THE ANTIMICROBIAL EFFECT OF ESSENTIAL OILS ON DERMATOPHYTES

By

Mohammed Nasir

A Thesis Submitted to Graduate Studies Program, Addis Ababa University in Partial Fulfillment of the Requirements for the Degree of Master of Science in Applied Microbiology.

JULY 2010
ACKNOWLEDGMENTS

First and foremost, I would like to praise the Almighty Allah for protecting me from any temptation and giving me the endurance to face any challenge in the light of success.

I take this golden opportunity to express my humble gratitude and respect to my research guide Dr. Dawit Abate for his constant invaluable guidance, constant encouragement and support throughout the year. I once again heartedly thank him for his marvellous support throughout my work. You have been a fantastic mentor and I sincerely appreciate all your efforts in helping me achieve this goal.

I would then like to extend my heartfelt thanks to Addis Ababa University, Faculty of Science, School of Graduate Studies and Department of Biology for giving me the opportunity to grasp a profound knowledge and the faculty to pursue my project in a successful manner. The staff members of the university had been overall helpful to me in many ways.

I express my gratitude and sincere thanks to W/t Zenebech Aytenew, assistant of the mycology Laboratory at A.A.U. for providing facilities, which enabled me to complete this work successfully.

It's my pleasure to express my grateful thanks to Ato Nega Asamene, Laboratory technician of Ethiopian Health and Nutrition Institute (EHNRI), for his willingness to isolate, culture and providing me.
Last but not least, I am indebted to my family members and colleagues for their encouragement and for being so proud of my efforts.
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<tr>
<td>A A U</td>
<td>Addis Ababa University</td>
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<tr>
<td>ADA</td>
<td>Agar Diffusion Assay</td>
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<tr>
<td>ANOVA</td>
<td>Analysis Of Variance</td>
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<tr>
<td>ATP</td>
<td>Adenosine tri-phosphate</td>
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<tr>
<td>CFU</td>
<td>Colony forming unit</td>
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<tr>
<td>0c</td>
<td>Degree Celsius</td>
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<tr>
<td>DMSO</td>
<td>Dimethylsulfoxide</td>
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<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
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<tr>
<td>EHNRI</td>
<td>Ethiopian Health and Nutrition Research Institute</td>
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<tr>
<td>g</td>
<td>Gram</td>
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<tr>
<td>hr</td>
<td>Hour</td>
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<tr>
<td>l</td>
<td>Litre</td>
</tr>
<tr>
<td>MIC</td>
<td>Minimal Inhibitory Concentration</td>
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<tr>
<td>MI</td>
<td>$10^{-3}$</td>
</tr>
<tr>
<td>mm</td>
<td>$10^{-3}$</td>
</tr>
<tr>
<td>NCCLS</td>
<td>National Committee for Clinical Laboratory Standard</td>
</tr>
<tr>
<td>PDA</td>
<td>Potato Dextrose Agar</td>
</tr>
<tr>
<td>RNA</td>
<td>Ribonucleic acid</td>
</tr>
<tr>
<td>SD</td>
<td>Standard Deviation</td>
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<tr>
<td>SDA</td>
<td>Sabouraud Dextrose Agar</td>
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<td>SPSS</td>
<td>Statistical Products and Service Solutions</td>
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ABSTRACT

Dermatophytes are pathogenic fungi that have a high affinity for keratinized structure like nails, skin or hair, causing superficial infection known as dermatopytosis in both human and animals. The antifungal drugs like the Azoles are routinely used for treatment of infection caused by dermatophytes. They are limited in their cellular targets. Essential oils, derived from plants have been reported to be active against fungi and bacteria. In this study, the in vitro activities of four plant essential oils (Thymus schimperi, Cinnamomum Zeylanicum, Citrus limon and Eucalyptus camaldulensis) were evaluated against dermatophytes (Tricophyton spp. and Microsporum spp.). The studies were carried out using Agar disk diffusion method for screening the most effective essential oils and Agar dilution to determine Minimum Inhibitory Concentration (MIC) of the essential oils. Results showed that essential oils of Thymus schimperi and Cinnamomum Zeylanicum were highly active against dermatophytes. Citrus limon showed moderate effects and E. camaldulensis had no or little effect. The MIC values of Thymus schimperi was at the range of 0.08µl/ml to 0.31µl/ml against Tricophyton spp. and Microsporum. The MIC values of the other essential oils were in the range of 0.31µl/ml to 0.16µl/ml for Cinnamomum, 1.25µl/ml to 2.5µl/ml for limon and 5µl/ml to 2.5µl/ml for Eucalyptus against dermatophytes. Thymus schimperi and Cinnamomum Zeylanicum oils also showed antimicrobial effect against Candida albicans, Aspegilus niger, Rhodotorula, E.coli., Shigella spp., Bacillus spp. and Streptococci. The results obtained suggest that the essential oil of Thymus schimperi is effective against dermatophytes in vitro.

Key words: Dermatophytes, Essential oil, Tricophyton, Microsporum, Minimum inhibitory concentration
1. INTRODUCTION

Fungi are parasitic or saprophytic organisms that occupy a unique niche in the plant and animal kingdoms. Fungi differ from plants in that they have no chlorophyll and differ from bacteria in that they have cell walls that contain either cellulose or chitin (John, 1995). Diseases caused by fungi, or mycoses, can be clinically classified as superficial, deep, or systemic. The superficial mycoses are caused by approximately 20 fungi, including dermatophytes, yeasts and non dermatophytes (John, 1995). They possess the ability of parasitizing keratin rich tissues and produce dermal inflammatory response and intense itching in addition to a cosmetically poor appearance (Grover and Roy, 2003).

Mycoses have recently shown an important increase in their incidence because the general population is more exposed to factors that favour mycosis infection (Ruben, 2010). This increase may be a result of frequent usage of antibiotics, immunosuppressive drugs and various conditions like organ transplantations, leukemia and human immunodeficiency virus (HIV) infections (Petmy, et al., 2004).

The prevalence of superficial mycoses (Dermatophytes) has been studied in different parts of the world (Akpolat, et al., 2005). The relative occurrence of the etiologic agents of these infections varies from country to country and from one climatic region to another (Korstanje, and Staats, 1995). In tropical countries, a warm and humid climate, crowded living and poor sanitary conditions promote the spread of these infections. In Ethiopia, only a few research studies are available on the prevalence and etiological agents of superficial Mycoses. According to Yimtubezinash, et al (2005) on Tulugudu Islands, Southern Ethiopia, of 135 samples from hair, skin and finger-nail, 74.1% were microscopically positive for dermatophytes, 73% were positive in culture, giving an overall prevalence of dermatophytes in 57.3% of all the children examined.

Absence of control measures and lack of knowledge about the disease among the population in less developed societies allow the expansion of the infection without much restriction. Although
the health effect on each individual is not profound, and not life-threatening the disease impairs quality of life may be long-lasting, taxing and painful (Woldeamanuel, et al., 2005). The antifungal drugs that are routinely used for treatment of superficial mycoses, particularly infections caused by dermatophytes, the azoles, allylamines, and griseofulvin are most commonly used (Richard, 2005). However, their cellular targets are limited because of the similarity existing between fungi and hosts, i.e., both are eukaryotic organisms with some exceptions (e.g., griseofulvin), the antifungal drugs in common usage are directed against the ergosterol biosynthetic pathway. In addition to this, the occurrence of drug resistance clinical isolates leads to failure in the treatment of mycosis. Thus, the effective control of dermatophytes will necessarily involve the development of a new generation of potent broad-spectrum antifungal with selective action against new targets in the fungal cells, without irreversible side effects in the host.

In this regard Essential oils will be the best candidates for two reasons: their natural origin generally means more safety to people and environment; and they can be considered at low risk for development of microbial resistance since they are mixtures of compounds which may present different mechanisms of antimicrobial activity (Daferera et al., 2003; Nostro et al., 2004).

Spice and herbs such as Thymus schimperi (Thymus), Cinnamomum zeylanicum (Cinnamon), Eucalyptus camaldulensis and Citrus limon (Lemon) are essential oil plants commonly grown in various parts of Ethiopia. At the same time they are extensively used by local people as food preservatives, cure for various ailments and food flavouring and seasonings (Fullas, 2003).

However, the antifungal activity of these plant extracts has not been widely investigated and a little quantitative data on the antimicrobial activities of most plant extracts is reported. Therefore, in order to fill this gap of information, essential oils of some traditional spices and herbs are to be investigated for their potential in the control of dermatophytes, yeast (Candida spp., ), other fungi (Aspergillus niger, Rhodotorula rubra) and different bacteria strains ( Escherichia coli, Bacillus spp, shigella spp. and Streptococci ) growth in vitro.
2. RESEARCH OBJECTIVE

2.1. General objective

- To extract, evaluate and determine the activity of some essential oils extracted from selected plant (Thymus schimperi, Cinnamomum Zeylanicum, Citrus limon and Eucalyptus camaldulensis) which are growing in different Part of Ethiopia against Dermatophytes.

2.2. Specific objectives

- To evaluate the effectiveness of the selected essential oil extracts in inhibiting the growth of dermatophytes using Agar disk diffusion method

- To determine the MIC (minimum inhibitory concentration) of the essential oil extracts using Agar dilution method

- To evaluate the effectiveness of the selected essential oil extracts in inhibiting the growth of different fungi and bacteria using Agar disk diffusion method
3. LITERATURE REVIEW

3.1. Dermatophytes

Dermatophytes are pathogenic fungi that have a high affinity for keratinized structures like nails, skin or hair, causing superficial infections known as dermatophytoses in both humans and animals (Luciene et al., 2008). When dermatophytes infect humans, they colonize the keratinized outermost layer of the skin, and usually do not invade the living tissue (Nilce et al., 2008). Proteinases are produced by dermatophyte in vitro and play an important role in the pathogenesis of fungal infections in vivo. It has been suggested by many workers that the pathogenicity of microorganisms is related to the production of proteinases which enable them to parasitize tissues such as the stratum corneum, nails and hair (Irene and Richard, 1995).

Reactions to a dermatophyte infection may range from mild to severe as a consequence of the host’s reactions to the metabolic products of the fungus, the virulence of the infecting strain or species, the anatomic location of the infection, and local environmental factors (Irene and Richard, 1995).

3.2. Etiologic Agents

The etiologic agents of the dermatophytoses are classified in three anamorphic (asexual or imperfect) genera, *Epidermophyton*, *Microsporum*, and *Trichophyton*, of anamorphic class Hyphomycetes of the Deuteromycota (Fungi Imperfecti) (Irene and Richard, 1995). On the basis of anamorph morphology, two species of *Epidermophyton*, approximately 18 species of *Microsporum* and 25 species of *Trichophyton* are considered valid members of these genera (Mucoma, 2000).

3.2.1. Epidermophyton

The genus is characterised by large macroconidia which are thin-walled, multicellular, club-shaped and clustered in bunches. Microconidia are not produced. The genus features are based
on *E. floccosum* (Mukoma, 2000). *E. floccosum* is one of two species belonging to the genus *Epidermophyton*. It is an anthropophilic species which means it tends to infect human more than animals. Microscopically examination of *E. floccosum* colony revealed the presence abounded of macroconidia with absence of microconidia (Ali Abdul Hussein, 2009).

### 3.2.2. Microsporum

The genus produces both micro- and macroconidia. Macroconidia are multiseptate, with a thin or thick echinulate cell wall, spindle shaped and may be numerous or scarce. However, the essential distinguishing feature of this genus is the echinulations on the macroconidial cell wall. The thickness of the cell wall and shape varies depending on the species (Mucoma, 2000). Microconidia are pyriform, about 2-3μm. *Microsporum gypseum* is a geophilic fungus with a world-wide distribution which may cause infections in animals and humans, particularly children and rural workers during warm humid weather. Usually produces a single inflammatory skin or scalp lesion (Gilino *et al.*, 2008). The zoophilics, such as *M. canis*, have adapted to animals and are found in the soil only rarely. *Microsporum canis* is the etiologic agent most frequently associated with dog and cat dermatophytosis (Gilino *et al.*, 2008). Human infection through contact with animals is fairly common.

### 3.2.3. Trichophyton

This genus produces smooth walled macroconidia and microconidia. Macroconidia are thin walled and cigar-shaped. Microconidia may be pyriform 2-3μm or irregular in form. Some species rarely produce macroconidia (Mucoma, 2000).

Members of the genus *Trichophyton* are the commonest agents of dermatophytoses. They are especially significant in onychomycosis but also invade skin and hair, causing infection associated with substantial morbidity (Ajello, 1974).
3.3. Epidemiology and Ecology

3.3.1. Epidemiology

The dermatophytes are among the commonest infectious agents of man and no persons or geographic areas are free of them. Among the most common scenarios of dermatophytosis are *Tinea capitis*, *Tinea cruris*, *Tinea pedis* and *Tinea unguium* (onychomycosis) (Rinaldi, 2000). The prevalence of dermatophytoses varies in different geographical locations. According to the anatomic sites of dermatophytosis infection, *Tinea corporis* in Yemen Republic (Mahmoud, 2002) *Tinea pedis* in Croatia, (Brajac et al., 2002) *Tinea capitis* in United States (Rinaldi, 2000) were the predominant clinical forms of dermatophytosis. The immigration of labour, troop movements, emigrations and other traveling played important roles in spreading of these fungi (Pakshir and Hashemi, 2006).

3.3.2. Ecology

Dermatophytes and their congeners have long been divided into anthropophilic, zoophilic, and geophilic species on the basis of their primary habitat associations (Irene and Richard, 1995). Individual dermatophytes differ considerably in their host range and importance as agents of disease in man and animals (Mucoma, 2000).

3.3.2.1. Geophiles

Geophilic dermatophytes have their habitat reservoir in the soil, the degraded keratin or organic substances which are constantly thrown into the soil. Some species are pathogenic to both humans and animals (Lima et al., 1999). The occurrence of dermatophytes in the soil is influenced by biological and non-biological factors, such as soil pH, temperature, humidity, environmental light, climate, chemical composition and amount of organic material in the soil (Kaul and Sumbali, 1999). A few geophiles do have the additional capacity to cause ringworm in some species of animals, including man. These dermatophytes are generally contracted directly from soil containing a high number of spores and are only rarely transmitted from man to man or lower animals to man (Mucoma, 2000).
3.3.2.2. Zoophiles

Zoophilic species are basically animal pathogens, often with a single preferred animal host or very limited host range, outside which they are found only in exceptional circumstances (English, 1972). The potential source of these fungi can be pets, farm animals, or wild animals. Farmers are believed to be at higher risk of zoophilic dermatophytoses because they are in regular contact with farm animals, as well as wild animals (rats, mice, etc.) which can also transfer fungal infection (Spiewak and Szosta, 2000). M. canis, T. verrucosum and T. mentagrophytes are common agents of ringworm in animals but are also frequently associated with human infection.

3.3.2.3. Anthropophiles

The anthropophilic species are perfectly adaptable to human skin and particularly to keratinized tissue (cornea layer, hair and nails), being responsible for most cases of dermatophytoses (DA Silvapontes and Oliveira, 2008). Anthropophilic dermatophytes are mainly associated with community life. Since transmission is man to man, contracting the disease therefore requires human contact. The spread of anthropophiles is more common in communities like schools, barracks, prisons and the family (Rippon, 1982). In concentrated communities, the use of facilities such as shower-rooms, and common headgear leads to rapid spread of infection of the anthropophilic *Trichophyton* species *T. rubrum* is a very common cause of *Tinea unguium, cruris, and pedis* (Rippon, 1982).

3.4. Clinical manifestation

3.4.1. Tinea barbae

*Tinea barbae* is a rare dermatophytic infection that is limited to the bearded areas of the face and neck (Bonifaz et al., 2003). Infection occurs almost exclusively in males – teenagers and adults. Typical clinical symptoms are severe pustular eruption, deep inflammatory plaques or non inflammatory superficial patches (Trotha et al., 2003). The most common inflammatory type is typically caused by zoophilic dermatophytes – *Trichophyton mentagrophytes var. granulosum* or
*Trichophyton verrucosum*. Infections caused by other zoophilic fungi e.g. *Microsporum canis* and *Trichophyton mentagrophytes var. intrdigitale* are rare (Trotha *et al.*, 2003).

### 3.4.2. Tinea capitis

*Tinea capitis*, or scalp ringworm, is an infection caused by dermatophyte fungi mainly found in pre-pubertal children. It is characterised by infection of the hair of the scalp and scalp skin associated with symptoms and signs of inflammation and hair loss (Elewski, 2000). Transmission is fostered by poor hygiene and overcrowding, and can occur through contaminated hats, brushes, pillowcases, and other inanimate objects. After being shed, affected hairs can harbour viable organisms for more than one year (Barry *et al.*, 2003). They are often created by *Microsporums* (*M. audovinii*) and sometimes appear as the areas with alopecia with black dots for which *Trichophyton Tonsurances* and *Trichophyton Violaceum* are the most Known causes (Shahla *et al.*, 2007).

### 3.4.3. Tinea corporis

*Tinea corporis*, or ringworm, typically appears as single or multiple, annular, scaly lesions with central clearing, a slightly elevated, reddened edge, and sharp margination (abrupt transition from abnormal to normal skin) on the trunk, extremities, or face. The border of the lesion may contain pustules or follicular papules (http: //www.cdc.gov/healthypets/diseases/ringworm.htm). Both zoophilic and anthropophilic dermatophytes are common in children, and on the neck and wrists of adults in contact with the child (Barry and Hainer, 2003). In other adults, *Tinea corporis* is often the result of chronic infection with *T. rubrum*, an anthropophilic dermatophyte. In many people, untreated *Tinea corporis* resolves within a few months, particularly if it is caused by a zoophilic or geophilic organism.
3.4.4. Tinea cruris

Tinea cruris, frequently called “jock itch,” is a Dermatophyte infection of the groin. This dermatophytosis is more common in men than in women and is frequently associated with *Tinea pedis*. *Tinea cruris* occurs when ambient temperature and humidity are high (Shahla *et al*., 2007). Occlusion from wet or tight-fitting clothing provides an optimal environment for infection. *Tinea cruris* affects the proximal medial thighs and may extend to the buttocks and abdomen (Barry and Hainer, 2003). It is seen mostly with *Trichophyton rubrum* and *Trichophyton mentagrophyte* (Shahla *et al*., 2007).

3.4.5. Tinea pedis

*Tinea pedis* (Athlete’s foot) is an infection of the foot, characterized by fissures, scales and maceration in the toe web, or scaling of the soles and lateral surfaces of the feet. Erythema, vesicles, pustules and bullae may also be present (http://www.cdc.gov/healthypets/diseases/ringworm.htm). These fungi may be spread from soil (geophilic), animals (zoophilic), or humans (anthropophilic) as well as through contact with fomites (http:pedsinreview aappublications org/cgi/content/full/19/11/368). Three species of fungi, *Trichophyton rubrum*, *Trichophyton mentagrophytes*, and *Epidermophyton floccosum* are together responsible for the vast majority of cases of *Tinea pedis* throughout the world. Of these keratinophilic organisms, *Trichophyton rubrum* is the most common pathogen associated with chronic *Tinea pedis* (Weinstein and Berman, 2002). A recent study showed that *T. rubrum* accounted for over 76% of all dermatophyte infections, including *Tinea pedis* (Weinstein and Berman, 2002) and may account for over 2/3 of all *Tinea pedis* infections.

3.4.6. Tinea manuum

*Tinea manuum* is a dermatophyte infection of one or, occasionally, both hands. In this form, the palms become diffusely dry, scaly and erythematous. It is most often caused by anthropophilic dermatophytes (cases may be an extension of Athlete’s foot) but is occasionally caused by zoophilic organisms (http://www.cdc.gov/healthypets/diseases/ringworm.htm). Most common

3.4.7. \textbf{Tinea unguium}

Tinea unguium is a dermatophyte infection of the nail. It is characterized by thickened, discolored, broken and dystrophic nails. The nail plate may be separated from the nail bed. It can be caused by anthropophilic or zoophilic dermatophytes (http://www.cfsph.iastate.edu.). 

\textit{Tinea unguium}, sometimes consequential to \textit{Tinea pedis} (Tullio \textit{et al}., 2007). There are striking geographical differences in the epidemiological and etiological pattern of \textit{Tinea unguium}, especially in the frequency with which each group of dermatophyte is responsible for infection (Sofia \textit{et al}., 2000). In general, fungal diseases of nail demonstrate major differences in different regions of the world.

![Fig.1. Common types of Dermatophyte diseases A= Tinea corporis (Ringworm), B= Tinea pedis (Athlete’s foot), C= Tinea unguium](image)

4. \textbf{Medicinal plants}

The use of medicinal plants as a source for relief from illness can be traced back over five millennia to written documents of the early civilization in China, India and the Near east, but it is doubtless an art as old as mankind. Neanderthals living 60,000 years ago in present day Iraq used plants such as holly back. These plants are still widely used in ethno medicine around the world (Stockwell, 1988). The ancient-Egyptians were familiar with many medicinal herbs and were aware of their usefulness in treatment of various diseases (Abu-Shanab \textit{et al}., 2004). In Ethiopia,
medicinal plants have been used as traditional medicine to treat different human ailments by the local people from time immemorial. These medicinal plants are estimated to be over 700 species (Abbink, 1995). There is a high expectation of enormous traditional knowledge and use of medicinal plant species in Ethiopia due to the existence of diverse cultures, languages and beliefs among the people.

Medicinal plants used in traditional medicines to treat infectious diseases seem to be an abundant source of new bioactive secondary metabolites (Jürgen et al., 2009). In the recent years, research on medicinal plants has attracted a lot of attentions globally. Large body of evidence has accumulated to demonstrate the promising potential of Medicinal Plants used in various traditional, complementary and alternate systems of treatment of human diseases (Cowan, 1999).

Aromatic plants possess odorous volatile substances which occur as essential oil, gum exudates, balsam and oleoresin in one or more parts, namely, root, wood, bark, stem, foliage, flower and fruit. Essential oils, derived from aromatic medicinal plants have been reported to be active against Gram-positive and Gram-negative bacteria as well as against yeasts, fungi, and viruses. It seems that the antifungal and antimicrobial effects are the result of many compounds acting synergistically (Hammer et al., 1999). Plants are rich in a wide variety of secondary metabolites such as tannins, terpenoids, alkaloids, flavonoids, etc, which have been found in vitro to have antimicrobial properties (Dahanukar et al., 2000).

4.1. Essential oils

Essential oils are volatile, natural, complex compounds characterized by a strong odour and are formed by aromatic plants as secondary metabolites. They are usually obtained by steam or hydro-distillation (Bakkali et al., 2008). The term essential oil is concomitant to fragrance or perfumes because these fragrances are oily in nature and they represent the essence or the active constituents of the plants. They are called volatile or ethereal oils as they evaporate when exposed to air at ordinary temperatures. Essential oils are highly concentrated, low volume, high value products (Joy et al., 2001).
Essential oils are extracted from various aromatic plants generally localized in temperate to warm countries like Mediterranean and tropical countries where they represent an important part of the traditional pharmacopoeia (Naeini et al., 2009). They are liquid, volatile, lipid and rarely colored, lipid soluble and soluble in organic solvents with a generally lower density than that of water (Bakkali, and Averbeck, 2008).

There are several methods for extracting essential oils. These may include use of liquid carbon dioxide or microwaves, and mainly low or high pressure distillation employing boiling water or hot steam. Due to their bactericidal and fungicidal properties, pharmaceutical and food uses are more and more widespread as alternatives to synthetic chemical products to protect the ecological equilibrium. In those cases, extraction by steam distillation or by expression, for example for Citrus, is preferred. For perfume uses, extraction with lipophilic solvents and sometimes with supercritical carbon dioxide is favoured. Thus, the chemical profile of the essential oil products differs not only in the number of molecules but also in the stereo chemical types of molecules extracted, according to the type of extraction, and the type of extraction is chosen according to the purpose of the use. The extraction product can vary in quality, quantity and in composition according to climate, soil composition, plant organ, age and vegetative cycle stage (Masotti et al., 2003; Angioni et al., 2006). Furthermore, the method used to asses antimicrobial activity and the choice of test organism varies between results of different publications (Janseen et al., 1987). So, in order to obtain essential oils of constant composition, they have to be extracted under the same conditions from the same organ of the plant which has been growing on the same soil, under the same climate and has been picked in the same season.

4.2. Chemical composition

Essential oils are very complex natural mixtures which can contain about 20–60 components at quite different concentrations. They are characterized by two or three major components at fairly high concentrations (20–70%) compared to others components present in trace amounts. For example, carvacrol (30%) and thymol (27%) are the major components of the Origanum compactum essential oil, linalol (68%) of the Coriandrum sativum essential oil, a- and b-thuyone
(57%) and camphor (24%) of the *Artemisia herba-alba* essential oil, 1,8-cineole (50%) of the *Cinnamomum camphora* essential oil, a-phellandrene (36%) and limonene (31%) of leaf and carvone (58%) and limonene (37%) of seed *Anethum graveolens* essential oil, menthol (59%) and menthone (19%) of *Mentha piperita* essential oil. Generally, these major components determine the biological properties of the essential oils. The components include two groups of distinct biosynthetical origin (Croteau et al., 2000; Betts, 2001). The main group is composed of terpenes and terpenoids and the other of aromatic and aliphatic constituents, all characterized by low molecular weight.

### 4.2.1. Terpenoids

The fragrance of plants is carried in the so called quinta essentia, or essential oil fraction. These oils are secondary metabolites that are highly enriched in compounds based on an isoprene structure. They are called terpenes, their general chemical structure is C10 H16 and they occur as diterpenes, triterpenes and tetraterpenes (C20, C30 and C40) as well as hemiterpenes (C5) and sesquiterpenes (C15). When the compounds contain additional elements, usually oxygen, they are termed terpenoids. Example of common terpenoids is menthol and camphor (monoterpenes) and farnesol and artemisin (sesquiterpenoids). Artemisin and its derivative alpha arteether (Bakkali et al., 2008).

Terpenenes or terpenoids are active against bacteria, fungi and viruses. The ethanol-soluble fraction of purple prairie clover yields a terpenoid called petalostemumol, which showed excellent activity against *Bacillus subtilis* and *Staphylococcus aureus* and lesser activity against gram negative bacteria as well as *Candida albicans* (Hufford et al., 1993).

### 4.2.2. Aromatic compounds

Derived from phenylpropane, the aromatic compounds occur less frequently than the terpenes. Aromatic compounds comprise of Aldehyde (cinnamaldehyde), Alcohol (cinnamic alcohol), Phenols (chavicol, eugenol), Methoxy derivatives (anethole, elemicine, estragole,
Methyleugenol) and Methylene dioxy compounds (apiole, myristicine, safrole). The principal plant sources for these compounds are anise, cinnamon, clove, fennel, nutmeg, parsley, sassafras, star anise, tarragon, and some botanical families (Apiaceae, Lamiaceae, Myrtaceae, Rutaceae) (Bakkali et al., 2008).

4.3. Potential suggested uses of essential oils

Essential oils have been largely employed for their properties already observed in nature, i.e. for their antibacterial, antifungal and insecticidal activities. At present, approximately 3000 essential oils are known, 300 of which are commercially important especially for the pharmaceutical, agronomic, food, sanitary, cosmetic and perfume industries (Silva et al., 2003).

Essential oils or some of their components are used in perfumes and make-up products, in sanitary products, in dentistry, in agriculture, as food preservers and additives, and as natural remedies. For example, d-limonene, geranyl acetate or d-carvone are employed in perfumes, creams, soaps, as flavour additives for food, as fragrances for household cleaning products and as industrial solvents. Moreover, essential oils are used in massages as mixtures with vegetal oil or in baths but most frequently in aromatherapy (Hajhashemi et al., 2003; Perry et al., 2003).

4.4. The importance of essential oils as potential Antimicrobials

For a long period of time, plants have been a valuable source of natural products for maintaining human health, especially in the last decade, with more intensive studies for natural therapies (Ellof, 1998). According to World Health Organization (31) medicinal plants would be the best source to obtain a variety of drugs. About 80% of individuals from developed countries use traditional medicine, which has compounds derived from medicinal plants. Therefore, such plants should be investigated to better understand their properties, safety and efficiency (Ellof, 1998).
4.4.1. Essential oils with Antibacterial activity

In a study carried out by Chakraborty, et al (1995) it has been observed that a carbazole alkaloid “clausenol” isolated from an alcoholic extract of the stem bark of *Clausena anisata* possesses antibacterial and antifungal activity. The acetone and alcoholic extracts of the leaves of *Cassia alata* showed significant in vitro antibacterial activity against *Staphylococcus aureus*, coagulase positive *Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus stearothermophilus*, *Escherichia coli*, *Salmonella typhi* and *Salmonella dysentriae*. Further, alcoholic extracts also inhibited the growth of *Klebsiella pneumoniae* where as the acetone extract inhibited the growth of *Vibrio cholerae* (Sakharkar, and Patil, 1998).

The Alcoholic extract of dry nuts of *Semecarpus anacardium* showed bactericidal activity in vitro against 3 gram negative strains (*Escherichia coli*, *Salmonella typhi* and *Proteus vulgaris*) and 2 gram positive strains (*Staphylococcus aureus* and *Corynebacterium diphtheriae*). Subsequent studies have shown that the alcoholic extracts of different parts of the plant (leaves, twigs, green fruits) also possess antibacterial properties especially the leaf extract (Nair, and Bhide, 1996).

When *Mycoplasma pneumoniae* was treated with 0.006% Tea Tree oil in ethanol (1%) for 12hr, the cells lost their typical ‘pear-shaped’ appearance and became rounded. The rounded shape resembles mutants which have lost their virulence as a result of this morphological change and the loss of their attachment site. Tea tree oil seems to affect the intracellular cytoskeletal structure in a way that *M. pneumoniae* cells become rounded and lose their virulence (Harkenthal et al., 2000).

In Argentina, a research tested 122 known plant species used for therapeutic treatments (Anesini, and Perez, 1993). It was documented that among the compounds extracted from these plants, twelve inhibited the growth of *Staphylococcus aureus*, ten inhibited *Escherichia coli*, and four inhibited *Aspergillus niger* and also reported that the most potent compound was one extracted from *Tabebuia impetiginosa*. A more detailed study on antimicrobial compounds was done
evaluating extracts from 120 plant species from 28 different families (Santos et al., 1990). It was documented that 81 extracts obtained from 58 plants were active against *S. aureus*, and five extracts from four other plants inhibited the growth of *Pseudomonas aeruginosa*.

### 4.4.2. Essential oils with antifungal activity

Essential oils play a fundamental role in traditional medicine in some countries. Among these, essential oils from natural plant origin have been shown to be active against some pathogenic microorganisms. As it is indicated in the result of some studies that the growth of *C. albicans* can be inhibited by essential oil (Pawar, and Thaker, 2006; Saikia et al., 2001; El-Shazly, and Hussein, 2004). In this matter a number of essential oils such as Lavender, Sage, Clove and Thyme were used (Saikia et al., 2001).

Rai, (1996) observed antimycotic activity against the test pathogen *Pestalotiopsis mangiferae* in 14 medicinal plants. Maximum anti-mycotic activity was shown by *Eucalyptus globulus* (88%) and *Catharanthus roseus* (88%) followed by *Ocimum sanctum* 85.50%, *Azadirachta indica* (84.66%). *Ricinus communis* (75%) and *Lawsonia inermis* (74.33%) while the minimum activity was exhibited by *Jatropha curcas* (10%) (Venugopal, 1994).

Essential oil obtained from the herb of *Santolina chamaecyparissus* showed significant antifungal activity both in vitro (against 13 strains of Candida albicans) and in vivo (experimentally induced vaginal and systemic candidiasis in mice) (Suresh et al., 1997). It also showed activity against experimentally induced superficial cutaneous mycosis in guinea pigs by the hair root invasion test (Suresh et al., 1997).

Various publications have documented the antimicrobial activity of essential oils and plant extracts including rosemary, peppermint, bay, basil, tea tree, celery seed and fennel (Lis-Balchin, and Deans, 1997). All the oils tested exhibited different degrees of antifungal activity against
Aspergillus fumigatus and A. niger. The maximum antimycotic activity was shown by Cymbopogon martinii followed by Eucalyptus globulus and Cinnamomum zylenicum.

Thymus pulegioids essential oil has potential as a topical antifungal agent against Dermatophytes, Aspergillus, and Candida (Eugenia, et al., 2006). Other species of the genus Thymus, such us T. zygis and T. vulgaris, with high amounts of phenols, also show a broad spectrum of activity against a variety of pathogenic yeasts and filamentous fungi, including fungi with decreased susceptibility to fluconazole (Eugenia, et al., 2006).

The in vitro activity of some essential oils (EO) (thyme red, fennel, clove, pine, sage, lemon balm and lavender) was determined against clinical Dermatophyte and environmental fungal strains. The minimal inhibitory concentrations were determined by a microdilution method and by a vapour contact assay, MICs values for Dermatophyte ranged from 0.0078% to 0.5% (Tullio et al., 2006).

Eucalyptus camaldulensis were investigated for in vitro antibacterial activities against Staphylococcus. The study was carried out using agar dilution method. The result showed that Eucalyptus camaldulensis inhibited with MIC of 1.95µl/ml (Morteza et al., 2010).

4.4.3. Essential oils with antiviral activity

There is considerable evidence emerging from in vitro studies and controlled trials of the potential of plant-derived phytoantiviral agents for the treatment of human viral infections. Many essential oils were investigated towards their antiviral activity. Most of them were tested against enveloped RNA and DNA viruses, such as herpes simplex virus type 1 and type 2 (DNA viruses), dengue virus type 2 (RNA virus), and influenza virus (RNA virus), whereas only few essential oils, e.g. oregano (Origanum vulgare) oil and clove (Syzygium aromaticum) oil, were also tested against non-enveloped RNA and DNA viruses, such as adenovirus type 3 (DNA virus), poliovirus (RNA virus), and coxsackie virus B1 (RNA virus) (Bacon et al., 2003).
Glycyrrhizin, a triterpenoid glycoside obtained from *Glycyrrhiza glabra* was tested against RNA viruses like the Chandripura virus, Measles virus, Polio vaccine virus type 1, 2 and 3, Polio wild type viruses 1, 2 and 3 as well as DNA viruses like the Herpes type 1 and 2 viruses in vitro. It inhibited the DNA virus’s plaque formation at lower concentrations while the RNA viruses were inhibited at higher concentrations (Badam, 1994).

**4.5. Mode of action of essential oils**

While essential oils were extensively tested against a broad spectrum of bacteria, yeasts, and fungi, the interaction between essential oils and microbes which ultimately induces the antimicrobial activity is not well understood. Previously, Takaisi-Kikuni *et al.*, (1996) studied the effect of various amounts of the essential oil of *Cymbopogon densiflorus* on the metabolic activity, growth, and morphology of *Staphylococcus aureus*. Relatively high concentrations of the oil impaired staphylococcal growth in a bacteriostatic manner (chloramphenicol-type), and in low doses metabolism became ineffective due to energy losses in the form of heat. Ultrastructural data revealed morphological changes characteristic of the induction of bacteriolysis by bactericidal antibiotics (penicillin-type).

Hammer *et al.*, (2004) investigated the antifungal effects of tea tree (*Melaleuca alternifolia*) oil and several of its components on *Candida albicans*, *Candida glabrata*, and *Saccharomyces cerevisiae*. TTO and its components were reported to alter both permeability and membrane fluidity of the yeasts tested. Based on these results, it was assumed that the essential oils may have antimicrobial activity by influencing bacterial and fungal targets involved in cytoplasmatic and cell wall metabolism. It is stated by several researchers that especially monoterpenes will increase cytoplasmic membrane fluidity and permeability, disturb the order of membrane embedded proteins, inhibit cell respiration, and alter ion transport processes (Reichling *et al.*, 2006).
Carvacrol and thymol are able to disintegrate the outer membrane of gram-negative bacteria, releasing lipopolysaccharides (LPS) and increasing the permeability of the cytoplasmic membrane to ATP (Helander et al., 1998). Studies with *Bacillus cereus* have shown that carvacrol interacts with the cell membrane, where it dissolves in the phospholipid bilayer and is assumed to align between the fatty acid chains. This distortion of the physical structure would cause expansion and destabilisation of the membrane, increasing membrane fluidity, which in turn would increase passive permeability (Ultee et al., 2002). However some studies showed that Gram-negative bacteria were more resistant to the essential oils present in plants than gram – positive bacteria (Smith-Palmer et al., 1998).

Although cinnamaldehyde (3-phenyl-2-propenal) is known to be inhibitive to growth of E. coli at similar concentrations to carvacrol and thymol, it did not disintegrate the outer membrane or deplete the intracellular ATP pool (Helander et al., 1998). The carbonyl group is thought to bind to proteins, preventing the action of amino acid decarboxylases in E. aerogenes (Wendakoon, and Sakaguchi, 1995).

4.6. General characteristics of Medicinal plants

4.6.1. Cinnamon (*Cinnamomum Zeylanicum*)

*Cinnamomum zeylanicum* Linn bark (family: Lauraceae) is commonly used as food additive all over the world with its major use in South Asia and China. It is commonly known as Cinnamon. It is also known as Ceylon or True cinnamon. Cinnamon is extensively used as spice and flavoring agent in commercially available products (Toothpaste) and is almost entirely obtained from cultivated plants. It is an evergreen and bushy tree. It occurs in long, slender, flexible sticks about 1 meter in length and 6 mm in width, each consisting of numerous channelled pieces or single quills, about 1-2 cm wide when flattened out. The individual pieces of bark are not more than 0.5mm thick and of a dull pale brown colour. The odour is delicate, fragrant and aromatic and the taste warm, sweet and agreeable (Kokate et al., 1994).
Cinnamon is a native of Sri Lanka (formerly Ceylon) and tropical Asia. The best cinnamon grows along the coastal strip near Colombo. It has been cultivated from ancient times. It appears to have reached Egypt and Europe by the fifth century BC. This tree occurs in South India up to altitudes of 500 meters but is more common at lower altitudes, even below 200 meters (Niphade, 2006).

4.6.1.1. Chemical composition

*Cinnamomum zeylanicum* bark contains about 0.5 -10% of volatile oil, 1-2 % of tannins (Phlobatannins), mucilage, calcium oxalate, starch and sweet substance in the form of mannitol. The volatile oil is the active constituent of the drug. It is light yellow (fresh distilled) in colour and changes to red on storage (Kokate *et al.*, 1994).

Cinnamon oil contains 60-75% w/w of cinnamic aldehyde. Genuine oil also contains 4-10% of phenols (mainly Eugenol), hydrocarbons (pinene, phellandrene and caryophyllene), bezaldehyde, cumin aldehyde and small amount of ketones, alcohols and esters. Oil distilled from fresh bark samples contained a high proportion of cinnamyl acetate (Evans, 2002).

4.6.1.2. Traditional uses

The oil obtained from leaves, bark, roots and flower buds has numerous uses in traditional medicine. It is mainly used as flavouring agent in astringent powders and tinctures; it has aromatic, antiseptic and mildly astringent properties (Evans, 2002). Cinnamon tree was known to ancient physicians even before 2700 BC. The Chinese used the bark of this tree as a medicine. The Romans also knew about the medicinal value of this bark (Niphade, 2006).

In Ethiopia, cinnamon sticks have been used to flavor tea. Its powder is used as an ingredient for “berbere” (red pepper spice mix) and “awaze” (red pepper paste) and to spice foods such as “mitin shiro”(spiced, hot, powdered peas) “yeminshet abish dabo” ( bread made with spiced minced beef sauce), and “ginfilfil” ( “injera” in sauce mixed over low heat) (Fullas, 2003). In
Ethiopia, although “qarafa” has been used in teas by people to treat cold symptoms, it appears that there are no medicinal uses reported in the literature.

4.6.1.3. Antimicrobial activity

The EO is active against four Gram-positive bacteria (Staphylococcus aureus, Bacillus cereus, Enterococcus faecalis, and Listeria monocytogenes), four Gram-negative bacteria (Escherichia coli, Yersinia enterocolitica, Salmonella choleraesuis, and Pseudomonas aeruginosa), and three fungi (a yeast, Candida albicans, and two molds, Penicillium islandicum and Aspergillus flavus) (Lopez et al., 2005). The antimycotic activity of cinnamon bark due to presence of cinnamaldehyde is well known (Viollon, and Chaumont, 1994).

A substance that inhibits the activity of bacterial endotoxin was found in cinnamon bark extract 67% ethanol/water. It is uncertain whether the inhibitor actually plays a role in the defense mechanism in cinnamon bark (Azumi et al., 1997). It is also being proved that the combination of nisin and cinnamon accelerates death of Salmonella typhimurium and E. coli in apple juice and so enhances the safety of the product (Yuste, and Fung, 2004).

4.6.2. Thymus (Thymus schimperi)

The genus Thymus includes about 350 species world wide and is distributed mainly in temperate Eurasia. Thymus schimperi is represented in Ethiopia and Eritrea, but seems to be absent in tropical Africa. However, T. vulgaris is cultivated in tropical areas. The Thymus species in Ethiopia and Eritrea are restricted to afromontane and afro-alpine regions and are represented by the two endemic species, T. schimperi Ronninger and T. serrulatus Hochst (Nigist Asfaw et al., 2000).

Thymus schimperi is a perennial herb, woody at the base and 5 to 40 cm high. They have a crowded inflorescence with pink corollas and have ovate to elliptic leaves with entire margins. Its name is derived from Greek, which refers to courage, sacrifice and fumigation. In Ethiopia, it is commonly called ‘Abyssinian thyme’. It was an essence used to purify temples. In ancient
Greek, it was regarded as a symbol of courage and bravery. It is believed that thymus was found in the hay and straw bed in which Virgin Mary and Chilled Christ rested (Fullas, 2003).

4.6.2.1. Chemical composition

The chemical composition of the essential oils of various *Thymus* species has been extensively studied. Knowledge of the composition of the essential oils of *Thymus* species endemic to Ethiopia and Eritrea is, however, limited (Nigist Asfaw et al., 2000). The composition of the oil of *T. schimperi* from Bale differed from those of the three other regions (Gonder, Shewa, Wello) in having carvacrol (63%) as the major constituent. Thymol (36-38 %) dominated the oils of *T. schimperi* from Gonder, Shewa and Wello. The amounts of γ-terpinene and p-cymene in the sample from Bale were notably lower than found in the samples from the other three regions. The difference in compositions might be due to geographic and climatic conditions (Friis, 1992). *Thymus vulgaris*, belonging to the Lamiaceae family, is a pleasant smelling perennial shrub, which grows in several regions the world (Davis, 1982). Characteristics compounds of *T. vulgaris* essential oil have been established, namely: thymol (44.4-58.1%, P-Cymene (9.1-18.5%), gamma-terpinene (6.9-18.9%) and Carvacrol (2.4-4.2%) (Baranauskine et al., 2003).

4.6.2.2. Traditional uses

Thyme is cultivated all over the world and has naturalized in some areas including the north eastern USA. Thyme is used for seasoning fish, poultry, soups and vegetables, for flavouring liqueur, in herbal teas prepared for colds and flues, as well (Musa, and Jean-Claude, 2004). Fresh and dried thyme leaves are used extensively for flavouring purposes. In Ethiopia the dried leaves of *T. schimperi* and *T. serrulatus* are used to flavour tea, coffee and different kinds of stew. They are also used in traditional medicine for the treatment of headache, cough, stomach-ache, earache, liver disease and gonorrhoea (Demissew, 1993)
4.6.2.3. Antimicrobial activity

The Thyme essential oils possess antibacterial antifungal, antiviral, antioxidant and wide spectrum of pharmacological activities (Imai et al., 2001). These properties of essential oils are used in pharmacy and food industry (Kosalec et al., 2005; Pitarokili et al., 2002). The essential oils became officinal drugs in many countries what has been documented in their pharmacopoeias (Imai et al., 2001). The essential oils have found the widest use in the treatment of infectious pathologies of the respiratory and gastrointestinal systems, urinary tract as well as at various skin diseases (Vila et al., 1999).

The main essential oil in thymol, is active against salmonella and Staphylococcus bacteria. The main constituents of thyme include thymol, carvacrol and flavonoids often thought to have antibacterial, anti-flatulent and anti-worm properties. It is also used to suppress coughing, ease chest congestion and stimulate production of saliva (Barnes et al., 2002).

Maria et al., (2008) studied antimicrobial activity of the seven essential oils from three Thymus species against Salmonella enteritidis, Salmonella typhimurium, Eschericcha coli, Shigella flexneri and Listeria monocytogens. The result showed essential oil from T. hyemalis inhibited with the range of 19.6 mm to 45 mm.

4.6.3. Lemon (Citrus limon)

The Lemon is a small, straggling tree about 11 feet high, irregularly branched, the bark varying in colour from clear grey on the trunk, green on the younger branches to a purplish colour on the twigs. The evergreen leaves are ovate-oval, about two inches long, the margin serrate with sharp spines in the axils of the stalks. The solitary, five petalled flowers, white inside and tinged with deep pink outside, grow on stems in the axils. The well-known fruit is an ovoid berry, about three inches long, nipple-shaped at the end, smooth, bright yellow, indented over the oil-glands, having an acid, pale yellow pulp. About forty-seven varieties are said to have been developed during the centuries of cultivation. The fruit is small, green to yellow in color and oval in shape. C.limon originated in southeast, probably in India, Asia and Southern China (Grieve, 2010).
4.6.3.1. Chemical composition

In total 42 components have been identified in lemon peel oil. Lemon peel oil has high content of monoterpenic hydrocarbons (89.9%) with limonene (61.8%), γ-terpinene (10.6%) and β-pinene (8.1%); sesquiterpenic hydrocarbons (3.3%) were second major class of substances. The most prominent sesquiterpenes in the lemon oil were β-bisabolene (1.6%), trans-α-bergamotene (1.0%) and β-caryophyllene (0.7%). The major oxygenated components (5.1%) of the oils were found; aldehydes components (2.4%); geranial (1.3%), neral (0.7%), octanal (0.1%), decanal (0.1%) and alcohol components (0.9%); linalool (0.2%), nerol (0.1%), geraniol (0.1%) and ester components (1.8%); neryl acetate (1.2%) and geranyl acetate (0.6%) (Gulay et al., 2009).

4.6.3.2. Traditional Uses of Lemon

Oil of lemon is one of the most important flavouring oils, used widely in all kind of beverages, soft drinks, soft drinks powders and tablets, and in baked goods, such as cakes, pastries, pie fillings, confectionery, soft and hard center candies, gelatine desserts, ice creams, etc. The oil is also employed in perfumes, toilet waters, and in cosmetics to which it imparts a refreshing top note (Gamarra et al., 2006). In Ethiopia, the juice is directly sucked from the fresh fruits. Lemon juice is used in the preparation of various Ethiopian foods, such as dried fish ("yasa quanta"), boiled beets ("yeqeyisir qiqil"), fresh tomatoes and green pepper ("teematim beqaria"), spiced chicken pea bread ("yeshimbira kitfo"), and fresh chopped tomatoes blended in ‘injera’ and spices (‘yetimatim fitfit’) (Fullas, 2003).

4.6.3.3. Antimicrobial activity

Lemons contain unique flavonoid compounds that have antioxidant and anti-cancer properties. Of special interest in lemons have been flavonoids called flavonol glycosides, including many kaempferol-related molecules. While these flavonoids have been shown to stop cell division in many cancer cell lines, they are perhaps most interesting for their antibiotic effects. In several
villages in West Africa where cholera epidemics had occurred, the inclusion of lemon juice during the main meal of the day was determined to have been protective against the contraction of cholera (www.whfoods.org).

The antimicrobial activities of Turkish Citrus peel oils were evaluated using the disk diffusion method toward 9 bacteria and the results compared with those for penicillin-g, ampicillin, cefotaxime, vancomycin, oflaxacin and tetracycline. Antifungal activities were reported for Kluyveromyces fragilis, Rhodotorula rubra, Candida albicans, Hanseniaspora guilliermondii and Debaryomyces hansenii yeasts, and the results were referenced against nystatin, ketoconazole and clotrimazole antifungal agents. The Citrus peel oils showed strong antimicrobial activity against the test organisms (Gulay-kirbasilar et al., 2009).

In addition to their unique phytonutrient properties, lemons are an excellent source of vitamin C, one of the most important antioxidants in nature. Since free radicals can damage blood vessels and can change cholesterol to make it more likely to build up in artery walls, vitamin C can be helpful for preventing the development and progression of atherosclerosis and diabetic heart disease (www.whfoods.org). The pectin content of lemon is hydrophilic and hence useful to treat vomiting and diarrhea, by thickening gastric contents and regulating transit. The bioflavonoid constituents strengthen the inner lining of blood vessels, thus helping in the management of varicose veins (Fullas, 2003).

4.6.4. *Eucalyptus camaldulensis*

Eucalyptus (River red gum) is a large genus of the Myrtaceae family that includes some 900 species and subspecies (Brooker, and Kleinig, 2004). Although Eucalyptus is widely grown in many countries all over the world, Australia is probably the only one where such a single group of plants dominate most of the landscape.

Eucalyptus is a large evergreen tree 24–40(-50) m high with stout trunk often short and crooked, to 2 m in diameter; crown open, widely spreading, irregular. Bark smoothish, white, gray, or buff. Twigs reddish, long, slender, angled, drooping. Trunk can form air roots. Root system is deep and spreading. Leaves
alternate, drooping, narrowly lanceolate, 8–22 cm long, 1–2 cm wide, often curved or sickle-shaped, tapering to long point, short-pointed at base, entire glabrous, dull pale green on both surfaces or occasionally grayish. Umbels single at leaf base, ca 2.5 cm long on slender stalk 6–19 mm long. Flowers 5–10, each on slender stalk 5–12 mm long from ovoid buds 6–10 mm long, 4–5 mm wide. Stamens many, threadlike, white, 5–6 mm long; anthers with small round gland. Pistil with inferior, long-pointed, 3–4-celled ovary and long, stout style. Capsules several, clustered, hemiglobose or ovoid, 7–8 mm long, 5–6 mm wide, light brown, with wide raised disk and 3–4 prominent triangular teeth almost 2 mm long. Seeds many, tiny, 1.5 mm long, light brown (Little, 1983).

4.6.4.1. Chemical composition

The composition of the essential oils from *E. camaldulensis*, especially from the leaves, has been widely studied. Thus, the first two main components were spathulenol and *p*-cymene detected in trees from Morocco 1,8-cineole and β- pinene from Mozambique, *p*-cymene and spathulenol from Jerusalem and 1,8-cineole and limonene from Burundi (Akin *et al.*, 2010).

4.6.4.2. Traditional Uses Eucalyptus

The *Eucalyptus* has been used in folklore medicine as a remedy for sore throat and other bacterial infection of the respiratory and urinary tracts. The inhalation of the decoction vapour of the leaves is used for catarrh and nasal congestion. Essential oils of the leaves are used in the treatment of lung diseases while the volatile oils are used as expectorant and cough stimulant. These oils were stated to have antitubercular properties (Oyedeji *et al.*, 1999). Topical ointments containing eucalyptus oil have also been used in traditional Aboriginal medicines to heal wounds and fungal infections (Su *et al.*, 2006). In Ethiopia, the oils are used as an inhalant with steam and other preparations for relief of colds and influenza symptoms. Because of its refreshing odour and its efficiency in killing bacteria, the oil is also used as an antiseptic. (http://www.worldagroforestry.org/af/treedb)

4.6.4.3. Antimicrobial activity

Crude methanolic extract of *E. Camaldu-lensis* has been reported to inhibit the growth of *Candida albicans* (Adebola *et al.*, 1999). Also, it has been shown that ethanolic leaf extract of
Eucalyptus camaldulensis had marked fungicidal effect against clinical dermatophytic fungal isolates; Microsporum gypseum and Trichophyton mentagrophytes (Nakayama et al., 1990). According to recent studies carried out by Pattnaik et al., (1995) Eucalyptus leaf and its essential oil showed a great antimicrobial activity. Study by Adebolla et al., (1999) designed to evaluate the antimicrobial effect of five different Eucalyptuses via diffusion in agar revealed that the obtained MIC for Candida Albicans and gram positive and negative bacteria was 5 mg/ml.
5. MATERIALS AND METHODS

5.1. Plant sample collection

Four various types of herbs and spice plants were collected around Addis Ababa and in the compound of Addis Ababa University from December, 2009 – February, 2010 (Fig.2. and Table 1). *Cinnamomum zylianicum* (bark) and *Citrus limon* (peel) were collected from Addis Ababa, Merkato. *Thymus schimperi* (leaf with aerial parts) was collected from Debrzeit and *Eucalyptus camaldulensis* (leaf) from the compound of Addis Ababa University. The plants were brought to the laboratory and thoroughly washed in running tap water to remove debris and dust particles and then rinsed in distilled water.

**Fig. 2.** Herbs and spice plants (A= *Cinnamom zylianicum*, B= *Citrus limon peel* C=*Thymus schimperi* and D= *Eucalyptus camaldulensis*).
5.2. SAMPLE PREPARATION

The collected plant materials were dried at room temperature and ground into a semi powder using a grinder.

Table 1. Plants used as a source of essential oils and Known components of the essential oils

<table>
<thead>
<tr>
<th>Species</th>
<th>Part of plant used</th>
<th>Code</th>
<th>source</th>
<th>Known components of the essential oils</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Eucalyptus camaldulensis</em></td>
<td>leaf</td>
<td>Eu</td>
<td>AAU</td>
<td>Cineole, Terpineol, Spathulenol, P- Cymene, Limonene</td>
</tr>
<tr>
<td><em>(Thymus schimperi)</em> (thymus)</td>
<td>Leaf and aerial part</td>
<td>Ty</td>
<td>Debrzeit</td>
<td>Carvacrol, γ-terpinene, P-Cymene</td>
</tr>
<tr>
<td><em>Citrus limon</em> (lemon)</td>
<td>peel</td>
<td>Lm</td>
<td>Addis Abeba Atiklit tera</td>
<td>Monoterpenes, γ-terpinene, β-pinene, Sesquiterpene, Linalool</td>
</tr>
<tr>
<td><em>Cinnamomum zeylanicum</em> (cinnamon)</td>
<td>Bark</td>
<td>Cn</td>
<td>Addis Abeba</td>
<td>Cinnamic aldehyde, Phenols (Eugenol), Hydrocarbons, Bezaldeye, Cuminaldehyde, ketones, Alcohols, Esters</td>
</tr>
</tbody>
</table>
Sources for known components of essential oils: (Akin et al., 2010; Friis, 1992; www.whfoods.org; Kokate et al., 1994)

5.3. Extraction of the essential oils

The extraction of the spice and herbs were conducted through the process of hydrodistillation at Insect Science Laboratory Department of Biology Addis Ababa University.

The air dried aerial parts of the plants were hydro distilled by a Clevenger apparatus. About 500 g of these materials were packed in a distillation flask with approximately four times water (w/v) in volume than the plant material. Each plant material was heated in hydro distillation mantle to 70°C – 80°C for 3-5hrs.

The extracted fractions of plant parts exhibited two distinct layers an upper oily layer and the lower aqueous layer. Both the layers were separated and oils were collected in clear vials, after removing water traces using anhydrous sodium sulphate, and they were stored at 4 °C until needed.

The yields of the oils as percent of plant material volume by weight were as follows: 0.76%, 0.85%, 0.65% and 1.4% for the essential oils from Cinnamomum zylianicum, Thymus schimperi, Citrus limon peel and Eucalyptus camaldulensis respectively.
5.4. Fungi sample collection and maintenance

Two *Tricophyton spp.*, one *Microsporum nannum* and *Candida spp.* isolated from recurrent cases of vaginal candidiosis were obtained from Ethiopian Health and Nutrition Institute (EHNRI) while local isolates of *Aspergillus niger* and *Rhodotorula spp.* and four Bacteria strains (*Escherichia coli*, *Bacillus*, *shigella* and *Strepto cocci*) were obtained from Addis Ababa University, Department of Biology, Mycology laboratory.

Sabouraud Dextrose Agar was used as growing medium for *Tricophyton spp.* and *Candida spp.* while Potato dextrose agar (PDA; Himedia Ltd., India) for *Aspergillus niger* and *Rhodotorula*. The Bacteria were grown in nutrient broth and Mueller – Hinton agar. Periodic transfers were done to keep the micro organism viable.
5.5. Inoculums Preparation

Sterile distilled water containing 0.05% Tween 80 was added to the surface growth and spores and hypae were scraped off with a sterile wire loop. A spectrophotometer set at 530 nm was used to adjust the suspension to 90% transmittance. This concentration approximates about $1 \times 10^6$ CFU/ml. The appropriate media were used for the inoculation.

The bacteria used for the study were prepared by inoculating isolates into nutrient broth and incubated at 37°C for 24 h using sterile normal saline; the cells from the above cultures were resuspended to a 0.5 McFarland standard (Optical density of 0.17 at 660 nm). The cell density was approximately $1 \times 10^8$ CFU/mL.

5.6. The antimicrobial assays

5.6.1. Disk diffusion assay

The antimicrobial activity test of the essential oils against Dermatophytes, Candida spp., Aspergillus niger, Rhodotorula and Bacteria were carried out by the agar diffusion method, which is normally used as a preliminary screening of efficient essential oils (Maidment, et al., 2000) following the procedure approved by NCCLS (2002) with little modification. The agar plate was prepared for each organism as follows: a suspension of the organism (20 μl and 0.1ml of inoculums suspension for fungi and bacteria respectively) of the standardized inoculum was mixed with 20 ml of sterile Sabouraud Dextrose ® Agar for dermatophytes, PDA for other fungi (Candida, Aspergillus and Rhodotorula) and Nutrient agar for bacteria (maintained at 45-50°C in a molten state) using a mixer and then poured into sterilized Petri dishes and set aside.

After solidified, sterile filter paper disc (Whatman no.1, 6 mm in diameter) impregnated with 5μl of each essential oil were placed over the middle of the plates already seeded test organism. Sterile distilled water but not oil was added on the discs to provide negative control and Griseofulvin (0.03mg/ml) and Chloramphenicol (0.01mg/ml) were used as a positive control for dermatophytes and bacteria respectively. The plates were then left at room temperature for 30
minutes (to favour diffusion over microbial growth) and then incubated at 37 °C for 10 days for dermatophytes and plates with *Candida* and *Aspergillus* for 48 hours and plates with bacteria were incubated for 24 hours. Each test was done in triplicates. The antimicrobial activity was evaluated by measuring the diameter of the zone of inhibition (including diameter of the disk) (Maidment, *et al.*, 2000).

### 5.6.2. Determination of minimum inhibitory concentration (MIC)

The essential oils that previously showed antimicrobial activity were screened for determination of MIC by Agar dilution method (Griffin, *et al.*, 2000). The agar dilution method followed that approved by the National Committee for clinical laboratory standards NCCLS (2000) with little modification.

Two fold serial dilutions in DMSO ranging from 0.019 to 20µ/ml were tested for essential oils. 1 ml of DMSO was added to each of 10 sterile test tubes (1-10). 20 µl of the essential oil was added to test tube one. The contents were mixed and 20µl transferred to test tube two. This serial dilution was repeated through to test tube nine. 20µl was discarded from test tube 9. Then the contents of each test tube mixed with 20 ml of melted (40-50) Sabouraud Dextrose ® Agar for dermatophytes and *Candida*, PDA for *Aspergillus niger* and *Rhodotorula spp.* and Nutrient Agar for bacteria poured into a 9 cm diameter Petri dish. After solidification of the medium, 20µl of inoculum were spread evenly onto each plate. The plates were incubated at 37 °C for 10 days for dermatophytes, 24 hours for bacteria and 48 hours for the rest of the organisms. At the end of the incubation period, the plates were evaluated for the presence or absence of microbial growth. The MIC was determined as the lowest concentration of oil inhibiting the visible growth of each organism on the plates (Griffin, *et al.*, 2000).

### 5.7. Data analysis

All the measurements were replicated three times for each assay and the results are presented as mean ± SD. The statistical analysis was performed by one-way analysis of variance (ANOVA)
followed by Post Hoc Multiple Comparison Tests using statistical software (SPSS) package version 15.0 for windows and P values < 0.05 were considered as significant.

6. Results

6.1. Screening for antimicrobial activity using disk diffusion assay

Preliminary screening of the antifungal activity \textit{in vitro} of the four essential oils was studied against fungal pathogens using the filter paper disc agar diffusion technique as shown in (Table 2). All the oils tested exhibited different degrees of antifungal activity against \textit{Tricophyton spp}, \textit{Microsporum} spp. and other tested fungi. The maximum antimycotic activity was shown by \textit{Thymus schimperi} (thymus) followed by \textit{Cinnamomum} \textit{zeylanicum} (cinnamon). \textit{Thymus schimperi} has shown the greatest inhibition zone diameter of 73.33 mm against \textit{Clinical} isolates of \textit{Tricophyton spp.} and \textit{Microsporum nanum}. The oils of \textit{Citrus limon} (lemon) exhibited moderate activity and the oils of \textit{Eucalyptus camaldulensis} showed comparatively low activity against the above listed tested organisms. Control disks with distilled water showed no inhibition in a preliminary test. The difference between the essential oils was statistically significant as proofed from the results of one-way ANOVA.

Bacteria susceptibility to the essential oils also determined by the agar diffusion method and showed that Thymus oil posses’ potential antibacterial activity against \textit{Escherichia coli}, \textit{Bacillus} \textit{spp.}, \textit{shigella} \textit{spp.} and \textit{Strepto cocci}. The highest antibacterial activity of Thymus oil was 90mm in \textit{E.coli}, \textit{Shigella} \textit{spp.} and \textit{Stripto cocci} and relatively least activity was recorded in \textit{Bacillus sp.} measured 63mm (Table3). \textit{Cinnamomum zeylanicum} exhibited almost the highest activity against all the tested bacteria which measured in the range of 36mm-43mm as shown in (Table 3) next to Thymus oil. The oils of \textit{Citrus limon} (lemon) exhibited moderate activity and \textit{Eucalyptus camaldulensis} which has less or no effect with similar concentration (5µl).

As to the standard drugs used in the test, the inhibition zone for Grisofulvin was 36mm for \textit{Tricophyton} (scalp isolate) where as the inhibition diameter recorded for the standard drug chloramphenicol was 44.33 mm against \textit{Shigella} \textit{spp.} (Table 2 and Table 3).
Table 2. Antifungal activity of four essential oils against the tested fungi using disk diffusion assay. (Mean inhibition zone diameter including disk diameter).

<table>
<thead>
<tr>
<th>Tested fungi</th>
<th>Inhibition zone diameter in mm (Mean ± SD)</th>
<th>Thymus EO</th>
<th>Cinnamon EO</th>
<th>Lemon EO</th>
<th>Eucalyptus EO</th>
<th>Grisiofulvin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>73.33±2.081a</td>
<td>61.00±2.000b</td>
<td>15.33±0.577e</td>
<td>5.66±1.155f</td>
<td>36.00±1.000d</td>
</tr>
<tr>
<td>Tricophyton spp. (scalp isolate)</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>Tricophyton spp. (nail isolate)</td>
<td>72.33±2.08a</td>
<td>62.66±5.27b</td>
<td>16.33±0.577e</td>
<td>6.66±1.527f</td>
<td>32.66±1.577</td>
<td></td>
</tr>
<tr>
<td>Microsporum nanum</td>
<td>73.00±1.000a</td>
<td>62.00±2.081b</td>
<td>14.66±0.577e</td>
<td>3.66±3.214f</td>
<td>34.00±2.000d</td>
<td></td>
</tr>
<tr>
<td>Candida albicans standard</td>
<td>35.66±1.527d</td>
<td>31.33±1.527</td>
<td>15.66±0.577e</td>
<td>0.00±0.00</td>
<td>N.T</td>
<td></td>
</tr>
<tr>
<td>Aspergillus niger Local isolate</td>
<td>62.66±1.527b</td>
<td>54.66±2.081c</td>
<td>15.33±1.527e</td>
<td>10.66±0.57</td>
<td>N.T</td>
<td></td>
</tr>
<tr>
<td>Rhodotorula Local isolate</td>
<td>88.66±2.309</td>
<td>54.00±1.000c</td>
<td>42.00±1.000</td>
<td>10.00±2.00</td>
<td>N.T</td>
<td></td>
</tr>
</tbody>
</table>

N.T. = not tested
* Means with the same superscripts are not significantly different (P < 0.05)

(−) Essential oils with less or no effect
(*) Essential oils having weaker effect
(**) Essential oils having moderate effect
(*** ) Essential oils having strong effect
Table 3. Antibacterial activity of four essential oils against the tested bacteria using disk diffusion assay (Mean inhibition zone diameter including disk diameter).

<table>
<thead>
<tr>
<th>Tested Bacteria</th>
<th>Inhibition zone diameter including in mm (Mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Thymus EO</td>
</tr>
<tr>
<td></td>
<td>Cinnamon EO</td>
</tr>
<tr>
<td></td>
<td>Lemon EO</td>
</tr>
<tr>
<td></td>
<td>Eucalyptus EO</td>
</tr>
<tr>
<td></td>
<td>Chlo</td>
</tr>
<tr>
<td>E.coli</td>
<td>90.00±0.00a ***</td>
</tr>
<tr>
<td></td>
<td>42.66±1.527b **</td>
</tr>
<tr>
<td></td>
<td>26.66±1.527c *</td>
</tr>
<tr>
<td></td>
<td>16.00±2.645d *</td>
</tr>
<tr>
<td></td>
<td>43.33±0.577b **</td>
</tr>
<tr>
<td>Bacillus spp.</td>
<td>63.00±7.2 ***</td>
</tr>
<tr>
<td></td>
<td>43.33±1.527b **</td>
</tr>
<tr>
<td></td>
<td>25.66±0.577c *</td>
</tr>
<tr>
<td></td>
<td>7.66±5.89-</td>
</tr>
<tr>
<td></td>
<td>34.00±1.000b **</td>
</tr>
<tr>
<td>Shigella spp.</td>
<td>90.00±0.00a ***</td>
</tr>
<tr>
<td></td>
<td>36.66±1.527b **</td>
</tr>
<tr>
<td></td>
<td>27.00±1.000c *</td>
</tr>
<tr>
<td></td>
<td>0.00±0.000-</td>
</tr>
<tr>
<td></td>
<td>44.33±0.577b **</td>
</tr>
<tr>
<td>Streptococci</td>
<td>90.00±0.00a ***</td>
</tr>
<tr>
<td></td>
<td>41.33±2.081b **</td>
</tr>
<tr>
<td></td>
<td>20.33±0.577c *</td>
</tr>
<tr>
<td></td>
<td>14.33±0.577d *</td>
</tr>
<tr>
<td></td>
<td>42.66±1.527b **</td>
</tr>
</tbody>
</table>

* Means with the same superscripts are not significantly different (P < 0.05)

(-) Essential oils with less or no effect
(*) Essential oils having weaker effect
(**) Essential oils having moderate effect
(***) Essential oils having strong effect
Fig. 5. Growth inhibition zone on Tricophyton spp. (A= Thymus oil, B=Cinnamon oil, C= Limon oil, D= Eucalyptus oil, E=Grisofulvin, F=Control).
Fig. 6. Growth inhibition zone on Candida spp. (A= Thymus oil, B= Cinnamon oil, C= Limon oil, D= control).

Fig. 7. Growth inhibition zone on Aspergillus niger (A= Thymus oil, B= Cinnamon oil, C= Limon oil, D= Eucalyptus, E= Control).
Fig. 8. Growth inhibition zone on *Rhodotorula* (A = Thymus oil, B = Cinnamon oil, C = Limon oil, D = Eucalyptus, E = Control).

Fig. 9. Growth inhibition zone on *E. coli* (A = Thymus oil, B = Cinnamon oil, C = Limon oil, D = Eucalyptus, E = Control).
6.2. Determination of minimum inhibitory concentration

The minimum inhibitory concentration (MIC) of all the essential oils was determined by agar dilution method (Table 4). Evaluation of MIC showed that the thymus and cinnamon oils were active against all the tested isolates. *T. schimpri* essential oil exhibited significant antifungal activity. MIC values ranged from 0.08µl/ml to 0.31µl/ml against *Tricophyton* spp., *Candida*, *Rhodotorula* and *Aspergilus niger* (Table 4).

Thymus oil showed the highest MIC values (0.08µl) against *Tricophyton* spp. (scalp isolate), *Microsporum nanum* and *Rhodotorula*. Cinnamon oil also has similar effect on *Candida*, *Aspergilus* and *Rhodotorula*. The lowest concentration of the lemon essential oil at which the tested organisms were unable to grow was found to be ≥1.25µl/ml. As can be noted from the table, Eucalyptus essential oils inhibited growth of the *Tricophyton* spp. (scalp isolate) and *Aspergilus niger* at 2.5µl/ml and for the rest of tested fungal strain at 5µl/ml that were higher concentration when compared to the other oils.

The data presented in Table 4 revealed variability in the inhibitory concentrations (MIC) of each extracts for tested bacteria. The essential oils from *Thymus* and *Cinnamon* showed activities in the range (concentration) from 0.31µl/ml to 0.63µl/ml and from 0.63µl/ml to 1.25µl/ml, respectively.
Maximum activity was observed by *Thymus* oil against *Shigella* with MIC of 0.31µl/ml. Lemmon oil exhibited modest antibacterial activity with 2.5µl/ml MIC values against *E. coli*, *Shigella spp.*, *Bacillus spp.* and *Streptococi*.

Eucalyptus oil was not as effective as others; it exhibited less antibacterial activity for all tested bacteria with MIC values of 5µl/ml (Table 4).

**Table 4.** Minimum inhibitory concentrations (MIC) of the selected essential oils against the tested microorganisms

<table>
<thead>
<tr>
<th>Test organisms and corresponding MIC (µl/ml)</th>
<th>Thymus schimperi</th>
<th>Cinnamomum Zeylanicum</th>
<th>Citrus limon</th>
<th>Eucalyptus camaldulensis</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Tricophyton</em> (nail isolate)</td>
<td>0.31</td>
<td>0.31</td>
<td>2.5</td>
<td>5</td>
</tr>
<tr>
<td><em>Tricophyton</em> (scalp isolate)</td>
<td>0.08</td>
<td>0.16</td>
<td>2.5</td>
<td>2.5</td>
</tr>
<tr>
<td><em>Microsporum nanum</em></td>
<td>0.08</td>
<td>0.16</td>
<td>1.25</td>
<td>5</td>
</tr>
<tr>
<td><em>Candida spp.</em></td>
<td>0.16</td>
<td>0.08</td>
<td>2.5</td>
<td>5</td>
</tr>
<tr>
<td><em>Aspegilus niger</em></td>
<td>0.16</td>
<td>0.08</td>
<td>2.5</td>
<td>2.5</td>
</tr>
<tr>
<td><em>Rhodotorula</em></td>
<td>0.08</td>
<td>0.08</td>
<td>1.25</td>
<td>5</td>
</tr>
<tr>
<td><em>E.coli</em></td>
<td>0.63</td>
<td>1.25</td>
<td>2.5</td>
<td>5</td>
</tr>
<tr>
<td><em>Shigella spp.</em></td>
<td>0.31</td>
<td>1.25</td>
<td>2.5</td>
<td>5</td>
</tr>
<tr>
<td><em>Bacillus spp.</em></td>
<td>0.63</td>
<td>1.25</td>
<td>2.5</td>
<td>5</td>
</tr>
<tr>
<td><em>Streptococci</em></td>
<td>0.63</td>
<td>0.63</td>
<td>2.5</td>
<td>5</td>
</tr>
</tbody>
</table>

MIC expressed in µl ml⁻¹ (v/v).
7. Discussion

In this study, the antimicrobial effect of essential oils from four plants, *Thymus schimperi*, *Cinnamomum zeylanicum*, *Citrus limon* and *Eucalyptus camaldulensis* was tested against dermatophytes, other fungi and bacteria. The results in the present study indicate that essential oils extracted by hydro-distillation applied with the same concentration have variable antimicrobial effect against dermatophyte, *Candida*, *Aspergillus niger*, *Rhodotorula sp.*, *E.coli*, *Bacillus spp.*, *Shigella spp.* and *Streptococci invivito*. Among the four plants *Thymus schimperi* which is endemic to Ethiopia was overall effective against all the test organisms.

In the preliminary screening of the essential oils, *Thymus schimperi* at 5µl concentration caused higher inhibition zone with the range of 35.66 mm to 88.66 mm against tested fungi and 63 mm to 90 mm against bacteria.

Similarly, Maria *et al.*, (2008) studied antimicrobial activity of the seven essential oils from three *Thymus* species against *Salmonella enteritidis*, *Salmonella typhimurium*, *Eschericcha coli*, *Shigella flexneri* and *Listeria monocytogens*. The result showed essential oil from *T. hyemalis* inhibited with the range of 19.6 mm to 45 mm. The existence of variations in the inhibition zone can be assumed that due to differences in the number of molecules and chemical type of molecules in the plant materials (Masotti *et al.*, 2003 and Angioni *et al.*, 2006).

In this study the essential oil extracted from *Cinnamomum zeylanicum* bark demonstrated strong antifungal and antibacterial activity next to *Thymus schimperi*. The antifungal activity of *Cinnamomum zeylanicum* exhibited 61 mm, 62.66 mm, 62 mm, 31.33 mm, 54.66 mm and 54 mm inhibition zone against Tricophyton spp. (scalp isolate), *Tricoophyton spp.* (nail isolate), *Microsporum nanum*, *Candida spp.* *Aspergillus niger* and *Rhodotorula spp.* respectively. It also showed inhibition zone of 42.66 mm, 43.33 mm, 36.66 mm and 41.33 mm against *E.coli*, *Bacillus spp.*, *Shigella spp.* and *Streptococci* respectively.

Similarly, Lopez *et al.*, (2005) reported antimicrobial activity of *Cinnamomum zeylanicum* against four Gram-positive bacteria (*Staphylococcus aureus*, *Bacillus cereus*, *Enterococcus*
faecalis, and Listeria monocytogenes), four Gram-negative bacteria (Escherichia coli, Yersinia enterocolitica, Salmonella choleraesuis, and Pseudomonas aeruginosa), and three fungi (a yeast, Candida albicans, and two molds, Penicillium islandicum and Aspergillus flavus). The antimycotic activity of cinnamon bark is due to presence of cinnamaldehyde is well known (Viollon, and Chaumont, 1994).

In the present study, the Lemmon peel oil inhibited the tested fungi and bacteria in the range of 14.66 mm to 42 mm and 20.33 mm to 27 mm respectively. Therefore, in this study the antimicrobial effect of lemmom oil taken as a moderate activity when compared to Thymus and Cinnamon oil against tested organisms.

In other studies, Gulay-Kirbasilar et al., (2009) the Citrus peel oil showed similar antimicrobial activity against Candida albicans, Rhodotorula, Escherichia coli, Staphylococcus aureus, and Bacillus cereus. The Lemmon peel oil inhibited the tested bacteria and fungi in the range of 13mm-16mm, 13mm-14mm respectively. The present study results were better than the previous study. This may be due to variations in climate and soil composition in which the plant growing (Masotti et al., 2003 and Angioni et al., 2006).

In this study, E. camaldulensis showed less inhibition zone against Shigella spp. and Bacillus spp. with the inhibition diameter of zero and 7.6 mm respectively. These results were recorded as the least antimicrobial effect against the tested organism. However, the result of E. coli and Strepto cocci in this study were 16mm and 14.3mm respectively and taken as a moderate activity.

In contrast to the present study, Babay, et al., (2004) tested the efficacy of methanol extract of E. camaldulensis against Salmonella typhi, Staphylococcus aureus and Bacillus subtilis and reported a higher inhibition zone in a range of 15mm-16mm. In this case, the solvent extract of E. camaldulensis is better than the hydrodistilled essential oil against tested organism. These differences come probably due to method of extraction. Masotti et al., (2003) and Angioni et al.,
(2006) reported that, the extraction product can vary in quality, quantity and in composition according to the type of extraction method.

The result obtained in the present study showed that the oil of Thymus did not show any selectivity towards the tested gram positive and gram negative bacteria. However, some oils appeared more active with respect to Gram reaction, exerting a greater inhibitory activity against Gram positive bacteria (Smith – palmer., 1998). In this case, Thymus schimperi seems to better than other essential oils.

Among the plants studied, the Thymus oil has been found to exhibit an overall superiority in its antimicrobial activity compared to other plant extracts according to the agar diffusion method. The MIC values of thymus were with the range of 0.08µl/ml to 0.031µl/ml against Tricophyton spp. (scalp isolate and nail isolate), Microsporum nannum and Rhoduturula spp.

Cinnamon oil also showed similar result with the same concentration on Microsporum nannum, non Dermatophyte fungi (Candida spp., Aspegilus niger and Rhodotorula). These results are in line with the work of Eugenia et al., (2006) who had reported that Thymys pulegioids essential oil showed the lowest MIC value in the range of 0.16µl/ml to 0.32µl/ml for dermatophyte and 0.32µl/ml to 0.64µl/ml for Candida. This indicates that plants rich in a wide variety of secondary metabolites such as tannins, alkaloids, terpenoids which have been found invitro to have antimicrobial property (Dahanukar et al., 2000).

The MIC of Citrus limon peel oil was 1.25µl/ml against Rhodotorula and Microsporum nannum. On the other hand, 2.5µL/ml was recorded for the rest of the tested organisms. Therefore, Rhodotorula and Microsporum nannum were more susceptible to limon peel oil than other tested organisms. However, the result obtained in limon oil was not comparable from the result of Thymys schimperi and Cinnamomum zeylanicum which showed better antimicrobial activity for all tested organisms. This variation was due to the difference in plant type and part (organ) used in the experiment (Masotti et al., 2003 and Angioni et al., 2006).
In the present study, MIC of the essential oil of *E. camaldulensis* was in the range of 2.5µl/ml to 5µl/ml against all the tested organisms. The obtained results were in contrast to Morteza *et al.*, (2010) who had recorded 1.95µl/ml against *Staphylococcus aureus*. This variation may due to the difference in the tested organisms. The method used to assess antimicrobial activity and the choice of test organism varies between results (Janssen *et al.*, 1987).

From the present study, it can be concluded that *Thymys schimperi* and *Cinnamomum zeylanicum* essential oils cold be an alternative source against dermatophytes and all other tested organisms. Therefore, further *invivo* study and identification of the chemical compound would be expected to enhance the observed activity.

In general, recent studies encouraged the investigation of antimicrobial properties of plant derived essential oils. Use of these essential oils in controlling skin disorder and other human diseases could reduce risk of drug resistance and also they are safe and target specific (Ellof, 1998).
8. Conclusion and Recommendations

8.1. Conclusion

The results of this study have shown that, *Thymus schimperi* and *Cinnamomum Zeylanicum* can be used as potential candidates for preparation of antidermatophytic drug formulations and thus may be useful in the treatment of different kinds of dermatophytes in both humans and animals.

Regarding the susceptibility of *Candida spp.*, *Aspegilus niger*, *Rhodotorula spp.*, *E.coli.*, *Shigella spp.*, *Bacillus spp.* and *Streptococci* to the *Thymus schimperi* essential oils tested, it was verified that all the tested organisms were susceptible to the Thymus oils. The oil also did not showed any selectivity between the tested Gram positive and Gram negative bacteria. Therefore, these results suggest that *Thymus schimperi* having the potential to be used as antimicrobial of human diseases.

Though, the antifungal activity of the remaining essential oils are less effective than the two most effective oils, their antimicrobial activity are not regarded as useless.
8.2. Recommendations

- Further research is needed to identify active elements in the essential oil of *Thymus schimperi*.
- Only a few genera of microorganisms were used in the study. Antimicrobial tests are recommended to be done on more strains of the same genera and more species that are of relevance to health.
- The results described above did not tell the possible mechanisms of action these antifungal essential oils and it require further investigation.
- Further studies are required to study the effect of these essential oils in experimental animals (*in vivo*) and to establish if they could be safely used as antifungal agent against dermatophytes and other infectious diseases.
- There is need for further in depth studies to formulate essential oils of common medicinal plants grown in Ethiopia into an efficient, cost effective and ecologically friendly to overcome the common dermatophyte disease and other microbial infections.
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DECLARATION

I, the undersigned, declare that this thesis is my own work in design and in execution. It has never been submitted in any institution and that all sources of materials used for this thesis have been duly acknowledged.

Name: Mohammed Nasir

Signature: ………………………

Date of submission: 23/06/2010

This thesis has been submitted to examination with my approval

Advisor: Dr. Dawit Abate

Signature: ………………………

Date received: