagrophytes*

	Sum of Squares	Df	Mean Square	F	P	N	Remark
Between Groups	1613.386	6	268.898				
Within Groups	72.971	28	2.606	103.180	0.000	35	Significant
Total	1686.356	34					

P value is computed at 3 decimal places

P value is computed at 95% confidence interval

Fig.92 Keratinisation comparison of *Acalypha wilkesiana* extracts with the positive control against *Malassezia furfur*.

a= Positive Control vs 0.5% medicinal plant extract alone

b=Positive Control vs 0.5% medicinal plant extract with emulsion

c=Positive Control vs 1% medicinal plant extract alone

d=Positive Control vs 1% medicinal plant extract with emulsion

e=Positive Control vs 2% medicinal plant extract alone

f=Positive Control vs 2% medicinal plant extract with emulsion

Table 126 Analysis of variance showing the difference in the keratinization between 6 different groups of *Acalypha wilkesiana* formulations against *Malassezia furfur*

	Sum of Squares	df	Mean Square	F	N	P	Remark
Between Groups	3648.317	6	608.053	318.527	35	0.000	Significant
Within Groups	53.451	28	1.909				
Total	3701.767	34					

p value is computed 3 decimal places
p value is computed at 95% confidence interval

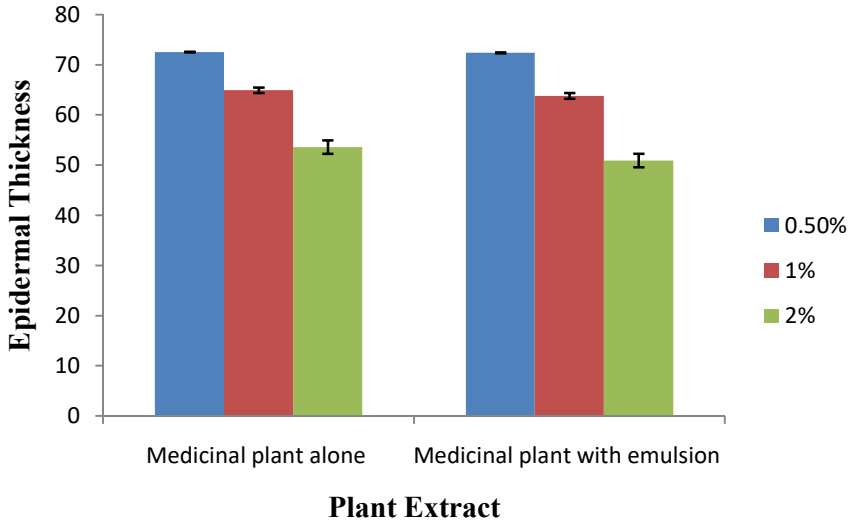

Fig.93 Epidermal thickness comparison of *Acalypha wilkesiana* extracts with the positive control against *Malassezia furfur*.

a= Positive Control vs 0.5% medicinal plant extract alone

b=Positive Control vs 0.5% medicinal plant extract with emulsion

c=Positive Control vs 1% medicinal plant extract alone

d=Positive Control vs 1% medicinal plant extract with emulsion

e=Positive Control vs 2% medicinal plant extract alone

f=Positive Control vs 2% medicinal plant extract with emulsion

Table 127 Analysis of variance showing the difference in the epidermal thickness between 6 different groups of *Acalypha wilkesiana* formulations against *Malassezia furfur*

	Sum of Squares	df	Mean Square	F	N	P	Remark
Between Groups	3689.351	6	614.892	314.002	35	0.000	Significant
Within Groups	54.831	28	1.958				
Total	3744.182	34					

p value is computed 3 decimal places

p value is computed at 95% confidence interval

ANIMAL CARE USE AND RESEARCH ETHICS COMMITTEE (ACUREC)

UNIVERSITY OF IBADAN

☎ 08176917269

E.mail: animaluseresearch@gmail.com / animaluseresearch@yahoo.com

Our Ref: ..... Your Ref: .....

Date: .....

U.I. ACUREC/FAB/29-12-2016

29th December, 2016

FAWEHINMI, Akinlolu B,
Department of Chemistry
Faculty of Science,
University of Ibadan
Ibadan.

NOTICE OF ETHICAL APPROVAL FOR A RESEARCH PROJECT PROPOSAL

On behalf of the University of Ibadan Animal Care and Use Research Ethics Committee (UI-ACUREC), I write to grant you an ethical approval to carry out your research project work titled:**"DEVELOPMENT, PRODUCTION AND EFFICACY TEST FOR HERBAL DERMATOSIS CREAM PRODUCED WITH SOME SELECTED NIGERIAN MEDICINAL PLANTS."**

refers: strictly as outlined in yourproposal submitted for assessment.

Please quote **UI-ACUREC/App/12/2016/06** as reference for this approval.

You are to note that **UI-ACUREC** reserves the right to monitor and conduct compliance visit to your research site without previous notification.

Thank you.

Prof. S.I.B. Cadmus
Chairman, UI-ACUREC

NB: The committee reserves the right to revoke this approval if there is non-compliance to the approved proposal concerning ACUREC guidelines

Chairman: Professor S. I. B. Cadmus (DVM, Ph.D)
Department of Veterinary Public Health and Preventive Medicine, Faculty of Veterinary Medicine, University of Ibadan, Nigeria