USMANU DANFODIYO UNIVERSITY, SOKOTO
(POSTGRADUATE SCHOOL)

ANTIMICROBIAL ACTIVITIES OF LEAF EXTRACTS OF *Mimosa pudica* Linn. (SENSITIVE PLANT) AGAINST MICROBIAL FLORA ASSOCIATED WITH RAZOR BUMPS IN SOKOTO, NIGERIA

A dissertation

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MUHAMMAD MUSA TANKO
(112103021241)
DEPARTMENT OF BIOLOGICAL SCIENCES

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DEDICATION

This work is dedicated to my late dad Mal. Muhammad, my mum Haj. Hajara Umar, and my elder brother Alh. Umar Musa Jega.

CERTIFICATION

This dissertation by Muhammad Musa Tanko (Adm. 112103021241) has met the requirement for the award of the Degree of Master of Science (Biology) of the Usmanu Danfodiyo University, Sokoto, and is approved for its contribution to knowledge.

…… …….

External Examiner Date

…….. …….

Dr. K. Abdullahi Date

Major Supervisor

……... …….

Dr. K. Shehu Date

Co-supervisor I

…… ……

Dr. (Mrs) S. A. Shinkafi Date

Co-supervisor II

…… ……

Dr. K. Shehu Date

Head of Department

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ABSTRACT

Research was carried out on "Microorganisms associated with razor bumps and their responses to sensitive plant leaf extracts *Mimosa pudica*''. All the bacterial species were isolated and identified based on biochemical test conducted. The fungal species isolated were identified based on morphological and cultural characteristics. Nine bacterial species and one genus were identified. The isolated bacterial includes, *Bacillus cereus, B. anthracis, B. mycoids, B. panthothencus, Staphylococcus aureus, S. capitis*, *Pseudomonas aeruginosa, P. putida, Proteus mirabilis* and *Citrobacter spp.* Eight fungal species were identified, the species includes; *Trichophyton verrucosum, Microsporum ferrugineum, T. schoenleinii, T. rubrum, T. concentricum, T. soudanense, M. canis*, and *M. gypseum*. Among the bacterial species isolated, *S. aureus* had the highest percentage frequency of occurrence (20.00%), bacteria with the least frequency was *B. panthothencus, B. mycoids and Citrobacter spp* (4.44%). The fungal isolate with the highest frequency of occurrence was *T. schoenleinii* (17.76%), the fungal isolate with the least frequency of occurrence was *M. ferrugineum, T. concentricum,* and *M. gypseum* (5.88%). The phytochemical analysis conducted revealed the presence of different phytochemical components such as alkaloids, saponin, tannins, flavonoids, glycosides, steroids, saponin glycosides, balsams and volatile oil. The antimicrobial activity of the extracts of *M. pudica* was tested against the isolated organisms using the agar well diffusion method at different concentrations of 150mg, 200mg, 250mg, and 300mg. *Citrobacter spp* and *P. mirabilis* revealed the highest diameter of zone of inhibition at different while *P. putida* and *B. mycoids* were not inhibited. *T. verrucosum and Trichophyton soudanense* revealed highest diameter of zone of inhibition while *M. canis* revealed the least diameter of zone of inhibition. *T. concentricum*, and *M. gypseum* with least frequency of occurrence. The presence of phytochemical components might be the reason for the inhibition in growth of some of the microorganisms. This indicates that *M. pudica* possess anti-microbial activity.

CHAPTER ONE

1.0 INTRODUCTION

Tinea barbae, is a mycosis usually caused by zoophilic and anthropophilic dermatophytes. The infection is more common in rural areas, and usually involves the hair and hair follicles of the beard and mustache. It is an exceptional tinea, limited only to adult males, and occurs in two modalities: mild superficial, very similar to the common tinea, and deep, which typically causes pustular folliculitis or severe kerion-like inflammation (Greer, 1990; Rippon, 1992). Tinea barbae is a rare dermatophytic infection that is limited to the beard area of the face and neck (Bonifaz *et al.*, 2003). Infection occurs almost exclusively in males– teenagers and adults. Typical clinical symptoms are severe pustular eruption, deep inflammatory plaques or non-inflammatory superficial patches (Trotha *et al.*, 2003; Szepietowski and Schwartz, 2004).

Bacterial infection of razor bumps is pseudofolliculitis barbae (PFB), it is a problem caused when the hair curves back into the skin due to thick curly nature of the hair which causes itching and tiny bumps that could later develop into big bumps and unsightly darkening of the skin. This problem is common among colored races including Africans, Caribbeans, Afro Americans and Hispanics. In medicine, it is termed as persistent irritation caused by shaving (Rapini *et al.*, 2007). The most common cause of bumps can be attributed to the use of infected and un-sterilized blades, clippers, bad and blunt clipper blades. Generally, bumps are formed through inflammation of the hair follicle. Shortly after a hair cut or close shave the hair begins to grow, it curls back into the skin where it bores a hole. When this happens inflammation of the skin occurs and it could result into bumps. Bumps can also

develop as a result of bacterial infections of cut sustained during or using a fake, unhygienic infected and un-sterilized blades or clippers (Rapini *et al.,* 2007).

Generally, tinea barbae is infrequent, but it is more common in areas where weather condition are tropical, characterized by high temperature and humidity (Shrum *et al*., 1994). Almost exclusively adult males are affected because this dermatophytosis is localized in the hairs and hair follicle of the beard and mustache. Dermatophyte infections in females and children are diagnosed as tinea faciei (Szepietowski and Schwartz, 2004). In the past, infection was often transmitted by barbers because of single-use razors blade. Nowadays this source of infection is almost eliminated and the old definition of tinea barbae, barber's itch, is forgotten (Kawada *et al*., 2000). In the rural regions, cattle, horses, cats and dogs are the main sources of infection. Recently, some authors reported infection as an autoinoculation from fingernails or tinea pedis (Beswich *et al*., 1999).

Tinea barbae is caused by zoophilic and antropophilic fungi. Zoophilic dermatophytes – *Trichophyton mentagrophytes var. granulosum* and *Trichophyton verrucosum* are most often responsible for inflammatory Kerion-like plaques and infection caused by them is more severe. Infections caused by other zoophilic fungi e.g. *Microsporum canis* and *Trichophyton mentagrophytes var. intrdigitale* are rare (Rippon, 1992; Drake *et al*., 1996). In recent years some authors described similar lesions caused by the antropophilic fungus *Trichophyton rubrum* (Ive, 2000). Pseudofolliculitis barbae is mostly common on the male face, but it can also happen on other parts of the body where hair is shaved or plucked, especially areas where hair is curly and skin is sensitive, such as genital shaving also termed pseudofolliculitis pubis.

After a hair has been shaved, it begins to grow back. Curly hair tends to curl into the skin instead of straight out of the follicle, leading to an inflammation reaction. Pseudofolliculitis barbae can make the skin look itchy and red, and in some cases, it can even look like pimples. These inflamed papules can form especially if the area becomes infected.

This is especially problematic for some men who have naturally coarse or tightly curling thick hair. Curly hair increases the likelihood of pseudofolliculitis barbae by a factor of 50 (Winter *et al*., 2004). If left untreated over time, this can cause keloid scarring in the beard area. Pseudofolliculitis barbae can further be divided into two types of in grown; transfollicular and extrafollicular. The extrafollicular hair is a hair that has exited the follicle and re-entered the skin. The transfollicular hair never exist the follicle, but because of its naturally curly nature curls back into the follicle causing fluid build-up and irritation.

Traditionally, the extracts of *Mimosa pudica* herbs incorporated with other herbs in the poly herbal formulation was used to treat wounds (Molina *et al*., 1999). The study of the plants also shows that methanolic extracts of the roots exhibit good wound healing activity and skin diseases due to phenols constituents (Kokane *et al*., 2009). The root is bitter, acrid, cooling, vulnerary, alexipharmic, and used in the treatment of leprosy, dysentery, vaginal and uterine complaints, inflammations, burning sensation, asthma, leucoderma, and fatigue and blood diseases (Renu, 2010). In some traditional Healthcare System, its root is resolvent, alternative, and useful in the treatment of diseases arising from blood impurities and bile, bilious fevers, piles, jaundice, and leprosy, etc. Decoction of the root is used with water to gargle to reduce toothache. It is very useful in treating diarrhea, amoebic dysentery, bleeding piles and urinary infections. It arrests bleeding and promote wound

healing process. It is mainly used in herbal preparations for gynecological disorders. It has been said to have medicinal properties to cure skin diseases. It is also used in conditions like bronchitis, general weakness and impotence. The content of *M. pudica* has a capacity to arrest bleeding and it fastens the process of healing of wounds. It is recommended in diarrhea, amoebic dysentery and bleeding piles. Some herbal doctors recommend it for bronchitis, general weakness and impotence. All the five parts of the plant that is, leaves, flowers, stems, roots, and fruits are used as medicines in the traditional healthcare systems. In India, different parts of the plant have been in popular use for treating various ailments since long. Recent researches show that the extract of this plant has contraceptive properties (Gordon and David, 2001).

Many researchers have revealed that *M. pudica* is a mood enhancer and improves circulation of the blood. Some believe Mimosa can reduce the onset of baldness. Due to its ability to promote healthy cell growth, it is used in shampoos, creams, capsules, and soaps which are applied as facial cleansers (Gordon and David, 2001). *M. pudica* root is used to treat bilious fevers, piles, jaundice, leprosy, dysentery, vaginal and uterine complaints, inflammations, burning sensation, fatigue, asthma, leucoderma, and blood diseases. In Western medicine, Mimosa root is used for treating insomnia, irritability, premenstrual syndrome, menorrhagia, hemorrhoids, skin wounds, and diarrhea (Nair *et al*., 2005). It is also used to treat whooping cough and fevers in children, and there is some evidence to suggest that Mimosa is effective in relieving the symptoms of rheumatoid arthritis. All parts of the Mimosa plant are reportedly toxic if taken directly. Its consumption is not recommended for pregnant or nursing ladies. Due to these reports, it seems to be best to consult a physician before using Mimosa internally (Ahmad and Beg, 2001). It is used in

parts of the southeastern Nigerian as herbal remedy for hyperglycemia. It produces liquid oleoresin, which has been used as medicine by indigenous people for more than 400 years. The oleoresin is produced in the tree's trunk, stem, and leaves and is traditionally used as an anti-inflammatory agent and in the treatment of a variety of genitor-urinary tract diseases and skin ailment.

1.1 Statement of the Problem

Razor bumps (Pseudofolliculitis barbae and tinea barbae) is a common occurring problem among black Africans, Caribbean and Hispanics. It causes rash and itch. Many drugs and medications have been shown to reduce swellings, redness and itchiness but not very effective. Antibiotics and antifungal gels or oral antibiotics were also used but are expensive and not readily available (Halder *et al*., 2006). Therefore, there is a need to device a cheaper traditional way of treating these infections.

1.2 Justification

Nigeria have a long history of culture that relies on herbal products as theurapeutics and today, herbal source is the most reliable foundation to discover new chemical entities for health care product (Christopher and Nkechi, 2010). Knowledge and application of ethno-medicinal properties of plants date back to about 300 years BC (Makhubu, 1998; Ogbonna *et al.,* 2007). About 80% of the world's inhabitants rely mainly on traditional medicines for their primary health care (Owolabi *et al.,* 2007). According to World Health Organization, medicinal plants would be the best source to obtain a variety of drugs. Therefore, such plants should be investigated to better understand their properties, safety, and efficacy (Nascimento *et al.,* 2000). More than 95% of traditional preparations in Nigeria are of plant

origin (Iwu, 1993). Plant products still remains the principal source of pharmaceutical agents used in traditional medicine and therefore this long history use of medicinal plants in Nigeria and its diverse plant species can be of paramount importance in future research and drug discovery. In view of this, the present study focused on *Mimosa pudica* leaf extracts against microbial isolates of razor bumps.

1.3 Aim and Objectives

The aim of the study is to isolate and identify the microbes associated with razor bumps and to determine the effects of leaf extract of *Mimosa pudica* on management of the associated microbes. The specific objectives are;

1. To isolate and identify the microorganisms associated with razor bumps.
2. To assess the antimicrobial activities of aqueous and ethanol leaf extracts of *Mimosa pudica* on the tested organisms.
3. To determine the phytochemical constituents of the *Mimosa pudica* leaves.

CHAPTER TWO

2.0 LITERATURE REVIEW

The history of herbal medicine was rather old and dates back to the time when the early man becomes conscious of his environment. Cultural man was said to have been on earth for some two or three million years and throughout the greater part of the period, he has struggled for his existence as a hunter-gatherer. Thousands of years of experience, by trial and error, have taught him to distinguish between useful and harmful plants with their properties as healing agents dawning on him much later (Baquar, 1995). Since then, medicinal plants have been used in virtually all cultures as a source of medicine (Lanfranco, 1999). The earliest record of human civilization and culture of China, Egypt, Assyria, and Indies valley reveals that the elders and wise men of those times used herbal medicines to treat various diseases.

Information regarding these medicinal herbs are available in the old literature, folklore, mythological stories, epic poems, medical treatises and thousands years old manuscripts. The plants found in the grave were later identified as having various medicinal properties (Banquar, 1995). One of the earliest records of the use of herbal medicine is that of Chaulmoogra oil from *Hydnocarpus gaertn*, which was known to be effective in the treatment of leprosy. Such a use was recorded in the pharmacopoeia of the Emperor of China between 2730 and 3000 B.C.

Similarly, the seeds of the opium poppy (*Papaver somniferum*) and castor seed (*Ricinus communis*) were excavated from some ancient Egyptian tombs, which indicated their use in

that part of Africa as far back as 1500 B.C. The records available in “Ebers papyrus” also confirm that medicinal plants were used at that time in Egypt (Lanfranco, 1999). According to medicinal records, the Materia Medica of Hippocrates, who is now referred to as the father of medicine consisted essentially of herbal recipes, some 400 simple remedies having been compiled and described by him. Theophrastus of Athens (370-287 B.C) was another famous Biologist-botanist who produced a number of manuscripts including the famous Historia plantarium. About 500 plants, mostly cultivated, were described in this manuscript (Evans, 2000). A Roman Naturalist and Philosopher described 1000 plants with their medicinal properties, anatomy and horticultural practices in his book, Historia Naturalis. Dioscorides (60 A.D) wrote “De Materia Medica” describing 600 plant species of medicinal value from Mediterranean region. Another manuscript, the Alicia Juliana Codex, was prepared for the daughter of Byzantine Emperor about 512 A.D from material originally compiled by Dioscorides (Douglas, 2001). In the middle ages, the writing of Galen (131 A.D) became popular. Galen is considered today to be the most distinguished physician of antiquity after Hippocrates. He treated diseases essentially by the use of herbs. Allopathic as well as homeopathic systems of medicine today are based on the doctrine explained by Galen (Lanfranco, 1999).

During the last decade, the use of traditional medicine has expanded globally and has gained popularity. It has not only continued to be used for primary health care of the poor in developing countries, but has also been used in countries where conventional medicine is predominant in the National health care system (Vickers, and Zollman, 1999). It has been confirmed by WHO that herbal medicines serve the health needs of about 80% of the world’s population, especially for millions of people in the vast rural areas of developing

countries (WHO, 2001). The practice of traditional medicine is widespread throughout Asia including China, India, Japan, Pakistan, Srilanka, and Thailand. In Japan, herbal medicinal preparations are more in demand than mainstream pharmaceutical products (Baquar, 1995). 60 to 70% of allopathic doctors in Japan prescribe traditional medicine for their patients. In Malaysia, traditional forms of Malay, Chinese and Indian medicine are used extensively (Der *et al*., 2000). China is the leading country for incorporating traditional herbal medicine into a modern health care system. In this country, a traditional medicine account for around 40% of all health care delivered and is used to treat roughly 200 million patients annually. According to a recent survey, almost 7,300 plants have been used in traditional Chinese medicine (Der *et al*., 2000; WHO, 2001).

The majority of African population relies on traditional medicine for the treatment of both human and animal diseases (Singh, 2002). In Tanzania, Uganda and Zambia, researchers have found a ratio of traditional medicine practitioners to population of 1:200 to 1:400. This contrasts starkly with the availability of allopathic practitioners, where the ratio is typically 1:20,000 or less. Survey conducted by WHO Roll Back Malaria Programme in 1998 showed that in Ghana, Mali, Nigeria and Zambia, more than 60% of children with high fever are treated at home with herbal medicine. One of the key reasons cited for this was the readily accessibility of herbal medicine in the rural areas (Der, *et al*., 2000).

In Nigeria, traditional remedies represent not only part of the struggle of the people to fulfill their essential drug needs but also they are integral components of the cultural beliefs and attitudes (Elujoba *et al*., 2005). It is customary to find medicinal plants in markets where food items and spices are sold. Fresh and dried leaves, flowers, roots, bark, seeds,

etc. of medicinal plants are displayed for sale in most markets in Nigeria along with spices such as pepper, cardamom, ginger, etc. Some of the common uses of the medicinal plants sold in markets include fumigation, vermifuge, pain relief, treating skin infections, etc. Antimicrobial and wound healing plants are among some of the major medicinal plants that are commonly available in markets (Iwu, 1993).

The Nigerian flora was estimated to be more than 6,500 and 7,000 species of higher plants of which about 3/4 are endemic. Nigeria is also a home for many languages, cultures and beliefs that have in turn contributed to the high diversity of traditional knowledge and practice of the people, which, among others include the use of medicinal plants. More than 95% of traditional preparations in the country are of plant origin (Iwu, 1983). Despite its significant contribution to society, traditional medicine has received very little attention in modern research and development and less effort has been paid to upgrade the traditional health practices in the country. But, the long history of use of medicinal plants in Nigeria and its huge biotic riches can be of paramount importance in future research and drug discovery.

Mimosa pudica Linn. is a native to South America and Central America. It has been introduced to many other regions and is regarded as an invasive species in Tanzania, South Asia, and South East Asia and many pacific Islands. Sharma *et al*., (2001) it is regarded as in parts of Australia. In other areas of the world like the Northern territory and Western Australia, it is declared as a weed. This plant was introduced to Nigeria, Mauritius and some other African countries, Seychelles and East Asia but is not regarded as invasive (Hartmut and Thorsten, 2006).

2.1 Keratin Polymorphism

A common polymorphism is a keratin gene (K6HF) has been linked to pseudofolliculitis barbae, suggesting that it may be a genetic risk factor (Robert, 2004). This sequence change leads to an amino acid substitution in the highly conserved helix initiation motif of the k6hf rod domain (Winter *et al.*, 2004). Carries of the A12T polymorphism are six times more likely to develop pseudofolliculitis barbae compared with people homozygous for the wild-type K6hf sequence (Halder *et al*, 2006). This suggest K6hf mutation structurally weakens the companion cell layer and increase the chances that a beard hair will in-grow (Maclean, 2004).

2.2 Biology of *M. pudica*

The plant is round, often woody stem, reddish brown or purple, short, and curved prickles. The plant bears leaves with dark green color, feathery, fern-like, which is divided into one or more pairs of segments near the end of the leaf stalk. The segment is divided into 10-25 pair of leaflets. These small leaflets close up when disturbed externally (Cheryld, 2006). The leaves are bitter, sudorific, tonic and are used in hydrocele, hemorrhoids, fistula, sucrofula, conjunctivitis, cuts, and wounds and hemorrhage (Vaidyaratanm, 2001; Pande and Pathak, 2010). The flowers are pale pink in fluffy balls, present on short stalks in leaf forks and develop into clusters of seed pods. As a member of family *Fabaceae*, the root system consists of a tap root and extensive fibrous roots with nodules (Loigier, 1988). The roots are bitter, astringent, acrid, cooling vulnerary, alexipharmic, resolvent, diuretic, antispasmodic, emetic, constipating, and febrifuge and are used in vitiated conditions of pifta, leucoderma, vaginopathy, metropathy, ulcers, dysentery, inflammations, burning sensation, hemorrhoids, jaundice, asthma, fistula, small pox, strangury, spasmodic,

affections and fever (Pande and Pathak, 2010). The plant is a well known weed of cultivated areas, such as road sides, and swampy areas. It grows on a wide variety of soil and can stand considerable shading. The seed remains viable for many years (Noman *et al*., 1999). Sensitive plant grows on most well drained soil, even scalped or eroded sub soil and soil with low nutrient concentrations. It requires disturbed soil to establish itself.

2.3 Major Constituents in *M. Pudica*

The constituents responsible for pharmacological actions of *M. pudica* are flavonoids, phytosterol, alkaloids, amino acids, saponins, tannins, phenolics, essential oils, fatty acids, courmarin, anthroquinone and quinines (Gandhiraja *et al*., 2009). Indoleamines like serotonin, melatonin, and calcium channels were indicated to be responsible for shoot organogenesis in *M. pudica*, it main role is physiological but in future it may be the basis for cellular signaling mechanism in plants (Ramakrishna *et al*., 2011).

2.4 Antimicrobial Effect

The leaf powder of *M. pudica* has been reported to have antibacterial activity against *Escherichia coli* and antifungal activity against *Aspergillus niger* when screened by disc diffusion method (Muthukumaran *et al*., 2011). Recently, a study reported antibacterial activity of the plant extract of *M. pudica* by well diffusion method as well as activity against *Aspergillus fumigatus*, *Citrobacter divergens*, and *Klebsiella pneumonia* (Gandhiraja *et al*., 2009). The chloroform extract of *M. pudica* was shown to possess the significant hypolipidemic activity due to presence of flavonoids and glycosides and the action was compared with standard atorvastatin (Rajendran and Krishnakumar, 2010). A poly herbal formulation containing *M. pudica* was used as an alternative therapy in the

management of polycystic ovary syndrome so as to reduce the side effects of modern drug in rats (De vane *et al*., 1975; Bhattacharya *et al*., 2005). The aqueous extracts of *M. pudica* leaves showed significant diuretic activity with increased electrolytes excretion in albino rats (Sangma *et al*., 2010). Another formulation was proved to be effective in minimizing the bleeding during tonsillectomy operation which contains the herb *M. pudica* and was proven clinically with no side effect.

2.5 Wound Healing Activity

The study on the plant showed that methanolic extract of root of *M. pudica* exhibited good wound healing activity due to phenols constituents (Kokane *et al*., 2009). Wound healing activity was studied in three types of model in rats viz; excision, incision and estimation of biochemical parameter in a study. In another study reported the shoot and root extracts of *M. pudica* was used to cause epithelization of wound in mice.

2.6 Common Dermatological Disorders and Their Prevalence

The skin is primarily an organ of protection. As the body's first line of defense, the skin is continuously subjected to potentially harmful environmental agents, including solid matter, liquids, gases, sunlight, and microorganisms (Mitscher *et al*., 1987). Although it has remarkable properties that allow for a continuous cycle of healing, shedding and cell regeneration, the skin is subjected to a very wide range of disorders (Simandi, 1990). An intact skin surface, its relative dryness, desquamation of cells, a surface pH between 5.0 and 6.0 and a normal flora of coagulase-negative *staphylococci* and other gram-positive bacteria such as diphtheroids are all barriers to infection. In addition, sebum produced by the sebaceous gland is converted to free fatty acids by the normal flora of the skin, and this

fatty acid inhibit the growth of pathogens such as group A *streptococcus*. Bacteria enter the skin via minor abrasions, surgical incisions or via the hair follicles. It is likely that conditions such as cellulitis may also arise as a result of an occult bacteraemia (Owen *et al*., 2003).

2.7.0 Infectious Skin Disorders

The skin is subject to attack by a number of microorganisms. Normally the skin flora, sebum, immune responses and other protective mechanisms guard the skin against infection. Depending on the virulence of the infecting agent and the competence of the host's resistance, infections may result (Mitscher *et al*., 1987).

2.7.1 Bacterial Infections

Bacteria are considered normal flora of the skin. Most bacteria are not pathogenic, however, when pathogenic bacteria invade the skin, superficial or systematic infections may develop. Bacterial infections are classified as primary, or superficial (e.g. impetigo) and secondary, or deep (e.g. infected ulcers) (Mitscher *et al*., 1987). The most common bacterial infections include impetigo, folliculitis, furuncles and carbuncles (Mitscher *et al*., 1987; Reynolds, 1996).

Cellulitis; this is a diffuse inflammation of connective tissue with severe inflammation of dermal and subcutaneous layers of the skin (David *et al*., 2009). Cellulitis can be caused by normal skin flora or by exogenous bacteria and often occurs where the skin has previously been broken; cracks in the skin, cuts, blisters, burns, insect bites, surgical wounds, intravenous drug injection or sites of intravenous catheter insertion (Maseleno and Hasan,

2012). Skin on the face or lower legs is most commonly affected by this infection, though cellulitis can occur on any part of the body. In adults and children, cellulitis is most often caused by *streptococcus, S. aureus,* and *bacillus* (Tevelli, 2001).

Cellulitis is a common bacterial infection of the skin which can affect all ages. It can occur by itself, or complicate an underlying skin condition or wound. In some rare cases, it can be caused by *Pseudomonas aeruginosa*. However, the lesion of cellulitis is erythematous, edematous, brawny, and tender, with borders that are poorly defined (Aly *et al.*, 1978). Folliculitis is the name given to a group of skin conditions which there are inflamed hair follicles which result into a tender red spot, often with a surface pustule. There are two categories on the basis of histologic location; superficial and deep folliculitis (Maibach and Aly, 1992). Impetigo is a highly contagious bacterial skin infection most common pre-school. It is primarily caused by *S. aureus* and sometimes *S. pyogenes* (Kumar *et al*., 2007). Impetigo tends to occur in areas of minor breaks in the skin such as insect bites, cuts, or abrasions. Often people harbor the *staphylococcus* bacteria inside their nose. However, direct contact with infected nasal fluid can infect other parts of the body or other people (Noble, 1981).

Erysipelas is an acute *streptococcus* bacterial infection of the deep epidermis with lymphatic spread (Habif, 2001). Erysipelas is a superficial form of cellulitis, a potentially serious bacterial infection affecting the skin. It most often affects infants and the elderly, but can affect any age group. Risk factors are similar to those for other forms of cellulitis. However, unlike cellulitis, almost all erysipelas is caused by group A beta haemolytic streptococci (*S. pyogenes*) and bacillus (Bisno and Stevens, 1996).

P. aeruginosa is associated with green nail syndrome, which is essentially a greenish discoloration of the nail plate (Shaffner *et al.*, 1987). Whether there is true invasion of the nail plate by the bacteria or just diffusion of the pigment in the nail plate is not certain. *Staphylococcus* and *streptococcus* may be found as secondary invaders (Noble, 1981). In burn injuries of the skin, it is estimated that about 75% of the mortality following the injuries is related to infections (Rajput *et al*., 2008). When a hole is created on the skin, microorganisms, usually the opportunistic organisms invade the holes and multiply leading to a delay in the healing process and finally infectious condition. The spectrum of infections ranges from asymptomatic colonization to bacteraemia and death (Abubakar, 2009). The common pathogens isolated from burn wound are *S. aureus, P. aeruginosa, S. pyogenes* and various coliform *bacilli* (Ahmad and Iranzo, 2003).

S. aureus is a versatile human pathogen. It was the predominant cause of burn wound infection in pre antibiotic era and still persists as an important pathogen, strongly considered as a major cause of nosocomial infection. Burn injuries have become major reservoir for *S. aureus* that has the special characteristics for spreading quickly (Gang *et al*., 2000). Edward-Jones and Greenwood (2003) mentioned that burns become infected because of the environment at the site of the wound which is ideal for the multiplication of the infecting organisms. This happens when there is plentiful supply of moisture and nutrients in the physical environment, the temperature, and gaseous requirement etc., are ideal for the growth, bacteria will proliferate rapidly.

2.7.2 Fungal Infections

Fungal infections of the skin can be superficial, intermediate or deep. Some are opportunistic and affect a susceptible host while some are truly pathogenic and can infect a healthy person. The superficial infections are called dermatophytoses and they are commonly known as tinea, or ringworm. Different forms of tinea affect different body areas. Tinea can affect the body (tinea corporis), scalp (tinea capitis), beard (tinea barbae), hands (tinea manus) feet (tinea pedis), nail (tinea unguium) or groin and upper parts of the thigh (tinea cruris). Individual species of three genera have been identified as the invading fungi in most forms of tinea: *Microsporum*, *Epidermophyton*, and *Trichophyton*. Intermediate fungal diseases invade both the superficial and deeper tissues, moniliasis caused by *Candida albicans* being an example. Deep fungal infections involve the epidermis, dermis and subcutis. Infections that are typically superficial may exhibit deep involvement in immune suppressed individuals (Mitscher *et al*., 1987; Reynolds, 1996).

2.8 Prevalence of Skin Disorders

Skin diseases have a high prevalence throughout the world. In developing countries, infectious diseases are more common, whereas in developed countries inflammatory disorders are more common (Thomas and Robert, 1991). Atopic dermatitis now affects 15 to 20% of children in developed countries and the prevalence in cities in developing countries undergoing rapid demographic changes is quickly following suit (Hailu, 2004).

Atopic eczema affects between 5 and 20% of children aged up to 11 years at one time or other (Williams, 2002). A research made on primary school children in Turkey revealed that infectious skin diseases were frequently observed: pediculosis capitis (9.4%), scabies

(2.2%), viral skin diseases (3.8%), and fungal infection (0.7%) (Fennesy *et al*., 2000). A similar study made in Aman, Jordan showed an overall prevalence of skin disorder to be 19.23% (Inanir *et al*., 2002).

A study made in rural Tanzania also reported that34.7% of 800 villagers had one or more skin diseases, the most common of which were tinea capitis, tinea corporis, scabies, acne and eczema (Shakkoury and Abu-Wndi, 1999). Although limited studies have been made on the prevalence of skin diseases, in Ethiopia, the available records are indicative of the high prevalence of this problem in the country. A study made at Black Lion hospital indicated that skin diseases are among the leading causes of hospital visits. The most common skin diseases were allergic skin diseases (25.5%), infections (25.4%), photodermatoses (22.9%), followed by papulo squamous diseases (11.4%) and pruritus of unknown origin (3.3%). The pattern of skin diseases observed in this analysis shows that allergic and infectious causes account for three-quarter of skin problems (Satima *et al*., 1999). The prevalence study among school children in Southwestern Ethiopia revealed that 80.4% of 112 children included in the study were found to have one or more skin diseases. Infestations were the most prevalent skin pathology (81.2%) followed by fungal infection (Sheibeshi, 2000). Similar study made in Northwest Ethiopia showed a prevalence rate of 49.2%.

Tinea capitis, Tinea sycosis, impetigo, infected wounds and verrucae were the most common skin infections (Figueroa *et al*., 1996). Another study made at 5 elementary schools in Dembia district, North Ethiopia indicated that infectious skin disorders are very common among the children and adults in the rural areas (5 to 15 years old). Only 16% of

all children were found to be free of infectious skin diseases (Dagnew and Erwin, 1991). A study on the prevalence of skin diseases in two different rural communities in Southwestern Ethiopia also showed that parasitic infestations (46%) were the commonest complaints among the case studies followed by bacterial and fungal infections (33%) (Dagnew and Gunther, 1990). A related study made at Kazanchis Health Center in Addis Ababa also reported that bacterial, fungal and viral infections were dominating, comprising 19.4%, 18.5% and 6.5% of the cases (Figueroa *et al*., 1998). The frequency of skin problems in patients with HIV infection and AIDS is well known. As HIV disease progresses, these skin manifestations may become more severe and varied. In advanced immune suppression, opportunistic pathogens may present as atypical cutaneous lesions (Chism, 1993; Hiletework, 1998). Cutaneous and mucosal complications eventually occur in nearly all individuals (up to 92%) with HIV infections. A cutaneous eruption is present in approximately 75% of cases. More than 90%of patients will have oral candidiasis at some stage of their disease. *Staphylococcus aureus* is the most common (50%) cutaneous and systemic bacterial pathogen in HIV infected adults. Superficial fungal infections with dermatophytes are also very common (Chism, 1993; Darley, 1998). Although generally less well known than most opportunistic infections, some of the noninfectious cutaneous disorders associated with HIV disease are remarkably prevalent. Seborrhetic dermatitis is seen in up to 85% of all HIV-infected individuals.

Psoriasis develops in 5% of patients with HIV infection, which is a much higher incidence than the 1 to 2 % reported for the general population. Pityriasis rubra pilaris may develop in 20 to 30% of HIV infected patients. Cutaneous drug reactions are the most common manifestations of drug hyper sensitivity. A recent study of 684 HIV-infected patients in

Boston revealed that 79% had one or more dermatologic diagnosis, 188 of which included cutaneous reactions to drugs (Tappers *et al*., 1995; Cockerell, 1995).

In the Northern flanks of West African countries of Guinea Conakry and Burkina Faso *T. violaceum*, *T. rubrum* and *T. soudanense* were the predominating etiological agents of dermatophytoses (Menan *et al*., 2002; Guiguemde*et al*., 1992). The report from Guinea Conakry indicated a shifting trend as it was noted earlier in 1959 that *T. soudanense* and *M. audouinii* were more prevalent in that country. The two agents were often seen in tinea capitis and tinea sycosis while the former was responsible for tinea corporis cases (Philpot, 1978).

Gaye *et al*., (1994) found that the poor school and home background along with other socioeconomic factors were responsible for the high prevalence of tinea capitis in Dakar, Senegal. In another study conducted in the Mycology laboratory of Hospital A. Le Dantec, Dakar, Senegal, 1512 patients aged from 14 days to 70 years with a mean age of 21.4 years including 882 with suspected tinea were screened and a prevalence of 25.7% was established. The main etiological agents were *T. soudanense* (47.3%), followed by *T. rubrum* (33.41%) and *Microsporum langeronii* (11.3%). Only a single case of *T. violaceum* was isolated. These species were earlier shown to be the predominating agents of dermatophytosis in Senegal. The prevalence of dermatophytosis seems to vary across various regions of Senegal. Develoux *et al.,* (2002) found a frequency of 26.4% in Dakar, where as Cremer *et al*., (1997) reported 11.4 and 11%, respectively. In a recent two year study involving three dermatology Centers in Senegal, 16% of HIV patients with dermatosis had dermatophytic infections suggesting that dermatophytoses could pose a

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

Studies conducted among 463 in the Greater Accra region showed that *T. violaceum* (26%) was the most prominent species in Ghana, followed by *T. tonsurans* (22%). The percentage occurrence of *M. audouinii* (15%) was relatively low compared to other studies performed in Africa. The prevalence of *T. rubrum* was 11% and no *T. soudanense* was recovered in the study (Hogewoning *et al*., 2006). Tietz *et al*., (2002) recovered a rare species of the *T. rubrum* complex (*Trichophyton raubitschekii*) in Germany from a set of four African immigrant patients who presented with typical lesions of tinea corporis. One of the patients was from Ghana and the other three were from Cameroon. In a similar incident in the USA, two African children internationally adopted from Liberia in West Africa, who were residing in Cincinnati, Ohio, presented with tinea capitis associated with *T. soudanense*, a dermatophyte that is not common in the whole of North America (Markey *et al*., 2003).

There are varying reports of dermatophytosis in different cities and communities of Nigeria (Nweze and Okafor, 2005). Considering its human and socioeconomic diversity and the staggering population, this is understandable. This author carried out an extensive survey of dermatophytosis in Nigeria's Northeastern state of Borno. The study involved 2193 children aged between 4-40 years in different local and urban communities of the state. Seven percent were proved to be positive for dermatophytosis. Incidence was significantly higher in young children aged 7-11 years and 4-6 years than in adult aged 18-45 years. Moreover, there was a significant difference in the incidence of dermatophytoses amongst

children in urban and rural areas, thereby emphasizing the role of locality in dermatophytoses.

Tinea capitis was the predominant clinical type followed by tinea corporis and tinea sycosis. *Trichophyton schoenleinii* was the most prevalent etiological agent (28.1%), followed by *T. verrucosum* (20.2%) and *M. gallinae* (18.4%). Other species recovered included *T. mentagrophytes* (16.7%), *T. tonsurans* (10.5%), *T. yaoundei* (4.4%) and *M. gypseum* (1.8%) (Nweze, 2001). In a similar study carried out in Kano State Nigeria, 2150 itinerant Quranic scholars were screened. Only 9.5% were found to be infected and the age group 10-14 years was most affected. *T. rubum* (50.2%) was the most prevalent followed by *M. audouinii* (26.5%). *T. rubrum* was the only dermatophyte that was recovered from all sites apart from the buttocks (Adeleke *et al*., 2008).

Out of a total of 6987 primary school children and teachers sampled across 4 schools in Jos, Plateau state Nigeria, only 3.4% were found to be infected by this disease. There was a high incidence of both scalp and foot ringworms among the infected children and some few beard cases. A large spectrum of fourteen species of dermatophytes was isolated from the ringworm cases, the scalp ringworm had the highest number of fungal isolates. *Trichophyton mentagrophyte* and *T. rubrum* had the highest frequencies of occurrence (Ogbonna *et al*., 1985).

A total of 2772 randomly selected junior secondary school pupils and teachers between the ages of 8-45 years from 60 schools were examined from Ogun state in South Western Nigeria. The prevalence of dermatophytosis was 23.21%. Etiological agents identified with

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

Earlier studies carried out between 1974-1977 in the old Anambra state of Nigeria, (now subdivided into two States: Enugu and the new Anambra states), 3478 primary and secondary school children aged 4-20 years made up of 1868 males and 1610 females were screened for dermatophytoses. A total of 303 (8.7%) mycologically proven cases of tinea capitis and a few case of tinea sycosis were detected. *Microsporum audouinii* was the commonest etiological agent (48.3%) followed next by *T. soudanense* (26.6%) and *T. tonsurans* (15.2%). Other dermatophytes occasionally represented were *M. ferrugineum* (3.4%), *T. violaceum* (3.7%), *T. yaoundei* (1.2%), *T. mentagrophytes* (0.9%) and *T. schoenleinii* (0.6%). Investigation of scalp carriage of dermatophytes by the authors showed that approximately 9% of children without any clinical signs of tinea capitis harbor dermatophytes in their scalp hair (Gugnani and Njoku-Obi, 1986).

More recently, further studies involving this author were conducted in the new Anambra State by screening 1624 children with clinically suggestive lesions. These children aged between 4 and 16 years were sampled in selected urban and rural areas of the State. Our data showed that tinea capitis was the predominant clinical type. *T. tonsurans* was the most prevalent etiological agent while *M. audouinii* was the least in occurrence (Nweze and Okafor, 2005). Emele and Oyeka (2008) in another larger study which involved a total of 47723 primary school children residing in different regions of Anambra State, found that

4498 (9.4%) had tinea capitis. The highest prevalence of the disease occurred in the Southern region of the state (12.6%). Schools in urban areas recorded lower prevalence of the disease. More so, tinea capitis occurred significantly more in children below 10 years of age than in those above this age. This agrees in part with the findings in Anambra state by Nweze and Okafor (2005). *M. audouinii* was the most prevalent (42%), followed by *M. ferrugineum* (17%) and *T. mentagrophytes* (16%).

Although, dermatophytes respond well to conventional antifungal agents (Weitzmann and Summerbell, 1995; Nweze *et al*., 2007) many patients usually cannot afford the cost of conventional antifungal and antibacterial antibiotics but use local medicinal plants to treat the infections. Our laboratory tested some of these plant extracts which are in use in Nigeria against dermatophytes recovered from patients and indeed found that some of them have good *invitro*activities against dermatophytes (Okafor *et al*., 2001; Nweze *et al*., 2004). Extracts of *Mimosa pudica* leaves has been shown to have medicinal effects on some of the listed microbes. It has also been shown to have effect on dermal surface reactions of various kinds.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Sample Collection

The leaves of the mimosa plant (*Mimosa pudica*) were collected around kwalkwalawa River along University Road, Sokoto. The plant material was authenticated by a plant Taxonomist in the Herbarium, Department of Biological Science, Usmanu Danfodiyo University, Sokoto.

The microbial isolates were collected from different individuals in different areas of Sokoto metropolis. Cotton wool and methylated spirit was used to surface sterilizes the identified position. The methylated spirit was left for some minute to dry up. Sterilized razor blade was used to scrape up the identified position. The sample collected was inoculated into culture media and labeled appropriately. Vaseline was used to cover the injured position. It was then brought to the Microbiology and Mycology Laboratory of Usmanu Danfodiyo University, Sokoto.

3.2.0 Media Preparation

The media used for the research were nutrient agar and potato dextrose agar. All the media were prepared according to manufacturer's instructions.

3.2.1 Nutrient Agar

Nutrient Agar was prepared by weighing twenty eight grams of the powder using a meter balance which was then transferred into a conical flask containing 1000mls of distill water. This was then heated using hot plate to uniformly dissolve the powder. This was followed

by autoclaving at 121^0C for fifteen (15) minutes. The prepared media was allowed to cool to about 40-37^0C and then dispersed into Petri-dishes and allowed to solidify (Cheesbrough, 2000).

3.2.2 Potato Dextrose Agar (PDA)

The media used in this research was Potato's Dextrose Agar (PDA). The media was prepared in accordance with the manufacturer's instructions. Thirty nine grams of Potato's Dextrose Agar (PDA) was weighed and dissolved in 1000ml of distilled water. The pH was adjusted to 5.6, the media was well shake and then autoclaved at 121^0C for fifteen (15) minutes. The sterile medium was allowed to cool to 45^0C before it was poured into sterile Petri-dish (Cheesbrough, 2000).

3.3 Serial Dilution

The procedure was adopted according to Allan *et al*., (1998). From the bottle containing the isolate, nutrient broth serial dilution was carried out by pipeting 1ml from the test tube and transferred to another tube containing only 9ml of nutrient broth. From this test tube subsequent dilution were carried out by repeating the procedure up to 5^{th} labeled (10^{-5}) tube 0.1ml or 1ml. The diluents in test tube labeled 10^{-5} were inoculated to the plates.

3.4 Inoculation of Culture Media

This is based on the procedure outlined by Allan *et al*., (1998). A small volume of bacteria suspension was transferred to the surface of a solidified medium in a Petri-dish. Glass rod was flame sterilized and allowed to cool. This was allowed to air dry and incubated at 37^0C for 24 hours.

3.5 Sub-Culturing of Bacteria

This was done to separate bacteria colonies (where colonies appeared). These were gram stained and sub-culture on a fresh media (nutrient agar) purified isolation were transferred on to slants in universal bottles. The slants were incubated and preserved in a refrigerator at 4^0C (Cheesbrough, 2000).

3.6 Gram Staining

This was carried out according to the modified version of Allan *et al*. (1998). A smear was prepared by placing a drop of normal saline in the middle of a clean slide. An inoculation loop was flame sterilized and allowed to cool. Bacteria colony was touched with wire loop and transferred to the middle of the slide containing a drop of normal saline and streaded into a thin smear along the slide. Smear was allowed to air dry and flamed (Fawole and Oso, 1988). The prepared smear was flooded with crystal violet for 1 minute. The crystal violet was poured off and the slide was flooded with Gram's iodine for 1minute. The slide was washed off with tap water. The slide was decolorized with acetone for 10 seconds. Safranin was added for 20 seconds and was poured off. The slide was rinsed with tap water then the smear was allowed to air dry. Viewing was done using oil immersion objective lens.

3.7 Spore Staining

A 24 hours old culture of organism was smeared with the aid of a sterile loop on a glass slide. The smear was heated fixed. A drop of 5% malachite green was added and washed off, 0.25% safaranin was added for 40 seconds, it was also washed off and left to dry. The

slide was viewed using oil immersion objective lens. The spore stained green while the other part of bacteria stained red or pink (Cheesbrough, 2000).

3.8.0 Biochemical Tests

Biochemical tests were carried out for the confirmation of isolation from the different samples analyzed. These tests varied with organism and they include; catalase oxide, coagulase, indole, urea, hydrogen sulphate, citrate utilization, triple sugar iron, methyl red-voges-proskarver (M.R.V.P) and motility tests.

3.8.1 Catalase Oxide Tests

This test was used to differentiate those bacteria that produce the enzyme catalase. A drop of hydrogen peroxide (H_2O_2) was placed on a clean sterile slide and a loop full of colonies from the Nutrient Agar slant was picked by using a wire loop and emulsified in the H_2O_2 drop. This was observed immediately for gas bubbles and a positive test was indicated by bubbling while a negative test was indicated by absence of bubbling (Cheesbrough, 2000).

3.8.2 Coagulase Tests

This test shows the ability of the organism to form coagulase. A drop of human plasma was made on a glass slide and transfer of heavy inoculums of the suspected organism was made on the drop of the serum. Coagulation of the serum indicated positive test (Cheesbrough, 2000).

3.8.3 Indole Tests

A 24 hours old culture was inoculated into a sterile tryptophan (1%) and incubated for 24 hours. 4 drops of chloroform and 5 drops of Kovac's reagent were added. This was allowed to stand for 20 seconds. A positive reaction was indicated by a red colour at the top layer (Cheesbrough, 2000).

3.8.4 Triple Sugar Iron Agar Tests

A 24 hour old culture was inoculated into a sterile triple sugar medium in test tubes. This was incubated for 24-48 hours. The reaction of both lactose and glucose were observed, for glucose positive result, the bottom of the tube was red while for lactose all tube was yellow. Similar reaction for sucrose was observed (Cheesbrough, 2000).

3.8.5 Hydrogen Sulphide Production Tests

A 24 hour old culture was stabled into the various tubes containing triple sugar iron (TSI) agar and incubated for 24-48 hours. A black line along the line of inoculation indicated a positive reaction (Cheesbrough, 2000). The sample procedure as in hydrogen sulphate was applied and creaking of the tube indicated a positive result.

3.8.6 Methyl Red-Voges-Proskaver (M.R.V.P)

A 24 hour old culture was inoculated into various sterile tubes containing M.R.V.P medium and incubated for 24-48 hours. Four drops of methyl red indicator was added to the M.R.V.P medium and allowed to stand for few seconds. The positive reaction was indicated by a red colour of the indicator while negative reaction was indicated by changing in colour from red to yellow colour (Cheesbrough, 2000). Then 0.5ml of 40% potassium

hydroxide was added which was followed by 1ml of alpha-napthol. This set up was allowed to stand for 40 minutes. A positive result was indicated by pink-red colour while negative reaction was indicated by a brown colour.

3.8.7 Urea Tests

Urea agar in universal bottle was inoculated with a 24 hours old culture and incubated for 24-48 hours at 37^0C. A positive reaction was indicated by a deep blue colour (Cheesbrough, 2000).

3.8.8 Motility tests

Bacteria were introduced into a semisoft agar medium by performing a stab with an inoculating needle. After inoculating the test tube, motility was determined by examining whether or not the bacteria have migrated away from the stab line and throughout the medium (Cheesbrough, 2000).

3.8.9 Citrate utilization

Bacterial colonies were picked up from inoculating loop and inoculated into slope of Simmons citrate agar and incubated for 24 hours at 37^0C. If the organism has the ability to use citrate, the medium changes its colour from green to blue (Cheesbrough, 2000).

3.8.10 Starch hydrolysis

Starch agar was used for cultivating microorganism being tested for starch hydrolysis. Flood the surface of a 48-hour culture on starch agar with gram iodine. Iodine solution (Grams) was an indicator of starch, when iodine comes in contact with a medium

containing starch, it turns blue. If starch was hydrolyzed and starch was no longer present, the medium will a clear zone next to the growth (Cheesbrough, 2000).

3.9 Isolation of Fungi

The samples were surface sterilized for 60 seconds in 1% sodium hydro chlorite and rinsed in three changes of sterile distilled water. Segment of the surface sterilized isolate of 5mm in diameter were placed on Potato's Dextrose Agar (PDA) (Ataga and Ota-Ibe, 2006) and incubated at room temperature (28 ± 2^0C) for 21 days at which time, the development and growth of the fungi was evident on the medium.

3.10 Sub-Culturing of Fungi

More Potato's Dextrose Agar (PDA) plates were prepared and allowed to solidify. A small portion of each of different fungal colony singly placed in the center of the Potato's Dextrose Agar (PDA) plate and allowed to inoculate at room temperature (28 ± 2^0C) for 21 days. Subculture was done to obtain the pure isolate. The developing fungal colonies were sub-cultured repeatedly on fresh PDA plate until pure cultures of the isolate were obtained (Chiejina, 2006).

3.11 Fungal Identification

The pure cultures of the isolate obtained were subjected to microscopic examination with the view to identify the organism present. Clean grease-free glass was used for identification. A drop of water was placed in the center of the slide, a small portion of the fungal culture was cut out with a sterile inoculating needles. The piece was put in the water droplet and teased out. A cover slip was then placed on the teased portion and mounted on

the microscope stage, clamped with the clips. The viewing was first done with the lower magnification (X_{40}) objective lens. The nature of the mycelium, the types of fruiting body and the spore structure served as the criteria for identification of the isolates. The isolates were identified and confirmed with the mycological atlas. The isolate were identified based on morphological and cultural characteristics in accordance with Barnett and Hunter (1999); Alexopoulos *et al*., (2002).

3.12.0 Preparation of Extracts

The fresh leaves were washed thoroughly 2-3 times with running tap water and once with sterile water, to remove dirt and air dried to constant weight for 5-7 days. The dried leaves were then blended using a household electric blender. The leaf powder was stored sealed in five labeled reagent bottles for further use. The bioactive components were extracted using the methods of Akerele *et al*., (2008).

3.12.1 Extraction of plants materials

One hundred grams of the powdered plant material (*M. pudica* leaves) was weighed on a weighing balance (mettler 166) and kept in a container. Five hundred milliliters of ethanol and water was transferred to the container of the powdered extract, respectively. This was shaken thoroughly and allowed to stay overnight. The solution was filtered and heated at 50^{o}C for 72 hours until the aqueous content evaporated completely. The dry extract was collected and weighed in varying concentration.

3.13 Determinations of Phytochemical Constituents

The freshly prepared extracts were subjected to standard phytochemical analysis for different constituents such as tannins, alkaloids, flavonoids, anthraquinones, glycosides, saponins, balsam, cardiac glycoside, steroids, and volatile oil as described by Jigna *et al.*, (2006).

3.13.1 Test for flavonoids

Mix 3 ml aliquot of the filtrated extracts and 1 ml of 10% sodium hydroxide, the presence of a yellow colour indicates the possible presence of flavonoids compound (EL-Olemy *et al.*, 1994).

3.13.2 Test for tannins

A drop (2-3ml) of the extracts was added to 5% ferric chloride solution and the colour produced was noted. It was observed that condensed tannins usually give a dark green colour and hydrolysable tannins give blue-black colour (El-Olemy *et al.*, 1994). .

3.13.3 Test for glycosides

Drops of H_2SO_4 (2.5ml) was added to $5cm^3$ of the extracts in a test tube. The mixture was heated in boiling water for 15 minutes. It was allowed to cool down and neutralized with 10% NaOH, add 5ml of fehling's solution and boil the mixture. A brick-red precipitate was observed which indicate the presence of glycosides (Jigna *et al.*, 2006).

3.13.4 Test for alkaloids

A portion of the extract was stirred with 2ml of 10% aqueous hydrochloric acid. 1ml was treated with a few drops of Wagner's reagent and the second 1ml portion was treated similarly with Mayer's reagent. The presence of turbidity or precipitation with either of these reagents indicates the presence of alkaloids (El-Olemy *et al.,* 1994).

3.13.5 Test for cardiac glycosides

To one portion of *M. pudica* extract, 2ml of 3.5% ferric chloride solution was added and allowed to stand for 1 minute, 1ml of concentrated of H_2SO_4 was carefully poured down the wall of the test tube so as to form a lower layer. A reddish brown ring at the interface indicates that cardiac glycoside was present (El-Olemy *et al.,* 1994).

3.13.6 Test for steroids

Five grams of the extract was dissolved in 2ml of chloroform, 2ml of sulphuric acid was carefully added to form lower layer. A reddish-brown colour at the interface indicated the presence of a steroidal ring (El-Olemy *et al.,* 1994).

3.13.7 Test for saponin glycosides

Fehling's solution of 2.5ml was added to 2.5ml of the extract, a bluish green precipitate showed the presence of saponin glycosides (El-Olemy *et al.,* 1994).

3.13.8 Test for balsams

The extract was mixed with equal volume of 90% ethanol, 2 drops of alcoholic ferric chloride solution was added to the mixture. A dark green colour indicates the presence of balsams (Jigna *et al.*, 2006).

3.13.9 Test for anthraquinones

A part (0.5g) of the plant extracts was shaked with 10ml benzene, and 5ml of 10% ammonia solution was added. The mixture was shaken and the presence of a pink, red, or violet color in the ammoniacal (lower) phase indicates the presence of anthraquenones (Jigna *et al.*, 2006).

3.13.10 Test for volatile oils

Mix 1ml of the extracts fraction with 1ml of dilute hydrochloric acid. A white precipitate was formed which indicated the presence of volatile oils (El-Olemy *et al.*, 1994).

3.14 Determination of Minimum Inhibitory Concentration

The Minimum Inhibitory Concentration (MIC) was determined as the least concentration that showed an inhibitory effect on test organism using the tube method. Two fold serial dilutions were made using nutrient broth. Then 5 ml of a solution of the extracts (250 mg/ml) was added aseptically to 5 ml of double strength medium and mixed by shaking. Using a fresh pipette, 5 ml of the mixture was transferred to test tube 2 which contained 5ml of the single strength medium. This too was mixed by shaking and from it 5 ml was taken into test tube 3 aseptically and mixed by shaking. The 9^{th} tube containing no test compound served as control. Finally, to each tube was added 0.2 ml inoculums of the test

organisms aseptically. The test tube were covered with cotton wool and incubated at 37^0C for 24 for bacteria hours and 21 days for fungi and then observed for turbidity. The lowest concentration that inhibited growth of test organism was noted as the MIC.

3.15 Antimicrobial Sensitivity Test

Antibacterial activities of the plant extracts were tested using Agar Well diffusion method (Bauer and Tittel, 1995). The prepared culture plates were inoculated with the isolated microbes. Wells were made on the agar surface with 6mm cork borer. The extracts were poured into the well using sterile syringe. The plates were incubated at 37 ± 2^OC for 24 hours for bacteria and 21 days for fungi. All the inoculated plates were also labeled with the name of the microbe culture. At the end of this period, all the plates were observed for any zone of inhibition. The result was read by observing the zone of inhibition of microbial growth in each plate. Plate showing zone of inhibition were measured with the aid of meter ruler. The diameter of the inhibition was measured and recorded respectively.

3.16 Statistical Analysis

The data obtained was statistically analyzed using student T- test and results were reported as means ± standard error of triplicate values. The difference between means was determined using the least significant difference (LSD) as described by Gomez and Gomez (1984).

CHAPTER FOUR

4.0 RESULTS

4.1 Bacteria Associated With Razor Bumps

The bacteria isolated and identified from the razor bumps are *Staphylococcus aureus, S. capitis, Pseudomonas aeruginosa, P. putida, Bacillus anthracis, B. cereus, B. mycoids, B. panthothencus, Proteus mirabilis* and *Citrobacter spp.* However, *Staphylococcus aureus* had the highest percentage of occurrence (20.00%), followed by *Pseudomonas aeruginosa* (17.77%), *B. cereus* (13.33%), *Proteus mirabilis* (11.11%), *S. capitis*, *P. putida*, (8.88%), and *B. anthracis* (6.66%), the least encountered bacteria were *B. panthothencus*, *B. mycoids* and *Citrobacter spp* with 4.44% frequency of occurrence each. Characteristics and frequency of the associated bacteria are shown in Table 1 and Table 2, respectively.

Table 1: Biochemical Characteristics of Bacteria associated with razor bumps

Gram reaction	Lac	Suc	Glu	Mot	Gas	H_2S	U	Cit	MR	VP	Indole	Starch	Cat	O	Coag	Bacteria Isolate
+ rod	**-**	-	+	+	-	-	+	-	-	+	-	+	+	NA	NA	**A**
+ rod	**+**	+	NA	NA	NA	NA	NA	NA	-	**+**	NA	NA	NA	-	+	**B**
- rod	**-**	-	+	+	+	-	+	+	+	-	-	-	NA	+	NA	**C**
+ rod	**-**	+	NA	NA	NA	NA	NA	NA	+	-	NA	NA	NA	-	-	**D**
+ rod	**-**	-	+	-	-	-	+	-	-	+	-	+	+	NA	NA	**E**
-rod	**-**	+	+	+	+	+	+	+	+	-	-	-	+	NA	NA	**F**
-rod	**-**	-	+	+	+	-	-	+	-	+	-	-	NA	+	NA	**G**
+rod	**-**	-	+	+	-	-	+	-	-	+	-	+	+	NA	NA	**H**
-rod	**+**	-	+	+	+	+	+	+	+	-	-	-	+	NA	NA	**I**
+rod	**-**	-	+	+	-	-	-	-	+	-	-	-	+	NA	NA	**J**

Key: NA = Not Applicable, **A** = *Bacillus cereus*, **B** =*Staphylococcus aureus*, **C** = *Pseudomonas aeruginosa*, **D** = *S. capitis*, **E** = *B. anthracis*, **F** =*Citrobacter spp,* **G** = *P. putida,* **H** = *B. mycoids,* **I** = *Proteus mirabilis,* **J** = *B. panthothencus*

Table 2: Relative frequency of occurrence of isolated bacteria associated with razor bumps

Identified bacteria	Number of sample Isolated	% frequency
P. aeruginosa	8	17.77
S. aureus	9	20.00
P. mirabilis	5	11.11
B. cereus	6	13.33
S. capitis	4	8.88
P. putida	4	8.88
B. anthracis	3	6.66
B. panthothencus	2	4.44
B. mycoids	2	4.44
Citrobacter spp	2	4.44

4.2.0 Phytochemical Screening

4.2.1 Qualitative Phytochemical Screening of *M. pudica*

The results of qualitative phytochemical screening of the *M. pudica* leaf extracts are presented in Table 3. This shows that the aqueous leaf extracts of *M. pudica* revealed the presence of Alkaloid, Flavonoids, Saponin, Tannins, Glycoside, Saponin glycoside, Steroids, Volatile oil and Balsams. However, Cardiac glycoside, and Anthraquinone were not detected in the extract.

Ethanol leaf extracts of *M. pudica* revealed the presence of Flavanoids, Alkaloids, Saponin, Tannins, Steroids, Glycosides, Volatile oil, Balsams, Cardiac glycoside, Anthraquinone while Saponin glycoside was not detected.

Table 3: Phytochemical Contents of *M. pudica* leaf

Component	**Leaf Extracts (Aqueous)**	**Leaf Extracts (Ethanol)**
Flavonoids	+	+
Tannins	+	+
Saponin	+	+
Glycosides	+	+
Alkaloids	+	+
Cardiac glycosides	ND	+
Steroids	+	+
Saponin glycosides	+	ND
Balsams	+	+
Anthraquinones	ND	+
Volatile oil	+	+

Key: + = Present**, ND** = Not Detected

4.3 Antibacterial activity of *M. pudica*

The result of the antibacterial test using the ethanol and aqueous extract of *M. pudica* indicated that the leave of the plant exhibited antibacterial activity against some isolated bacteria at four different concentrations of 150mg, 200mg, 250mg and 300mg. The potential sensitivity of the extract was obtained against some of the bacterial isolates tested; *B. cereus, B. anthracis, B. panthothencus, S. aureus, P. mirabilis*, and *Citrobacter spp* was sensitive to both ethanolic and aqueous extracts. *Pseudomonas aeruginosa* and *S. capitis* were only sensitive to aqueous and ethanol extracts respectively. *B. mycoids* and *P. putida* was not sensitive to any of the extracts at any concentration. The zone of inhibition was recorded and presented in Table 4 below.

Table 4: Diameter of Zone (mm) of Inhibition of Ethanol and Aqueous Extracts of *M. pudica* leaf on Bacterial Isolates

Test Organism	**Ethanol Conc. Mg/ml**				**Aqueous conc. Mg/ml**			
	150	200	250	300	150	200	250	300
B. cereus	0	3	5	3	3	1	2	0
B. anthracis	6	5	5	10	6	5	7	8
B. mycoids	0	0	0	0	0	0	0	0
B. panthothencus	0	2	3	5	2	0	4	2
S. aureus	0	2	2	2	2	0	5	2
P. mirabilis	6	10	12	15	8	8	10	0
P. aeruginosa	0	0	0	0	5	2	4	0
Citrobacter spp	10	10	11	9	3	9	2	1
S. capitis	9	11	10	8	0	0	0	0
P. putida	0	0	0	0	0	0	0	0

Diameter of cork borer used is 14mm

4.4 Minimum Inhibitory Concentration (MIC)

Table 5 shows minimum inhibitory concentration for both ethanol and aqueous extracts of *M. pudica* on test isolates. It shows that *B. cereus* was inhibited at 200 mg/ml and 190 mg/ml, *B. anthracis* was inhibited at 210 mg/ml and 150 mg/ml, *B. panthothencus* was inhibited at 200 mg/ml and 180 mg/ml, *S. aureus* was inhibited at 250 mg/ml and 220 mg/ml, *P. mirabilis* was inhibited at 230 mg/ml and 250 mg/ml and *Citrobacter spp.* was inhibited at 300 mg/ml and 200 mg/ml respectively while *P. aeruginosa* was only inhibited at 200 mg/ml of aqueous extract and *S. capitis* was only inhibited at 300 mg/ml of ethanolic extract. These revealed that at these concentrations, these bacterial isolates from razor bumps growth can inhibited.

Table 5: Minimum Inhibitory Concentration of Ethanol and Aqueous Extracts of *M. pudica* leaf against bacteria associated with razor bumps

Test Organisms	**MIC (mg/ml) Ethanol**	**MIC (mg/ml)Aqueous**
B. cereus	200	190
B. anthracis	210	150
B. panthothencus	200	180
S. aureus	250	220
P. mirabilis	230	250
P. aeruginosa	-	200
Citrobacter spp	300	200
S. capitis	300	-

Key: (-) = Not Inhibited

4.5 Fungal Associated with Razor Bumps

Mycological analysis of the razor bumps revealed it was infected with fungi. Eight different fungal species were isolated from the razor bumps. These isolated fungal species are *Trichophyton verrucosum, Microsporum ferrugineum, T. schoenleinii, T. rubrum, T. concentricum, T. soudanense, M. canis* and *M. gyseum*. The cultural and morphological features of the isolated fungi are shown in Table 6.

Table 6: Colonial and Morphological Characteristics of isolated Fungi associated with razor bumps

Fungal Isolates	Colonial and Morphological Characteristics
Trichophyton verrucosum	Colonies grow slowly, but are small, or disc-shaped, white to cream-colored, with a suede-like, a raised center, and flat periphery with a submerged growth. Reverse pigment vary from non-pigment to yellow. Broad, irregular hyphae with a terminal and chlamydospores are present. The tips of some hyphae are broad and club-shaped, and occasionally divided.
Microsporum ferrugineum	Colonies grow, forms a waxy, convoluted thallus with a cream to buff-colored surface with reverse pigmentation. Micro conidia or macroconidia are not produced. Irregular branching hyphae with prominent cross wall and chlamydospores are seen.
Trichophyton shoenleinii	Colonies grow slowly, waxy with a deep folded honey comb-like Thallus and some sub-surface growth. The thallus is cream-colored to yellow to Orange brown. Cultures are difficult to maintain in their typical convoluted form, which rapidly becomes flat and downy. No reverse pigmentation is present. No macroconidia and micro conidia are seen in routine cultures, however numerous chlamydospores may be present in older cultures.
Trichophyton rubrum	Colonies were flat to slightly raised, white to cream, with a yellowish-brown to wine-red reverse. Most cultures show scanty to moderate numbers of slender clavate to pyriform micro conidia. Macroconidia were usually absent, however closterospore-like projections were present in some mounts.
Trichophyton concentricum	Colonies grow, raised and folded, suede-like, mostly white to cream-colored, but looks orange-brown-colored, deeply folded into the agar which may produce splitting of the medium in some cultures. Reverse is buff to yellowish-brown to brown in color. Cultures consist of broad, much branched, irregular, often segmented, septate hyphae which may have antler tips resembling *T. schoenleinii*. Chlamydospores are often present in older cultures. Micro conidia and macroconidia are not usually produced, although some isolates produce occasional clavate to pyriform microconidia.
Microsporum canis	Colonies are flat, spreading, white to cream-colored, with a dense cottony Surface which may show some radial grooves. Colonies usually have a bright golden yellow to brownish yellow reverse pigment, but non-pigmented strains may also occur. Macroconidia are typically spindle-shaped with 5-15 cells, verrucose, thick-walled and often have a terminal knob, 35-110×12-25µm. A few pyriform to clavate microconidia are also present.
Trichophyton soudanense	Colonies are slow-growing with a flat to folded, suede-like surface. Often there is a Broad fringe of submerged growth. The surface mycelium and reverse pigment are characteristically a deep apricot-orange in color. Microscopically, the hyphae often show reflexive or right-angle branching. Pyriform microcondia may occasionally be present and numerous chlamydospores are often found in older cultures.
Microsporum gypseum	Colonies are flat, spreading, with a deep cream to tawny-buff to pale cinnamon-colored surface. Many cultures develop a central white downy umbo (dome) or a fluffy white tuft of mycelium and some also have a narrow white peripheral border. A yellowish-brown pigment, often with a central darker brown spot, is usually produced on the reverse, however a reddish-brown reverse pigment may be present in some strains. Cultures produce abundant, symmetrical, ellipsoidal, thin-wall, verrucose, four-to six-celled macroconidia. The terminal or distal ends of most macroconidia are slightly rounded, while the proximal ends (point of attachment to hyphae) are truncates

4.6 Frequency of Occurrence of Isolated Fungi

Out of these isolated fungi, *T. schoenleinii* was the most frequently isolated fungus with 23.52% of occurrence, followed by *T. rubrum* and *M. canis* with 17.64% of occurrence, followed by *T. verrucosum* and *T. soudanense* with 11.76% of occurrence and the least isolated fungi were *M. ferrugineum, T. concentricum* and *M. gypseum* with 5.88%. This is shown in table (7).

Table 7: Relative frequency of occurrence of fungi species associated with razor bumps

Fungi Identified	Frequency of Occurrence	Percentage Of Occurrence (%)
Trichophyton verrucosum	2	11.76
Microsporum ferrugineum	1	5.88
Trichophyton schoenleinii	4	23.52
Trichophyton rubrum	3	17.64
Trichophyton concentricum	1	5.88
Trichophyton soudanense	2	11.76
Microsporum canis	3	17.64
Microsporum gypseum	1	5.88

4.7 Antifungal activity of *M. pudica*

Result of antifungal test using the ethanol and aqueous extracts of *M. pudica* revealed that the leaves of the plant exhibited antifungal effect against some of the isolated fungi from the razor bumps. Using four different concentrations of 150mg, 200mg, 250mg and 300mg. the potential sensitivity of extract was obtained against some of the fungal isolates tested. *T. verrucosum, M. ferrugineum, T. shoenleinii, M. canis, T.soudanense* and *M. gyseum* were sensitive to ethanolic extract while *T. concentricum and T. rubrum* wasnot. *T. verrucosum, M. ferrugineum, T. shoenleinii, T. rubrum, T. concentricum, T. soudanense* and *M. canis* were also sensitive to aqueous extract while *M. gyseum* was not. The zone of inhibition was recorded and presented in Table 8 below:

Table 8: Diameter Zone (mm) of Inhibition of Ethanol and Aqueous Extracts of *M. pudica* leaf on Fungal Isolates from razor bumps

Test organism	**Ethanol extract conc. Mg/ml**				**Aqueous extract conc.Mg/ml**			
	150	200	250	300	150	200	250	300
Trichophyton verrucosum	2	4	3	5	3	3	4	4
Microsporum ferrugineum	3	0	0	1	4	1	2	0
Trichophyton schoenleinii	0	2	0	0	2	1	3	4
Trichophyton rubrum	3	0	5	0	1	2	1	0
Trichophyton concentricum	0	0	0	0	2	2	3	4
Trichophyton soudanense	2	4	6	5	1	3	5	7
Microsporum canis	1	1	0	0	0	0	1	0
Microsporum gypseum	3	5	0	4	0	0	0	0

Key: Diameter of cork borer is 14mm

4.8 Antibacterial Activity of *M. pudica* in Aqueous and Ethanol extracts

The result in Table 9 showed that leaf extracts with different solvent inhibited the growth of all the bacteria isolate. At 300mg/ml of 3.90±1.80, highest growth inhibition was recorded in ethanol extract. The least growth inhibition was also recorded in aqueous leaf extracts at 150mg/ml at 0.99±1.85. This was similar in all the result, showing that the result is significant ($p>0.005$).

Table 9: Antibacterial activity of Aqueous and Ethanol extracts of *M. pudica* leaf

Extracts	Conc. (mg/ml)	Leaf extract
Ethanol	150	1.00±1.87
	200	1.80±1.79
	250	1.40±1.81
	300	3.90±1.80
Aqueous	150	0.99±1.85
	200	1.83±1.80
	250	1.38±1.81
	300	3.90±1.81

Values are means ± standard error

4.9 Antifungal Activity of *M. pudica* in Aqueous and Ethanol extracts

The result in Table 10 showed that leaf extract with different solvent inhibited the growth of all the bacteria isolate. At 300mg/ml of 0.50±1.27, highest growth inhibition was recorded in ethanol extract. The least growth inhibition was also recorded in aqueous leaf extract at 150mg/ml at 0.12±0.67. This was similar in all the result, showing that the result is significant ($p>0.005$).

Table 10: Antifungal activity of Aqueous and Ethanol extracts of *M. pudica* leaf

Extracts	Conc. (mg/ml)	Leaf extract
Ethanol	150	0.12±0.70
	200	0.50±0.84
	250	0.62±1.08
	300	0.50±1.30
Aqueous	150	0.12±0.67
	200	0.52±0.86
	250	0.60±1.08
	300	0.50±1.27

Values are means ± standard error

Plate 1 Infected portion of razor bumps (A, B and C)

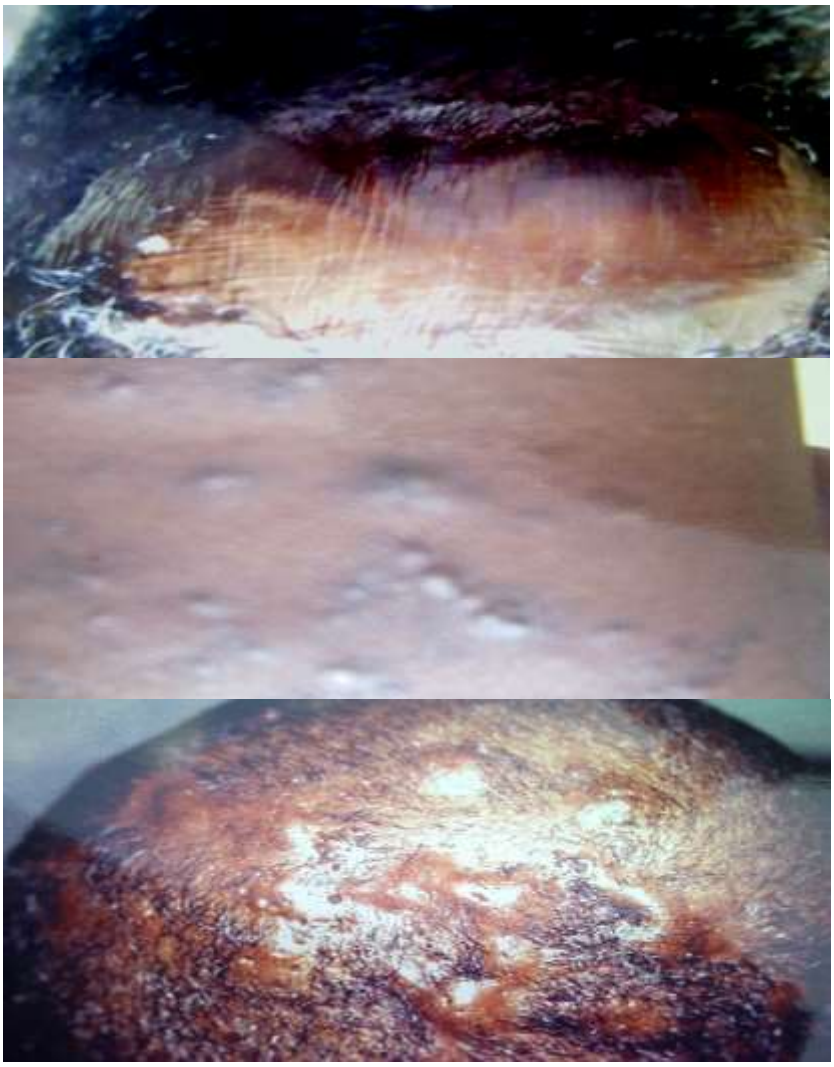

Plate 1(A, B, and C)

Plate 2 Fungal growth on P D A.

Plate 2 (A, and B)

Plate 3 Bacterial growth on different growth media

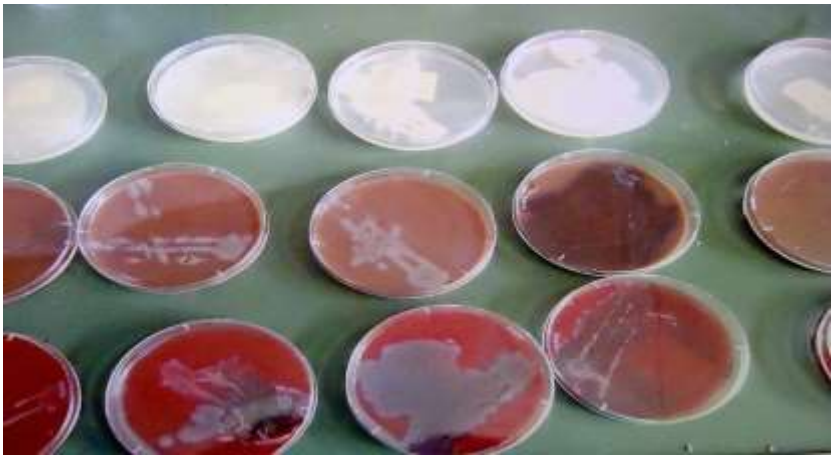

Plate 3

Plate 4 Zone of growth inhibition of *M. pudica*

Plate 4

CHAPTER FIVE

5.0 Discussion

The results obtained from this research shows the presence of some species of bacteria and fungi which are the primary cause of razor bumps. The microbial isolates react with the skin cells and cause the inflammation of the skin (Ahmad and Iranzo, 2003). Same results were obtained by Tevelli (2001) from cellulitis while Ahmad and Iranzo (2003) also obtained similar results from burn wounds. A significant number of fungal isolates were obtained from the research carried out by Menan *et al.,* (2002) in the Northern flanks of African countries of Burkina faso and Guinea Conakry. The microbial isolates were responsible for itching, and making the skin around the razor bumps wet (Nweze and Okafor, 2005).

The present study revealed the presence of various secondary metabolites like tannins, saponins, alkaloids, flavonoids and others in qualitative analysis which indicates the medicinal importance of *M. pudica*. This findings are similar to those reported by Edeoga *et al.,* (2005); Nyananyo *et al.,* (2010) on different plants species in Niger delta area of Nigeria. The secondary metabolites are responsible for the inhibition of growth of the microbial isolates. Several plants rich in tannins, alkaloids, glycosides, flavonoids, saponin and steroids have been shown to possess antibacterial activities against a number of microorganisms, this is shown in the investigation of the antimicrobial activity of leaf extracts of *Dichrostahys cinerea* and *M. pudica* (Mohan *et al.,* 2011 ; Venkataswany *et al.,* 2010).

The extracts of *M. pudica* leaf shows highest zone of inhibition against *Bacillus anthracis*, *Proteus mirabilis* and *Citrobacter spp.* it shows minimum zone of inhibition on other

bacterial isolates excluding *Pseudomonas aeruginosa* and *Staphylococcus capitis* in different concentrations. The extracts of *M. pudica* leaves also shows highest zone of inhibition against *Trichophyton verrucosum* and *T. soudanense* but not effective against other isolates. The presence of phytochemical compounds of the studied plants part, the inhibitory zone and concentrations at which values were effective on the tested organisms highlight that there were variations in the antimicrobial potency of the plants. The variation in sensitivity could also be attributed to differences in growth rate of isolated organisms nutritional requirement, temperature and inoculums size (Kalimuthu *et al.,* 2010). Similar results were obtained by Gangai *et al.,* (2014), whose results revealed maximum zone of inhibition when *M. pudica* leaf extracts were used against *E. coli, Lactobacillus* and *Salmonella typhi*.

Among gram positive and gram negative bacteria, gram negative bacteria strains were susceptible to the *M. pudica* extracts when compared to gram negative bacteria and this may be attributed to the fact that these two groups differ in their structure of cell wall components (Mohan *et al.,* 2011). The ability of the compounds to cause the bacterial colonies to disintegrates, probably results from their interference with the bacterial cell wall thereby inhibiting the microbial growth. Although a significant number of studies have been used to obtain purified plant chemicals, very few screening program have been initiated on crude plant materials. It has also been widely observed and accepted that the medicinal value of plants lies in the bioactive phytocomponents present in them (Veermuthu *et al.,* 2006).

The extracts indicates significant difference ($p<0.05$) inhibitory activity of aqueous and ethanol extracts. Antimicrobial activity of the ethanol extracts appeared to be more effective than aqueous extracts since ethanol could extract a wide variety of active components as

compared to aqueous Kaur *et al.,* (2011). Rekha and Sundarajan, (2010) reported similar results from their research and recorded a significant zone of inhibition when *M. pudica* leaf extracts was used against *S. aureus* and *P. aeruginosa*.

In the present era, medicinal herb resources are abundant, but these resources are dwindling fast due to the onward march of civilization (Vogel, 19991). The presence of alkaloids, flavonoids, tannins, saponins, steroids, balsams, and volatile oil might, explain the therapeutic properties of this plant since plants containing these properties have long been used by humans for medicinal purpose (Sofowara, 1993).

Conclusion

The tested bacteria strains were found to be more sensitive to the extracts than fungi. The reason for the difference in sensitivity between the bacteria and fungi might be ascribed to the differences in morphological constitutions between these microorganisms. The cell wall of the fungi is more complex in lay out than the bacteria. In spite of this permeability differences, however, some of the extracts have still exerted degree of inhibition against some fungi as well. In the present study, *M. pudica* leaf extract possesses antimicrobial activity against some of the tested microorganisms and the plant contains potential antimicrobial component for the therapy of infections. From the studies, it is concluded that traditional plants may represent new sources of antimicrobials with stable, biologically active components that can establish a scientific base for the use of plants in modern medicine and the compounds are known to be biologically active, therefore, aid the antimicrobial activity. These local ethno-medical preparations and prescriptions of plant sources should be scientifically evaluated and then disseminated properly and the knowledge about the botanical preparations of traditional sources of medicinal plants can be extended for future investigation into the field of pharmacology, phytochemistry, ethno botany and other biological actions for drug discovery.

Recommendations

Based on the results of this study, the following recommendations are made:

1. Considering the antimicrobial potentials of *M. pudica*, further research should be carried on skin diseases and how effective it can be against it.

2. Other parts of *M. pudica* like the stem and root should be included with the leaf against microbes of the razor bumps.

3. Further investigation should be carried on the sources of this infection.

4. Other solvents should be employed and investigated on its effectiveness on the isolated microbes.

REFERENCES

Abubakar, E. M. (2009). The use of *Psidium guajava* Linn. In treating wound, Skin and soft Tissue infections. *Scientific Research and Essay*. **4** (6) 605-606.

Adeleke, S. I., Usman, B. and Ihesiulor, G. (2008). Dermatophytoses among itinerant Quaranic scholars in Kano (Northwest Nigeria). *Nig. Med. Practitioner*. **53**: 33-35.

Ahmad, I. and Beg, A. Z. (2001). Antimicrobial and Phytochemical studies on45 Indian medicinal plants against multi-drug resistant human Pathogen . *Journal of Ethnopharmacol*. **74**:113-123.

Ahmad, S. I. and Iranzo, O. G. (2003). Treatment of post burns bacterial Infections by fention reagent, particularly the ubiquitous multiple drug Resistant *Pseudomanas* species. *Medical hypothesis*. **61** (4): 431-434.

Akerele, Z. O., Obasuyi, O., Ebomoyi, M. I, Oboh, I. E., and Uwumarongie, O. H. (2008). Antimicrobial activity of ethanol extract and fractions of the seeds of *Garcinia kola* heckle (Guttiferae*). African Journal of Biotechnology*. **7** (2): 169-172.

Alexopoulus, C. J., Mims, C. W., and Blackwell, M. (2002). *Introductory Mycology*.4th Edition; John Wiley and Sons Inc. Singapore. Pp. 869.

Allan, J. Rob, P., and Jonathan, W. (1998): Practical skill in Biology. 2nd Edition; published by Pearson Education, Higher Education. Pp. 15-90.

Ataga, A. E., and Ota-Ibe, G. (2006). Seed-borne fungi of the wild mango (Ogbono) (Irvingia gabonensis (Aubry-Leconte Ex on Rorke) Bail) and their effects on food composition. *Nigeria Journal of Botany*. **19** (1):54-60.

Aly, R., Maibach, H. I., and Charles, C. T. (1978). Chemical skin Microbiology.

Baquar, S. R. (1995). The Role of Traditional Medicine in Rural Environment, In: Traditional Medicine in Africa, Issaq, S. (Editor), East Africa Educational Publishers Ltd., Nairobi, Pp. 141-142.

Barnet, H. L., and Hunter, B. B. (1999). Illustration genera of imperfect fungi; 4th Edition. The American Phytopathological Society st, Minnesota U.S.A. Pp. 218.

Bauer, R. and Tittel, G. (1995). Quality assessment of herbal preparations as a precondition of pharcological and clinical studies. *Phytomedicine*. **2**: 193-198.

Beswich, S. J, Das, J., Lawrence, C. M., and Tan, B. B. (1999). Kerion formation due to *Trichophyton rubrum*. *Britain Journal of Dermatology* . Pp. 953-4.

Bhattacharya, D., Mandal, S. K., Mukherjee, S., and Pradhan, M. (2005). Clinical correlation with Biochemical status in polycystic ovarian syndrome. *Journal* of *obstetrics and gynaecology*. **55** (1).

Bisno, A. L., and Stevens, D. L. (1996). Streptococcal infection of skin and soft tissues. *England Journal of Medicine*. **334**: 240-245.

Bonifaz, A., Ramirez-Tamayo, T., and Saul, A. (2003). Tinea barbae, experience with nine cases. *Journal of Dermatology*. **30**: 898-903.

Cheesbrough, M. (2000). *District laboratory practice in tropical countries*, 2nd Edition, part 2. Pp 2-71.

Cheryld, L. E. (2006). *Mimosa pudica* as an experimental organism for Botany Lab. Division of Biology, Alfred University, Alfred. New York. Pp. 351.

Chiejina, N. V. (2006). Potentials of the leaf extracts of *Azadirachta indica*, S. Juss and *Ocimum gratissimum* L. for the control of some potato (*Solanum tuberosum* L.) *Fungal disease. Nigeria Journal of Botany*. **19** (1):68-73.

Chism, J. (1993). Treatment Issues: The Gay Men's Health Crisis, Newsletter of Experimental AIDS Therapies, **7** (6): 1-3.

Cremer, G., Bournerais, I., Vandemeleubroucke, E., Houin, R., and Revuz, J. (1997). Tinea capitis in adults: misdiagnosis or reappearance. *Dermatology*. **194**: 8-11.

Christopher, M. D., and Nkechi, E. O., (2010). In *vitro* Antimicrobial Assay and Phytochemical Analysis of Ethanol extracts of *Voacananga Africana* seeds. *Journal of American Science.* **6** (6).

Cockerell, C. J. (1995), Noninfectious Skin Disorders in HIV Infected Patients. *Dermatology of Clinical Microbiology*. **9**: 531-541.

Dagnew, M. B., and Erwin, G. (1991). Epidemiology of Common Transmissible Skin Diseases among Primary School children in Northwest Ethiopia. *Tropical and Geographical Medicine*. **43** (1-2): 152-155.

Dagnew, M. B., and Gunther, E. (1990). Epidemiology of Communicable Skin Diseases in School children of a Rural Area in North Ethiopia. *Dermatology of Monatsschrift*. **176** (4): 219-223.

Darley, C. (1998). The Prevalence of Skin Diseases in HIV Infection, AIDS Patient Care, **12**(11): 849-842.

Der, A. H., Kratz, A. M. and Riedlinger, J. E. (2000), Complementary and Alternative Medicine Health Care, In: Remington, the Science and Practice of Pharmacy, 20th Edition. Lippincott Williams and Wilkins, U.S.A. P. 1769.

De Vane, G. W., Czekala, N. M., Juff, H. L., and Ven, S. S. C. (1975). Circulatory gonado tropins, estrogens and androgens in polycystic ovarian disease .*American Journal of Obstetrics and Gynecology*. **121** (4): 496-500.

Develoux, M., Dieng, M. T., Ndiaye, M., Dir, O. N., and Diaye, B. N. (2002). Les teignes de l`adulte au senegal. Etude prospective et retrospective. *Journal of Mycological Medicine*. **12**: 25-29.

Douglas, K. A. (2001). Pharmacognosy in the 21st Century. *Journal of Pharmacology*. **53**: 135-148.

Drake, L. A., Dinehart, S. M., and Farmer, E. R. (1996). Guide-Lines of care superficial infections of the skin: tinea capitis and tinea barbae, *Journal of Academical Dermatology*. **34**: 290–294.

Edeoga, H. O., Okwu B. E., and Mbaebie, B. O. (2005). *African Journal of Biotechnology*.Vol. 4(7). Pp. 685-688.

Edward-Jones, V., and Greenwood J. E. (2003). What new in burns microbiology. *Burns*. **29**: 15-25.

Elujoba, A. A., Odeleye, O. M., and Ogunyemi, C. M. (2005). Traditional medical development for medical and dental primary health care delivery system in Africa. *African Journal of Traditional Complementary and Alternative Medicine.* **2** (1): 46-61.

El-Olemy, M. M., Al-Muhtad, F. J., and Affi, A. A. (1994). *Experimental Phytochemistry*. A laboratory Manual, King Saudi University press Riyadh, Saudi Arabia. Pp. 3-137.

Emele, F. E. and Oyeka, C. A., (2008). Tinea capitis among primary school children in Anambra state of Nigeria. *Mycoses*, **51**: 536-541.

Evans, W. C. (2000), Trease and Evans Pharmacognosy, 15th Edition, W. B. Sounders, London. Pp.488-491.

Fawole, M. O., and Oso, B. A. (1988). Laboratory manual of microbiology.

Fennesy, M., Coupland, S., Popay, J. and Naysmith, K. (2000). The Epidemiology and Experience of Atopic Eczema during Childhood: a Discussion Paper on the

Implication of Current Knowledge for Health Care, Public Health Policy and Research. *Journal of Epidemiology in Communty Health*. **54**: 581-589.

Figueroa, J. I., Fuller, L. C., Abraha, A. and Hay, R. J. (1996). The Prevalence of Skin Diseases Among School Children in Rural Ethiopia - a Preliminary Assessment of Dermatological Needs in Pediatrics. *Dermatology*. **13** (5): 378-81.

Figueroa, J. I., Fuller, L. C., Abraha, A. and Hay, R. J. (1998). Dermatology in Southwest Ethiopia: Rational for a Community Approach. *International Journal of Dermatology*. **37** (10): 752-758.

Gandhiraja, N., Sriram, S., Meenaa, V., Kavitha, J., Sasikumar, C., and Rajeswari, R. (2009). Phytochemical screening and Antimicrobial activity of the Plant extracts of *Mimosa pudica* against selected microbes. *Ethnobotanical leaf*. **13**: 618-624.

Gangai, S. K. A., Sudha, K. M., Misha, D. M., and Nirmala, P. D. (2014). The Microbial Activity of *Mimosa pudica* L. *International Journal of Ayurveda and Pharmacological Research.* **2**(1): 105-108.

Gang, R. K., Sanyol, S. C., Bay, R. L., Mokaddas, E., and Lari A. R. (2000). Staphylococcal septicaemia in burn. *Burns*. **26** (4): 359-366.

Gaye, O., Ndir, O., Kane, A., Belmachi, R., and Ndiaye, M. (1994). Intestinal parasitic diseases and tinea of the scalp in Dakar school population: Influence of environmental factors on the infestation level. *Dakar Medical Health*. **39**: 57-61.

Gomez K. A., and Gomez A. A. (1984). *Statistical procedure for agricultural research*, 2nd ed. Pp. 20-30.

Gordon, M. C. and David, J. N. (2001). Natural product drug discovery in the next millennium. *Journal of pharmaceutical Biology*. **39**:8-17.

Greer, D. L. (1990). Dermatophytosis, in Jacobs PH, Nall L (eds): *Antifungal Drug Therapy, A Complete Guide for the Practitioner,* Marcel Dekker Co, New York. P. 5-6.

Gugnani, P. H. C. and Njoku-Obi, A. N. (1986). Tinea capitis in school children in Eastern Nigeria. Mykosen, **29**: 132-140.

Guiguemdeet, T. R., Tapsoba,G. P., and Pare, J. L.(1992). Preliminary data on dermatomycoses in Ouagadougou (Burkina Faso). *Medicine in Tropics*. **52**: 151-155.

Habif, T. P., (2001). Skin disease, diagnostic and treatment. Mosby.

Hailu, T. (2004). Phytopharmaceutical studies of some selected medicinal plants locally used in the treatment of skin disorder. Pp. 15.

Halder, R. M, Robert, C. I, Nootheti, P. K, and Kelly, A. P (2006). Dermatologic Disease in blacks. *Dermatology and dermatological therapy of pigmented skin*. Pp. 331-355.

Hartmut, K., and Thorsten, E. E., (2006). Rapid hydro passive opening and subsequent active stomatal closure follow heat-induced electrical signals in *Mimosa pudica*. *Journal of Experimental Botany*. **5** (9): 2087-2092.

Hiletework, M. (1998), Skin Diseases Se4en in Kazanchis Health Center, Ethiopia. *Medical Journal*. **36** (4): 245-54.

Hogewoning, A. A., Duijvestein, M., Boakye, D., Amoah, A. S., and Obeng, B. B. (2006). Prevalence of symptomatic tinea capitis and associated causative organisms in the Greater Accra Region, Ghana. *Britain Journal of Dermatology*. **154**: 784-786.

Inanir, I., Sahin, M., Gunduz, K., Turel, A., and Ozturkan, S. (2002), Prevalence of Skin Conditions in Primary School Children in Turkey: Difference Based on Socioeconomic Factors. *Pediatric Dermatology*. **19** (4): 307-311.

Ive, F. A. (2000). Kerion formation caused by *Trichophyton rubrum*. *Britain Journal of Dermatology*. **142**: 1065-6.

Iwu, M. M. (1993): Handbook of African medicinal plants, 1st edition, CRC press Incorporation, Florida. Pp. 239-239.

Iwu, M. M. (1983). Traditional Igbo medicine. Institute of African studies publication, University of Nigeria, Nsukka.

Jigna, P., Nehal, K., and Sumitra, C. (2006).Evaluation of anti-bacterial and phytochemical analysis of *Bauhinia variegate* L. bark. *African Journal of Biomedical Resource*. **9** (1): 53-56.

Kaur, P., Nilesh, K., Shivananda, T. N., and Gagandeep, K. (2011). *Jounal of Medicinal Plants Research.* **5** (22): 5356-5359.

Kawada, A., Argane, Y., Maeda, A., Yudate, T., Tezuka, T., and Hiruma, M. (2000). Tinea barbae due to *Trichophyton rubrum* with possible involvement of autoinoculation. *Britain Journal of Dermatol*. **142**: 1064-1065.

Kalimuthu, K., Vijayakumar, S., and Senthilkumar, R. (2010). Antimicrobial activity of the Biodiesel plant, *Jatropha curcas. International journal of Pharmaceutical Biological Science.* **1** (3): 1-5.

Kokane, D., More, R., Kale, M., Nehete, M., Mehandale, P., and Gadgoli, C. (2009). Evaluation of wound healing activity of root of *Mimosa pudica*. *Journal of Ethnopharmacology*. **124**: 311-315.

Kumar, V., Abbas, A. K., Fausto, N., and Mitchell, R. N. (2007). *Robbins Basic Pathology* (8th Edition). Saunders Elsevier. P. 843.

Lanfranco, G. (1999). Invited Review Article on Traditional Medicine, E J B, **2** (2): 1-3.

Loigier, H. A. (1988). Descriptive flora of Puerto Rico and adjacent islands. *Spermatophyte*.Volume editorial de la Universidad de Puerto Rico, Rio Piedras. P. 481.

Maclean, W. H. I. (2004). *Journal of Investigative Dermatology* **130** (3): 440-455.

Maibach, H. I., and Aly, R. (1992). Bacterial infections of the skin. *Dermatology*, 3rd edition. WB Saunders, Philadelphia.

Makhubu, L. (1998). Bioprospecting in an African context. Science, **282**: 41 – 43.

Markey, R. J., Staat, M. A., Gerrety, M. J., and Lucky, A. W. (2003). Tinea capitis due to *Trichophyton soudanense* in Cincinnati, Ohio, in internationally adopted children from Liberia. *Pediatric Dermatology*. **20**: 408-410.

Menan, E. I., Zongo-Bonou, F., Rouet, P. C., Kiki-Barro, W., Yavo, F. N., N`Guessan, O., and Kone, M. (2002). Tinea capitis in school children from lvory Coast (Western Africa). A 1998 and 1999 cross-sectional study. *International Journal of Dermatology*. **41**: 204-207.

Meseleno, A., and Hasan, M. (2012). Skin diseases expert system using Dempster-shafer theory. *International Journal of Intelligent systems and Applications*. **4** (5): 38-44.

Mitscher, L. A., Drake, S., Gollapudi, S. R., and Okwute, S. K. (1987). A Modern Look at Folkloric Use of Anti-infective Agents. *Journal of National Production*. **50** (6): 1025-1040.

Mohan, G., Anand, S. P., and Doss, A. (2011). Efficacy of Aqueous and Methanol extracts of *Caesapinia sappan* L. and *Mimosa pudica* L. for their potential Antimicrobial activity. *South Associate Journal of Biological Science* **1**(2): 48-57.

Molina, M., Contreras C. M., and Alcantara, P. (1999). *Mimosa pudica* may possess antidepressant actions in rat. *Phytomedicine*. **6**(5): 319-323.

Monsel, G., Ly, F., Canestri, A., Diousse, P., Ndiaye, B., and Caumes, E. (2008). Prevalence of skin disorders in HIV patients in Senegal and relationship to degree of immune suppression. *Annual Dermatology of Venereology*. **135**: 187-193.

Muthukumaran, P., Padmapriya, P., Salomi, S., Umamaheshwani, R., Kalaiarasan, P., and Malarvizhi, C. (2011). In vitro antimicrobial activity of leaf powder .*Asian Journal Pharmacological Resource*. **1**(4): 108-110.

Nair, R., Kalariya, T., and Chanda, S. (2005). Antibacterial activity of some selected Indian Medicinal Flora. *Turkish Journal of Biology*. **29**: 41-47.

Nascimento, G. G. F., Lacatelli, J., Freitas, P. C., and Silva, G. L. (2000). Antibacterial activity of plant extracts and phytochemicals on antibiotic-resistant bacteria. *Brazil Journal of Microbiology*. **31**(4): 886-891.

Noble, W. C. (1981). *Microbiology of Human skin*. Lloyd-Luke, London.

Noman, R., Harry, H. S., and Gail, B. (1999). WHO Monographs on selected Medicinal plants, Genva. World Health Organisation. Pp.1-2.

Nweze, E. I. and Okafor, J. I. (2005). Prevalence of dermatophytic fungal infections in children: A recent study in Anambra State, Nigeria. *Mycopathologia*, **160**: 239-243.

Nweze, E. I. and Okafor, J. I. (2007). Increasing cases of tinea capitis dermatophytoses in Children of school age in Nigeria.2nd Pan African Medical Mycology Society Conference, Cape Town, South Africa.

Nweze, E. I. (2001). Etiology of dermatophytes amongst children in north eastern Nigeria. *Medical Mycology*. **39**: 181-184.

Nweze, E. I., Okafor, J. I. and Njoku, O. (2004). Antimicrobial activities of methanolic extracts of *Trema guineensis* (Schumm and Thorn) and *Morinda Lucida* Benth used

in Nigeria herbal medicinal practice. *Journal Biological Resource in Biotechnology*. **2**: 36-39.

Nyananyo, B. L., Mensah, S. I., and Achama, C. (2010). *Scientia Africa*, Vol. 9(No. 1) Pp. 173-177.

Ogbonna, C. I. C., Robinson, R. O. and Abubakar, J. M. (2007).The distribution of ringworm infections among primary school children in Jos, Plateau State of Nigeria. *Mycopathology*. **89**: 101-106

Okafor, J. I., Nweze, E. I. and Njoku, O. U. (2001). Antimicrobial activities of the methanolic extracts of *Zapotica portericensis* Benth and *Cissus quadrangularis* Linn. *Nigeria Journal of Pure and Applied Science*, Volume 1, Issue 2.

Owen, E., David, G. P., John, C. David, P. B. (2003). *Clinical examination*, third edition.

Owolabi, J., Omogbai, E. K. I.,and Obasuyi, O. (2007). Antifungal and antibacterial activities of the ethanol and aqueous extract of Kigelia africana (Bignoniaceae) stem bark. *African Journal of Biotechnology*. **6** (14): 882-85.

Pande, M., and Pathak, A. (2010). Preliminary pharmacognostic evaluations and phytochemical studies on roots of *Mimosa pudica. International journal of pharmaceutical sciences Review and Research*. **1** (1): 50-52.

Philpot, C. M. (1978). Geographical distribution of dermatophytes: a review. *Journal of Hygiene*. **80**: 301-313.

Popoola, T. O. S., Ojo, D. A., and Alabi, R. O. (2006). Prevalence of dermatophytosis in junior secondary school children in Ogun State, Nigeria. *Mycoses*, **49**: 499-503.

Rajendran, R., and Krishnakumar, E. (2010). Hypolipedemic Activity of chloroform extract of *Mimosa pudica* leaves. *Avicenna Journal Medical Biotechnology*. **2** (4): 215-221.

Rajput, A., Singh, K. P., Kumar, V., Sexena, R., and Singh, R. K. (2008). Antibacterial resistance pattern of aerobic bacteria isolate from burn patients in tertiary care hospital. *Biochemical research*. **19** (1): 1-4.

Ramakrishna, A., Giridhar, P., and Ravishankar, G. A. (2011). Phytoserotonin. *Plant signaling and behavior*. **6** (6): 800-809.

Rapini, R. P., Bolognia, J. L., and Jorizzo, J. L., (2007). Dermatology: 2-volume set.

Raynolds, J. E. F. (1996). Martindale, the Extra Pharmacopoeia, 31st Edition. *The Royal Pharmaceutical Society*, London. Pp. 160-163.

Rekha, R., and Sundararajan, R. (2010). Preliminary Phytochemical Analysis and Antibacterial Activity of *Mimosa pudica* L. leaf. *International Journal of Pharmacological and Biological Sciences*. Pp. 5-8.

Renu, S. (2010). Some medicinal plants with antibacterial activity. *International Journal of Comprehensive Pharmacy*. **4** (10): 1-4.

Rippon, J. W. (1992). Tinea barbae. The Pathogenic Fungi and the Pathogenic Actinomycetes. *Medical Mycology*. Pp. 215-216.

Robert, W. E, (2004). Chemical peeling of ethnic/dark skin. *Dermatologic therapy* **17**(2): 196-205.

Sangma 1, T., Meitei, U., Sanjenbam, R., and Khumbongmayum, S. (2010). Diuretic property of aqueous extract of leaves of Mimosa pudica on experimental albino rat. *Journal of Natural Products*. **3**: 172-178.

Satima, F. T., Mc Birde, S. R., and Leppard, B. (1999). Prevalence of Skin Disease in Rural Tanzania and Factors Influencing the Choice of Health Care, Modern or Traditional. *Archives of Dermatology*. **134** (11): 1050-1055.

Shaffner, W., Lowy, D. R., Flitzpatrick, T. B., Eisen, A. Z., and Wolff, K. (1987). Rickettal and Viral diseases with cutaneous involvement. Dermatology in General Medicine. 3rd edition. McGraw-Hill, New York.

Shakkoury, W. and Abu-Wndi, E. (1999). Prevalence of Skin Disorders Among Male School children in Aman, Jordan. *Archives of Dermatology*. **5** (5): 955-959.

Sharma, P. C., Velne, M. B., and Dennis, T. T. (2001). Data base on Medicinal Plants Govt. of India, Janakpuri, Delhi. Pp. 369-379.

Sheibeshi, D. (2000). Patterns of Skin Diseases at the University Teaching Hospital, Addis Ababa, Ethiopia. *International Journal Dermatol*. **39** (11): 822-825.

Shrum, J. P., Millikan, L. E., and Bataineh, O. (1994). Superficial fungal infections in the tropics. *Dermatology in Clinic*. **12**: 687-93.

Silverberg, N. B.,Weinberg, M. J., and de Leo, V. A. (2002). Tinea capitis: focus on African American woman. *Journal of Academy in Dermatology*. **46**: 120-124.

Simandi, C. (1990). Alteration in Skin Function and Integrity, In: Pathophysiology, Concepts of Altered Health States, 3rd Edition, Porth, C. M. (Editor), J. B. Lippincott Company, U.S.A. Pp. 108-140.

Singh, A. P. (2002). Emerging Trends in the Herbal Pharmaceutical Industry and Practice, Perspectives. Pp. 1-2.

Sofowara, A. (1993). Medicinal plants and traditional medicine in Africa. Spectrum Books Ltd. Ibadan, Nigeria. P. 289.

Szepietowski, J. C., and Schwartz R. A. (2004). Tinea barbae. e-Medicine. *Journal Dermatology*. Pp. 60-62.

Szepietowski, J. C., Bielicka, E, and Maj, J. (2002). Inflammatory tinea barbae due to *Trichophyton rubrum* infection–autoinnoculation from fingernail onychomycosis. Case Report in Clinics, Practical Revision. **3**: 68-70.

Tappers, J. W., Perkins, B. A., and Gerger, T. G. (1995). Cutaneous Manifestations of Opportunistic Infections in Patients with the Human Immuno deficiency Virus. *Clinical Microbiology Review*. **8**: 440-450.

Tevelli, B. (2001). Infectious diseases of the skin. Noble text book of primary care Medicine, 3rdedition. New York. Pp. 787-788.

Thomas, J. L., and Robert, A. S. (1991). Common Skin Disorders, In: Harrison's Principles of Internal Medicine, 12th Edition, Mc Graw-Hill, Incorporation., New York, P. 309.

Tietz, H. J., Hopp, M., and Graser, Y. (2002). First isolation of *Trichophyton raubitschekii* (syn. *T. rubrum*) in Europe. *Mycoses*. **45**: 10-14.

Trotha, R., Graser, Y., Platt, J., Koster, A., Konig, B., Konig, W., and Freytag, C. (2003). Tinea barbae caused by a zoophilic strain of *Trichopyton interdigitale*. *Mycoses*. **46**: 60-3.

Vaidyaratanm, P. S. (2001). Indian Medicinal Plant data base, Kottakal, Orient Long man. *Arya Vidyashala*. **2** (1): 27-36.

Veermuthu, D., Muniappan, A., and Savarimuthu, I. (2006). Antimicrobial activity of some ethno medicinal plants used by paliyar tribe fromTamilnadu, India. B M C Complement. Alternative Medicine. **6**: 35.

Venkataswany R., Doss A., Sukumar M., Muhamed M. H., 2010. Preliminary Phytochemical Screening and Antimicrobial Studies of *Lantana indica* Roxb. *Indian Journal of Pharmacological Science*. **72** (2): 229 -231.

Vickers, A., and Zollman, C. (1999). A B C of Contemporary Medicine, Herbal Medicine. *Britain Medical Journal*. **319** (16): 1050-1053.

Vogel, H. G., (1991). Similarities between various system of traditional medicine. *Journal of Ethnopharmacology*. **35**: 179-190.

Weitzman, I. and Summerbell, R. C. (1995). The dermatophytes. *Clinical Microbiology*. *Review*. **8**: 240-259.

Williams, H. (2002). New Treatments for Atopic Dermatitis. *Britain Medical Journal*. **324**: 1534-1535.

Winter, H., Schissel, D., Parry, D. A. D., Smith, T. A., Liovic, M., Birgitte, L. E., Edler, L., and Langbein, L. (2004). *Journal of Investigative Dermatology.* **122** (3): 652-657.

World Health Organization (WHO), 2001.General Guidelines for Methodologies on Research and Evaluation of Traditional Medicine. P. 1.