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PHYTOPHARMACEUTICAL STUDIES OF SOME SELECTED MEDICINAL PLANTS LOCALLY USED IN THE TREATMENT OF SKIN DISORDERS

BY

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PHYTOPHARMACEUTICAL STUDIES OF SOME SELECTED MEDICINAL PLANTS LOCALLY USED IN THE TREATMENT OF SKIN DISORDERS

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By

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ACRONYMS

AIDS:	Acquired Immunodeficiency Virus
ATCC:	American Type Culture Collection
BP:	British Pharmacopoeia
DACA:	Drug Administration and Control Authority
DNA:	Deoxyribonucleic acid
DPPH:	Diphenylpicrylhydrazyl
EHNRI:	Ethiopian Health and Nutrition Research Institute
EI:	Edema Inhibition
EV:	Edema Volume
FDA:	Food and Drug Administration
GC:	Gas Chromatography
HIV:	Human Immunodeficiency Virus
HPLC:	High Pressure Liquid Chromatography
MIC:	Minimum Inhibitory Concentration
MS:	Mass Spectrometry
NDA:	New Drug Application
PEG:	Poly Ethylene Glycol
Rf:	Retention Factor
SD:	Standard Deviation
SEM:	Standard Error of the Mean
SSTI:	Skin and Soft Tissue Infections
TLC:	Thin Layer Chromatography

TM:	Traditional Medicine
TS:	Test Solution
TSY:	Tryptone Soya
USD:	United States Dollar
USP:	United States Pharmacopoeia
UV:	Ultra Violet
WHO:	World Health Organization

ABSTRACT

In this study, eight species of traditionally used medicinal plants namely *Acokanthera schimperi* (Apocynaceae), *Calpurnia aurea* (Fabaceae, Leguminosae), *Kalanchoe petitiana* (Crassulaceae), *Lippia adoensis* (Verbenaceae), *Malva parviflora* (Malvaceae) *Olinia rochetiana* (Oliniaceae), *Phytolacca dodecandra* (Phytolaccaceae) and *Verbascum sinaiticum* (Scrophulariaceae), were screened for antimicrobial activity against different strains of bacteria and fungi which are known to cause various types of skin infections. Among these plants, *L. adoensis* and *O. rochetiana*, which showed better antimicrobial activity in the initial screening test, were selected for further investigations. Fractionation and antimicrobial activity tests of the fractions, anti-inflammatory activity tests, phytochemical screening, evaluation of topical formulations, and preliminary standardization studies were carried out on the two species of plants.

The results of the initial antimicrobial screening test indicated the potential of these herbal drugs in treating bacterial and fungal infections of the skin. Almost all species of plants were found to have activity on at least one strain of bacteria and/or fungi. This might justify their claimed uses in the treatment of various skin disorders the majority of which are of infectious origin. Among the different fractions (petroleum ether, chloroform, acetone and methanol) tested for antimicrobial activity, the non-polar fractions were found to be more active than the polar fractions. The phytochemical screening tests carried out on *L. adoensis* and *O. rochetiana* indicated the presence of tannins, flavonoids and saponins in both species of plants. The anti-inflammatory activity test results however have indicated that the two species of plants do not have demonstrable anti-inflammatory activity.

Performance evaluation of topical formulations of the crude extracts in different vehicles revealed that extracts incorporated into creams (especially the hydrophilic ones) are superior in performance than those incorporated in to ointments. In addition, crude extracts formulated into water soluble ointment (PEG ointment) demonstrated higher performance compared to lipophilic ointments. The most lipophilic formulation of the crude extracts, petrolatum ointment, was found to be devoid of any activity against all the tested strains of bacteria and fungi indicating that the active compound(s) could not be released from this vehicle. Although evaluation of the quality of the two herbal drugs was not possible due to absence of published data for comparison, the most commonly employed standardization/quality control parameters including ash values, solvent extractable matters, loss on drying and TLC fingerprints were determined for the two herbal drugs in an attempt to provide such base line data as an indication of their quality attributes.

1. INTRODUCTION

1.1 Herbal Drugs in Medical Health Practices

1.1.1 Historical Perspectives

The history of herbal medicine is rather old and dates back to the time when the early man became conscious of his environment. Cultural man is 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 year's experience, by trial and error must have taught him to distinguish between useful and harmful plants with their properties as healing agents dawning on him much later [1]. Since then, medicinal plants have been used in virtually all cultures as a source of medicine [2].

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 is available in the old literature, folklore, mythological stories, epic poems, medical treatises and thousands years old manuscripts, palm leaves and copper plates and other records on these cultures which are preserved even today. The excavation of Shanidar cave in Iraq in 1963 revealed the grave of Neanderthal man buried sixty thousand years ago along with many flowers of his time. The plants found in the grave were later identified to having various medicinal properties [1].

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 [3].

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 [4]. Pliny, the elder (23-79 A.D.), 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 [1,4]. In the middle Ages, the writing of Galen (131 A.D.) becomes 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 [3].

The ancient use of plants for healing purposes forms the origin of much of modern medicine. Many conventional drugs originate from plant sources: a century ago, most of the few effective drugs were plant based. Examples include aspirin (from willow bark), digoxin (from foxglove), quinine (from cinchona bark), and morphine (from opium poppy). The development of drugs from plants continues, with drug companies engaged in large-scale pharmaceutical screening of herbs [5].

During the last decade, the use of TM (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 [6]. 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 [7].

1.1.2 Current Status of Herbal Drugs: Global Perspective

For centuries, right up to the 19th century, herbs were the major sources of drugs. While they were put aside with the rapid advance in synthetic organic chemistry for around 50 years in the past, they still occupy an important place in medicine. With upswing consumer interest since 1960s, high demand has been seen for contemporary alternative medicine, to the extent that health foods and herbals have become a several billion dollars per year business [8].

The practice of TM 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 [2]. 60 to 70% of allopathic doctors in Japan prescribe TM for their patients. In Malaysia, traditional forms of Malay, Chinese and Indian medicine are used extensively [9]. China is the leading country for incorporating traditional herbal medicine into a modern health care system. In this country, TM accounts 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 [7-9].

In Latin America, the WHO regional office for the Americas reported that 71% of the populations in Chile and 40% of the population in Colombia use TM. In developed countries, alternative medicine is becoming more and more popular. Various reports state that the percentage of the population that uses alternative medicine is 46% in Australia, 49% in France and 70% in Canada [9]. This situation has undergone the most dramatic changes during the last few years. For instance, usage of herbalism by the general US population has increased by 380% between 1990 and 1997. In 1990, the 1-year prevalence was 2.5% while in 1997, it had risen to 12.1% [10].

Many people in the US are turning to herbal medicine to treat their ills. It has been estimated that up to 50% of the prescription presently dispensed in the US may contain one or more natural product drugs [11]. Over 20,000 herbal and other natural products are available in the United States [12,13]. In Europe, some 1,500 species of medicinal and aromatic plants are widely used in Albania, Bulgaria, Croatia, France, Germany, Hungary, Poland, Spain, Turkey, and the United Kingdom [14]. Germany and France together represent 39% of the global retail markets [15].

The majority of African population relies on TM for the treatment of both human and animal diseases [16]. In Tanzania, Uganda and Zambia, researchers have found a ratio of TM 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 [9].

The world market for herbal remedies in 1999 was US$ 19.4 billion, with Europe in the lead (US$ 6.7 billion), followed by Asia (US$ 5.1 billion), North America (US$ 4 billion), Japan (US$ 2.2 billion) and the rest of the world (US$ 1.4 billion) [17,18]. In Europe, Germany has the largest market (US$ 3.5 billion) followed by France (US$ 1.8 billion) and Italy (0.7 billion) [19]. In Canada, it is estimated that a total of US$ 2.4 billion was spent in 1997 on alternative medicine [9]. In 1999 among the 25 best selling drugs in the world, 30% came from natural products [7].

In Ethiopia, 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 [20]. 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 Ethiopia 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 [21].

1.1.3 Potential of Herbal Remedies as Sources of New Drugs

Historically, plants have provided a source of inspiration for novel drug compounds, as plant derived medicine have made large contribution to human health and well-being. Their role in the development of new drugs could be either by serving as a natural blueprint for the development of new drugs, or as a phytomedicine to be used for the treatment of disease. It is estimated that, plant materials have provided the models for 50% of the western drugs. Many commercially

proven drugs used in modern medicine were initially used in crude form in traditional or folk healing practice [22].

Since the beginning of the 19th century, a large number of biologically active secondary metabolites of plant origin have been found to have commercial application as drugs. Recently, there has been an upsurge of interest in the use of plants with folkloric reputations as sources of potentially useful compounds [23]. Analysis of the number and sources of anticancer and anti infective agents, reported from 1984 to 1995, indicates that over 60% of the approved drugs and pre-NDA (New Drug Application) candidates (for the period 1989-1995), excluding biologics, developed in these disease areas are of natural origin. A recent review reported that at least 119 compounds derived from 90 plant species could be considered as important drugs currently in use in one or more countries, with 77% of these being derived from plants used in traditional medicine. Further evidence of the importance of natural products is provided by the fact that close to half of the best selling pharmaceuticals in 1999 were either natural products or their derivatives [24-25].

Several new small molecules of natural product-derived drugs have been introduced into therapy in western countries in recent years, including acarbose, artemether, capsaicin, docetaxel, dronabinol, galanthamine, irinotecan, paclitaxel, tacrolimus, and topotecan. This trend is likely to continue in the future, at least for the treatment of disease states such as cancer and infectious diseases. In a recent statistical survey, it was pointed out that the origin of 30,000 bioactive natural products could be divided between animals (13%), bacteria (33%), fungi (26%), and higher plants (27%) [11].

It is estimated that there are 250,000 to 500,000 species of higher plants on earth. Only small proportion (1-10%) of these is used as foods by both humans and other animal species. It is possible that even more are used for medicinal purposes [26]. But, only a relatively small percentage (5-15%) has been systematically investigated for the presence of bioactive compounds. Plants offer the scientist searching for novel bioactive compounds the added advantage of ethnobotanical observations, since many species are used in systems of TM. It has been estimated that nearly 75% of about 120 biologically active plant derived substances used throughout the world were discovered by following up on leads from TM [11].

The Ethiopian flora is estimated to contain between 6,500 and 7,000 species of higher plants of which about 12% are endemic. Ethiopia 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 [27,28]. Despite its significant contribution to society, TM 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 Ethiopia and its huge biotic riches can be of paramount importance in future research and drug discovery.

1.1.4 Herbal Products as Antimicrobial/Antiinfective Agents

Worldwide, infectious diseases are the leading causes of death accounting for approximately one-half of all deaths in tropical countries. They are also becoming a significant problem in developed nations. It is estimated that infectious diseases are the underlying causes of death in 8% of the

deaths occurring in US [29]. Development of new antimicrobials is among the proposed solutions to curb this problem [30]. In this regard, plants could provide a good alternative in search for new chemical agents with a wide-ranging antimicrobial activity.

The use of higher plants and preparations taken from them for the treatment of infections predates written records. Certainly, some of the earliest surviving accounts of medical practice such as Pen Tsao of 3000 B.C., the Ebers papyrus of 1500 B.C and Celsus "De Materia," for example, record such usage [31]. The isoquinoline alkaloid emetine obtained from *Cephaelis ipecacuanha* and related species, has been used for many years as amoebicidal drug as well as for the treatment of abscesses due to *Escherichia histolytica* infections. Another important drug of plant origin with a long history of use is quinine, which occurs naturally in the bark of cinchona tree. The bacteriostatic and fungicidal properties of Lichens, the antibiotic action of allicine in *Allium sativum*, the antimicrobial action of berberine in *Hydrastis canadensis* are also examples of higher plants that have been used as sources of antibiotics [22].

Many medicinal plants of Africa have been investigated for their chemical components and some of the isolated compounds have been shown to possess interesting biological activities. *Garicinia cola*, *Aframomum melegueta, Xylopia aethiopica*, *Cryptolepis sanguinolent* and *Chasmanthera dependens* are among the most widely used species that are found to possess different groups of compounds with wide ranging anti-inflammatory and antimicrobial activities [22].

All these are indicative of the fact that plant based antimicrobials represent a vast untapped source for medicines. Plant based antimicrobials have enormous therapeutic potential. They are effective in the treatment of infectious diseases while simultaneously mitigating many of the side

effects that are often associated with synthetic antimicrobials. Phytomedicines usually have multiple effects on the body. Their actions often act beyond the symptomatic treatment of disease. For example, *Hydrastis canadensis* not only has antimicrobial activity, but also increases blood supply to the spleen promoting optimal activity of the spleen to release mediating compounds [22].

1.2 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 [32]. 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 [33].

1.2.1 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 [32].

1.2.1.1 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) [32]. The most common bacterial infections include impetigo, folliculitis, furuncles and carbuncles [32,34].

Impetigo is an acute bacterial infection that occurs superficially on the skin as serous and purulent vesicles that later rupture and forms a golden crust. The causative organisms include β-hemolytic streptococci and coagulase-positive staphylococci. Folliculitis is also bacterial infection of the skin originating within the hair follicle. Staphylococci are the usual causative organisms. Furuncles frequently develop from a preceding staphylococcal folliculitis and are usually located in body areas containing hair follicles. Carbuncles are larger staphylococcal abscesses that drain through various points [34].

1.2.1.2 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 *Trychophyton*. 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 immunosuppressed individuals [32,34].

1.2.1.3 Viral Infections

Viruses are intracellular pathogens. They rely completely on live cells for reproduction. The viruses seen in skin lesion disorders tend to be DNA-containing viruses. Viruses invade the keratinocyte, begin to reproduce, and cause cellular proliferation or cellular death. The rapid increase in viral skin diseases has been attributed to the use of birth control medication and corticosteroid drugs, which have immunosuppressive property, and the use of antibiotics, which alter the bacterial flora of the skin [32]. Herpes simplex, Varicella (also known as chickenpox), Herpes zoster, Warts (Verrucae), Rubeola (Measles), and Rubella (also known as German measles) are some of the most common infectious skin disorders of viral origin [34].

1.2.2 Inflammatory and Allergic Skin Disorders

The inflammatory skin diseases are usually localized to the skin and are rarely associated with a specific internal disease. They produce marked variations in normal skin, usually papulosquamous in nature. Inflammation and erythema are common. Acne, lichen planus, psoriasis, and pityriasis resea, are among the most common skin disorders of this type. Allergic skin responses involve the body's immune system and are caused by hypersensitivity reactions. They include contact dermatitis, atopic and cummular eczema and drug reactions [32,34].

Acne vulgaris is a common, chronic, inflammatory disease of the sebaceous glands and hair follicles of the skin, also known as pilosebaceous ducts. Lichen planus on the other hand is a relatively common chronic, pruiritic disease involving inflammation and popular eruption of the skin and mucous membranes [32]. Psoriasis is another chronic inflammatory skin disorder characterized by variably pruritic rashes [35]. Pityriasis rosea is a self-limiting rash seen in

adolescents and young adults. Eczema describes an acutely inflamed weeping skin with vesicles. It is synonymous with the term 'dermatitis' and the two words are interchangeable [36]. Itching may be severe and secondary infections are common [32].

1.2.3 HIV-Associated Skin Disorders

HIV infection commonly causes significant dermatological problems. A rash may even be the presenting feature of the underlying HIV infection. It is estimated that 90% of the HIV positive patients will suffer with a mucocutaneous disorder during the illness. It is also estimated that up to 30% of people with AIDS will suffer from three different dermatoses. These rashes can often be clinically atypical and difficult to diagnose. On top of this many of the skin problems are resistant to standard treatments. The dermatoses may be arbitrarily divided into cutaneous and opportunistic infections, inflammatory dermatoses, autoimmune dermatoses, drug rashes, cutaneous tumours and specific HIV dermatoses [36-38].

1.2.4 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 [36]. 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 [39]. Atopic eczema affects between 5 and 20% of children aged up to 11 years at one time or other [40].

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%) [41]. A similar study made in Aman, Jordan showed an overall prevalence of skin disorder to be 19.23% [42]. A study made in rural Tanzania also reported that 34.7% of 800 villagers had one or more skin diseases, the most common of which were tinea capitis, tinea corporis, scabies, acne and eczema [43].

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 papulosquamous 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 [44].

The prevalence study among school children in South West 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 [45]. Similar study made in Northwest Ethiopia showed a prevalence rate of 49.2%. Tinea capitis, impetigo, infected wounds and verrucae were the most common skin infections [46]. Another study made at 5 elementary schools in Dembia district, North Ethiopia indicated that infectious skin disorders are very common among the children (5 to 15 years old). Only 16% of all children were found to be free of infectious skin diseases [47]. 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%) [48]. 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 [49].

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 immunosuppression, opportunistic pathogens may present as atypical cutaneous lesions [38,50]. 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 [38,51].

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 hypersensitivity. 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 [52-53].

1.3 Treatment of Skin Disorders

The skin has many essential functions, including protection, thermoregulation, immune responsiveness, biochemical synthesis, sensory detection, and social and sexual communication. Therapy to correct dysfunction in any of these activities may be delivered topically, systemically, intralesionally or through ultraviolet radiation [54].

1.3.1 Conventional Approaches

1.3.1.1 Treatment of Infectious Skin Disorders

Bacterial Infections: Antibiotics are used empirically for the treatment of bacterial skin infections with consideration for resistance patterns. Current antibiotics recommendations include penicillinase-resistant penicillins, azithromycin, clarithromycin, amoxycillin-clavulanic acid, fluoroquinolones and cephalosporins [55].

Systemic antibiotics including tetracycline, doxycycline, minocycline, erythromycin, trimethoprim-sulfamethoxazole, and clindamycin are commonly used medications in most bacterial infections. Topical antibiotics commonly are used in acne and superficial secondary infections. Superficial cutaneous staphylococcus infections are treated with mupirocin [54]. Neomycin and gentamycin are active against gram-negative organisms, including *E. coli*, proteus, klebsiella, and enterobacter. Gentamycin is also active against staphylococci and group A beta-hemolytic streptococci [56].

Fungal Infections: The choice of an antifungal agent is dependent on the site involved, the extent of clinical infection, the age and general health of the patient and concomitant drug

therapy. Superficial infections of the skin and nails may be managed either topically or systemically [57].

The treatment of superficial fungal infections caused by dermatophytic fungi may be accomplished with topical antifungal agent (e.g. clotrimazole, miconazole, econazole, ketoconazole, oxiconazole, sulconazole, ciclopirox olamine, naftifine, terbinafine, and tolnaftate) or with orally administered agents (i.e. griseofulvin, terbinafine, ketoconazole, fluconazole, and itraconazole). Superficial infections caused by candida species may be treated with topical applications of clotrimazole, miconazole, econazole, ketoconazole, oxiconazole, ciclopirox olamine, nystatin, or amphotericin B [56].

Viral Infections: Few effective medications are available for viral diseases. Acyclovir, famciclovir and valacyclovir frequently are used systemically to treat herpes simplex and varicella infections. Acyclovir and penciclovir are available for topical use in treating mucocutaneous herpes simplex. Podophyllin (25% solution) and podofilox (0.5% solution) are used to treat moist warts of condyloma. The immune response modifier imiquimod, which induces interferon production, is approved for treatment of condyloma and is efficacious for the treatment of verrucae, molluscum contagiosum, and superficial basal cell carcinomas [54].

1.3.1.2 Treatment of Inflammatory and Allergic Skin Disorders

Urticaria: Urticaria is a common reaction pattern characterized by the presence of itchy or 'burning' edematous swellings (wheals) occurring anywhere in the body [58]. Antihistamines are still the main stay of treatment of chronic urticaria, although they tend to be more effective in

suppressing itching than healing [59]. Oral antihistamines (H_1 blockers) are the most important part of treating the idiopathic cases. Angioedema of the mouth and throat may require urgent treatment with intravenous steroids and subcutaneous adrenaline [36].

Eczema: The basis of direct treatment of eczema is to suppress the symptoms and control or prevent complications. Anti-inflammatory topical steroids are the drugs of choice. In children, when the eczema is very active, stronger steroids such as betamethazone valerate may be required. Normally, clobetasone or mometasone may be adequate for treating areas other than the face; 1% hydrocortisone is the main steroid for the face [60]. Acute flares are often induced by staphylococcal super infection. Systemic antibiotics (flucloxacillin or erythromycin) should be used. As second line of treatment corticosteroids should be used very seldom. Ultraviolet light and photo chemotherapy with psoralen and long wavelength ultraviolet irradiation may be used in older children and adults. When second line treatment fails or is not suitable, further treatment options include cyclosporin A and azathioprine [60-61].

Psoriasis: The selection of therapy for psoriasis is multifactorial. The overall health status of the patient and the percent of body surface area involved should be taken into account. Topical therapy for psoriasis includes multiple options the first of which are emollients to soften and moisturize psoriatic plaques. Topical keratolytic agents, formulated with urea or salicylic acid, also are useful in the treatment of localized or limited psoriasis. Topical steroids are the mainstay of treatment for localized psoriasis. A vitamin D analog, calcipotriene, is useful for the topical treatment of psoriasis, as a solution, an ointment or a cream. Anthralin and the topical retinoid tazarotene also are beneficial. The use of systemic medications for the treatment of psoriasis may be indicated by the extent or severity of the disease. Some cancer chemotherapeutic agents have

been used with good results in psoriasis, especially methotrexate, thioguanine and hydroxyurea [54].

Acne: Treatment of acne is aimed at decreasing sebum production, decreasing bacteria, normalizing duct keratinization or decreasing inflammation [36]. Topical treatment of acne involves the use of retinoids and antimicrobials. Tretinoin is a comedolytic agent that loosens existing comedones and prevents the formation of new comedones. When combined with topical retinoids, treatment with antimicrobials is more effective than if either medication is used alone. Commonly used topical antimicrobials include erythromycin, clindamycin and benzoyl peroxide. Systemic therapy in acne is most often reserved for inflammatory lesions. Systemic treatment includes retinoids, antibacterials and hormones [54].

1.3.1.3 Limitations of Conventional Approaches

Conventional medications are often unsatisfactory for many patients with chronic skin disorders such as eczema because of adverse effects on long-term use [62]. Chronic use of very high potency topical glucocorticoids in the treatment of eczema can lead to cutaneous atrophy and often accompanied by loss of effectiveness. In patients with chronic atopic eczema, therapy with systemic glucocorticoids will generally clear the skin only briefly, but cessation of the systemic therapy will invariably be accompanied by return, if not worsening, of dermatitis. The side effects of daily doses of systemic glucocorticoids preclude chronic use in virtually all patients with atopic dermatitis, and the efficacy of alternate-day regimens in this disease is limited [34,54].

Most patients with localized plaque-type psoriasis can be managed with midpotency topical glucocorticoids, but their long-term use is often accompanied by loss of effectiveness. The

combination of ultraviolet A (UV-A) radiation and either oral or topical psoralens is also effective for the treatment of psoriasis but the photosensitizing potential of psoralens and unknown long term toxicity may limit the use of this therapy. The systemic retinoid etretinate is effective in some patients with severe psoriasis, but is a potent teratogen with an extremely long tissue half-life, thus precluding its use in women of childbearing age [35].

Dermatologists rely heavily on antibiotics to treat their patients for a variety of conditions. But treating bacterial pathogens with antibiotics has suffered enormous setbacks since the introduction of these drugs. In 1950, the antibiotic arsenal included penicillin, streptomycin and sulfonamides followed by tetracyclines, chloramphenicol and erythromycin. Today, bacterial resistance has compromised the effectiveness of these antimicrobials. Until the last decade, the development of new antibiotics has roughly kept pace with the continuing evolution of bacterial resistance to antimicrobials. Today, the problem of resistance has become far more serious, escalating to crisis proportions [63].

A study conducted in Latin America hospitals to evaluate the frequency of occurrence and antimicrobial susceptibility of bacterial isolates collected from patients with skin and soft tissue infections (SSTI) indicated that 31% of *S. aureus* isolates were resistant to methicillin [64]. In a similar study made at 20 hospitals in 12 different European countries, methicillin resistance in *S. aureus* averaged 22% across Europe, only slightly less than that in isolates derived from blood [65]. Topical antibiotics are widely used but resistance has been encountered with tetracycline, gentamycin and fusidic acid. Systemic toxicity and contact allergy is also a possibility, for example the topical use of neomycin in patients with extensive skin damage may result in deafness [33].

1.3.2 Phytopharmaceutical Approaches

1.3.2.1 Herbal Drugs Commonly Used in the Treatment of Dermatological Disorders

The traditional practice of topically treating dermatological conditions with plant-derived therapeutic preparations predates the cultures of ancient Egypt and remains vital today in our life [66]. Alternative therapies are becoming increasingly popular, particularly among patients with chronic skin disorders such as eczema, which are not cured by standard treatments [67]. The American Indians used roots of *Polemonium caeruleum* in prescriptions for piles and to treat eczema. The root of *Sanguinaria candensis* is used as a local application in chronic eczema. *Centaurium erythraea* is a highly praised plant by American Indians as a remedy for wound, eczema and all types of sores. *Trifolium pratense* has long been recognized amongst herbalists as a useful remedy in eczema and psoriasis [68].

Thuja accidentalis has been used successfully for the treatment of psoriasis, rheumatism and for warts. *Smilax aristolochiaefolia* has anti-inflammatory properties, which would further relieve the discomfort of psoriasis. *Stellaria media* contains saponin glycosides, coumarins and hydroxycoumarins, flavonoids, carboxylic acids, triterpenoids and Vitamin C. These constituents give the plant antipruritic, vulnerary, emollient and antirheumatic properties, which when used in an ointment or poultice is excellent for eczema, psoriasis, ulcers and boils. *Anaphalis margaritaceae* is used by American Indians for rheumatism, burns, sores, bruises, swellings and psoriasis. Interestingly a related species found in Nepal, *Anaphalis triplinervis* is used as an antiseptic on wounds. Numerous other plant species including *Artemisia tridentate, Trillium erectum, Grindelia robusta, Clematis virginiana* and *Eupatorium perfoliatum* are used worldwide for the treatment of wounds, cuts and other skin problems [68].

In the hot and humid lowlands of Mexico, half of all recorded species of plants (49%) are used to treat dermatological disorders, and about one fifth of the use-reports fall into this group. The fresh leaf sap from *Hamelia patens* is applied to stop the bleeding of a wound. The closing of a wound is accelerated by dried leaf powder of S*olanum torvum*, *Solanum rudepannum* or the bark extract of *Heliocarpus* species. *Tithonia diversiflora* is used for scabies and for washing wounds. The use of this species for these purposes can presumably be explained by the presence of sesquiterpene lactones, which are potent inhibitors of inflammatory processes and have antibacterial effects [69].

The African continent is a rich source of plants used for treating various skin diseases. The juice from the aerial parts of *Borreria verticillata* is used in west cost of Africa to treat various skin diseases including eczema. Another species *B. campacta* is used similarly in southern Africa. *B. natalensis* is used by the Zulus for the treatment of leprosy and furuncles. *Anogeissus leiocarpus* is used in Nigeria for the treatment of skin diseases and the itch of psoriasis. The powdered bark is applied to wounds, sores, boils, cysts, diabetic ulcers and acne with good results. *Andira inermis* is also used to treat psoriasis and ringworm of the scalp. Extracts of *Ammi visnaga* have been used to treat psoriasis, where khellin is the major component responsible for the beneficial action [70].

Plumbago zeylanica is a plant, which is extremely popular throughout Africa and Asia as a remedy for parasitic skin diseases, especially leprosy, scabies, acne vulgaris, sores and leg ulcers. The root contains plumbagin, which is responsible for its antimicrobial activity. Plumbagin is lethal to a wide spectrum of pathogenic bacteria and fungi at very low concentrations. The Negritos of Philippines use *Artemisia vulgaris* or *Artemisia absinthus* in fomentations for skin

diseases including ulcerative sores and wounds. The leaves are used in eczema, herpes and purulent scabies. *Artemisia herba-alba* is extremely popular throughout much of North Africa; being used for a variety of skin problems such as burns, wound, abscesses, excessive bleeding, herpes lesions, acne and curbuncles. *Artemisia afra* is also popular for skin ailments. It is used externally for boils, carbuncles, and large acne pimples. A poultice of the leaf is applied locally to relieve neuralgia, to the swelling in mumps, and to any glandular or skin inflammation [70].

1.3.2.2 Scientific Studies Made on Topically Used Herbal Drugs

The traditional practices of topically treating dermatological conditions with plant-derived medicines are supported by recent scientific studies. With the growing interest in alternative and complementary therapies, clinical studies and collected observations will help define specific indications for choice of herbal treatment based on both the skin disorder and the unique characteristics of the patient involved [71].

Many scientific studies show that plants possess a vast complex arsenal of phytochemicals that calm, restore and heal the skin. At the simplest level, soothing and emollient herbal remedies are found to contain mucilage, polysaccharides, complex sugars and starch derivatives that relieve dryness, and provide a soothing membrane that covers the skin. Protection of the skin hydration is achieved using seed oils rich in fatty acids and triglycerides that reduce the transepidermal water loss and so increase skin hydration. It is known that those plants with anti-inflammatory properties often have a high level of flavonoids, those that are used to firm and tone the skin are rich in tannins and those that have cicatrizing and vulnerary properties often have a high level of plant sterols. Often the skin healing is compromised by opportunistic infections and in these cases the use of plants can provide a complex array of antimicrobial and antifungal biocides [72].

Different fractions of herbal drugs used in the folk medicine of Central America against skin disorders (*Aristolochia trilobata*, *Bursera simaruba*, *Hamelia patens*, *Piper amalago*, and *Syngonium podophyllum*) were evaluated for their topical anti-inflammatory activity. Most of the extracts induced a dose-dependent edema reduction. The chloroform extract of almost all the drugs exhibited interesting activities [73]. The anti-inflammatory activity of *Thymus willdenowii* Boiss (Labiatae) leaves has been studied and the chloroform extract was found to have potency similar to that of indomethacin [74]. Evaluations of different extracts of *Corrigiliola telephiifolia*, *Echinops spinosus, Kundmania sicula, Tamarindus indica* and *Zygophyllum gaetulum* for their anti-inflammatory activity have also shown good results, thus, supporting the traditional use of these plants in North-African countries [75].

A study made to determine the microcirculatory and wound healing effects of *Aloe vera* in rats showed that this plant could exhibit the actions of both anti-inflammation and wound healing promotion [76]. In animal studies of skin inflammation, both topical and oral *Aloe vera* have proven to be beneficial in decreasing inflammation and promoting cellular repair [77-78]. Topical *Aloe vera* has facilitated wound healing in controlled human research as well [79]. In another preliminary study, “gotu kola” extract was proven to help the healing of infected wounds [80]. Gotu kola extract was also reported to be helpful in preventing and treating enlarged scars [81]. Animal studies have shown that constituents of gotu kola, called asiaticosides, increase antioxidant levels during wound healing and facilitate repair of connective tissues [82-83].

Asiaticoside derived from the plant *Centella asiatica* is known to possess good wound healing activity. Since antioxidants have been reported to play a significant role in wound healing process, the investigators studied the effect of asiaticoside on the levels of certain antioxidants in

the wound. It appears from their result that asiaticosides enhanced induction of antioxidant levels at an initial stage of healing which may be an important contributory factor in the healing properties of this substance [83]. The fruits of *Thespesia populnea* have been traditionally used to treat a variety of skin ailments including wounds. The aqueous extract of *T. populnea* fruit showed significant wound healing activity in rats following both topical and oral administration [84].

Plant chemicals are useful for infection control and until the advent of antibiotics they were the only remedies available. The current role of plants in this area could be summarized as treating minor acute infections, topical therapy for skin or wound infections and perhaps a supportive role in chronic infection. With increasing resistance of bacteria, viruses and malaria to standard therapy, alternative treatments are being re-explored [85].

The antibacterial potential of *Ocimum gratissimum* essential oils was studied using liquid and semisolid formulations of the oil. Remarkable antibacterial effects, higher than those of commercial antiseptics, were demonstrated at 2% ocimum oil concentration in some bases [86]. The essential oil of *Eucalyptus pauciflora* also showed strong antifungal activity against different human pathogenic fungi *in vitro*. Subsequent topical testing of this oil in the form of an ointment (1% v/v) on patients resulted in complete recovery in 60% and significant improvement in 40% of all patients at the end of treatment [87].

In Africa, the active components of many traditional preparations are often of plant origin and more than 25 plants have been described as useful in relation to burns and wound healing. *Carica papaya* is currently used in Gambia as major component of burns dressings, where it is well

tolerated by the children. It appears to be effective in desloughing necrotic tissue and preventing burn wound infection. Possible mechanisms of action include the activity of proteolytic enzymes chymopapain and papain, as well as an antimicrobial activity [88].

Research on the bioactivity of tropical medicinal plants has demonstrated that most are safe and effective therapies. Tropical rural communities, have a very low access to modern pharmaceuticals due to their high cost. Therefore, locally available medicinal plants can contribute to health care needs and generate economic benefits for tropical rural communities. The WHO Traditional Medicine Programme and other research programmes have conducted various researches on tropical medicinal plants and the results have demonstrated safety and efficacy for the treatment of common tropical diseases including malaria and infections of the skin, lungs and gastrointestinal tract [89]. Therefore, the wise and scientific use of these resources can contribute to the health of large number of the rural community in these areas that have limited or no access to modern health care.

1.4 Review of Some Topically Used Herbs Included in this Study

1.4.1 *Acokanthera schimperi*

A. schimperi (Apocynaceae) is an evergreen tree or shrub growing up to 6 m in height. The plant occurs in both savanna and rainforest vegetation. It grows in Ghana, Nigeria, Zaire, and parts of East Africa [90]. *Acokanthera* is the primary source of dreaded African arrow poison, which is considered so dangerous that even minute quantities transmitted through small injuries could lead to fatal cardiac arrest within a few minutes of reaching the blood stream [90]. The bark of the root is used for spasmodic dysmenorrhoea, to alleviate pain of the spleen and as purgative [91].

Different species of the same genus were also reported to have medicinal properties for treating skin, ectoparasitic, abdominal, and venereal diseases [92].

In Ethiopia, *A. schimperi* (locally called "Mrenz") is used alone or in combination with other species of plants for the treatment of headache (root and bark), epilepsy, amnesia, eye disease, scabies, leprosy (leaf), syphilis (leaf), Tinea capitis (leaf), wound (leaf), eczema (unspecified part), swelling (root), warts (unspecified part), common cold (leaf), rheumatic pain (stem) and elephantiasis (root) [93].

The wood of African *Acokanthera* species yields very potent cardiotonics of which the principal compound is ouabain [90]. An arrow poison prepared by traditional method from *A. schimperi* in the Maasai plains of Kenya was shown to contain acolongifloroside K as its major active principle and smaller amounts of ouabain and acovenoside A [94].

1.4.2 *Calpurnia aurea*

There are about 650 genera and 18,000 species included under the family Leguminosae distributed in most parts of the world. It has three sub-families. The sub family Papilionoideae to which the genus *Calpurnia* belongs includes about 440 genera and 12,000 species widely distributed in most parts of the world, but with greatest diversity in tropical and subtropical regions. The genus *Calpurnia* is represented by shrubs or small trees, and has about 7 species all of which except one are confined in South Africa [95]. *C. aurea* (Leguminosae) is a yellow flowered leguminous small tree or shrub (1 to 10 m tall) widely distributed in Africa from Cape Province in South Africa to Ethiopia and which also occurs in Southern India [95].

In Ethiopia, *C. aurea* (locally known as "Digita") is used in indigenous medicine as an insecticide as well as for the treatment of amoebic dysentery and diarrhea in animals. The extracts of the leaves in particular are used for killing head lice in humans and ticks in cattle. The head is washed with the juice from the leaves to treat Tinea capitis. Powdered seeds are pasted with honey and swallowed to treat syphilis and the ground fruits are mixed with butter and applied topically for treating scabies [96-97]. In addition, the leaves are used for the treatment of diarrhea, leishmaniasis, Tinea capitis, wound and as an insecticide, seeds for eczema, toothache, root for lung TB, swelling, cough and snake bite [93].

The pharmacological activity of virgiline (an alkaloid isolated from *C. aurea*) was studied and it was found to have a potent molluscicidal activity against *Biomphalaria glabrata* [98]. Phytochemical investigation of *C. aurea* has led to the isolation of different alkaloids including calpurmenine [99], virgiline and virgiline pyrrolecarboxylic acid ester [168], digittine and its amino alcohol [100], lupanine, calpaurine, lupinine, calpurmenine and its pyrolecarboxylic acid ester, 13-hydroxylupanine and its tiglate, and calpurnine [101]. It is also reported to have lectins, non-protein aminoacids and tannins [97].

1.4.3 *Kalanchoe petitiana.*

Crassulaceae is a cosmopolitan family with perhaps 35 genera and 1,500 species. Many members of the family are cultivated as ornamentals. The genus *Kalanchoe* includes about 60 species, mostly in Africa and Madagascar. *K. petitiana* (Crassulaceae) is one of these species and is distributed around the forest margins and open evergreen bush land, often in disturbed areas 2000-3000 m above sea level [95].

In Ethiopian traditional medicine, the leaves of *K. petitiana* (locally known as "Endohahila") are used in the treatment of tapeworm, trachoma, syphilis and different swellings; the roots for ascariasis, epilepsy, rabies, constipation, and to induce labor; the whole plant is used to treat hemorrhoid [93-94,102]. 24-Alkylsterols were isolated from the aerial parts of *K. petitiana*. These sterols were known to be present as mixtures of the 24α- and 24β-epimers [103]. This is the only constituent of this species reported so far in the literature.

1.4.4 *Lippia adoensis*

L. adoensis is included under Verbenaceae that is a large family with about 70 to 80 genera and over 3,000 species; distributed throughout the world mainly in the tropics and temperate regions. In Ethiopia, the family is represented by 9 genera and 30 species. *Lippia* is a genus with 200 species in tropical Africa and America. Five species have been described in the country [104]. *L. adoensis* (locally known as "Kesse") is a shrub having a height of 1 to 3 meters. Two varieties are recognized in Ethiopia, the wild variety (var. *adoensis*) and the cultivated variety (var. *koseret*). The fragrant leaves are used by the Gurage and Oromo tribes as one of the condiments in the preparation of spiced butter. The special taste and flavor of the "Gurage Kitfo" is attributed to the oils imparted by the leaves [104].

In Ethiopia, the wild variety (var. *adoensis*) is used for washing kitchen utensils to impart fresh and spicy fragrance [105]. The leaves of *L. adoensis* are used in Ethiopian traditional medicine for the treatment of various skin diseases including eczema and superficial fungal infections [102].

Scientific studies dealing with the pharmacological activities of this particular species is non-existent except one study that investigated the antioxidant activity of the volatile oil and the major terpenoids therein. The result indicated that the essential oil possess a significant radical scavenging property when assessed in the DPPH (diphenylpicrylhydrazyl) assay. As free radical oxidative stress is implicated in the pathogenesis of a variety of human diseases including inflammation, the traditional uses of the plant for the treatment of various kinds of inflammatory skin diseases is partly justified [106].

The chemical compositions of *Lippia adoensis*, investigated so far are essential oils. The oils were predominantly monoterpenoids with minor sesquiterpenoid fraction. In one study, out of more than 10 compounds isolated, linalool comprised 81.30% and 94.56% of the leaf and flower oils, respectively [107]. In a report of a similar study, 46 compounds were identified from the same species [106].

1.4.5 *Malva parviflora*

The family Malvaceae includes about 90 genera and 200 species. About 30 species are included in the genus *Malva* distributed mainly in the temperate and Mediterranean regions extending into central Asia. A number of species are now widespread cosmopolitan weeds. *M. parviflora* (Malvaceae) is a decumbent or an ascending annual herb having stems up to 50 cm long. It grows in degraded and overgrazed mountain bush land, lakeshores, and roadsides [108].

In Ethiopian traditional medicine, *M. parviflora* (locally known as “Lit”) is used in the treatment of wounds and asthma [102]. Three potent antimicrobial proteins were purified from the seeds *M. parviflora*. These proteins showed different antimicrobial spectrum and potency [109]. In

addition, two novel antifungal proteins were purified and characterized. These proteins possess very potent antifungal activities, and more interestingly the inhibition is fungicidal [110].

1.4.6 *Olinia rochetiana*

O. rochetiana is a plant included under the family Oliniaceae which is reported to have a single genus, *Olinia*, with one species known to occur in Ethiopia. *Olinia* is a small genus of probably 5-6 species (usually estimated at about 10) in Eastern and Southern Africa. O. *rochetiana* is a shrub, small tree or less often a large tree, evergreen, usually (1.2-) 4-16 meters tall, but occasionally said to reach 27 meters [111].

In Ethiopia, the leaves of O. *rochetiana* (locally known as "Tife") are used traditionally (either as powder, infusion or in the form of ointment) for the treatment of eczema, acne and scabies [93]. A phytochemical investigation made on this plant has led to the isolation of the cyanogenic glucoside prunasin (2-β-D-glucopyranosyloxy-2-phenylacetonitrile) from its leaves and twigs [112]. Report on the biological activities of this plant could not be found in the literature.

1.4.7 *Phytolacca dodecandra*

About 16 genera and 100 species, mostly in tropical and subtropical America are included under the family Phytolacaceae. The genus *Phytolacca* includes about 35 species in tropical and subtropical regions, mostly in America. *P. dodecandra* (Phytolaccaceae) is a semi-succulent straggling or scrambling shrub having a height of 10 m or more.

It is widely used as soap, vermifuge and as a molluscicide for the control of snails that are the vectors of bilharzia. The species is regarded as highly poisonous to stock in East Africa but apparently less so in Ethiopia [111]. The roots of *P. dodecandra* are used in African traditional medicine as purgative, taenifuge, cathartic, and for the treatment of venereal diseases particularly syphilis [91]. The fruits and leaves are also used as fish poison, purge and taenifuge [90].

In Ethiopia, different parts of *P. dodecandra* (locally known as "Endod")are used alone or in combination with other plants for the treatment of pruiritus, eczema and ascariasis (fruits), gonorrhoea, malaria, rabies, sore throat and rheumatic pain (roots), Jaundice (bark) and syphilis and vitiligo (leaves) [93,96,102]. The molluscicidal properties of *P. dodecandra* have been extensively studied. A large species of plants have been tested for this purpose and more than 50 molluscicidal compounds have been isolated from plants, including saponins, terpenes, flavonoids, naphtoquinones and tannins. The sapponins from *P. dodecandra* are the most active and have been successfully employed in field tests [113-118]. The molluscicidal sapponins have also been shown to be spermicidal, with potency comparable to that of a commercial spermicide [96].

1.4.8 *Verbascum sinaiticum*

The family Scrophulariaceae is a cosmopolitic family with 300 genera and about 5400-5500 species, mainly in the holarctic and the tropical mountains. *Verbascum* is a genus having about 360 species in Europe and Asia. *V. sinaiticum* is a biennial plant, 60 to 150 cm tall. It is distributed on cultivated ground and on grassland [94].

In Ethiopia, *V. sinaiticum* (locally known as "Ketetina") is used in the treatment of abdominal dropsy (root), anthrax (root and leaves), postpartum hemorrhage (leaves), diarrhea (root) and superficial fungal infections (flower and roots) [96,102]. The root is also used in the treatment of mental illness, amnesia, tape warm, syphilis, gonorrhoea, relapsing fever, rheumatic pain and elephanthiasis, while the leaf is used in the treatment of wound, measles and infections with *Tinea decalvans* [93].

Investigation of the leaves of *V. sinaiticum* has afforded two flavonolignans, hydrocarpin and the novel sinaiticin, as well as two flavones, chrysoeriol and luteolin. All compounds exhibited dose dependent cytotoxicity against leukaemia cells [119]. A study conducted on the ethanolic extracts of *in vitro* cultures of twelve plant species including *V. sinaiticum* indicated that both plants exhibited broad-spectrum antibacterial activity [120].

1.5 Performance Evaluation of Topical Formulations

Topical preparations applied to the skin may be designed for surface, local or systemic effects. In order to formulate an effective and efficient topical preparation, consideration must be given to the intended purpose. This is directly concerned with the site of action and the desired effect of the preparation [121]. In some cases the base may be used alone for its therapeutic properties such as emollient, soothing, or protective action. Many topical preparations, however, contain a therapeutically active ingredient, which is dispersed or dissolved in the base [122]. Important considerations in selection of a base include the solubility of the active agent in the vehicle; the rate of release of the agent from the vehicle; the ability of the vehicle to hydrate the stratum corneum, thus enhancing penetration; the stability of the therapeutic agent in the vehicle; and interactions of the vehicle, stratum corneum and active agent [56].

Since a vehicle makeup the greatest portion of a topical formulation, it has a significant impact on the absorption and hence therapeutic effect of the active drug. Factors that determine the choice of vehicle and the transfer rate of a drug across the skin are the drug's hydrophobic/hydrophilic partition coefficient, molecular weight, and water solubility [54].

The release of a drug molecule from a vehicle into the skin and subsequent diffusion across the skin is therefore a complex process controlled by physicochemical factors sensitive to the molecular weight of the permeant, the vehicle and the skin. The drug delivery process is identified to be influenced by four categories of interactions: drug-vehicle, drug-skin, drug-vehicle-skin and vehicle skin interactions. Drug-vehicle interactions are those in which physicochemical interactions between the drug and the vehicle kinetically or thermodynamically govern the release of the drug into the skin. Such interactions can become the rate controlling factors and be clinically highly important when the stratum corneum is impaired as a consequence of disease or injury, in which case dissolution and diffusion in the vehicle may be slow relative to skin penetration. For the more usual case, in which the permeability of the intact stratum corneum is low, partitioning of the drug into the skin can still exert a profound, if not dominant, influence on the rate of delivery. Drug-skin interactions include alteration of the surface structure of the skin by the drug components of the formulations as well as binding of drugs to constituents of the skin as they diffuse through the tissue field. A vehicle skin interaction occurs when the vehicles main components effects a change in the physical state of the skin, in turn, affecting the skin's permeability [123].

The ultimate systems for establishing the therapeutic efficacy of topical formulations are the qualitative and quantitative clinical trials. Since these therapeutic appraisals are full of variables

and very costly to perform, they should be reserved only for careful evaluation of preselected candidates using double blind clinical comparison between new and marketed products. With the development of bioassays for topical antimicrobials, antimitotics, antiperspirants, sunscreens, antidandruff formulations, anaesthetics, antipruritics, antiwart formulations and corticosteroids, it is now possible to evaluate the inherent potencies of various topically active chemicals. It is also possible to evaluate the drug delivery characteristics of various vehicles [124].

The *in vitro* release test methods serve primarily as a quality control tool to ensure batch-to-batch uniformity and screen experimental formulations during product development. Neither universal release testing procedures nor universal test conditions exist. Rather, the release test must be tailored to a formulation i.e. suitable test conditions can usually be developed [125]. Although these *in vitro* methods are of limited predictive value, they are a means of assessing the ability of a vehicle or base to liberate medicament under the conditions of the test. Those reported are of comparative nature and most are empirical which prevent results from being compared with those from other techniques. Different authors hold that the study of ointment bases by *in vitro* methods is essentially a study of diffusion rates, neglecting the importance of the base as an emollient or protective. The methods seem to fall into two categories, diffusion methods that use a membrane and those that do not. In both these categories, chemical, physical and microbiological estimations have been devised [126].

Diffusion methods using membranes are conducted using natural or artificial membranes in an attempt to simulate *in vitro*, the barrier, which is presented by the skin, to a topical application. The assumption is that the process of penetration in the skin is similar to the quantitative diffusion through a membrane. This however does not make any allowance for differences in

physicochemical properties of dead membranes and living tissues, the latter presenting a much more complex system both physically and chemically [126]. Simulated skin membranes such as cellulose acetate, polydimethylsiloxane, egg shell and hydrogel membranes and natural skin membranes from a variety of animals, including rats, mices, rabbits, guinea pigs, pigs and monkeys have generally been used in diffusion cells [127-128].

According to suggestions of FDA, the membranes selected for use should be commercially available, have little capacity to bind a drug, have little tendency to interact with the releasing medium and offer the least diffusional resistance. Generally, in the release test a layer of the semisolid dosage form is placed in contact with the reservoir that is separated by a membrane and diffusion of the drug out of the semisolid and into the medium of the reservoir is followed [125]. In this system, the receiver phase is usually stirred and both compartments are kept at constant temperature [128]. This method has been used to study the release of drugs from different vehicles [129-130].

The other method that has closer resemblance to the above method which has been frequently used to assess the release of a drug from a vehicle involves the release of the drug from the vehicle directly into the stirred solvent. The receiver solvent is usually present in a volume that allows sink conditions to be approximated throughout the course of the experiment. In this design, the vehicle slab is in direct contact with the receptor phase and therefore, the solvent is chosen so that it does not dissolve the vehicle. This method has been used to study the release of topical steroids from various vehicles using isopropylmyristate as a receptor phase [133-134]. In the *in vitro* diffusion methods that do not use membranes, the assumption made is that the

distribution of medicament between the vehicle or base and the area under treatment will be similar to the distribution between vehicle and medium of the test [135].

Since many of the topical applications are often intended to be antiseptic, a number of methods and techniques have been devised which depend up on a form of microbiological assay. The tests aim at assessing the antiseptic value and also give an indication of the rate and degree of release of the medicament from the vehicle, but, of course different organisms and bases are used and comparisons should be made only when all the conditions of bioassay are met [126]. The first experiments that dealt with the release of drugs from topical formulations involved the measurement of the antiseptic properties of ointments containing various drugs. The experiments were carried out by applying the ointments to an agar media that had been inoculated with *Staphylococcus aureus*. After incubation, the agar plates are assessed to determine the presence of zone of inhibition. Thus, a crude measurement of the ointments ability to liberate a drug was achieved [123].

The criteria for drug release from a topical antimicrobial formulation differ from those for other topical products in that penetration through the stratum corneum is not necessarily the major consideration. Clinical indications for topically applied antimicrobials include infections of the skin surface, damaged skin, open wounds and burns and of the anterior nares. Hence measurement of drug release into an appropriate aqueous medium at physiological pH may be a more realistic *in vitro* method than release and penetration through a membrane system representing the stratum corneum. Agar diffusion presents itself as a model for comparison of clinical efficacy of antimicrobial products [135]. This method is widely used to study the

performance of different topical antimicrobial formulations [136-140]. Therefore, the same method was used in this study to evaluate the performance of topical formulations of the crude extracts of *L. adoensis* and *O. rochetiana* in different vehicles.

1.6 Objectives

1.6.1 General objective

To verify the claims of selected medicinal plants traditionally used for the treatment of skin disorders and recommend the most suitable topical formulation for the herbal drug(s) with promising biological activities.

1.6.2 Specific Objectives

1. To investigate the antimicrobial activities of some selected topically used traditional herbal drugs,
2. To study the anti-inflammatory activities of the plant(s) selected for further studies on the basis of their antimicrobial activities,
3. To conduct phytochemical screening on the most active plant(s),
4. To develop suitable topical formulation for the active plant(s),
5. To evaluate the performance of the developed formulation using appropriate *in vitro* method, and
6. To perform preliminary semi-quantitative standardization for the most active species(s).

2. EXPERIMENTAL

2.1 Materials and Methods

2.1.1 Materials

Chemicals and Solvents

Methanol (LOBA CHMIE Pvt. Ltd., India), Ethanol absolute (Avondale Laboratories Ltd., England), Acetone (Fisher Scientific International Company, United Kingdom), Chloroform (E. Merk, Stockholm), Ferric chloride (Hayashi Pure Chemical Industries Ltd., Japan), Potassium Iodide (Hayashi Pure Chemical Industries Ltd., Japan), Formic acid (LAB PAK Ltd., United Kingdom), Petroleum ether (80 ^{0}C to 100^{0}C), Ethyl acetate, Dichloromethane, Sulphuric acid, Hydrochloric acid, Acetic acid glacial, Ammonia Solution, Acetic acid, Diethyl ether, Butan-1-ol, Calcium hydroxide, Potassium hydroxide, Vanillin, Toluene, Benzene, Lead acetate, Bismuth nitrate, Mercuric Chloride, Potassium Ferrocyanide, Glyceryl monostearate, Cetostearyl alcohol, Cetomacrogol 1000 BPC, Polyethylene glycol 4000 (all from BDH Chemicals Ltd., England), Sodium lauryl sulphate (AVONCHEM Ltd., UK), Liquid Paraffin BP (Germany), Petrolatum white, USP (Ethiopian Pharmaceutical Manufacturing, Addis Ababa, Batch No. 101087-1), Polyethylene glycol 400 (Sigma-Aldrich Chemie GMbH, Germany), were all used as received. Carrageenan Lambda (SIGMA CHEMICAL CO. USA) was obtained from Ethiopian Health and Nutrition Research Institute (EHNRI). Reference drugs, ketoconazole, gentamycin sulphate and Indomethacin (all Working Standards) were kindly supplied by Department of Quality Control and Toxicology, DACA.

Medias

Nutrient agar (DEFCO Labodratories, USA), Peptone bacteriological (BDH Chemicals Ltd., England), Tryptone Soya Broth and Sabauraud dextrose agar (both from OXOID Ltd., England),

Yeast Extract (UNIPATH Ltd., England) and Sodium Chloride (East Anglia Chemicals, United Kingdom) were obtained from Ethiopian Health and Nutrition Research Institute (EHNRI).

Test Strains and Animals

Staphylococcus aureus (ATCC 6538), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Aspergillus niger* (ATCC 10535), *Trichophyton mentagrophytes* (ATCC 18748) all American Type Culture Collections and *Candida albicans* (clinical isolate) were obtained from Ethiopian Health and Nutrition Research Institute (EHNRI). Departments of Pharmacology at School of Pharmacy and Faculty of Medicine kindly supplied albino rats of wistar strain. The animals were housed in cages in the animal house at the School of Pharmacy with 12 hours light-dark cycle.

Topical Antiseptic Products

The topical antibacterial and antifungal products namely Faban ointment (2% Sodium fusidate, HOE Pharmaceuticals, Lot No. 01021015, Malaysia), Bactroban ointment (2%mupirocin, SmithKline Beecham Pharmaceuticals, Batch No 2352/040818, England), Tetracycline hydrochloride ointment, USP (3% tetracycline, Ambalai Sarabhai Enterprise Ltd., Batch No 02JUL1, India), Faban cream (2% fusidic acid, HOE Pharmaceuticals, Lot No 649A0005, Malaisia), Sagestan topical cream (0.1% gentamycin PT SANBE FARMA, Batch No BA355, Indonesia), Dazor cream (2% ketoconazole, HOE Pharmaceuticals, Lot No. 51282015, Malaysia), Ketoral cream (2% ketoconazole, BİLİM PHARMACEUTICALS, Lot No. 0202003, Turkey), Kenazol cream (2% ketoconazole, DOMINA PHARMACEUTICALS, Lot. No. 83, Syria), Fungoral cream (2% ketoconazole, İLAN İLTAS FabricasI, Batch No. 9011610, Turkey),

Clotri-Denk cream (1% clotrimazole, Denk Pharma, Batch/Lot. No. 11880, Germany), Sha Hsein Ghin cream (1% clotrimazole, SHANGHAI MEDICINES & HEALTH PRODUCTS Import and Export Corporation, Batch No. JW02C01, China), Nizoral cream (2% ketoconazole, JANSSEN Pharmaceutical Ltd., Lot No. 430BA, South Africa), Canesten cream (1% clotrimazole, Bayer, Batch No. BXB55S1, Germany) were all purchased from local markets in Addis Ababa.

2.1.2 Methods

2.1.2.1 Collection and Preparation of the Plant Material

The leaves of *Lippia adoensis*, *Verbascum sinaiticum*, and *Olinia rochetiana*, the fruits of *Phytolacca dodecandra* and the roots of *Malva parviflora* were collected from Northern Shoa, Lallo Mama Woreda (260 km North of Addis Ababa) in November 2002. The leaves of *Kalanchoe petitiana* were collected from Addis Ababa, Entoto area in October 2002 and the leaves of *Calpurnia aurea* and *Acokanthera schimperi* were collected from around Debrezeit (50 km South of Addis Ababa) in December 2002. All parts of the plant materials were dried in an open air protected from direct exposure to sunlight. The dried plant materials were separately powdered to suitable size and made ready for extraction. The identities of each plant specimen were confirmed at the National Herbarium, Department of Biology, Faculty of Science, Addis Ababa University. A voucher specimen of each plant was deposited at the National herbarium with voucher numbers HT01 (*Verbascum sinaiticum*), HT02 (*Kalanchoe petitiana*), HT03 (*Lippia adoensis*), HT04 (*Calpurnia aurea*), HT05 (*Acokanthera schimperi*), HT06 (*Malva parviflora*), HT07 (*Phytolacca dodecandra*) and HT08 (*Olinia rochetiana*).

1.1.1.1 Extraction

1.1.1.1.1 Preparation of Crude Extracts

A known weight of each powdered plant material was extracted with 80% methanol using maceration method of extraction. Maceration was continued for 48 hours with frequent agitation and the resulting liquid is filtered using filter paper (Whatman No 3, Whatman Ltd., England). Extraction was repeated five times and the filtrates of all portions were combined in one vessel. The organic solvent was removed by evaporation using rota vapor (BÜCHI Rota-vapor R-205, Switzerland) at not more than 40 ^{0}C. The aqueous residue was then placed in an oven at 40 ^{0}C for about 48 hours to remove the water. The resulting dried mass was then powdered, packed into a glass vial and stored in a desiccator over silica gel until use.

1.1.1.1.2 Preparation of the Fractional Extracts

L. adoensis (100 g) and *O. rochetiana* (100 g) were sequentially extracted with petroleum ether, chloroform, acetone and methanol by the use of soxhelt extraction technique. Each solvent was removed by evaporation using a rota vapour and the fractions were then placed in an oven at not more than 40 ^{0}C for about 24 hours to remove any residual solvent. The resulting semisolid mass of each fractional extracts was stored in a desiccator until use in the same way as the crude extract.

1.1.1.2 Antimicrobial Screening of the Crude Extracts and Fractions

The antibacterial and antifungal activities of the hydro-alcoholic extracts of the 8 plant species were determined using agar well diffusion method which is commonly used for screening of the antimicrobial activities of herbal drugs [141-144]. The two plants *L. adoensis* and *O. rochetiana*, were fractionated into different solvents and the resulting fractions were also tested using the

same procedure. The initial antimicrobial screening test for the crude extracts was conducted at three concentration levels (100, 50 and 25 mg/ml). The test was carried out by dissolving petroleum ether and chloroform fractions in chloroform and acetone and methanol fractions in methanol. The fractions were tested at two concentration levels (25 and 5 mg/ml) in parallel with the same concentration of the crude extracts dissolved in 80% methanol for comparison. 80% Methanol, methanol and chloroform were used as negative controls during the whole test on bacteria and fungi. All tests were performed in triplicate and results were reported as averages of the triplicates. The diameter of zone of inhibitions reported in all cases includes the diameter of the wells. The procedures followed for the antibacterial and antifungal tests are described as follows:

1.1.1.2.1 Screening for Antibacterial Activity

In this study, one gram-positive (*S. aureus*) and two gram-negative (*E. coli* and *P. aeruginosa*) standard bacterial strains of human pathogens were used. All bacterial cultures were first grown on 5% sheep red blood agar plates at 37 ^{0}C for 18 to 24 hours prior to inoculation onto the nutrient agar. Few colonies (4 to 5) of similar morphology of the respective bacteria were transferred with a sterile inoculating loop to a liquid medium (TSY broth) and this liquid culture was then incubated until adequate growth of turbidity equivalent to McFarland 0.5 turbidity standard was obtained. The inocula of the respective bacteria were streaked on to the nutrient agar plates using a sterile swab in such a way as to ensure thorough coverage of the plates and a uniform thick lawn of growth following incubation. Wells of 11 mm in diameter were formed on to nutrient agar plates using a sterile cork borer. The wells were filled with the test agents (100 µl each) and the plates were then allowed to stay for 1 to 2 hours at room temperature. Finally, the

plates were incubated at 37 ^{0}C (Heraeus GmbH, D-6450, Germany) for 18 to 24 hours. The resulting diameters of zones of inhibition were measured and results were recorded in mm. Gentamycin was used as a positive control at a concentration of 0.1 mg/ml.

1.1.1.2.2 Screening for Antifungal Activity

The fungal strains used in this study were *C. albicans*, *T. mentagrophytes* and *A. niger*. The required amounts of each fungal strain were removed from the stock and suspended in 2 ml of Sabauraud dextrose broth. This suspension was uniformly spread on petriplates containing Sabouraud dextrose agar media using sterile swabs. After applying the samples into the wells formed by using the same technique for tests on bacteria, the plates were incubated at 25 ^{0}C for three days in case of *C. albicans* and *A. niger* and seven days in case of *T. mentagrophytes*. The plates were then examined for the presence of zones of inhibition and the results were recorded in mm. Ketoconazole was used as a positive control at a concentration of 0.3 mg/ml.

2.1.2.3.3 Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration of the crude extracts of *L. adoensis* and *O. rochetiana* were determined by agar dilution method [135,145-147]. The growth media, nutrient agar (for bacteria) and sabauraud dextrose agar (for fungi) were first prepared in a usual fashion and sterilized by autoclaving (Webco GmbH & Co. KG Bad Schwartau, Germany). The sterilized media was allowed to cool to 50 ^{0}C and 18 ml of the molten agar was added to test tubes which contained 2 ml of different concentration of the test drugs (crude extract) and the control (80% methanol). The mixture of the media and the test drugs were thoroughly mixed and poured into pre-labeled sterile petridishes on a level surface. Additional petridishes containing only the growth media were prepared in the same way so as to serve for comparison of growth of the

respective organisms. The concentrations of the extracts used in this test ranged from 20 mg/ml to 0.156 mg/ml. The plates were then set at room temperature and dried. The suspensions of the respective microorganisms having density adjusted to 0.5 McFarland turbidity standard were inoculated onto the series of agar plates using standard loop. Three loopful of the suspension were transferred into each plate. The plates were then incubated at 37 ^{0}C for 24 hours in case of bacteria and 25 ^{0}C for three to seven days in cases of fungi. The lowest concentration which inhibited the growth of the respective organisms was taken as MIC. All tests were carried out in triplicate.

2.1.2.4 Screening for Anti-inflammatory Activity

The anti-inflammatory property of crude extracts of *L. adoensis* and *O. rochetiana* were investigated using carrageenin induced rat paw edema test method, which is widely used [148-155] for evaluating the edema inhibition properties of plant extracts and drugs. Because of limitation of resources, this test was conducted in two phases i.e. first phase (test for *L. adoensis*) and second phase (test for *O. rochetiana*). In both cases male and female albino rats, weighing 90 to 130 g (in the first phase) and 115 to 175 g (in the second phase) were divided into groups of 5 per dose. The test group received 750 mg/kg and 100 mg/kg of the crude extract dissolved in distilled water through the oral route. The control groups were given distilled water through the same route. Acute inflammation or edema was induced by the injection of carrageenin (0.1 ml of 1% solution in saline) into the plantar surface of the right hind paw of the rats one hour after the administration of the test drugs. The volume of the paw was then measured by volume displacement method using plethysmometer (UGO BASILE Biological Research Apparatus, Italy) before and one, two, three and four hours after carrageenin injection. Edema was expressed

as a mean increase in paw volume with respect to the control group and inhibition as a percentage decrease in edema volume.

1.1.1.1 Phytochemical Screening

Extracts of the two plant species namely *L. adoensis* and *O. rochetiana*, which were found to have comparatively better antimicrobial activity, were subjected to phytochemical screening using standard screening procedures [156-160].

Test for Alkaloids [156-157]

a. About 0.5 g of the crude extract was stirred with 5 ml of 1% HCl on a steam bath. 1 ml of the filtrate was treated with a few drops of Mayer's reagent and another ml was similarly treated with Dragendorff's reagent. Turbidity or precipitation with both reagents was taken as preliminary evidence for the presence of alkaloids.
b. As a confirmatory evidence of the presence of alkaloids, 1 g of the extract was treated with a 40% $Ca(OH)_2$ solution until the extract was distinctly alkaline to litmus paper. The mixture was then shaken with 2 portions of 10 ml chloroform. The chloroform portions were combined and reduced to a small volume and then chromatographed on silicagel G (Pre-coated TLC plates, Silicagel, 60 F_{254}, MERK, Germany) using the following solvent systems:
    i. Ethyl acetate – methanol – water (150:26:19)
    ii. Chloroform – ethanol (9:1)
    iii. Ethanol – ammonia (9:1)

    The chromatograms were sprayed with freshly prepared Dragendorff's reagent and the presence of brown, orange brown colored spots was considered as positive test for alkaloids.

Test for Saponins [157-160]

c. **Frothing Test:** About 0.5 g of each crude plant extract was shaken with water in a test tube. Frothing which persists on warming was taken as preliminary evidence for the presence of saponins.

d. **Blood Haemolysis Test:** About 0.5 g of each crude plant extract was dissolved in 5 ml of distilled water and 100 µl of each was applied on 5% blood agar plates and this was left to stand for 6 hours. Complete haemolysis of the blood around the extract was taken as an indication for the presence of saponins.

e. **Chromatography:** 1 g of the powdered drug was extracted by heating on a water bath for 10 min with 5 ml of methanol and filtered. The filtrate was evaporated to about 1 ml, mixed with 0.5 ml of water and then extracted with 3 ml of n-butanol (saturated with water) and the butanol phase was used for TLC investigation. The chromatogram was developed on silicagel G using Chloroform – glacial acetic acid – methanol – water (64:32:12:8) as a mobile phase and vanillin-sulphuric acid as a spraying reagent for detection. Formation of a blue, blue violet, red or yellow brown zone is considered as positive test for sap onins.

Test for Tannins [157-159]

About 0.5 g of the crude extract was stirred with 10 ml of distilled water and filtered. The addition of $FeCl_3$ reagent to the filtrate resulting in blue, blue-black, green or blue-green coloration or precipitation was taken as evidence for the presence of tannins.

Test for Anthraquinones [157-160]

a. **Free Anthraquinones:** A sample (5 g) of each plant extract was shaken with 10 ml of benzene and filtered. A 10% ammonium hydroxide solution (5 ml) was added to the filtrate, and the mixture was shaken. The presence of a pink, red or violet color in the ammoniacal phase was taken as an indication of the presence of anthraquinones.
b. **Combined Anthraquinones:** A sample (5 g) of plant extract was boiled with 10 ml of 1% HCl and filtered while hot. The filtrate was shaken with 5 ml of benzene. The benzene layer was removed and to it was added 10% ammonium hydroxide (equal to half the volume of benzene). A pink, red or violet color in the ammonia phase indicated the presence of anthraquinone derivatives.
c. **Chromatography:** 1 g of the powdered drug was extracted by heating on a water bath for 10 minutes with 5 ml of methanol and the filtrate was used for TLC investigation. The chromatogram was developed on silicagel G using ethyl acetate – methanol – water (100:13.5:10) as a mobile phase. The chromatogram was then sprayed with Potassium hydroxide reagent (10%) for detection. The formation of yellow and red spots was taken as a positive test for anthrones and anthraquinones, respectively.

Test for Polyphenols (Phenolic Compounds) [157-158]

To 2 ml of the aqueous solution of the crude extract, 3 drops of a mixture of 1 ml 1% $FeCl_3$ and 1 ml 1% $K_3Fe(CN)_6$ were added. Formation of green blue color was taken as an indication of the presence of polyphenols.

Test for Flavonoids [156-160]

a. To 2 ml of the alcoholic solution of the crude extract 4 drops of 2% lead acetate solution were added. The development of yellow or orange color was taken as an indication of the presence of flavonoids.
b. 1 g of the powdered drug was extracted with 10 ml of methanol by heating on a water bath for 10 minutes and the filtrate was used for TLC investigation. The chromatogram was developed on silicagel G using ethyl acetate – formic acid – glacial acetic acid – water (100:11:11:26) as solvent system.

Test for Coumarins [160]

1 g of the powdered drug was extracted with 10 ml of dichloromethane by heating under reflux for 15 minutes. The filtrate was evaporated to dryness and the residue dissolved in 0.5 ml of toluene was used for TLC investigation. Diethyl ether – toluene (1:1; saturated with 10% acetic acid) was used as a mobile phase and ethanolic 10% potassium hydroxide as a spraying reagent for detection. Appearance of blue or brown fluorescence before or after spraying the reagent was taken as an indication of the presence of coumarins.

1.1.1.2 Formulation of the Herbal Drugs and *In Vitro* Evaluation of Their Performance

Topical formulations of the crude extracts of the two herbal drugs (*L. adoensis* and *O. rochetiana*) were prepared at strength of 10% by using five different formulation bases (Table 2.1).

Table 2.1: List of Formulation Bases and their Compositions Used as Vehicles for the Preparation of Topical Formulations of *L. adoensis* and *O. rochetiana*.

Code No	**Name of the base**	**Components of the base**	**Proportions (in percent)**
Base 1	Sodium Laurate Monostearin Cream Base	Glyceryl monostearate	5
		Sodium lauryl sulfate	3
		Cetostearyl alcohol	2
	Liquid paraffin	25	
		Water to	100
Base 2	Macrogol Cream Base	Cetomacrogol emulsifying wax BP	9
		Liquid paraffin	6
		White petrolatum	15
		Water	70
Base 3	Gibson Ointment Base	Sodium lauryl sulfate	1
		Cetyl alcohol	16
		White soft paraffin	40
		Water	43
Base 4	PEG Ointment Base	PEG 4000	40
		PEG 400	60
Base 5	White Petrolatum Ointment Base	White petrolatum	100

1.1.1.2.1 Preparation of Bases

The formulation bases were prepared manually by melting the fatty phases at 70 ^{0}C in a water bath and stirring continuously to room temperature in the case of anhydrous bases. In preparing hydrous vehicles, the aqueous phase was also heated to 70 ^{0}C and added to the melted fatty phase and stirred continuously to room temperature. PEG ointment was prepared by heating the two ingredients on a water bath to about 65 ^{0}C and then allowing the mixture to cool with continuous stirring until congealed. These bases were then used immediately for the preparation of topical products of the herbal drugs.

1.1.1.2.2 Preparation of the Topical Formulations

The crude extracts of the two herbal drugs (*L. adoensis* and *O. rochetiana*) were separately incorporated into the already prepared vehicles (formulation bases) while cold by levigation method. The crude drug was finely powdered in a mortar and then mixed with an equal amount of the base and levigated on an ointment slab until a smooth greet-free mixture was obtained. The rest of the base was then added in gradual increments. The strength of the final product was made to be 10% by incorporating 2 g of the powdered crude extract in 18 g of the respective bases to get 20 g of final preparation. The resulting preparation was finally packed into an ointment jar and stored at room temperature until it was repacked into a syringe for performance evaluation study.

2.1.2.6.3 Performance Evaluation of the Topical Formulations

The performances of topical formulations of the crude extracts of the two herbal drugs were evaluated using the agar well diffusion technique [86,161-162]. Sterilized and cooled nutrient agar (40 ml) were added to a sterile petridish and allowed to solidify. Equidistant holes were

formed on to the agar plates by the use of a sterile cork borer. Following removal of the agar plugs, the designated topical agents were added to each well until full (approximately equal to 0.1 ml or ≈ 0.2 g) by means of a 5 ml syringe. A volume of 20 ml of nutrient agar (which was previously melted and maintained at 50 ^{0}C) were inoculated with 0.5 ml of a microbial suspension equivalent to 0.5 McFarland standard. The broth suspension of the organisms was thoroughly mixed in an agitator and poured onto the previously prepared test plates containing the antimicrobial topical formulations. After solidification of the agar, the plates were inverted and incubated at 37 ^{0}C for 18 to 24 hours. The magnitude of susceptibility of the respective organisms was determined by measuring the diameter of the zones of inhibition in mm (including the diameter of the wells) around each well containing the topical antimicrobial agents.

The performance evaluation of the formulations against fungi was carried out in the same way mentioned above using Sabauraud dextrose agar as a growth media. The plates were incubated at 25 ^{0}C for 3 to 7 days and the resulting zones of inhibition were recorded accordingly. In addition to the stated herbal formulations, the formulation bases (vehicles) and different commercial topical antiseptics available in the market with different brands (Table 2.2) including gentamycin and ketoconazole were tested in the same manner as above serving as negative and positive controls, respectively.

Table 2.2: List of Topical Antibacterial and Antifungal Products Used as Positive Controls During the Performance Evaluation of Topical Formulations.

Code No	Product Name	Source Country	Antimicrobial Substance	Concen-tration	Bases
	Antibacterial Products				
1	Faban	Malaysia	Sodium fusidate	2%	Ointment
2	Bactroban	England	Mupirocin	2%	Ointment
3	Tetracycline Hydrochloride	India	Tetracycline	3%	Ointment
4	Faban	Malaysia	Fusidic acid	2%	Cream
5	Sagestan	Indonesia	Gentamycin	0.1%	Cream
	Antifungal Pruducts				
1	Dazor	Malaysia	Ketoconazole	2%	Cream
2	Ketoral	Turkey	Ketoconazole	2%	Cream
3	Kenazol	Syria	Ketoconazole	2%	Cream
4	Fungoral	Turkey	Ketoconazole	2%	Cream
5	Clotri-Denk	Germany	Clotrimazole	1%	Cream
6	Sha Hsein Ghin	China	Clotrimazole	1%	Cream
7	Nizoral	South Africa	Ketoconazole	2%	Cream
8	Canesten	Germany	Clotrimazole	1%	Cream

1.1.1.3 Preliminary Standardization

The determination of all standardization parameters was carried out using standard procedures recommended by WHO, Pharmacopoeia and/or other texts [145,163-165].

1.1.1.3.1 Determination of Ash Values

a) **Total Ash:** About 3 g of the dried and powdered leaves of *L. adoensis* and *O. rochetiana* were accurately weighed in a previously ignited and tarred crucible. The powder was spread in an even layer and was ignited at a temperature of 550 ^{0}C in a furnace (Nuber Industrieofunbau, D-2804 Lilienthal/ Bermen, West Germany) until it was white. The crucible was then allowed to cool in a desiccator and weighed. The content of total ash was finally calculated in terms of percentage with reference to the air-dried powdered drug.

b) **Acid – Insoluble Ash:** To the crucible containing the total ash, 25 ml of HCl (70 g/l) TS was added and it was covered with a watch glass and boiled gently for 5 min. The watch glass was rinsed with 5 ml of hot water and the liquid added to the crucible. The insoluble matter was collected on an ashless filter paper (Schleicher & Schuell, filter paper circles, ϕ 185 mm, D-3354 Dassel, W-Germany) and washed with hot water until the filtrate is neutral. The filter paper containing the insoluble matter was transferred to the original crucible, dried on a hot plate and ignited to constant weight. The residue was then allowed to cool in a desiccator for 30 min and weighed. The percentage of acid-insoluble ash was finally calculated with reference to the air-dried drug.

c) **Water – Soluble Ash:** Distilled water (25 ml) was added to the crucible containing the total ash and boiled for 5 min. The insoluble matter was collected in an ashless filter paper and washed with hot water. It was then ignited in a crucible for 15 min at a temperature less than 450 ^{0}C. The content of water-soluble ash was calculated by subtracting the weight of this residue from the weight of total ash and it was recorded in percentage with respect to the air-dried material.

2.1.2.7.2 Determination of Extractable Matter

The extractable matter of the two species of plants *L adoensis* and *O. rochetiana* was determined in different solvents by using a cold maceration method [163,164]. The solvents used in this determination were: petroleum ether, chloroform, acetone, ethanol, methanol, 80% methanol and water. The air-dried and powdered plant material (3 g) was accurately weighed in a stoppered conical flask and macerated with 100 ml of the solvent for six hours, with frequent shaking. This was allowed to stand for 18 hours and filtered rapidly by taking care so that no solvent is lost. The filtrate (25 ml) was transferred to a tarred flat-bottomed dish and evaporated to dryness on a water bath. Then it was dried at 105 ^{0}C to constant weight and the content of extractable matter was calculated as percentage with reference to the air-dried material.

2.1.2.7.3 Determination of Moisture Content

The water content of the two herbal drugs was determined gravimetrically by calculating the loss in weight after drying. The accurately weighed air-dried material (3 g) was placed in previously dried and tarred flat-bottomed dish. The sample was dried at 105 ^{0}C to constant weight. Then the percentage loss in weight was calculated with reference to the air-dried material.

2.1.2.7.4 Thin-Layer Chromatography Finger Print Analysis

Preparation of samples: Prior to testing, the crude extract and various fractions (i.e. petroleum ether, chloroform, acetone and methanol) of *L. adoensis* and *O. rochetiana* were prepared at a concentration of 0.5 % by dissolving the crude extracts in 80% methanol, the petroleum ether and chloroform extracts in chloroform and the methanol and acetone fractions in methanol.

Saturation of the chromatographic chamber: To achieve saturation, the inside wall of the TLC chamber was lined with filter paper and 100 ml of the mobile phase was poured into it. The chamber was then closed and allowed to equilibrate for one hour at room temperature.

Application of the test solutions: Using a 2 µl micropipette, the test solutions (plant extracts) were placed onto the base line in the form a horizontal band having a width of about 15 mm, which was parallel to and about 2 cm above the lower edge of the plate. A total volume 8 µl of each solution was applied in portions, drying between each application.

Development of chromatograms: The applied bands of the test solutions were allowed to dry and the plates were placed vertically into the chamber, ensuring that the points of application are above the surface of the mobile phase. The chamber was closed and the chromatogram was allowed to develop at room temperature. The mobile phase was allowed to ascend 15 cm from the starting line and the plates were then removed and dried.

Observation and interpretation of the chromatograms: The developed bands were observed in daylight, then under short wave (254 nm) and long-wave (366 nm) ultraviolet light. The

position of each band was marked and the distance from the center of each band to the point of application was measured and recorded. Finally, the bands were sprayed with vanillin sulfuric acid spraying reagent, and the resulting bands were recorded in the same way as above.

3. RESULTS AND DISCUSSION

3.1 Yields of the Crude (80% Methanol) Extracts and Different Fractions

Different parts of eight species of plants belonging to eight different families were extracted with 80% methanol using maceration technique and these extracts were used for the initial antimicrobial screening test. The percentage yields of these crude extracts are given in Table 3.1. Maximum and minimum yields were obtained from the fruits of *P. dodecandra* and roots of *M. parviflora*, respectively. From among the leaves, *A. schimperi* afforded maximum yield (35.3%) followed by *O. rochetiana* (32.6%) and *C. aurea* (29.5%), while minimum yield was obtained from *K. petitiana* (16.4%), which is almost comparable to the yield obtained from the roots of *M. parviflora* (15.2%). The fresh leaves of this plant have very high water content and shrinks extremely to a light weight dried mass with a partial loss of its green color. In general, the yields obtained from these plants are quite adequate thereby making further development of these herbal drugs economically feasible.

Table 3.1: Percentage Yields of the 80% Methanol Extracts of the Dried and Powdered Plant Materials (n = 3).

Plant Species	**Part Extracted**	**Percentage Yield (w/w) (Average $\pm$ SD)**
A. schimperi	Leaf	35.3 $\pm$ 3.1
C. aurea	Leaf	29.5 $\pm$ 2.4
K. petitiana	Leaf	16.4 $\pm$ 1.2
L. adoensis	Leaf	20.7 $\pm$ 0.8
M. parviflora	Root	15.2 $\pm$ 1.5
O. rochetiana	Leaf	32.6 $\pm$ 1.6
P. dodecandra	Fruit	39.5 $\pm$ 2.8
V. sinaiticum	Leaf	24.8 $\pm$ 2.4

3.2 Antimicrobial Activities

3.2.1 Antimicrobial Activities of the Crude Extracts

The hydroalcoholic extracts of the eight species of plants were screened for biological activity against different strains of bacteria and fungi. All species of plants included in this study were selected on the basis of data obtained from the literatures and reports of their local traditional uses for the treatment of various skin disorders. The antimicrobial activity-screening tests were carried out on organisms that are known to be among the most common causative agents of both primary and secondary infectious skin disorders.

The results of the initial antimicrobial screening assay of the crude extracts of all species of plants on the selected microbial strains are shown in Table 3.2. As can be seen from the results, *L. adoensis* is the most active species against bacteria and *O. rochetiana* against fungi. In addition, *L. adoensis* and *O. rochetiana* have shown some degree of activity against fungi and bacteria, respectively. All species of plants included in the present study were also found to be active on at least one of the selected microbial strains.

When the antimicrobial activities of these herbal drugs were compared to that of the positive controls, many of them (e.g. *C. aurea*, *K. petitiana*, *L. adoensis* and *O. rochetiana* at a concentration of 100 mg/ml) were found to have almost comparable activity to the standard gentamycin against bacteria. Similarly, *O. rochetiana* at 100 mg/ml showed comparable activity against the fungi *T. menthagrophytes* to that of ketoconazole. In some cases, antimicrobial activities even greater than the positive controls was observed, for example, *L. adoensis* against *S. aureus* and *O. rochetiana* against *T. menthagrophytes*. The concentrations of the positive controls were set based on information obtained from the literature and the amount loaded on

Table 3.2: Antimicrobial Activities of the Crude Extracts against Different Strains of Bacteria and Fungi.

Test Samples	Conc. (Mg/ml)	Zone of Inhibition (mm)					
		Bacterial Strains			Fungal Strains		
		Sa	Ec	Pa	Ca	Tm	An
A. schimperi	100	24±0.5	-	17±0.0	-	22±0.5	-
	50	20±0.3	-	16±0.6	-	17±0.6	-
	25	18±1.3	-	14±.2	-	15±0.8	-
C. aurea	100	21±0.8	21±1.0	18±0.3	-	-	-
	50	21±1.3	19±0.3	18±0.6	-	-	-
	25	19±1.0	15±0.3	17±0.5	-	-	-
K. petitiana	100	26±2.8	14+0.8	21±0.6	-	-	-
	50	20±0.8	13+0.6	20±1.3	-	-	-
	25	19±1.0	-	19±0.5	-	-	-
L. adoensis	100	31±0.5	16±0.3	24±1.3	-	22±0.8	-
	50	27±0.8	14±0.9	22±0.3	-	16±0.3	-
	25	20±2.4	14±0.4	20±1.5	-	15±0.4	-
M. parviflora	100	20±0.0	-	16±0.3	-	28±0.8	-
	50	17±0.9	-	-	-	19±1.0	-
	25	15±0.0	-	-	-	16±0.8	-
O. rochetiana	100	25±0.0	19±0.8	22±1.0	18±1.5	54±4.0	-
	50	20±0.3	15±1.0	19±0.8	15±1.0	31±1.5	-
	25	19±0.9	13±0.8	17±0.9	14±0.7	18±1.2	-
P. dodecandra	100	-	-	16±0.9	-	-	-
	50	-	-	15±0.5	-	-	-
	25	-	-	14±0.3	-	-	-
V. sinaiticum	100	25±0.6	-	20±0.5	-	-	-
	50	21±1.0	-	18±1.0	-	-	-
	25	19±0.6	-	16±0.3	-	-	-
Gentamycin*	0.1	29±0.0	22±1.3	21±0.5	NT	NT	NT
Ketoconazole*	0.3	NT	NT	NT	37±1.6	23±2.4	-

Sa = *S. aureus*, Ec = *E. coli*, Pa = *P. aeruginosa*, Ca = *C. albicans*, Tm = *T. mentagrophytes*, An = *A. niger*, NT = not tested, - = no activity, * = positive controls.

standard antimicrobial sensitivity testing discs. The negative controls 80% methanol and distilled water were found to be devoid of any antimicrobial activity.

The antimicrobial activity profile of all species of plants (except *P. dodecandra*) against the tested strains indicated that *S. aureus* was the most susceptible bacterium of all the bacterial test strains. Similarly, *T. menthagrophytes* was found to be the most sensitive fungus of all the tested fungal strains although some species such as *C. aurea, K. petitiana, P. dodecandra* and *V. sinaiticum* were found to be inactive against it. From all the fungal strains included in the test, *A. niger* was found to be virtually insensitive to all plant extracts and *C. albicans*, which is an isolate, was found to be the least inhibited fungus.

On the other hand, *E. coli* was found to be the most insensitive strain of all bacteria. In fact, gram-negative bacteria are frequently reported to have developed multi drug resistance to many of the antibiotics currently available in the market of which *E. coli* is the most prominent [166-167]. Therefore, it is not surprising to learn that *E. coli* is the least responding bacterial strain to the tested plant extracts. However, some species of plants are still of special interest for further studies in this regard as in the case of *C. aurea*, which showed exceptionally stronger activity against *E. coli* than other plant extracts yet having poor activity on gram positive bacteria, a trend not observed for other species of plants.

In general, among the tested microbial strains, bacteria were found to be more sensitive to many of the test agents than fungi. The antibacterial activity was more pronounced on the gram-positive bacteria (*S. aureus*) than the gram-negative bacteria (*E. coli* and *P. aeruginosa*). The reason for the difference in sensitivity between gram-positive and gram-negative bacteria might be ascribed

to the differences in morphological constitutions between these microorganisms, gram-negative bacteria having an outer phospholipidic membrane carrying the structural lipopolysaccharide components. This makes the cell wall impermeable to antimicrobial chemical substances. The gram-positive bacteria on the other hand are more susceptible having only an outer peptidoglycan layer which is not an effective permeability barrier. Therefore, the cell walls of gram negative organisms are more complex in lay out than the gram positive ones acting as a diffusional barrier and making them less susceptible to the antimicrobial agents than are gram positive bacteria [168-169]. In spite of this permeability differences, however, some of the extracts have still exerted some degree of inhibition against gram-negative organisms as well.

Several reports [44-47] have indicated that infectious skin disorders are very common in Ethiopia. Among the pathogens most commonly known to cause infectious disorders of the skin is *S. aureus* [170-171]. Thus, the fact that all species of the tested plants except *P. dodecandra* showed activity against *S. aureus* might justify the extensive use of these agents for the treatment of skin disorders.

In all species of plants tested for antimicrobial activity, the zone of inhibitions increased with an increase in concentration i.e. stronger activity was observed at 100 mg/ml than lower concentrations. In some cases, activity was observed only at higher concentrations (100 and 50 mg/ml). For example, *Malva parviflora* is active against *P. aeuginosa* only at 100 mg/ml. As these herbs are widely used for treating topical skin disorders, it would be necessary to test them at higher concentrations before concluding that their use is unjustified. In addition, since the extracts are crude, the active component might be present in a very low concentration as to inhibit the growth of microbes at high dilutions, but it might still be of paramount importance in the

development of new drug either by serving as a drug in its own right or as a lead for the synthesis of more active compounds.

Although the objective of the initial antimicrobial screening was to choose one out of the eight species that could be used for further studies, two species of plants were selected for this purpose, because one was found to be more active against bacteria (*L. adoensis*) and the other against fungi (*O. rochetiana*). Therefore, *L. adoensis* and *O. rochetiana* were selected for further investigations i.e. fractionation, anti-inflammatory activity tests, phytochemical screening, formulation studies and preliminary standardization.

3.2.2 Antimicrobial Activities of the Fractions

Based on the results of the initial preliminary antimicrobial screening tests, *L. adoensis* and *O. rochetiana*, the crude extracts of which showed better activity against the selected strains of bacteria and fungi, were further fractionated into different solvents using soxhelt method of extraction. The percentage yields obtained from successive extraction of these plants (Table 3.3) indicated that increasing polarity of the extracting solvent increases the yield except chloroform which afforded quite a low yield compared to petroleum ether. As a result, methanol, which is the most polar of all solvents used for fractionation, afforded the maximum yield.

Table 3.3: Percentage Yields of Different Fractions of *L. adoensis* and *O. rochetiana*.

Plant Species	**Part Extracted**	**Solvent**	**Percentage Yield (w/w)**
L. adoensis	Leaf	Pet. ether	2.76
		Chloroform	1.62
		Acetone	7.29
		Methanol	13.3
O. rochetiana	Leaf	Pet. ether	6.15
		Chloroform	2.63
		Acetone	15.12
		Methanol	23.33

These fractions were then tested against the selected bacterial and fungal strains on which the crude extract showed activity. The zone of inhibitions recorded for different fractions of each herbal drug is indicated in Table 3.4. The results illustrated that the non-polar fractions (i.e. petroleum ether and chloroform) were stronger in their activity compared to the relatively polar fractions (i.e. acetone and methanol). Antimicrobial activities were found to decrease with increasing polarity indicating that the active compounds responsible for antibacterial and antifungal activities of the extract reside in the non-polar fractions in relatively higher concentrations.

Table 3.4: Antimicrobial Activities of Different Fractions of *L. adoensis* and *O. rochetiana* against Selected Strains of Bacteria and Fungi.

Test sample	Fraction	Conc. (Mg/ml)	Zone of Inhibition (mm)				
			Bacterial Strains			Fungal Strains	
			Sa	Ec	Pa	Ca	Tm
L. adoensis	Pet. ether	25	27±1.2	17±3.0	15±1.9	NT	18±2.0
		5	24±1.7	15±2.6	15±1.8	NT	14±1.2
	CHCl3	25	26±1.2	15±1.0	16±1.0	NT	16±0.0
		5	22±1.0	13±0.3	14±1.3	NT	13±0.5
	Acetone	25	24±1.5	15±0.5	17±0.9	NT	-
		5	17±0.6	13±0.6	14±1.3	NT	-
	Methanol	25	20±0.5	14±0.8	16±0.3	NT	-
		5	13±0.6	-	14±1.3	NT	-
	Crude	25	20±2.4	14±0.4	20+1.5	NT	15+0.4
		5	15±1.3	-	15±0.6	NT	-
O. rochetiana	Pet. ether	25	27±0.3	21±0.6	21±2.3	21±1.9	32±1.8
		5	22±2.3	20±0.6	19±1.8	15±0.4	18±0.8
	CHCl3	25	27±2.3	20±1.0	19±1.0	15±0.3	44±3.6
		5	22±0.8	19±1.0	15±0.5	-	17±1.3
	Acetone	25	24±1.0	15±0.5	18±0.9	-	16±1.6
		5	17±0.6	13±0.3	13±0.5	-	13±1.0
	Methanol	25	23±1.6	14±0.3	16±0.8	-	-
		5	15±0.5	-	14±0.5	-	-
	Crude	25	19±0.9	13±0.8	17±0.9	14±0.7	18±1.2
		5	16±0.3	-	15±0.6	-	13±0.3

Sa = *S. aureus*, Ec = *E. coli*, Pa = *P. aeruginosa*, Ca = *C. albicans*, Tm = *T. mentagrophytes*, NT = not tested, - = no activity.

Comparison of the antimicrobial activities of the fractions with that of the crude extract indicated that the non-polar fractions in many cases are stronger in activity at the two concentration levels than the crude extract. These results are expected because 80% methanol, being highly polar, is unable to extract as much of the active compounds (which are non polar) as can be extracted with non-polar solvents like petroleum ether and chloroform.

The antimicrobial activity of the non-polar fractions of the two species showed similar activity profile on the selected strains to that of the crude extract, i.e. the petroleum ether and chloroform fractions of *L. adoensis* were more active against the bacteria and that of *O. rochetiana* against the fungi. In addition, the activity of the petroleum ether fraction of *O. rochetiana* at 25 mg/ml is almost equivalent to the activity of the crude extract at 100 mg/ml. This result supports the fact that the active compounds are more concentrated in this fraction. It also underlines the importance of testing activities of the different fractions before reporting that such type of herbal drugs are inactive by simply looking at the results of the crude extract, especially for those drugs having a long history of use by the local people.

In general, results of antimicrobial activity tests of the fractions indicated that further studies carried out on the petroleum ether and chloroform fractions of these two species might lead to the isolation of the desired active compound(s) should this be necessary.

1.1.1 Minimum Inhibitory Concentrations (MIC) of the Crude Extracts

The preliminary screening assays for antimicrobial activity can largely be considered as qualitative assays and are used for identifying the presence or absence of bioactive constituents in the extracts. However, these methods of assay offer little other information on these compounds.

Minimum inhibitory concentration (MIC) is a quantitative assay and provides more information on the potency of the compounds present in the extracts.

Thus, the MIC values of crude extracts of *L. adoensis* and *O. rochetiana* were determined so as to demonstrate the potency of the two species against the selected strains of bacteria and fungi. The MICs of the extracts are shown in Table 3.5.

The MIC values indicated that extracts of *L. adoensis* were more potent against bacteria than against fungi and similarly, extracts of *O. rochetiana* were found to be more active against fungi than against bacteria. The results were in agreement with the initial preliminary antimicrobial screening test results. The least MIC value observed was 1.25 mg/ml, which was the MIC value of the hydroalcoholic extracts of *O. rochetiana* on *T. mentagrophytes*. On the other hand, the highest MIC value was registered for *E. coli* (the least sensitive bacterial strain) to the crude extracts of both *L. adoensis* and *O. rochetiana*, i.e. 10 mg/ml. *P. aeruginosa* was more sensitive to the antimicrobial agents from among gram-negative bacteria being inhibited at 2.5 mg/ml by both *L. adoensis* and *O. rochetiana* crude extracts.

The MIC values of the two extracts on *S. aureus* were found to be 2.5 mg/ml (*L. adoensis*) and 5 mg/ml (*O. rochetiana*). *C. albicans* was inhibited by *O. rochetiana* at a concentration of 10 mg/ml. The figures of the MIC values of the two species seem to be relatively higher. But the overall antimicrobial activity screening results is still indicative of the potential of these herbal drugs as effective medicaments in the treatment of infectious skin disorders.

Table 3.5: Minimum Inhibitory Concentration (MIC) Values of the 80% Methanol Extracts of *L. adoensis* and *O. rochetiana* on the Tested Strains.

		Presence/Absence of Growth				
Plant	**Conc.**	**Bacterial Strains**			**Fungal Strains**	
Species	**(Mg/ml)**	**Sa**	**Pa**	**Ec**	**Ca**	**Tm**
L. adoensis	20	-	-	-	NT	-
	10	-	-	-	NT	-
	5	-	-	+	NT	-
	2.5	-	-	+	NT	+
	1.25	+	+	+	NT	+
	0.625	+	+	+	NT	+
O. rochetiana	20	-	-	-	-	-
	10	-	-	-	-	-
	5	-	-	+	+	-
	2.5	+	-	+	+	-
	1.25	+	+	+	+	-
	0.625	+	+	+	+	+

(+) = presence of growth, (-) = absence of growth, Sa = *S. aureus*, Ec = *E. coli*, Pa = *P. aeruginosa*, Ca = *C. albicans*, Tm = *T. mentagrophytes*, NT = not tested.

1.2 Anti-inflammatory Activities of the Crude Extracts

Inflammation is a normal and essential response to any noxious stimulus that threatens the host and may vary from a localized response to a generalized response [172]. The search for new and effective treatment modalities requires availability of reasonably precise and accurate screening tests. Although no model adequately reflects the real events that occur in human inflammatory conditions, several *in vivo* and *in vitro* assays are used. One of the most commonly used *in vivo* animal assays is the one that measures the ability of anti-inflammatory agents to inhibit edema induced in the rat paw by carrageenan [148-155,172]. The development of edema in the paw of the rat, after the injection of carrageenan has been described as a biphasic event. The initial phase, observed during the first hour, is attributed to the release of histamine and serotonin; the second one is due to the release of prostaglandin-like substances [153].

The results of this study indicated that the crude extracts of *L. adoensis* (Table 3.6) and *O. rochetiana* (Table 3.7) are devoid of any inhibitory effect on edema formation at 100 and 750 mg/kg dose levels, respectively. However, *L. adoensis* has shown some level of bioactivity during the second and third hours at a dose of 750 mg/kg, the maximum inhibition of edema formation being observed at the third hour (i.e. EI of 30%). None of the herbal extracts has shown anti-edematous response at all doses during the first hour of carrageenan induced paw edema, suggesting that the edema inhibitory effect is not related to inhibition of the release of active pain substances such as histamine and serotonin which occur in the early phase of edema formation. The relatively marked inhibition of edema by *L. adoensis* at the third hour may suggest an inhibition of the release of kinins or cycloxygenase enzymes which are involved in the formation of prostaglandins that is responsible for the induction of inflammatory processes at the later stage [149,153].

Table 3.6: Anti-inflammatory Activity of the Crude Extract of *L. adoensis* on Rat Hind-Paw Oedema Induced by Carrageenin.

	Time After Carrageenin Administration							
	1 hour		**2 hours**		**3 hours**		**4 hours**	
Experimental group	**EV**	**EI (%)**	**EV**	**EI (%)**	**EV**	**EI (%)**	**EV**	**EI (%)**
(-) Control	0.44+0.02		0.78±0.12		0.79±0.10		0.79±0.09	
L. adoensis								
100 mg/kg	0.52±0.06	(-)	0.81±0.08	(-)	0.83±0.04	(-)	0.96±0.08	(-)
750mg/kg	0.48±0.06	(-)	0.72±0.10	7.69	0.55±0.08	30.38	0.80±0.09	(-)

Oedema is expressed as increase in paw volume (ΔV)±S.E.M., EV = Edema Volume, EI = Edema Inhibition, N=5

Table 3.7: Anti-inflammatory Activity of the Crude Extract of *O. rochetiana* on Rat Hind-Paw Oedema Induced by Carrageenin.

	Time After Carrageenin Administration							
	1 hour		**2 hours**		**3 hours**		**4 hours**	
Experimental group	**EV**	**EI (%)**	**EV**	**EI (%)**	**EV**	**EI (%)**	**EV**	**EI (%)**
(-) Control	0.22±0.04		0.28±0.05		0.33±0.09		0.40±0.12	
O. rochetiana								
100 mg/kg	0.24+0.03	(-)	0.28±0.06	0	0.27±0.05	18.18	0.34±0.04	15
750mg/kg	0.30±0.03	(-)	0.35±0.05	(-)	0.43±0.03	(-)	0.49±0.05	(-)

Oedema is expressed as increase in paw volume (ΔV)±S.E.M., EV = Edema Volume, EI = Edema Inhibition, N=5.

In such type of biological tests, a dose dependent edema inhibition would be expected when using different concentrations [73,150] the strength of inhibition being higher at higher concentrations. However, edema reduction was not observed at 750 mg/kg in the case of *O. rochetiana*. Although the inhibition of edema by this plant was not marked, little edema reduction was observed at 100 mg/kg during the third and fourth hours. The crude extract is expected to contain several compounds and hence this unusual result might be explained by the fact that a compound in the crude extract with antagonistic action may act simultaneously with compounds having anti-inflammatory activity. It is also possible that, the presence of one compound in large quantities prevents or impedes the absorption of the other active compound there by inhibiting its activity.

The overall anti-inflammatory activity test results of this study indicate that the crude extracts of both species of plants did not have significant edema inhibition properties ($P > 0.05$) at the two dose levels. However, if similar tests were conducted on different solvent fractions of the two species of plants, different results might have been observed as is the case for the antimicrobial activity test results of the same plant drugs. Such variations in anti-inflammatory activities between different fractions of the same plant are reported [173-174]. On the other hand, many phytochemicals are poorly absorbed after oral administration [175] and may therefore have poor bioavailability to exert the desired systemic biological activity, yet may be effective after topical administration (a common way of drug delivery in the traditional practice for treating skin disorders). Thus, the topical anti-inflammatory activities of these herbal drugs need to be checked to verify if such discrepancies really exist.

1.3 Phytochemical Constituents of the Herbal Drugs

Recognition of the biological properties of myriad natural products has fueled the current focus of this field, namely, the search for new drugs including antibiotics [176]. The ultimate goal of surveying plants for biological activity is thus to isolate one or more biologically active compounds that may be potentially useful in treating certain disease conditions or serve as a structural analogue (template) from which better synthetic modifications can be derived. Chemical characterization and compositional analysis of traditional medicines provide the necessary scientific basis for the discovery and development of new drugs of natural origin. Should isolation of the active ingredients be unnecessary as is the case in the current trends of herbal therapy, knowledge of the type of chemical constituents found in a given herbal drug can be very helpful for standardization and quality control purposes. To this end, phytochemical screening can be a valuable aid.

Therefore, the leaves of *L. adoensis* and *O. rochetiana* were screened for the presence of different phytochemical compounds of therapeutic interest using both chemical (Table 3.8) and chromatographic (Table 3.9) methods with the object of finding out the possible class of compounds present in the respective plants.

According to the results of the phytochemical screening study, both species were found to show a positive test for the presence of saponins, polyphenols, flavonoids and tannins. While *L. adoensis* showed positive test for the presence of alkaloids, *O. rochetiana* showed a negative result for the same test. The test for coumarins (Table 3.9) indicated the absence of these secondary metabolites in both plants.

Table 3.8: Results of Preliminary Phytochemical Investigation of the Leaves of *L. adoensis* and *O. rochetiana* Using Chemical Test Methods.

Metabolites Tested for		Plant Species	
		L. adoensis	***O. rochetiana***
Alkaloids		+	-
Saponins	A (Froth)	+	++
	B (Hemolysis)	+	+
Tannins		++	+
Anthraquinones	A (Free)	-	-
	B (Combined)	-	+
Polyphenols		++	+
Flavonoids		+	+

- = negative, ++ = strongly positive, + = positive

Numerous studies conducted on the antimicrobial activities of the class of compounds listed above reported the potential of each class of compounds in inhibiting the growth of wide ranges of microorganisms. Phenolics and polyphenols are among these classes of compounds reported in the literature for having such potential. Eugenol [26], Caffeic acid [26,177], catechol and pyrogallol [178] for instance were found to be effective against bacteria and fungi. Reaction with sulfahydryl groups or more non-specific reactions with proteins is thought to be the possible mechanism for phenolic toxicity to microorganisms [179]. Quinones are known to complex irreversibly nucleophilic amino acids in proteins, often leading to inactivation of the protein and

loss of function. As a result, there is a great potential for quinones to serve as antimicrobial agents. Hypericin (an anthraquinone from *Hypericum perforatum*) [26] and another anthraquinone from *Cassia italica* [180] were reported to have general antimicrobial properties.

Flavonoids and flavonoid-derived plant natural products have long been known to function as antimicrobial defense compounds [181]. Different *in vitro* studies have shown that they are effective antimicrobial substances against a wide spectrum of microorganisms [182-183]. To mention a few, the flavonoids diosmetin and isoluteolin (isolated from *Soroseris hookreriana*) [184], quercetin and naringenin [185], the mixture of catechin compounds (found in oolong and green teas) [186-190] and galangin (3,5,7-trihydroxyflavone) (derived from *Helichrysum aureonitens*) [191-192] have been shown to exert antimicrobial activity against a wide range of microorganisms.

Consumption of tannin-containing beverages, especially green teas and red wines, was suggested to cure or prevent a variety of ills including wide ranges of infections [193-194]. One of the molecular actions of tannins is by complexing proteins through the so-called nonspecific forces such as hydrogen bonding and hydrophobic effects, as well as by covalent bond formation [26]. A review on the antimicrobial properties of tannins indicated that they inhibit growth and protease activity in many ruminal bacteria. It is reported that tannins bind to cell coat polymers in all strains. They also cause morphological changes in the organisms indicating that the cell wall is the main target of tannin toxicity [195].

Table 3.9: Results of Preliminary Phytochemical Investigation of the Leaves of *L. adoensis* and *O. rochetiana* Using Chromatographic Methods.

Metabolites Tested for	**Solvent System**	**Detection**	**Result**	
			L. adoe	***O. roch***
Alkaloids	EtOAc:MeOH:H_2O (150:26:19) CHCl3:EtOH (9:1) EtOH: NH3 (9:1)	Dragendorff's reagent	+	-
Anthraglycosides				
Anthraquinones	EtOAc:MeOH:H_2O (100:13.5:10)	Ethanolic KOH	+	+
Anthrones	"	"	-	+
Flavonoids	EtOAc:FA:GAA: H_2O (100:11:11:26)	UV-254	+	+
		UV-366	+	+
Saponins	$CHCl_3$:GAA:MeOH: H2O (64:32:12:8)	Vanillin H_2SO_4	+	+
Coumarins	DEE:toluene (1:1; saturated with 10% HAC)	Ethanolic KOH and UV 366	-	-

EtOAc = Ethyl acetate, MeOH = Methanol, EtOH = Ethanol, FA = Formic acid, GAA = Glacial acetic acid, DEE = Diethyl ether, HAC = Acetic Acid, *L. adoe* = *Lippia adoensis*, *O. roch* = *Olinia rochetiana.*

The role of coumarins in plants appears to be mainly defense-related, given their antimicrobial, antifeedant, UV-screening, and germination inhibitor properties [176]. Coumarins have a variety of bioactivities including antimicrobial, anti-inflammatory, molluscicidal, anthelmintic activities [196]. A series of simple coumarins evaluated for their antimicrobial activity against a range of microorganisms were reported to show broad diversity of inhibitory activity on the tested standard bacteria [197]. But, the phytochemical screening test of this particular study indicated the absence of these metabolites in the two species of plants. This might show that the antimicrobial activity of the plants in question is not associated with coumarins.

Terpenoids and essential oils are other groups of compounds reported to have antimicrobial activities. Results of numerous studies indicate that terpenes and terpenoids are active against bacteria [198-202] and fungi [203-208]. The diterpenoids and sesquiterpenoids obtained from *Salvia sclarea* were found to be active against *S. aureus* and *C. albicans* [209]. The terpenoid constituents capsaicin and petalostemumol were also shown to have an excellent activity against various strains of bacteria and fungi [210-212]. The essential oil of thyme as well as two of their constituents, thymol and carvacrol [213] have been found to antagonize the propagation of several bacteria. On the other hand sesquiterpene lactones isolated from *Centaurea thessala* and *C. attica* were shown to have great antifungal activity [214]. The phytochemical test for the presence of volatile oil constituents in *L. adoensis* and *O. rochetiana* was not addressed in this study. But, review of the literature has indicated the presence of quite a large number of these metabolites [106-107] in *L. adoensis*. Although one cannot for sure tell whether the antimicrobial activity of *L. adoensis* is related to the volatile oil constituents, their contributions to the activity however may be speculated as many of the essential oils are reported to have antibacterial and antifungal properties.

The other groups of compounds that are of interest for their antimicrobial activity are alkaloids. Several alkaloids, isolated from the plants of the Ranunculaceae family and the genus *Strychnos* (Loganiaceae) are known to have antimicrobial properties [26,145]. Dimeric tertiary toxiferine type alkaloids (active compounds found in some African chewing sticks), harmane-type alkaloids and solanum alkaloids have also been reported to exhibit antimicrobial activity against a series of microorganisms, including gram-positive and gram-negative bacteria, dermatophytes and yeasts [145]. The alkaloids, sanguinarine and chelerythrine (isolated from the roots of *Sanguinaria canadensis*) [215] and tingenone and 22 β-hydroxytingenone (isolated from khat callus cultures) [216] were shown to have significant antimycobacterial activity. The marine alkaloids haminol A and haminol B as well as 17 related compounds were also found to be active against at least one bacterial or fungal strain [217].

Although the antimicrobial activity of some herbs is attributed to a specific chemical compound, labeling the activity of some others, especially those commonly used in traditional therapy, to a single compound is a difficult undertaking and it is very unlikely that the activity is due to a single compound only. The possible explanation one can propose for their effectiveness in treating various infectious diseases is that each class of compounds might act synergistically contributing their own share to the total activity of the herbal drug.

This idea has been evidenced by a number of studies conducted on different traditionally used medicinal plants. For example, chewing sticks, widely used in African countries as oral hygiene aid, come from different species of plants and one stick may contain heterogeneous chemically active components. Crude extracts of one species used for this purpose, *Serindeia werneckei*,

inhibited the periodontal pathogens *Porphyromonas gingivalis* and *Bacteroides melaningenicus in vitro*. The milky sap or latex of *Carica papaya* is composed of mixtures of chemicals of which papain (a well-known proteolytic enzyme) and the alkaloid carpaine are among the chief components and may contribute to its antimicrobial properties [26]. The sap, seed and pulp of *Carica papaya* were found to be bacteriostatic against several enteropathogens [218]. Propolis (bee glue) has a very complex chemical composition including terpenoids, flavonoids, aromatic acids and caffeate esters. Propolis was found to have antibacterial, antifungal and antiviral activities [219].

More over, the empirical application of honey on open wounds, burns or the use of honey in syrups does show that it stops the growth of many organisms. Some chronic debilitating conditions resulting from pressure sores, infected wounds, burns and gas gangrene have been found to respond favorably to honey treatment. Various investigations have shown that honey stops the growth of both bacteria and fungi. Honey contains a mixture of several compounds including phenols, peroxidases, fatty acids, lipids, amylases and ascorbic acid. The ability of honey to kill microorganisms has thus been attributed to its content of the mixture of these compounds [220].

Therefore, the antimicrobial activity of *L. adoensis* and *O. rochetiana* might be attributed to either the individual class of compounds present in each herb, as confirmed by the phytochemical screening, or to the synergistic effect that each class of compounds exert to give the observed biological activity. Hence, further in-depth investigations should be carried out to resolve this issue. The fact that both plants contain saponins might contribute to their antimicrobial activity.

These compounds are known to disrupt the cell wall and cell membranes of microorganisms [221] causing lysis of the microbial cells. Although the antimicrobial activities of these herbal drugs are less likely to be due to polar compounds like saponins, the surface active property of these compounds may still contribute to the activity by reducing surface tension and facilitating the penetration of another active agent into the protoplasm. Nevertheless, since polar solvent extracts were relatively less active as compared to non-polar solvent extracts, the activity might be ascribed to lipophilic phytochemicals.

1.4 Performance Profiles of Topical Formulations of the Herbal Drugs

The release and diffusion of therapeutically active ingredients from topical preparations may be influenced by the base or vehicle into which they are incorporated [126]. Numerous preparations applied topically are intended to treat superficial infections of the skin. These topical antimicrobial formulations differ from other topical products in that penetration through the skin is not a necessary requirement. Thus, the use of membrane systems, which are meant to represent the *stratum corneum*, as *in vitro* model for studying the release of the medicament from its vehicle, seems to be less relevant. Since the availability and diffusion of the drug from the vehicle to the target site is of great importance in treating infections of the skin, the use of *in vitro* agar diffusion model is a more realistic representation and allows the objective comparison of antimicrobial topical products over a wide spectrum of microorganisms.

Thus, the same model was used in this study to evaluate the performance profile of topical formulations of *L. adoensis* and *O. rochetiana*. The main objective of this part of the work was to evaluate the antimicrobial activity profile or to assess indirectly the release profile of the active

components from topical formulations of the crude extracts of the two herbal drugs. Therefore, the formulation bases so chosen were made to be of widely different hydrophilicity/lipophilicity so as to exhaust the possible effect of changing the polarity of the base on the performance of the final product.

The results of the evaluation studies (Table 3.10) indicated that water miscible (hydrophilic) formulations are superior in performance than the water immiscible (hydrophobic) ones. In other words, while formulations 1 to 4 showed different degrees of activities against the tested strains of organisms, formulation 5, which was the most lipophilic ointment, showed virtually no activity indicating that the active component(s) of the herbal drugs could not be released from this formulation to the media thus failing to inhibit the growth of microorganisms surrounding the point of application.

The performance profile of the formulations was therefore found to be influenced by the type and nature of the vehicle (base) into which the drugs were incorporated. In general, all formulations containing water as one of the components of the vehicle showed some degree of activity, the strength of the activity being directly related to the degree of hydrophilicity of the vehicle. Similarly, from among one-phase systems (i.e. ointments), the water-soluble ointment (formulation 4) was found to have better activity profile than the hydrophobic ointment (formulation 5). All the above results signify that the release of the medicinally active component(s) from the hydrophilic bases is more intensive than from the lipophilic bases.

These results are in agreement with the results of other studies conducted using the same model or other models that utilize membrane systems. Release of many of the medicinal substances was

Table 3.10: Antimicrobial Activity Profiles of Topical Formulations of Crude Extracts and the Formulation Bases (Negative Controls)

Test Sample	Code No	Zone of Inhibitions (mm)				
		Bacterial Strains			Fungal Strains	
		Sa	Ec	Pa	Ca	Tm
10% *L. adoensis* in:						
Base 1	Formn.1	25±0.9	15±0.8	14±1.2	NT	38±1.8
Base 2	Formn.2	16±0.5	-	13±1.0	NT	29±1.2
Base 3	Formn.3	13±0.5	-	14±0.9	NT	18±1.5
Base 4	Formn.4	16±1.0	-	15±1.0	NT	15±1.0
Base 5	Formn.5	-	-	-	NT	-
10% *O. rochetiana* in:						
Base 1	Formn.1	23±0.3	13±0.3	14±0.6	22±1.6	65±2.5
Base 2	Formn.2	18±1.3	15±0.8	15±1.3	19±2.8	49±2.1
Base 3	Formn.3	17±0.8	13±0.6	14±1.0	19±2.0	42±3.1
Base 4	Formn.4	-	-	-	21±1.5	53±3.0
Base 5	Formn.5	-	-	-	-	-
Negative Controls (Formulation Bases)						
Na Laurate Monostearin Cream Base	Base 1	17±0.4	-	-	-	29±2.5
Macrogol Cream Base	Base 2	-	-	-	-	-
Gibson Base	Base 3	-	-	-	-	-
PEG Ointment	Base 4	-	-	-	-	15±1.8
White Petrolatum Ointment	Base 5	-	-	-	-	-

Formn. = Formulation, Sa = *S. aureus*, Ec = *E. coli*, Pa = *P. aeruginosa*, Ca =*C. albicans*, Tm = *T. mentagrophytes*, NT = not tested, - = no activity.

shown to be higher from the hydrophilic bases. It was found out that the release of indomethacin, chloramphenicol, acetyl salicylic acid and neomycin sulfate from hydrophilic bases is faster than from lipophilic bases [222]. Similarly, ocimum oil in lipophilic semisolid bases (petrolatum and simple ointment) exhibited much lower or no antibacterial activity compared to its formulation in the more hydrophilic macrogol blend ointment [86].

The sensitivity pattern of the microorganisms to the formulations was found to have of similar trend with the initial antimicrobial screening test results in that *E. coli* was still the least sensitive organism from among bacteria and *C. albicans* from among the fungi employed in the test. Formulation 1 was found to have the highest activity profile against all the tested strains. However, the base of this formulation showed activity against *S. aureus* (17 ± 0.4) and *T. mentagrophytes* (29 ± 2.5). Expectedly, the increased activity of this formulation was attributed to the additional activity exerted by the vehicle itself.

The antimicrobial activity of this vehicle (Base 1) may be due to the presence of sodium lauryl sulfate as one of the components of the base serving as a surface-active agent. Pharmaceutically, sodium lauryl sulfate is used as a protective skin cleaner, having bacteriostatic action against gram-positive bacteria, and is also used in medicated shampoos [223]. More over, all fatty acids and their salts are known to have variable fungicidal properties [221]. Therefore, the intrinsic antimicrobial property of sodium lauryl sulfate might have contributed to the remarkable antibacterial and antifungal activities of formulation 1.

Formulation 4 of *O. rochetiana* was shown to have significantly higher activity on *T. mentagrophytes* compared to the rest of the formulations. Similar trend was observed on its

activity against *C. albicans*. However, this trend was not observed for the same formulation of *L. adoensis* against *T. mentagrophytes*. This discrepancy might actually be related to the type and nature of the active components present in each herbal drug that are responsible for the antimicrobial activity. The active components found in *O. rochetiana* might interact with PEG to yield a more active product or a product that is more diffusible than the active component leading to a higher zone of inhibition.

The consequences of these drug vehicle interactions have been described in one study [137]. The study was made to evaluate the release properties of various drugs from different PEG ointment bases. It was indicated that the molecular size, shape and degree of interaction with the incorporated drugs are among the major factors affecting the rate of diffusion of the drugs from these bases. Accordingly, formulation base containing PEG 6000 was found to be the best vehicle for chloramphenicol compared to those formulated using PEG 4000 and PEG 2000. This increase in drug diffusion was reported to be due to direct interaction between chlormphenicol and PEG 6000, which is characterized by more surface tension lowering effect than PEG 4000 and PEG 2000.

In the case of neomycin sulfate however, diffusion was more pronounced in the less viscous PEG 2000 compared to PEG 4000 and PEG 6000. It was proposed that neomycin sulfate might interact to some extent with polyethylene glycol macromolecules, and this interaction may affect the extent of drug diffusion. Since PEGs influence the polarity, pKa and the apparent partition coefficients of drugs, it was evident that neomycin sulfate diffused more favorably from preparation containing PEG 2000. Usually, the bigger the dielectric constant, the greater the

diffusion rate will be, but if the dielectric constant is small and the drug is firmly bound to macromolecules, then the availability of free diffusible drug may be much less and therefore there is an apparent reduction in drug diffusion. For ampicillin trihydrate, the best release was obtained from preparation containing PEG 400:2000. Since ampicillin is an ionic drug i.e. more hydrophilic, it was expected that higher diffusion would be obtained with the lower molecular weight PEGs. It was also concluded that the more diffusible the PEG/ampicillin complex, the more it diffuses through the agar [137].

In another study, topical formulations containing ciprofloxacin and tinidazole in lanolin petrolatum base, emulsion (water washable) base and PEG (water soluble) base were prepared and evaluated for drug release. It was reported that the release of ciprofloxacin and tinidazole from the PEG base was maximum, the release of tinidazole being more than that of ciprofloxacin. The antimicrobial activities of the formulation containing PEG was found to be higher than the other formulations [224]. In fact, macrogol blend ointment base was also reported to have antibacterial properties [86]. It was found to have some degree of antifungal activity in this study as well on at least *T. mentagrophytes* (15 ± 1.8). The exceptionally higher antifungal activity of this formulation might therefore be linked to the inherent activity of the blend base or the synergistic or potentiation of antimicrobial activity it imparts upon to the active drug.

On the contrary, formulation 4 of *O. rochetiana* has shown no antibacterial activities against both gram-negative and gram-positive bacterial strains. This result in fact seems to negate the above argument. But it might still be justified if one can think of how diversified types of compounds having complex chemical nature one can have in one crude herbal drug. From the experience

learnt in conventional drugs, a single compound is less likely to have both antibacterial and antifungal properties. If that is the case, the antibacterial and antifungal activity of this herbal drug is more likely to be due to the presence of different types of compounds each having either antibacterial or antifungal activities. Macrogol ointment bases are known to be incompatible with a range of chemicals including phenols, sorbitol, tannic acid, etc. These bases are also known to reduce the antimicrobial activity of quaternary ammonium compounds and methyl and propyl *p*-hydroxybenzoates. They are also reported to rapidly inactivate penicillin and bacitracin [123]. Therefore, the lack of antibacterial activity of *O. rochetiana* formulated in macrogol ointment base might be due to the inactivation of the active compound(s) responsible for the antibacterial activity through interaction with the base which might lead to the formation of an inactive compound or a complex that fails to diffuse through the agar media.

Compared to the positive controls (Table 3.11), i.e. topical antimicrobial products marketed for the treatment of different topical skin infections, some formulations of the studied drugs (*L. adoensis* and *O. rochetiana*) have shown relatively comparable activities. For instance, Formulation 1 of both herbal drugs at a concentration of 10% showed activity against *S. aureus* comparable to that of gentamycin cream. Similarly, *O. rochetiana* formulations 1 and 4 at the same concentration showed by far better activity profile than all the marketed topical antifungal agents (Table 3.12) against *T. mentagrophytes*.

The higher performance of the marketed antiseptic products over some of the strains, for example, mupirocin and tetracycline on bacterial strains and ketoconazole and clotrimazole on *C. albicans* may even be related to the type of bases used to incorporate the designated drugs in

addition to the active components. Because, some components of topical vehicles like sodium lauryl sulfate, possess certain degree of antimicrobial activities as described previously. Cationic surfactants, which are commonly used components of topical products, are also known to exert a bactericidal action against a broad spectrum of gram-positive and gram-negative bacteria. They

Table 3.11: Antibacterial Activity Profiles of the Marketed Topical Antibacterial Agents in Comparison with Some Formulations of the Crude Extracts.

Code No	**Antimicrobial Substance**	**Cencen-tration**	**Base Type**	**Zone of Inhibitions**		
				Sa	**Ec**	**Pa**
1	Sodium fusidate	2%	Ointment	41±0.5	-	-
2	Mupirocin	2%	Ointment	57±3.1	30±0.9	16±1.2
3	Tetracycline HCl	3%	Ointment	43±2.1	33±0.6	19±1.0
4	Fusidic acid	2%	Cream	37±1.7	-	-
5	Gentamycin	0.1%	Cream	21±1.0	23±1.5	24±0.3
Form.1	*L. adoensis*	10%	Cream	25±0.9	15±0.8	14±1.2
Form.4	*L. adoensis*	10%	Ointment	16±1.0	-	15±1.0
Form.1	*O. rochetiana*	10%	Cream	23±0.3	13±0.3	14±0.6
Form.2	*O. rochetiana*	10%	Cream	18±1.3	15±0.8	15±1.3

Formn. = Formulation, Sa = *S. aureus*, Ec = *E. coli*, Pa = *P. aeruginosa*, - = no activity

are also active against several pathogenic species of fungi and protozoa. For example, benzalkonium chloride is employed as antiseptic for application to the skin, mucous membranes, burns and wounds [221,223,225].

Table 3.12: Antifungal Activity Profiles of the Marketed Topical Antifungal Agents in Comparison with Some Formulations of the Crude Extracts.

Code No	Antimicrobial Substance	Cencen-tration	Base Type	Zone of Inhibitions	
				Ca	Tm
1	Ketoconazole	2%	Cream	44±0.7	27±1.3
2	Ketoconazole	2%	Cream	48±4.8	31±1.5
3	Ketoconazole	2%	Cream	45±2.0	30±0.5
4	Ketoconazole	2%	Cream	43±3.5	30±2.0
5	Clotrimazole	1%	Cream	45±2.5	47±2.5
6	Clotrimazole	1%	Cream	44±4.0	39±2.5
7	Ketoconazole	2%	Cream	45±1.5	31±0.9
8	Clotrimazole	1%	Cream	51±2.6	31±1.3
Formn.1	*L. adoensis*	10%	Cream	NT	38±1.8
Formn.2	*L. adoensis*	10%	Cream	NT	29±1.2
Formn.1	*O. rochetiana*	10%	Cream	22±1.6	65±.5
Formn.2	*O. rochetiana*	10%	Cream	19±2.8	49±2.1
Formn.4	*O. rochetiana*	10%	Ointment	21±1.5	53±3.0

Formn. = Formulation, Ca = *C. albicans*, Tm = *T. mentagrophytes,* - = no activity, NT = not tested.

Besides, topical preparations, particularly those containing water are known to contain a preservative in order to prevent microbial growth and spoilage of the product [122]. Many of the marketed topical formulations might as well contain preservatives, and this by itself might contribute to the overall antimicrobial activity of the marketed products. The herbal formulations

on the other hand did not have any preservative included in the vehicle indicating that the reported activity is merely due to the inherent potential of these herbal products to kill or inhibit the growth of bacteria and fungi.

Lastly, one of the challenges encountered during the formulation study was stability of the cream formulation i.e. formulation 2 of *L. adoensis,* where it tended to break upon storage. Contamination with fungus of this formulation for the two herbal drugs was also encountered upon storage. If we have a close look into the composition of this formulation base, it consists of cetomacrogol emulsifying wax as an emulsifier, which is not found in other formulation bases. Cetomacrogol is known to be incompatible with phenols [123]. During the phytochemical screening test, *L. adoensis* has shown a strongly positive test for the presence of phenols. The instability of this formulation might thus be due to the incompatibility of the herbal drug with cetomacrogol. The fungal growth observed in this formulation is obviously due to the presence of relatively large quantities of water, which facilitates the growth of microorganisms.

By and large, the traditional claims attributed to these herbal drugs by the local people for the treatment of topical skin disorders is partly justified by the different degrees of antimicrobial activities exhibited by these products against the selected strains of bacteria and fungi which are known to be common causative agents of different types of skin infections. The overall results of this study have clearly indicated that the speed and degree of release and hence the activity or performance of a given drug may be modulated by the type and nature of the formulation. Therefore, in order to optimize the performance of the product, the choice of formulation should be based on whether or not the formulation delivers the drug at the target site so as to achieve the

desired therapeutic concentration. Because, a formulation that fails to release the drug to the required extent is likely to be ineffective.

As shown in the activity profile, creams especially the hydrophilic ones are generally superior in performance than ointments. The nature of the resulting profile reveals information as regards to the availability of the drug from the base and its rate of diffusion. Therefore, if development of the stated herbal drugs for clinical uses is desired, this information can be utilized to optimize the antimicrobial performance of topical formulations of these botanicals through rational selection of the formulation bases.

1.5 Preliminary Standardization of the Herbal Drugs

It is assumed that as soon as people began to trade in herbal medicines, detecting adulteration was an important aspect of quality evaluations. The works of many of the ancient Greek and Roman scholars discussed not only the therapeutic properties of natural medicines but also various aspects of their quality. The tests advocated during those periods to detect quality deficits were primarily organoleptic, although some physicochemical tests were also described. Later the importance of learning how to distinguish good quality products based upon organoleptics, pharmacological potency and geographical source were emphasized. As advances were made in organic chemistry, qualitative and then quantitative chemical assays were gradually introduced [226].

Standardization of herbal products is therefore not a new concept. Herbalists have defined standards of how and when to harvest medicinal herbs as well as how they may be dried, powdered and extracted in water or alcohol. All of these are processes of standardization [227].

Being natural products, herbs do not have consistent, standardized composition. Different parts of the plant (e.g. roots, leaves, bark) possess a variable profile of constituents, and the content and concentration of constituents can be influenced by several factors including climate, growing conditions, time of harvesting, storage conditions (e.g. light, temperature and humidity) and processing (e.g. extraction, drying) [228].

The process of standardization was thus originally introduced to produce more consistent botanical products [226]. Broadly speaking there are two types of standardization. One is based on identifying and quantifying an extract to a characteristic chemical marker compound. The second identifies and concentrates one or more as active constituents, making it closer to the level of chemical isolate [229]. This can however be a complex subject with herbal products, the activity of which is not due to a single chemical entity but to a mixture of constituents [230]. On the other hand the active ingredients or the quantity necessary for effectiveness have not been determined for several herbal products. Standardizing the content of one active component or a marker component (e.g. chemical characteristic of the herb or chemicals present in large quantities), while maintaining the relative contents of all other constituents, is considered as a satisfactory preliminary approach to achieve acceptable consistency among products [12,228].

This undertaking requires a method, which is capable of determining the proposed component (active or marker) for standardization in the plant. The technique, which revolutionized the chemical analysis of small quantities of plant material, was chromatography, particularly Thin Layer Chromatography (TLC) [231]. TLC has been widely used for the analysis of medicinal plants and it is included as a method for identification in monographs of herbal drugs in most pharmacopoeias throughout the world. Unsurpassed flexibility due to a large number of

parameters, which can influence the chromatographic results, is one of the inherent advantages of the method [232].

TLC enabled small amounts of material to be extracted and the components separated into a sequence of discrete zones of the range of compounds present, commonly called the zone profile to give a "fingerprint" distinctive for individual plant species. Compounds can thus be characterized by two criteria, i.e. the distance they travel in a particular TLC system and their appearance after visualization. In addition to a vast amount of qualitative information, TLC gives a semi quantitative assessment of constituents of a mixture since the color intensities of the separated zones reflect the concentration of substances present [231].

The use of such fingerprinting analysis for quality control and standardization of medicinal herbs has attracted intense interest in herbal drugs research in recent years. In herbal drugs, such a profile is dependent not only on the preparation processes but also on the quality of the crude drug, which varies according to the origins of the herb, sources, harvest times and pretreatment processes. The consistency and stability of the chemical constituents observed in the profiles thus reflect not only just the conditions of the drug preparation process, but also the source and quality of the raw herbs. For example, TLC has been the most widely used classical method for fingerprinting analysis in Chinese medicines. Chromatographic profiles of major components are used to evaluate herbal growers and suppliers, to standardize raw materials and to control formulation and tablet content uniformity [233-234].

Extracts of herbal drugs can therefore be analyzed by this technique for the presence of desired constituents and absence of impurities or compounds characteristic of common adulterants. Even

if the precise identification of the constituents is difficult, the pattern of zones may be used to characterize particular drugs [231]. In addition to TLC, there are also other crude forms of quantitation. As mentioned previously, the raw materials play a major role in the production of phytopharmaceuticals of standard quality. Evaluation of the raw material using different parameters for the determination of identity, purity and quality is of paramount importance to maintain the quality and purity of herbal drugs. The evaluation of these parameters gives a clear idea about the specific characteristic of the crude drug under examination. The most commonly employed parameters adopted to get information about the purity and standard of a crude drug include the determination of ash values, solvent extractive values and moisture content [145,163-164].

In this study, an attempt has been made to determine some standardization and/or quality control parameters for the two herbal drugs (*L. adoensis* and *O. rochetiana*) as part of the biological activity screening and formulation studies, with the object of generating essential preliminary data on the quality attributes of the herbal drugs in question. To this end, the two herbal drugs were subjected to preliminary standardization procedures such as determination of ash values, solvent extractable matter, moisture content and TLC fingerprinting analysis. Although complete chemical profiling of each extract was not possible at this stage, useful information about the drugs was obtained from the determination of the above values and also from thin layer chromatographic studies.

The Ash values of the two herbal drugs are shown in (Table 3.13). The ash of any organic material is composed of their non-volatile inorganic components. Controlled incineration of crude drugs results in an ash residue consisting of an inorganic material (metallic salts and silica).

This value varies within fairly wide limits and is therefore an important parameter for the purpose of evaluation of crude drugs. In certain drugs, the percentage variation of the weight of ash from sample to sample is very small and any marked difference indicates a change in quality [145].

Table 3.13: Ash Values of the Dried and Powdered Leaves of *L. adoensis* and *O. rochetiana*.

	Ash Values (In Percent ± SD)	
Parameters	***L. adoensis***	***O. rochetiana***
Total Ash	14.6 ± 0.4	5.7 ± 0.1
Acid Insoluble Ash	11.8 ± 1.4	0.7 ± 0.3
Water Soluble Ash	6.4 ± 1.1	1.8 ± 0.4

The solvent extractable values of the two herbal drugs are shown in Table 3.14. This value indicates the amount of active constituents extracted with solvents from a given amount of medicinal plant material. It is employed for materials for which as yet no suitable chemical or biological assay exists [163]. The British Pharmacopoeia [164] recommends the determination of water-soluble and alcohol soluble extractives as a means of evaluating crude drugs which are not readily estimated by other means. The use of a single solvent can thus be used as a means of providing preliminary information on the quality of a particular drug sample [145]; for example, a crude drug, which has initially been extracted with water, will yield a very low soluble matter upon subsequent extraction with the same solvent.

Table 3.14: Solvent Extractable Values of the Dried and Powdered Leaves of *L. adoensis* and *O. rochetiana*.

	Percent Extractable Value (Average ± SD)	
Type of Solvent	***L. adoensis***	***O. rochetiana***
Petroleum ether	1.5 ± 0.4	2.2 ± 0.2
Chloroform	3.9 ± 0.3	11.0 ± 0.6
Acetone	4.1 + 0.5	16.0 + 0.8
Ethanol	7.3 + 0.4	20.1 + 0.2
Methanol	15.8 + 0.9	37.3 + 1.2
Water	23.4 + 0.5	37.3 + 0.3

The test for loss on drying (Table 3.15) determines both water and volatile matter in crude drugs. An excess of water in medicinal plant materials will encourage microbial growth, the presence of fungi or insects, and deterioration following hydrolysis. Limits for moisture content should therefore be set for every given plant material. This is especially important for materials that absorb moisture easily or deteriorate quickly in the presence of water [163].

Table 3.15: Loss on Drying of the Dried and Powdered Leaves of *L. adoensis* and *O. rochetiana*.

Plant Species	**Percentage Loss on Drying**
L. adoensis	10.6 ± 0.0
O. rochetiana	10.4 ± 0.1

As described above, TLC is an ideal technique for screening of herbal drugs because of its low cost, easy maintenance and selectivity of detection reagents. Therefore, the extracts of the two herbal drugs were analyzed by TLC on silica gel in order to obtain information on the active compounds from the components separated into a sequence of discrete zones or the fingerprints distinctive for individual plant species. In such conditions compounds can be characterized by the distance they travel in a particular TLC system and the appearance of each zones after visualization. Thus, a data set of R_f values for the separation of various constituents in the crude extracts and different fractions of the two herbal drugs and their corresponding appearance under daylight and UV illumination is shown in Table 3.16 (*L. adoensis*) and Table 3.17 (*O. rochetiana*).

The 80% methanol, methanol, acetone, chloroform and petroleum ether extracts displayed a series of constituents on the chromatogram. The non-polar fractions showed quite a different fingerprint compared to the polar fractions both before and after treatment with the spray reagent. The photographs of the developed chromatograms after spraying with chemical reagent are depicted in Figures 3.1, 3.2 and 3.3.

As can be seen from the finger print profiles, maximum number of spots was obtained in the non-polar fractions especially under illumination with UV light. The polar fractions and crude extract of *O. rochetiana* yielded almost no visible spots after spraying with vanillin sulfuric acid. The crude extract of *L. adoensis* however has given at least five distinct zones after treatment with spraying chemical reagent. The methanol and acetone fractions have also displayed at least two and three distinct zones, respectively, after spraying. For *O. rochetiana*, the petroleum ether fraction yielded the maximum zones (six) followed by chloroform fraction (three).

Finally, the data given on different parameters of preliminary standardization/quality control procedures may not be considered as final and precise monographs for the stated herbal drugs. Additional data on the same species of plants grown under different conditions and collected from different sites is required in order to establish a range of values so as to serve for quality control purposes and also for identification. If this can be achieved, the results of each parameter for a similar species tested under the same conditions should fall within the established range and any deviation could be considered as an indication of poor quality or of different plant species. It is believed however that this data can serve as a starting point or as an additional information for more intensive research activities to be carried out on these two species of plants.

Table 3.16: TLC Fingerprints of the Crude (80% Methanol) Extract and Various Fractions of *L. adoensis*.

		Observations	
Type of Extract & Solvent System	**Light Source**	**No of Spots**	**R_f Values (Corresponding Colors)**
Crude Extract	Before Spraying*	-	-
MeOH:$CHCl_3$:Ac	After Spraying*	5	0.33 (blue), 0.48 (yellow), 0.48 (violet), 0.67 (violet), 0.8 (greenish brown)
(56:42:2)	UV 254	3	0.38, 0.57, 0.8
	UV 366	5	0.33 (blue), 0.55 (deep blue), 0.71 (light blue) 0.81 (red), 0.86 (light red)
Methanol Fraction	Before Spraying*	-	-
MeOH:$CHCl_3$:Ac	After Spraying*	2	0.28 (blue), 0.49 (violet)
(56:42:2)	UV 254	-	-
	UV 366	3	0.26 (light blue), 0.43 (light blue), 0.81 (light red)
Acetone Fraction	Before Spraying*	1	0.6 (light yellow)
MeOH:$CHCl_3$:Ac	After Spraying*	3	0.48 (violet), 0.58 (yellow), 0.65 (violet)
(56:42:2)	UV 254	1	0.61
	UV 366	5	0.58 (deep blue), 0.67 (light red), 0.69 (blue), 0.79 (red), 0.83 (red)
Chloroform Fraction	Before Spraying*	5	0.02 (yellow), 0.04 (green), (light brown), 0.6 (light yellow), 0.68 (light green)
$CHCl_3$:PE:Ac	After Spraying*	1	0.03 (green)
(58:38:4)	UV 254	2	0.03, 0.65
	UV 366	6	0.03 (red), 0.09 (brown red), 0.43 (red), 0.49 (red), 0.57 (red), 0.64 (red)
Pet. Ether Fraction	Before Spraying*	3	0.14 (yellow), 0.37 (light yellowish green), 0.62 (light greenish yellow)
PE:$CHCl_3$:EtOAc	After Spraying*	4	0.33 (violet), 0.58 (violet brown), 0.66 (brown), 0.73 (dark violet)
(40:54:6)	UV 254	2	0.59, 0.65
	UV 366	6	0.02 (yellowish red), 0.24 (red), 0.37 (deep red), 0.45 (red), 0.5 (red), 0.59 (deep red)

*= Daylight, UV= ultraviolet light, MeOH = methanol, $CHCl_3$= chloroform, Ac= acetone, PE = petroleum ether, EtOAc= ethyl acetate.

Table 3.17: TLC Fingerprints of the Crude (80% Methanol) Extract and Various Fractions of *O. rochetiana*.

		Observations	
Type of Extract & Solvent System	**Light Source**	**No of Spots**	**R_f Values (Corresponding Colors)**
Crude Extract	Before Spraying*	-	-
MeOH:$CHCl_3$:Ac	After Spraying*	1	0.8 (light brown)
(56:42:2)	UV 254	2	0.4, 0.59
	UV 366	3	0.4 (blue), 0.6 (blue), 0.8 (red)
Methanol Fraction	Before Spraying*	-	-
MeOH:$CHCl_3$:Ac	After Spraying*	-	-
(56:42:2)	UV 254	2	0.41, 0.61
	UV 366	2	0.38 (light blue), 0.83 (light red)
Acetone Fraction	Before Spraying*	2	0.6 (light yellow), 0.83 (yellowish green)
MeOH:$CHCl_3$:Ac	After Spraying*	1	0.81 (light yellowish green)
(56:42:2)	UV 254	2	0.41, 0.61
	UV 366	3	0.41 (blue), 0.54 (bright blue), 0.81 (red)
Chloroform Fraction	Before Spraying*	3	0.05 (light yellow), 0.58 (yellow), 0.65 (green)
$CHCl_3$:PE:Ac	After Spraying*	3	0.33 (violet), 0.58 (yellow), 0.63 (green)
(58:38:4)	UV 254	5	0.05, 0.11, 0.45, 0.57, 0.63
	UV 366	5	0.02 (blue), 0.05 (blue), 0.47 (light red), 0.57 (deep red), 0.63 (deep red)
Pet. Ether Fraction	Before Spraying*	3	0.35 (light yellowish green), 0.52 (light green), 0.6 (green)
PE:$CHCl_3$:EtOAc	After Spraying*	6	0.26 (light violet), 0.29 (violet), 0.47 (brown), 0.6 (green), 0.66 (light brown), 0.73 (violet)
(40:54:6)	UV 254	1	0.59
	UV 366	6	0.35 (light brown), 0.47 (red), 0.51 (red), 0.59 (deep red), 0.67 (red), 0.74 (red)

*= Daylight, UV= ultraviolet light, MeOH = methanol, $CHCl_3$= chloroform, Ac= acetone, PE = petroleum ether, EtOAc= ethyl acetate.

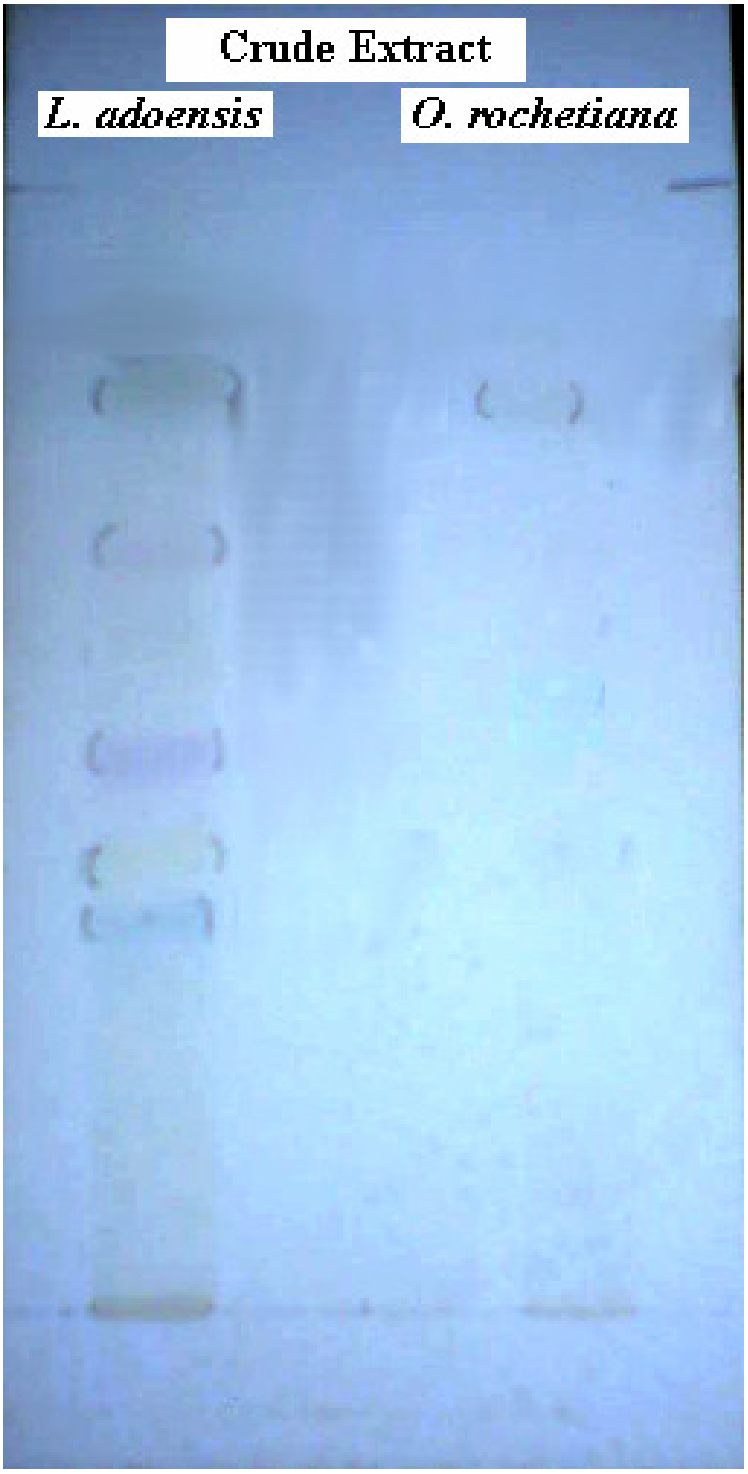

Fig. 3.1: Chromatogram of the Crude Extracts of *L. adoensis* and *O. rochetiana*.

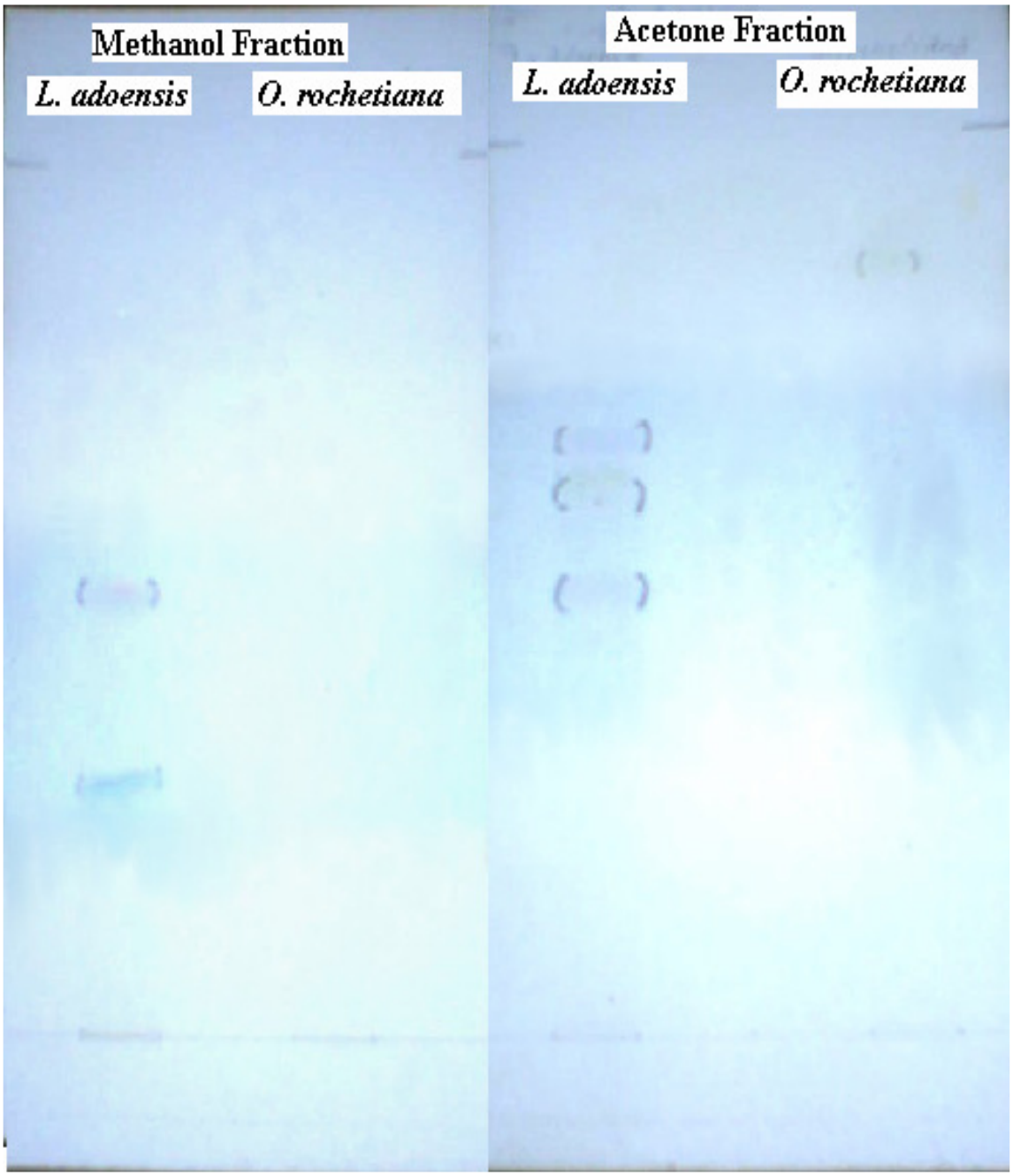

Fig. 3.2: Chromatogram of Methanol and Acetone Fractions of *L. adoensis* and *O. rochetiana*

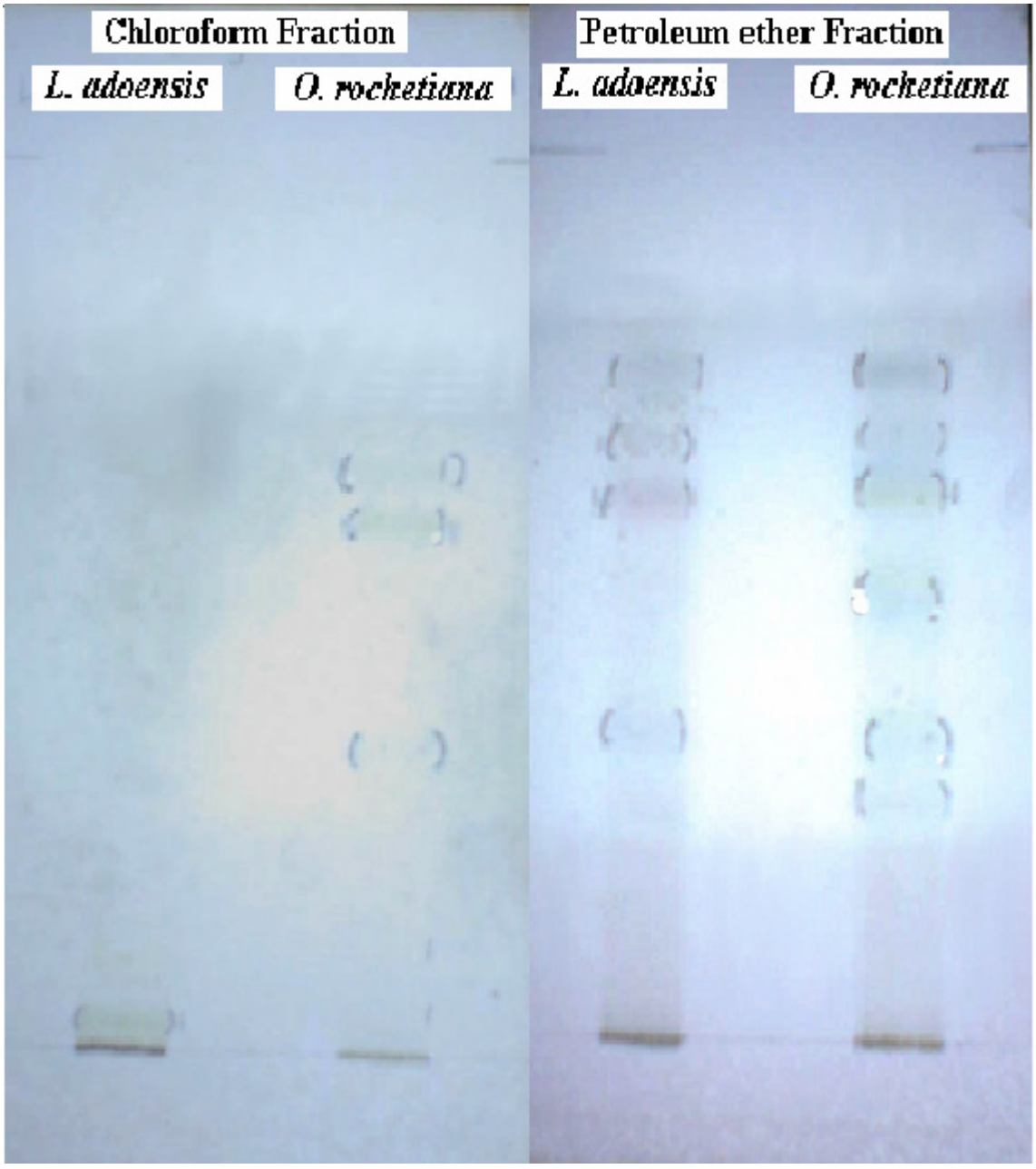

Fig. 3.3: Chromatogram of Chloroform and Petroleum Ether Fractions of *L. adoensis* and *O. rochetiana*

4. CONCLUSIONS

Therapy with herbal drugs is an old tradition and plants have been used over the years for the treatment of numerous health problems including infectious and non-infectious skin diosrders. This is because plants contain a number of phytochemical agents having potentially useful therapeutic values.

The use of conventional medications is often unsatisfactory for many patients with chronic skin disorders such as eczema, because of adverse effects and loss of effectiveness on long term uses. As a result, current trends indicate that people are shifting to non-conventional therapy. Moreover, the problem of resistnace to current antibiotics has become far more serious. Therefore, it has become necessary to search for an alternative and equally effective means of tackling the problem. As part of the efforts in searching for new alternatives, upgrading and integration of traditional medicine with the modern health care delivery system is considered to be one among the possible solutions. To this end, scientific studies aimed at proving the claims of effectiveness of medicinal plants having folkloric reputations are expected to yield fruitful results.

The herbal drugs included in the present study were selected on the basis of their local uses in the treatment of various skin disorders and the results of this study have indicated the potential of these botanicals in treating infectious skin diseases. Although the anti-inflammatory test results were not promising, the antimicrobial test results were quite impressive. The extensive use of the studied herbal drugs by the local people in treating various types of skin disorders might therefore be justified by their antimicrobial activities against different strains of bacteria and fungi, which are known to be responsible for causing varieties of skin infections.

Performance evaluation of the topical formulations of crude extracts of the herbal drugs has indicated that extracts formulated into creams are superior in performance than the ones formulated into ointments. Therefore, creams could be the best topical drug delivery alternatives for the herbal drugs in question. The values of some preliminary standardization and/or quality control parameters determined in this study for the two herbal drugs are believed to serve as baseline data providing information about the quality of the botanicals under study.

Lastly, the findings of this study suggest that *L. adoensis* and *O. rochetiana* may be considered as useful natural alternative therapy for patients with topical infectious skin disorders either alone or in combination with other suitable antimicrobial agents. Thus, further in-depth investigations on such herbal drugs with the aim of isolating the active compound(s) and optimizing the formulation for topical delivery are justified.

SUGGESTIONS FOR FURTHER WORK

1. The crude extracts of some of the herbal drugs included in this study have shown quite good antimicrobial activity indicating their potential in treating infectious diseases of the skin. Thus, fractionation of these herbal drugs and consequent antimicrobial screening of the fractions is recommended.
2. The antimicrobial screening was conducted on limited bacterial and fungal strains. So the same work should be carried out on large variety of microbial strains so as to have a clear picture of the spectrum of antimicrobial activity of the herbal drugs.
3. Anti-inflammatory test was conducted for the two herbal drugs, which showed better antimicrobial activity. The same test is recommended to be carried out for other species of plants as well. The anti-inflammatory activities of *L. adoensis* and *O. rochetiana* were not significant but similar study using large sample size of animals and other models is recommended in order to get better picture of their activity.
4. The antiviral, antioxidant and wound healing activities of the plants should be studied in order to have a conclusive idea about the application of these botanicals in the treatment of skin disorders.
5. The use of these herbal drugs over the years by the local people might be an indicative of their safety for the claimed uses. However, toxicity and skin sensitivity studies are suggested to be carried out so as to have a firm scientific ground on their safety.
6. As has been made clear in this study, the type of vehicle has a significant influence on the performance of the herbal drugs. Therefore, conducting the performance evaluation by using different types of formulation bases might give more information on the release profiles of the respective drugs from each preparation and thus assist in the selection of the best vehicle for topical delivery of the herbal drugs.

7. In order to establish a range of values that can serve as a standard monograph for the stated herbal drugs, additional data are required for each standardization parameters and thus effort should be made to generate these data and others so as to have adequate parameters for quality evaluation and standardization of the herbs.
8. It is suggested that further investigation be carried out on the identification of the active compound(s) or marker compound(s) that can be of value in the standardization of the botanicals or for the development of the active constituent into a drug.
9. All the standardization parameters determined in this study are conducted on plants collected from the same locality. Therefore, similar work is recommended to be conducted on the same species collected from different localities in the country so as to assure reproducibility of results or have information on the effect of geographical variation on the constituents and hence biological activity.

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