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Antimicrobial activity of Ethiopian medicinal plants

by

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Certification

I, Eva Bernášková, declare that this diploma thesis, submitted in partial fulfillment of the requirements for the degree of Master of Science, in the Faculty of Tropical AgriSciences of the Czech University of Life Sciences Prague, is wholly my own work unless otherwise referenced or acknowledged.

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Abstract

In vitro antimicrobial activity of eighteen Ethiopian medicinal plant species that were selected based on ethnobotanical information on their traditional use to treat infectious diseases was determined by the broth microdilution method. The antimicrobial activity of ethanol extracts of selected plants against potentially pathogenic microorganism such as *Bacillus cereus*, *Bacteroides fragilis*, *Candida albicans*, *Clostridium perfringens*, *Enterococcus faecalis*, *Escherichia coli*, *Listeria monocytogenes*, *Pseudomonas aeruginosa*, *Salmonella enteritidis*, *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Streptococcus pyogenes* was determined. The minimum inhibitory concentrations were obtained by measurement of bacterial and yeast growth. Seventeen from twenty-two ethanol extracts showed antimicrobial activity at least against one of twelve microorganisms. Among all plants tested *Embelia schimperi*, *Ocimum lamiifolium* and *Rubus steudneri* showed the most promising antimicrobial properties, inhibiting most Gram-positive bacteria with minimum inhibitory concentrations greater or equal to 64 and 128 μg/ml. These results call for further investigations focused on isolation and characterization of compounds, which are responsible for the antimicrobial activity of the most effective medicinal plants.

Keywords: Antimicrobial activity, Ethiopian medicinal plants, broth microdilution method.

Abstrakt

V rámci této práce byla zkoumána antimikrobiální aktivita osmnácti etiopských léčivých rostlinných druhů, jenž byly vybrány na základě ethnobotanických informací o jejich použití v tradiční medicíně k léčbě infekčních onemocnění. *In vitro* antimikrobiální aktivita byla stanovena pomocí bujonové mikrodiluční metody. Antimikrobiální aktivita ethanolových extraktů z vybraných rostlin byla testována proti potencionálně patogením mikroorganismům jako jsou např. *Bacillus cereus, Bacteroides fragilis, Candida albicans, Clostridium perfringens, Enterococcus faecalis, Escherichia coli, Listeria monocytogenes, Pseudomonas aeruginosa, Salmonella enteritidis, Staphylococcus aureus, Staphylococcus epidermidis* nebo *Streptococcus pyogenes.* Minimální inhibiční koncentrace byly získány na základě spektrofotometrického stanovení růstu testovaných mikroorganismů. Sedmnáct z dvacetidvou ethanolových extraktů prokázalo antimikromiální aktivitu přinejmenším na jednom z dvanácti mikroorganismů. *Embelia schimperi, Ocimum lamiifolium* a *Rubus steudneri* prokázaly největší antimikrobiálních účinek inhibicí většiny grampozitivních bakterií s minimálními inhibičními koncentracemi vyššími nebo rovny 64 a 128 µg/ml. Výsledky vyžadují další výzkum zabývající se izolací chemických látek odpovědných za silnou antimikrobiální aktivitu účinných léčivých rostlin.

Klíčová slova: Antimikrobiální aktivita, etiopské léčivé rostliny, bujonová mikrodiluční metoda.

Contents

List of Abbreviations

AD	*Anno domini*
ATCC	American Type Culture Collection
BC	Before Christ
CBD	Convention on Biological Diversity
CFU	Colony-forming unit
CJB	The Conservatory and Botanical Garden of the City of Geneva
DL	Discover Life
DMSO	Dimethyl sulfoxide
EOL	Encyclopedia of life
ICRAF	International Center for Research in Agroforestry
MIC	Minimum inhibitory concentrations
NTBG	The National Tropical Botanical Garden

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1 Foreword

Infectious diseases caused by bacteria, fungi, viruses and parasites are still a major threat to public health, despite tremendous progress in medicine. Their impact is particularly huge in developing countries like Ethiopia due to the relative unavailability of pharmaceuticals and the emergence of widespread drug resistance.

Plants have been resisting the continuous attacks of microorganism such as bacteria, fungi and viruses for ages. They developed chemical defensive mechanism based on producing secondary metabolites, such as tannins, terpenoids, alkaloids, flavonoids and others. It is well known that, these compounds have antimicrobial properties.

Man used the antimicrobial drugs against microbes since times immemorial. Fossil records revealed that the humans 60 000 years ago in Mesopotamia (Iraq) used a medicinal plant such as hollyhock (*Alcea rosea*) (Cowan, 1999), which also possess antimicrobial effect. The applications medicinal plants have been employed as remedy long before the development of western medicine. In 1929 Alexander Fleming isolated antibacterial compounds from *Penicillium* sp. and called this antibiotic penicillin. Discovery of penicillin led to later discoveries of antibiotics such as aureomycin, chloromycetin, streptomycin and many others. Scientists relied more and more on synthetic and semi-synthetic antibiotics and the use of plant derivatives as antimicrobials has been neglected. Unfortunately, when antibiotics are dose incorrectly, the bacteria will directly adapt and develop resistance. With its rapid multiplication, bacteria passes resistant genes through plasmid exchange and this leads to an increasing prevalence of multi-drug resistant infections. Recently, the global problem of dramatic development of bacterial resistance to synthetic antibiotics has led researchers to consider the use of other natural products.

Although soil microorganisms or fungi produce the most antibiotics, higher plants are also a source of antimicrobial agents. Artemisinin from sweet wormwood (*Artemisia annua*) and quinine from bark cinchona tree (*Cinchona* sp.) treat malaria caused by *Plasmodium falciparum*. Other drug, quinidine can be also used for treatment malaria but is not considered to be a principal medicine. Another source of antimicrobial agents is the monosaccharide fructose in cranberry and blueberry juices. This component competitively inhibited the

adsorption of pathogenic *E. coli* to urinary tract epithelial cells (Ciocan and Bara, 2007). Cranberry has been effective *in vitro* and *in vivo* in animals for the prevention of urinary tract infection. Alkaloid emetine obtained from the underground part of ipecacuanha (*Cephaelis ipecacuanha*), and related species, has been used for many years as amoebicidal drug and for treatment of liver abscesses due to the spread of *Entamoeba histolytica* infections (Ciocan and Bara, 2007). Many other plant species with antimicrobial activity are used in medicine, food or cosmetic industry. The most mentioned are basil (*Ocimum basilicum*), bearberry (*Arctostaphylos uva-ursi*), clove (*Syzygium aromaticum*), eucalyptus (*Eucalypthus citriodora*), garlic (*Allium sativum*), ginger (*Z. officinale*), goldenseal (*Hydrastis canadensis*), jambol (*Syzygium cumini*), lemon balm (*Melissa officinalis*), lemongrass (*Cymbopogon citratus*), Mexican mint (*Plectranthus amboinicus*), oregano (*Origanum vulgare*), peppermint (*Mentha piperita*), rosemary (*Rosmarinus officinalis*), rosemary-pepper (*Lippia sidoides*), Ceylon cinnamon (*Cinnamomum zeilanicum*), St. John's wort (*Hypericum perforatum*), tarragon (*Artemisia dracunculus*), tea tree (*Melaleuca alternifolia*) and thyme (*Thymus vulgaris*) (Belay *et al*., 2011; Ciocan and Bara, 2007; Farnsworth *et al*., 1985; Rios and Recio, 2005).

Plants are the largest source of biochemical and pharmaceutical compounds ever known on our planet and human use only a small portion of these compounds. The most of these components, which have still an enormous potential for medicine, remain unexploited. According to Lewis and Ausubel (2006) and Sibanda and Okoh (2007) plant antimicrobial agents are individually relatively weak but function in synergy. Plants could be also a source of compounds that can increase the sensitivity of bacterial cells to antibiotics and thereby they can help in a battle against drug resistant bacteria and fungi (Hostettmann *et al.*, 2000).

The documentation of medicinal uses of plants is becoming increasingly urgent because of the rapid loss of the natural habitats and anthropogenic activities, which are threats to the survival of many potential valuable medicinal plants. In Ethiopia, ethnoveterinary and ethno-medicinal surveys conducted in different parts of the country show use of different medicinal plants for treatment of various infectious diseases by traditional healers.

Reports of antimicrobial uses of indigenous plants have been published from many regions, but the direct antimicrobial effects of these plant species are still poorly known. The aim of this study is carry out antimicrobial screening of principal medicinal plant species from the

Ankober district, North Shewa Zone, Amhara Region, Ethiopia and thereby verify traditional uses of these plants for antimicrobial properties.

2 Introduction

2.1 African Traditional Medicine

Africa is considered to be the cradle of mankind and its traditional medicine is the oldest and perhaps the most diverse of all medicinal systems. As well as Chinese, Indian and Japanese traditional medicinal systems, African folk medicine has also its characteristic features. In contrast to western medicine, which is technically and analytically based, traditional African medicine takes a holistic approach: good health, disease, success or misfortune are believed to arise from the actions of individuals and ancestral spirits according to the balance or imbalance between the individual and the social environment (Bodeker, 2009; Cunningham, 1993). African healer is not only medicine man, but also diviner, adjudicator, protector against natural and supernatural forces and enhancer of success (Kebede *et al.*, 2006). African traditional healers are experts of the mind. They have enormous wisdom, expertise, and knowledge regarding the psyche, society, tradition and social problems (Kiteme, 1976). Very important in the therapy process is the power of the word from a doctor´s mouth. The religion and historical development has also significant influence on form of traditional medicine. Most of African traditional medicine consists of mixtures of various herbs, whereas European drugs are mainly isolated compounds obtained from single plants. Another characteristic is the dominant use of edible plants as medicines, in contrast with the modern medicine in which drugs are essentially poisons if they are taken in regulated doses (Iwu, 1994).

2.1.1 Ethiopian Traditional Medicine

The Ethiopian folk medicine is a subcategory of the African traditional medicine with some influence from Egypt, Greece and Arabia that has its own characteristic features. The widespread use of traditional medicinal plants is characteristic with various levels of sophistication. It is connected with religious thinking and various beliefs (Bekele, 2007; Fullas, 2007). Traditional medical knowledge is passed on from generation to generation. Practices and remedies are recorded in medico-religious manuscripts and traditional

pharmacopoeias. Traditional medicine is largely practiced by medicine practitioners, but also at home by elders and mothers.

Religious practices such as praying and going to church play a large part in the healing process. Holy water (*tsebel*) for Orthodox Christians, (*zemzem*) for Moslems is also frequently used for a wide variety of illnesses. Ethiopians believe that holy water cures when it is drunk or bathed in (Kebede *et al.*, 2006). They also believe in protecting power of amulets and arm rings. Hairstyle, eye make-up, cultural rituals and scarification are also supposed to protect from the evil eye (*buda*) (bewitchment) and evil spirits (Kebede *et al*., 2006).

In general, the traditional medicine in Ethiopia includes mainly medicinal preparations from plant, animal and mineral substances, as well as spiritual healing, traditional midwifery, hydrotherapy, massage, cupping, counter-irritation, surgery and bone setting.

Traditional Ethiopian medicine practice can be divided into preventive and curative. To the preventive approach belong for example deserting places where the epidemics occurred, isolating people with contagious diseases and sweeping or covering floors with particular plants. Curative practice is used to treat a variety of disorders and diseases of gastrointestinal tract (constipation, diarrhea, vomiting, dysentery, gastritis), respiratory tract (sinusitis, tonsillitis, epiglottitis, tuberculosis), lymphatic system (mumps, elephantiasis), integumentary system (tinea versicolor, tinea capitis, ringworm, eczema, skin lesion, wounds, allergic rushes, scabies, smallpox, abscess, leishmaniasis), sensory system (eye infection, trachoma, otorrhoea (discharge from ears)), circulatory system (hypertension), musculoskeletal system (rheumatism), disorders caused by intestinal parasites (ascariasis, taeniais, roundworm) and disease caused by parasites (malaria), metabolic diseases (diabetes), sexually transmitted infections (syphilis, gonorrhea) and other disorders and diseases like *Mich*, which is febrile disease characterized by fever, headache, sweating and sometimes lip sores (Giday *et al*., 2009a; Teklehaymanot *et al.*, 2007), epidemic disease *Tesbo Beshita*, cancer *Nagarssa*, swellings, headache, cold, cough, malaise (feeling of discomfort), mental disorders and others.

Healers obtain their drugs from natural substances mainly from plants. Only certain selected parts of plants, mainly leaves, roots and stems are processed fresh or dried. Squeezing, powdering, crushing, chewing, decoction and infusion are the most frequently used methods for preparation of traditional medicines. Water is the most frequently used diluent in the

preparation of the remedies. In very few cases, cow urine, a local beer (*tella*) or a fermented drink (*tej*) are also used (Giday *et al*., 2007).

Certain plants are mentioned to be applied only in combination with other medicinal substances for synergy the action of the drugs. In some cases are remedies for oral administration flavored by salt, sugar, honey or boiled coffee (Wabe *et al*., 2011). Ointments and liquid medicinal substances can be mixed with such additives as raw butter, honey or animal fat (Abebe, 1986).

Healers use various units of measurement such as finger length (for root, bark and stem), pinch (for powdered plant parts), volume of a coffee cup and numbers (for leaves, seeds, fruits and flowers) to estimate the dosage of the medicine. For example, Teklehaymanot *et al*. (2007) mention that people around Debre Libanos monastery in central part of Ethiopia dose for preparation certain remedies seven young leaf of *Calpurnia aurea* and six seeds of *Maesa lanceolata*. Naturally, the most important features for estimation the dosage are age and physical appearance of individuals.

Medicaments are prepared in various forms including liquids, ointments, powders and pills. Less common is bathing and smoking. Remedies are administered using different routes, the main are dermal, oral and respiratory route. Drugs are stored usually in containers such as bottles, papers, pieces of cloth, leaves and horns, and are kept anywhere at home. In the research of Gedif and Hahn (2002) from rural central Ethiopia, the most commonly stored plants at home are herbs such as *Lipidium sativum*, *Ocimum lamiifolium*, *Ruta chalpensis* and *Zingiber officinale*.

Traditional remedies are sold in every open market in Ethiopia, especially in the rural areas. Market vendors selling these materials are most often women. Traditional remedies are usually sold to the public together with other materials such as spices, salt and other food items. Most of the local trade of open market is dominated by a few species including e.g. *Allium sativium*, *Artemisia* spp., *Embelia schimperi*, *Glinus lotides*, *Hagenia abyssinica*, *Nigella sativa*, *Ruta chalepensis* and *Z. officinale*. The export trade includes *Catha edulis* and gums from various species of *Boswellia* and *Commiphora* but these plants are known to be primarily traded for their non-medicinal uses.

Although remedies are available in a market, most of the families prefer grow or gather medicinal plants in their vicinities of homes. Mesfin *et al.* (2009) mention frequent occurrence of plants such as *Artemisia abyssinica*, *Artemisia afra*, *Rhus vulgaris*, *R. chalepensis*, *O. lamiifolium* and *Thymus serrulatus* in home gardens.

In Ethiopia up to 80 % of the population use traditional medicine due to the cultural acceptability of healers and local pharmacopeias, the relatively low cost of traditional medicine and difficult access to modern health facilities (Bishaw, 1991; Kebede *et al*., 2006).

2.1.2 History of Ethiopian Traditional Medicine

Already 5000 years ago ancient Egyptians, Greeks and Romans traded unique commodities like frankincense, myrrh and other plant products originated from Ethiopia and also used these substances for medicine preparation. To these dates, the medicinal use of frankincense and myrrh is well documented. The history of cultivation and use medicinal plants in Ethiopia can be traced back to the reign of Queen Sheba (992 BC) when spices and essential oil bearing plants were used (Bekele, 2007). In the time of the Axumite kingdom (7th-11th AD), about 8000 plants were used as medicinal agents. Many manuscripts attest to this fact. These documents are now in the custody of the Ethiopian National Traditional Medicine Preparation and Therapy Association. The period of the Axumite kingdom was followed by the Zagwe dynasty (11th-13th AD), during which time about 2800 medicinal plants were recorded to have been used. Ethiopia's ancient church practices have documented some of the knowledge as inscribed in Parchments, which partly characterize the traditional medical system written in Geez manuscripts of the 15th century (Fullas, 2007). Other ancient written sources include the book of remedy (*Metsehafe Fews*) from the 17th century, which contains a wide range of medicinal plants prescription. These are the medical traditions of the followers of Coptic Christianity (Bekele, 2007). During the era of Gondarine kingdom (1636-1865), about 2900 medicinal plants were used. Another manuscript dated to the era of King Menelik II over 100 years ago was also recovered from the ruins of Aba Jifar´s palace in Jimma. It includes about 589 plants that were used as therapeutic agents. It has also been recorded that about 700 medicinal plants have been used during the reigns of King Hailemelekot through Emperor

Haile Selassie I (1870-1974) (Fullas, 2007). The number of plants may have been exaggerated, especially in the older manuscripts, considering the fact that only about 7500 plant species are known to exist in Ethiopia today. It is also possible that many of the plants may have been counted more than once or some plant species could have been extinct since the times of the manuscripts (Fullas, 2007).

Selassie (1971), where are described the uses of over 200 plants, Strelcyn (1973), where are listed the medical applications of 300 plants, Getahun (1976), where are described more than 120 medicinal and poisonous plant, Abate (1989), included descriptions of over 250 medicinal herbs, Abebe and Ayehu (1993), where are described uses of about 240 medicinal plants, belong to the publications which listed currently used Ethiopian medicinal plants. According CBD (2009) in Ethiopia are recorded about 887 plant species used in human medicine. The majority of these medicinal plants are herbs, followed by shrubs and trees. Twenty four (2.7 per cent) of the medicinal plant species are endemic (Bekele, 2007).

2.1.3 General Approaches

As well as Africa would appear to be extremely heterogenous with over 2000 distinct tribes (Iwu, 1994), many languages and dialects, Ethiopia with 90 languages of ethnic groups is also extremely various (Lewis, 2009). Each ethnic minority corresponds to its unique cultural and indigenous knowledge of medicinal plants. This knowledge is unequally distributed among community members. Several studies report the ethnomedicinal plant knowledge of different Ethiopian ethnic groups such as Shinasha, Agew-awi and Amhara (Giday *et al.*, 2007), Bench (Giday *et al.*, 2009a), Berta (Flatie *et al*., 2009), Kafficho people (Awas and Demissew, 2008), Me'e (Abbink, 1993), Meinit (Giday *et al*., 2009b), Oromo (Yineger *et al*., 2008b), Zay (Giday *et al*., 2003) and people in Dek Island (Teklehaymanot, 2009).

Peoples in different location with different religions (Christian, Islamic or indigenous religions), linguistic and cultural backgrounds have their own specific knowledge about use of plants. Getahun (1976) mentions that e.g. priests, students of the church or farmers are doctors when it is necessary.

2.1.4 Ethiopian Medicinal Plants

Ethiopian plants have shown very effective medicinal value for some ailments of human and domestic animals. In traditional veterinary medicine, anthelmintic plants such as *Allium sativum*, *Capsicum annuum*, *Lagenaria siceraria*, *Ricinus communis* and *Z. officinale* are used (Mesfin *et al.*, 2009). Many species of genus *Maytenus* and *Phytolacca dodecandra* are used in veterinary and human medicines (Bekele, 2007). Getahun (1976) mentions that investigations of *Maytenus ovatus* have shown that this plant is also an important source of anticancer drug. Many species of this genus have antimalarial properties. *P. dodecandra* is known for the control of schistosomiasis, which claims thousands of lives in Ethiopia every year. The efficacy of this plant has been scientifically determined (Bishaw, 1991; Girma, 1998). Various parts of this plant are also used against ascariasis, scabies, gonorrhea, malaria, rabies and syphilis.

Some species e.g. *Lantana camara*, *Momordica foetida*, *O. lamiifolium*, *Vernonia amygdalina* and *Withania somnifera* are used in medicine for treatment malaria. *W. somnifera* is also for treatment of joint infection and arthritis. Studies have shown that this plant indeed exhibits antibiotic, anti-inflammatory and antimalarial activities (Fullas, 2007). In the ethnobotanical research of Mesfin *et al.* (2009), *V. amygdalina* was the most effective drug against malaria.

In the same report, plants used for treatment of skin disorders such as *C. aurea*, *Croton macrostachyus*, *Datura stramonium*, *Eucalyptus globulus*, *Euphorbia candelabrum*, *Euphorbia tirucalli*, *P. dodecandra*, *Prunus africana* and *Ricinus communis* are mentioned. *R. communis* is also used for the oil from the seeds, which serve as a purgative (Fullas, 2007). In Ethiopia, there are over 55 species of *Eucalyptus*, whereas *E. globulus* is the most abundant species. The vapor of this plant obtained from boiling the leaves is inhaled as a common household remedy to treat common cold symptoms (Fullas, 2007; Gedif and Hahn, 2003). In the study of Yineger *et al.* (2008a), *Heracleum abyssinicum*, *Olea europae* subsp. *Cuspidata* and *Solanum anguivi* are mentioned as plant preferred in the treatment of eczema. Rheumatism is mostly treated with *Cassipourea malosana*, *Hypericum revolutum*, *Myrica salicifolia*, *Nuxia congesta*, *Peperomia tetraphyla*, *Schefflera volkensii* and *Senecio syringifolius*. Typical medicinal plants of Ethiopia include many *Aloe* species. The fresh juice

of the thick leaves has laxative properties and it is used in the treatment of fever, spleen and liver troubles. It is also used for healing eye problems and knee troubles in old age (Getahun, 1976). Plant as *Aloe macrocarpa*, *Olea europae* subsp. *Cuspidata*, *Ranunculus multifidus* and *S. anguivi* are used in treatment hemorrhoids.

Some species like *E. schimperi*, *Glinus lotoides* and *Hagenia abyssinica* are used in the treatment of tapeworm (Bishaw, 1991). *G. lotoides* is effective against *Moniezia* spp. and *Thysaniezia* spp. and *H. abyssinica* against *Moniezia* spp. (Mesfin and Obsa, 1994). The efficacy of both these plants has been scientifically determined (Bishaw, 1991; Girma, 1998). Some plants used in traditional medicines as taenicides, are widely known to be toxic. For example, blindness and changes in central nervous system function have repeatedly been found in people who took over dosage of *H. abyssinica* (Kebede *et al.*, 2006).

Some medicinal plant species of Ethiopia are reported to have been threatened by the overuse and over harvesting for marketing as medicine. An example is *Taverniera abyssinica* whose slender roots are desirable for market. This plant has been used to treat sudden illness characterized by fever and stomachache. Both uses of the plant have been investigated scientifically and the conclusions support them (Fullas, 2007). *T. abyssinica* is critically endangered in the Red List of Endemic Trees and Shrubs of Ethiopia. Another medicinal plant threatened is *P. africana*, which bark is used in internationally medicine. It showed positive results *in vitro* studies and mouse models of prostate cancer.

2.1.4 Ethnobotanical and Ethnopharmacological Studies

A number of inventory studies focused on documentation of the local utilization of indigenous plants have previously been carried out in different regions of Ethiopia: Amhara (Abebe, 1986; Giday *et al.*, 2007; Teklehaymanot and Giday, 2007; Teklehaymanot *et al.*, 2007; Teklehaymanot, 2009), Benishangul-Gumaz (Flatie *et al.*, 2009), Oromia (Bussmann *et al.*, 2011; Giday *et al.*, 2003; Lulekal *et al.*, 2008; Parvez and Yadaw, 2010; Wabe *et al.*, 2011; Wondimu *et al.*, 2007; Yineger and Yewhalaw, 2007;Yineger *at al.*, 2007; 2008a; 2008b; Zerabruk and Yirga, 2012), Southern Nations, Nationalities and Peoples (Awas and Demissew, 2008; Bekalo *et al.*, 2009; Gedif and Hahn, 2003; Giday *et al.*, 2009a; 2009b; 2010; Mesfin *et al.*, 2009;), Tigray (Wilson and Mariam, 1979; Yirga, 2010) and other regions

(Abbink, 1993; Getahun, 1976). Nevertheless in Ethiopia are regions where only few or no ethnobotanical studies of medicinal plants have been done. The documentation of medicinal uses of African plants is becoming increasingly urgent because of the rapid loss of the natural habitats and anthropogenic activities, which are threats to the survival of many potential valuable medicinal plants (Giday *et al*., 2010; Kebede *et al*., 2006).

2.1.5 Antimicrobial Activity of Ethiopian Plants

A number of reports have been published about antimicrobial activity of different extracts or essential oils from selected Ethiopian plants against different microorganisms. The most used method in studies was the diffusion method, less used microdilution and least macrodilution method. The most common diluents are: methanol, water and ethanol. Some authors used two or more diluents. In some cases were used acetone, butanol, chloroform, dichlormethane, essential oil, ethyl acetate, hexane and petroleum ether. The antimicrobial activity in previous studies was determined on *Aspergilus niger*, *B. cereus*, *Bacillus subtilis*, *C. albicans*, *E. coli*, *Klebsiella pneumonia*, *P. aeruginosa*, *S. aureus*, *S. pyogenes* and *Salmonella typhimurium*. Some authors mention also *Micrococcus kristiane*, *Shigella dysenteriae*, *S. epidermidis* and many others.

The antimicrobial activity of selected medicinal plants has previously been determined: from Ethiopia by Belay *et al*. (2011), Desta (1993), Gebrehiwot and Unakal (2013), Geyid *et al*. (2005), Habtamu *et al*. (2010), Tadeg *et al*. (2005), Taiwo *et al*. (1999), Taye *et al.* (2011); from Ivory Coast by Bolou *et al.* (2011); from Kenya by Awino *et al*. (2008), Okemo *et al*. (2003), Wagate *et al.* (2010); from Nigeria by Akinpelu (1999), Okigbo and Mmeka (2008), Uzoigwe and Agwa (2011); from Rwanda by Cos *et al.* (2002), Sindambiwe *et al*. (1999); from South Africa by Adedapo *et al.* (2008), Erasto *et al.* (2006), Madikizela *et al*. (2012), Mativandlela *et al.* (2008); from Tanzania by De Boer *et al*. (2005), Runyoro *et al*. (2010); from Zimbabwe by Kambizi and Afolayan (2001); from India by Ghosh *et al.* (2003), Sangeetha *et al*. (2013), Sanwal and Chaudhary (2011), Sharma *et al*. (2008) and from Pakistan by Hussain *et al.* (2010).

Antimicrobial activities of some Ethiopian medicinal plants were confirm in studies of Belay *et al.* (2011), Desta (1993), Geyid *et al.* (2005), Tadeg *et al.* (2005) and more others. It has been found that *Albizzia gummifera*, *Artemesia abyssinica*, *Calotropis procera*, *Clematis sinensis, Combretum molle*, *Cucumis prophetarum*, *Ferula communsis*, *Gardenia lutea*, *Kalanchoe petitiana*, *Lippia adoensis*, *Rosa abyssinica*, *Rumex steudelii* and *Syzygium guineense* are species with potent antimicrobial properties.

2.1.6 Characteristics of Studied Plant Species

2.1.6.1 *Bersama abyssinica* Fresen. (Melianthaceae)

Vernacular name: *Azamir*

Botanical description: Medium-sized evergreen tree 6-12 (25) m, but also sometimes shrub 1.5-8 m tall with light brown bark (Hyde *et al.*, 2013). When bark is slashed it weeps copious watery yellowish sap. Leaves imparipinnate, 5-10 pairs of leaflets and a terminal leaflet. Length of compound leaves is between 0.3-1 m. The central rhachis is distinctly winged. The leaflet margins could be round or toothed. In time of flowering from November to December greenish to cream flowers are visible in spike-like inflorescences. Fruit is a velvety spherical capsule, splitting into 4 valves, revealing bright red seeds.

Natural habitat and geographic distribution: *Azamir* grows in variety of habitats: secondary evergreen rain and semi swamp forests, grassland with scattered trees, grassland, woodland, along streams and at the margins of evergreen forest. *B. abyssinica* occurs from Sudan and Nigeria to Zimbabwe (Biegel, 1977).

Traditional use: Extract from shoots of *Azamir* is used for treatment dysentery, roots for sinusitis and stem bark for tonsillitis. The leaf, root and stem bark extracts treat malaria, wounds, rheumatism and largely is this plant used for anthelmintic properties (Desta, 1995; Getahun, 1976; Giday *et al*., 2009b; Lulekal *et al*., 2008; Wabel *et al*., 2011; Yineger *et al*., 2008b).

Antimicrobial activity: Geiyd *et al*. (2005) reported MIC of aqueous and ethanol extracts from root bark and methanol extract from stem bark on *B. cereus* 1 mg/ml, *S. aureus* 0.5 mg/ml, *S. pyogenes* and *E. coli* >2 mg/ml. MIC of ethanol extract from root on *P. aeruginosa*

is 2.5 mg/ml (Bolou *et al.*, 2011). Desta (1993) notes that aqueous root extract of *B. abyssinica* at a concentration 1 mg/ml had equal activity to standard antibiotic neomycin at a concentration 0.5 mg/ml against *S. aureus.*

2.1.6.2 *Calpurnia aurea* (Lam.) Benth. (Leguminosae)

Vernacular name: *Digita*

Botanical description: Multi-stemmed slender tree or woody shrub up to 3-4 (10) m tall with grey-brown bark. Leaves imparipinnate, 8-30 elliptic leaflets (10-55 x 5-25 mm), slightly hairy to hairless. Petiole and leaf axis is 50-250 mm long. Golden-yellow flowers are 10-20 mm long in hanging sprays, calyx sharply tapering. Flowering period from December to February. Fruit is 50-140 mm long flat pod occurring from March to June.

Natural habitat and geographic distribution: This tree is widespread in bushland, grassland and forest margins above 900 m from South Africa northward into tropical and to sub-Saharan Africa (Brummitt, 1967; Schmidt *et al*., 2002) and occurs also in India (Zorloni *et al.*, 2010).

Traditional use: Seeds and juice of fresh leaves of *Digita* are widely used in treatment of gastro-intestinal disorders like diarrhea, stomachache, vomiting and medicine from root cure amoebic dysentery and giardiasis. Seeds are often used for cure eye infection and eye diseases, less frequently root and leaf remedy treat toothache and leaves serve for cure earache. Leaves can be taken to treat various diseases and disorders like malaria, swelling, diabetes, tapeworms, wounds, snake bite, rheumatism, skin diseases and mental disorders. The medicine used to cure epidemic disease called *Tesbo Beshita* is prepared from its stem. Root serves for treatment smallpox, anthrax and seeds for healing of abscesses, hypertension and cough (Abebe, 1986; Bekalo *et al*., 2009; Desta, 1995; Giday *et al.*, 2007; Mesfin *et al*., 2009; Teklehaymanot, 2009; Teklehaymanot and Giday, 2007; Teklehaymanot *et al.*, 2007; Wabel *et al*., 2011; Wilson and Mariam, 1979; Wondimu *et al*., 2007; Yineger and Yewhalaw, 2007; Yineger *et al.*, 2008b; Zerabruk and Yirga, 2012).

Antimicrobial activity: According to Adedapo *et al.* (2008) methanol extract from leaves had MICs of *B. cereus*, *S. aureus*, *S. epidermidis*, *S. pyogenes* and *E. coli* 5 mg/ml. Tadeg *et al.* (2005) mention MICs of leaf methanol extract on *S. aureus*, *P. aeruginosa* and *E. coli* 25

mg/ml and MIC of *C. albicans* >25 mg/ml. Desta (1993) notes that aqueous and residual aqueous extracts from seeds of *C. aurea* had at a concentration 1 mg/ml equal activity to standard antibiotic neomycin at concentration 0.5 mg/ml against *P. aeruginosa* and higher activity than standard antibiotic against *S. aureus.*

2.1.6.3 *Carissa spinarum* L. (Apocynaceae)

Vernacular name: *Agam*

Botanical description: An erect thorny 2-3 m high shrub with forked branches. A light brown to green bark can be stripped off longitudinally by hand, exposing the white to light green very hard wood underneath. Thorns are 3.2 cm long, brown to greenish at the base and deep brown towards the tip. Leaves are ovate, 4.5 cm long and 2.5 cm broad. Leathery leaves have round margin. Petiole reach 3 mm long. When leaves are plucked from the stem it exudes a white latex. White flowers are bisexual, actinomorphic, sweetly scented. Inflorescence is terminal or a corymbose cyme, with about 10 flowers. Time of flowering is from September to December. Fruit is an ovoid berry, 9 x 6 mm with lanceolate black seeds, 5-6 x 4 mm (Parmar and Kaushal, 1982).

Natural habitat and geographic distribution: This shrub occurs at altitude 1060-1580 m near river and in dry woodland often associated with termite mounds. It is widespread in tropical Africa, Asia from Yemen to India, Thailand and the Indian Ocean islands (Hyde *et al.*, 2013).

Traditional use: *Agam* root is used in treatment rabies, skin and nasal infection, snakebite, stomachache and mental disorders. Stem of *Agam* use people with kidney problems and muscle cramps. Medicine from root and stem protect people from evil eye. Also, is this plant used in treatment malaria and leaf remedy is sedative of stabbing pain (Bekalo *et al*., 2009; Lulekal *et al.*, 2008; Teklehaymanot *et al.*, 2007; Wondimu *et al*., 2007; Yineger *et al.*, 2007; 2008b).

Antimicrobial activity: Sanwal and Chaudhary (2011) mention MIC of root methanol extract on *S. aureus* 0.11 mg/ml; *E. coli* 0.125 mg/ml and *Streptococcus* sp. 0.165 mg/ml.

2.1.6.4 *Clematis hirsuta* Guill. & Perr. (Ranunculaceae)

Vernacular name: *Azo hareg*

Botanical description: A perennial climber up to 15 m in length. Stems are more or less softly hairy but usually becoming glabrous or nearly so. Stems are longitudinally ribbed and furrowed. Leaves pinnate with 5 leaflets, rarely bipinnate. Leaves are from almost glabrous to hairy on lower surface, glabrous to more or less pubescent on upper surface. Inflorescence is generally many-flowered. Pedicels are 1-3 cm long. Flower buds are spherical to ellipsoid, rounded to acuminate. Sepals are 1-2.7 cm long. This climber is a decorative plant with white or cream to pale pink flowers (Turrill and Milne-Redhead, 1952).

Natural habitat and geographic distribution: *Azo hareg* grows on clearings in the galleried forest and in open or wooded savanna from 1000 m up to 3000 m altitude. It is widespread in tropical Africa (Burkill, 1985; Turrill and Milne-Redhead, 1952).

Traditional use: The most used part of *Azo hareg* is a leaf. It serves for cure eye and ear diseases, herpes zoster, wounds, leishmania, elephantiasis, swellings, cough, evil eye and febrile disease. Leaves and fruit cure hemorrhoids, dry seeds amoebiasis and root is used for its anthelmintic properties (Bekalo *et al*., 2009; Giday *et al.*, 2007; 2009b; Parvez and Yadav, 2010; Teklehaymanot and Giday, 2007; Teklehaymanot *et al.*, 2007; Yineger and Yewhalaw, 2007; Yineger *et al*., 2008b).

Antimicrobial activity: Geid *et al*. (2005) report MICs of leaf methanol extract on *B. cereus*, *S. aureus*, *S. pyogenes* and *E. coli* >2 mg/ml. Cos *et al.* (2002) mention MICs of leaf methanol extract on the same bacteria and *P. aeruginosa* >0.5 mg/ml and MIC of *C. albicans* 0.031 mg/ml.

2.1.6.5 *Clutia abyssinica* Jaub. & Spach.(Euphorbiaceae)

Vernacular name: *Fiyele fej*

Botanical description: Shrub or rarely a small tree, up to 4 m. Leaves, ovate to lanceolate, are 1-6 cm wide and up to 16 cm long. Leaves are light green above and somewhat blue-green below, mostly hairless with entire margin. Some orange-red leaves often present. Petiole is 5-23 mm long. Stems and leaves are hairless or almost hairless. Small yellowish to greenish

flowers are in axillary clusters, unisexual on different trees. Flowering period is from March to July. Fruit is a capsule 5 mm in diameter, almost round and almost hairless, pale green with whitish pustules. Seeds are 3 x 2.5 mm.

Natural habitat and geographic distribution: *Fiyele fej* grows in clearings and along margins of evergreen forest, riverine forest and high rainfall woodland at altitude 300-2300 m. This shrub occurs from Sudan and Somalia southwards to Angola and South Africa (Carter *et al.*, 1993; Hyde *et al.*, 2013).

Traditional use: Leaves of *Fiyele fej* are used to treat skin infection and rheumatism. Root remedy cure bloody diarrhea (Lulekal *et al.*, 2008; Yineger *et al.*, 2008b).

Antimicrobial activity: According to Cos *et al.* (2002) MICs of leaf ethanol extract on *B. cereus*, *S. aureus*, *S. pyogenes*, *E. coli*, *P. aeruginosa* and *C. albicans* are >0.5 mg/ml. De Boer *et al*. (2005) note that the root methanol extract at a concentration 0.02 mg/ml was able to inhibit growth of *S. aureus* by 50-80 %.

2.1.6.6 *Croton macrostachyus* Hochst. ex Delile (Euphorbiaceae)

Vernacular name: *Bisana*

Botanical description: A shrub or deciduous tree 7 to 15 meters high but can grow up to 30 m high. Crown is rounded and it is open with large spreading branches. Pale grey or grey-brown bark is finely reticulate, fairly smooth, finely fissured with age. When bark is slashed it is reddish. Shoots are densely and shortly hairy. Leaves large, green, turning to orange before falling. Shape of leaves ovate, base subcordate or rounded, apex acuminate. Size of leave is 5-19 x 3.5-15 cm. Veins prominent with 2 stalked glands just visible at the base of the leaf, paler below due to soft hairs. Texture more or less furry, margin slightly toothed. Flowers are creamy to yellow-white, sweetly scented, up to 3 mm long, dioecious or at least on separate shoots. Flowers are in erect spikes. Male inflorescence is up to 25 cm long, flowers stalked. Female inflorescence is usually less than 10 cm long and subsessile. In Nigeria, flowering occurs in March to May and fruiting from January to March. After pollination by insects, fruit development takes 3-5 months. Young fruits are green and turning grey at maturity, on drooping spikes up to 30 cm long. Fruits are 3-lobed, 8-9 x 8-10 mm, stellate, pubescent

covered at 1 end by a soft, creamy envelope. Fruits mature when still on the tree, splitting open with a sharp noise to release seeds. Each pea-sized capsule contains 3 shiny grey seeds with a soft, cream aril. The generic name is derived from the appearance of the seed, for „croton“ is based on the Greek word for a tick. The specific epithet is from the Greek macro (large) and stachyus (relating to a spike) hence „with a large spike” (ICRAF, 2013).

Natural habitat and geographic distribution: *Bisana* is common in secondary forests, on forest edges along rivers, around lakes, in moist or dry evergreen upland forests, wooded grasslands or clump bushland and along roadsides. It is associated with *Juniperus-Podocarpus* habitats and also occurs in the warmer parts of the mountain rain forests and semi-tropical rain forests. *Bisana* grows at altitude of 200-2000 m in most parts of Eritrea, Ethiopia, Kenya, Nigeria, Tanzania and Uganda (Belay *et al.*, 2011; Getahun, 1976).

Traditional use: Leaf milky sap of *Bisana* is widely used in treatment skin problems like ringworm, scabies, eczema, allergies and wounds. Latex is also used as blood coagulant. Root remedy treat gastro-intestinal disorders like stomachache, constipation and amoebiasis. Root and leaf extracts cure hepatitis and *Mich.* All parts of *C. macrostachyus* cure tapeworms, malaria and sexually transmitted diseases like gonorrhea. Seeds are used to cause abortion (Abebe, 1986; Awas and Demissew, 2008; Bekalo *et al*., 2009; Bussmann *et al*., 2011; Desta, 1995; Gedif and Hahn, 2003; Getahun, 1976; Giday *et al.*, 2003; 2007; 2009b; Lulekal *et al.*, 2008; Mesfin *et al*., 2009; Parvez and Yadav, 2010; Teklehaymanot and Giday, 2007; Teklehaymanot *et al.*, 2007; Wabel *et al*., 2011; Wilson and Mariam, 1979; Wondimu *et al*., 2007; Yineger and Yewhalaw, 2007; Yineger *et al*., 2008b; Yirga, 2010; Zerabruk and Yirga, 2012).

Antimicrobial activity: MICs 0.4 mg/ml of essential oil from seeds and fruit on *B. cereus* was determined by Belay *et al.* (2011), methanol extract from seeds and fruits had MIC >2 mg/ml (Geiyd *et al*., 2005) and leaf methanol extract had 15.6 mg/ml (Wagate *et al*., 2010). MICs of essential oil from seeds and fruit on *S. aureus* was 0.4 mg/ml (Belay *et al.*, 2011), methanol extract from seeds and fruits >2 mg/ml (Geiyd *et al*., 2005) and leaf aqueous and methanol extract >500 mg/ml (Taye *et al.*, 2011). *S. pyogenes* had MICs of essential oil from seeds and fruit 1.6 mg/ml (Belay *et al.*, 2011), methanol extract from seeds and fruits >2 mg/ml (Geiyd *et al*., 2005) and leaf aqueous and methanol 7.81mg/ml (Taye *et al.*, 2011). *L. monocytogenes*

had MIC of essential oil from seeds and fruit 0.1 mg/ml (Belay *et al*., 2011). MICs of Gram-negative bacteria: *E. coli* was inhibited by methanol extract from seeds and fruits at a concentration >2 mg/ml (Geiyd *et al*., 2005), essential oil from seeds and fruit 6.3 mg/ml (Belay *et al.*, 2011), leaf methanol extract 250 mg/ml (Wagate *et al*., 2010) and leaf aqueous and methanol extracts >500 mg/ml (Taye *et al.*, 2011). Essential oil from seeds and fruit inhibited *P. aeruginosa* at MIC 0.4 mg/ml (Belay *et al.*, 2011), leaf methanol extract had MIC of the same bacteria at a concentration 250 mg/ml (Wagate *et al*., 2010) and leaf aqueous and methanol extracts showed MIC >500 mg/ml on *P. aeruginosa* (Taye *et al.*, 2011).

2.1.6.7 ***Dodonaea angustifolia*** **L. f. (Sapindaceae)**

Synonyms*: Dodonaea viscosa* Jacq. var. *angustifolia* (L.f.) Benth., *Dodonaea viscosa* Jacq. f. *angustifolia* (L.f.) Sherff, *Dodonaea viscosa* Jacq. var. *angustifolia* (L.f.) Benth.

Vernacular name: *Kitkita*

Botanical description: An evergreen shrub or small tree 2-8 m tall. Branchlets are rusty red and resinous. Bark is dark grey, fissured and peeling. This shrub has alternate simple leaves lanceolate in shape, dark glossy green and very shiny. Leaves are 5-10 cm long and 5-8 mm wide. Sometimes sticky resinous coating on surface. The small, inconspicuous, yellow-green unisexual flowers without petals appear in spring and fall. Time of flowering from March to September. A fruit is inflated capsules with 3 or 4 rounded wings. The capsules are initially green and fleshy, becoming yellow green and tinged with red or pink and drying to a papery brown. Each capsule contains 1-3 small round, black seeds.

Natural habitat and geographic distribution: *Kitkita* occurs at altitude 0-2800 m with mean annual rainfall 450 mm and often on rocky sites or poor soils (ICRAF, 2013). It is native to subtropical and tropical areas worldwide, where it typically grows in dry regions (NTBG, 2013).

Traditional use: Roots of *D. angustifolia* are used for treatment hemorrhoids. Leaves are taken to treat skin disease and like anthelmintic. Fruit and seeds are used against ectoparasites, malaria and lymphatic swelling. It treats also dysentery, wounds and epidemic disease *Tesbo Beshita* (Desta, 1995; Giday *et al.*, 2007; Lulekal *et al*., 2008; Mesfin *et al*., 2009;

Teklehaymanot, 2009; Teklehaymanot *et al.*, 2007; Yineger *et al*., 2008a; Zerabruk and Yirga, 2012).

Antimicrobial activity: According to our best knowledge, no reports on antimicrobial activities of *D. angustifolia* were found in literature.

2.1.6.8 *Embelia schimperi* Vatke (Myrsinaceae)

Vernacular name: *Inkoko*

Botanical description: A climbing shrub or liane 2-5 m long with long trailing branches. The climbing aided by short hardened lateral branchlets. A bark is smooth red-brown. Leaves spirally arranged, crowded at the ends of branches, very variable in shape. Leaves are up to 6 cm long, thinly fleshy, hairless, rounded or notched at the apex. The margin of leaves is entire. Greenish to yellowish flowers are in spikes on the old lateral shoots. Flowers are unisexual on the same plant, sometimes bisexual flowers occur. Fruit somewhat depressed-spherical, circa 5 mm in diameter, scarlet when ripe. This species is very variable, particularly in leaf shape (Hyde *et al*., 2013).

Natural habitat and geographic distribution: *Inkoko* grows near roadsides, thickets, forest edges at altitude 1000-3200 m with mean annual rainfall 800-2000 mm. This shrub grows in a wide range of soil types and it is widespread in highland areas of Africa (Ruffo *et al*., 2002).

Traditional use: *E. schimperi* is mainly used as folk anthelmintic remedy. Seeds, fruits, and sometimes stem or root are often used for its anthelmintic properties. Fruit is also used in treatment constipation, gonorrhea, whereas root is remedy for treatment of leprosy (Awas and Demissew, 2008; Bussmann *et al*., 2011; Desta, 1995; Gedif and Hahn, 2003; Getahun, 1976; Giday *et al.*, 2007; 2009b; 2010; Mesfin *et al*., 2009; Wabel *et al*., 2011; Wilson and Mariam, 1979; Zerabruk and Yirga, 2012).

Antimicrobial activity: Desta (1993) notes that methanol extract from fruits of *E. schimperi* at a concentration 1 mg/ml had greater activity than standard antibiotic neomycin at concentration 0.5 mg/ml against *S. aureus* and aqueous and residual aqueous extract has equal activity to standard antibiotic against *P. aeruginosa*. Awino *et al*. (2008) determined MICs of ethyl acetate extract from stem bark on *E. coli*, *P. aeruginosa* and *S. aureus* > 0.1 mg/ml.

2.1.6.9 *Jasminum abyssinicum* R.Br. (Oleaceae)

Vernacular name: *Abita*

Botanical description: Strong woody climber shrub with main stems up to 13 cm in diameter. Leaves are opposite, 3-foliolate. Leaflets are ovate with a distinct drip-tip, dark glossy green, hairless except for hair-tuft domatia in the axils of the main veins below. Flowers are in terminal and axillary heads, white, tinged with pink on the outside, sweetly scented. Corolla with 5 or sometimes 6 elliptic lobes. Fruit is a berry 7 mm long, glossy black, sometimes 2-lobed.

Natural habitat and geographic distribution: Occurring in mountain evergreen forest from Ethiopia to South Africa (Hyde *et al*., 2013).

Traditional use: Leaves of *Abita* are used in treatment snakebite and leaves are also used for its anthelmintic properties. Fresh root tips are chewed to relieve tonsillitis (Getahun, 1976; Teklehaymanot *et al.*, 2007).

Antimicrobial activity: According to Geiyd *et al*. (2005) MIC of leaf methanol extract on *B. cereus* is >2 mg/ml. Habtamu *et al*. (2010) mention MIC of leaf aqueous and methanol on *S. aureus* >1 mg/ml and Geiyd *et al*. (2005) report MIC of leaf methanol extract >2 mg/ml. The same author determined MIC of *S. pyogenes* >2 mg/ml. *E. coli* has according Habtamu *et al*. (2010) MIC >1 mg/ml and according Geiyd *et al*. (2005) >2 mg/ml.

2.1.6.10 *Maesa lanceolata* Forssk. (Myrsinaceae)

Vernacular name: *Kelewa*

Botanical description: Evergreen shrub or much-branched small tree, 2-10 m, with a redbrown bark and large leaves. Dark brown resin exudes from the veins of the leaves if it is broken or slashed. The petiole is 1.5-3 cm long and lamina is 6-17 cm long. Leaves ranging from lanceolate to broadly elliptic, glabrous or pubescent especially on the nerves. The margin is slightly revolute, very shallowly to distinctly serrate. Corolla 1.5-1.8 mm, pale yellow or

whitish, scented. Flowering period is from November to August. Fruit has 3-4 mm in diameter, greenish to pale pink. Seeds are blackish, angular.

Natural habitat and geographic distribution: *M. lanceolata* grows at the margins of forest, in high altitude forests as an understory tree, occasionally in mountain grassland at altitude range 980-2000 m (Hyde *et al*., 2013).

Traditional use: Stem is used to treat eye diseases, skin diseases and anthelmintic. Medicine from leaves is applied on snakebite. Root is used in treatment of anthrax. Seeds and fruit are applied against skin infection, scabies and they are used as anthelmintic (Awas and Demissew, 2008; Mesfin *et al*., 2009; Teklehaymanot *et al.*, 2007; Wabel *et al*., 2011; Wilson and Mariam, 1979; Yineger and Yewhalaw, 2007; Yineger *et al.*, 2008b).

Antimicrobial activity: Two authors determined antimicrobial activity of leaf ethanol extract of *Kelewa*. Sindambiwe *et al.* (1999) determined MICs of *B. cereus*, *S. aureus*, *S. epidermidis*, *E. coli*, *P. aeruginosa* and *C. albicans* >10 mg/ml. Madikizela *et al.* (2012) report MICs of *S. aureus* 6.25 mg/ml and *E. coli* 0.78 mg/ml.

2.1.6.11 *Ocimum lamiifolium* Hochst. (Lamiaceae)

Vernacular name: *Dama kessie*

Botanical description: An erect perennial herb, with very hairy stems. Leaves distinctly petioled, ovate, regularly crenate. Leaves are 2.5-5cm long slightly pubescent on both sides. Racemes are long, lax, branched at the base. Flowers is whorls, bracts broadly ovate, cuspidate, deciduous, bright-colored, pedicels 4 mm. Calyx is 1.2 mm long. Upper tooth is ovate, obtuse, rather shorter than the tube. Lower tooth is with two large cusps. Corolla-tube is as long as the calyx. Stamens are much exerted, filaments of to upper pediculate above the base (Baker, 1900).

Natural habitat and geographic distribution: According to Baker (1900) this herb grows among shrubs on the high banks of rivers. *Dama kessie* occurs in Ethiopia, Tanzania and Cameroon (EOL, 2012).

Traditional use: The common medicinal use of *O. lamiifolium* is to treat febrile disease. To prepare remedy are taken leaves and juice of fresh leaves. Not so often leaves are used to treat

cough, headache, eye and ear diseases, diarrhea, leishmania and respiratory tract infection. Fresh crushed leaves and bark paste are used to treat wounds (Awas and Demissew, 2008; Bekalo *et al*., 2009; Desta, 1995; Giday *et al.*, 2007; 2009a; 2009b; 2010; Mesfin *et al*., 2009; Parvez and Yadav, 2010; Teklehaymanot, 2009; Teklehaymanot and Giday, 2007; Teklehaymanot *et al.*, 2007; Wabel *et al*., 2011; Wondimu *et al*., 2007; Yineger and Yewhalaw, 2007; Yineger *et al.*, 2008b).

Antimicrobial activity: MICs of essential oil from leaves on *S. aureus* is 1.95 mg/ml (Runyoro *et al*., 2010) and MIC of leaf ethanol extract is 200 mg/ml (Gebrehiwot and Unakal, 2013). *S. epidermidis* was inhibited at MIC 1.75 mg/ml (Runyoro *et al*., 2010). MICs of Gram-negative *E. coli* is 4.9 mg/ml (Runyoro *et al*., 2010) and 100 mg/ml (Gebrehiwot and Unakal, 2013); *P. aeruginosa* 2.75 mg/ml (Runyoro *et al*., 2010) and 100 mg/ml (Gebrehiwot and Unakal, 2013). Desta (1993) notes that leaf aqueous extract of *O. lamiifolium* at a concentration 1 mg/ml had equal activity to standard antibiotic neomycin at concentration 0.5 mg/ml against *P. aeruginosa.*

2.1.6.12 *Olinia rochetiana* A. Juss. (Oliniaceae)

Vernacular name: *Tife*

Botanical description: Small gnarled shrub 1.2 m high or less often a large tree 4-16 m high. Occasionally it can reach 27 m high. The bark is grey, sometimes slightly peeling, often fissured. Branchlets are reddish when are very young, later pale, mostly squarish, which together with the leaf venation renders sterile twigs easily identifiable. Leaves are opposite or ternate, often bright red when young. Blade is broadly rounded-elliptic or rounded-rhombic, 0.6-12 cm long and 0.5-4.5 cm wide. Reddish petiole is 2-9 mm long. Inflorescences globose or pyramidal, it has 1.5-7.5 cm in diameter. Usually many-flowered, the branches often with fine hairs. Pedicels are about 1 mm long. Thin, veined, ovate or oblong, cucullate and pubescent bracts are deciduous or rarely persistent. Flowers scent sweetly. Calyx is mostly carmine, glabrous or puberulous and 2.5-7 mm long. Petals are yellowish cream, white, pink or carmine, often white at first and becoming red later. The petal is 2.3-5 mm long and 1-2.5

mm wide. Fruit is pink or dark carmine, globose or ovoid and it is 0.5-1 cm long. Seeds are brown, ovoid, 2.5-3 mm long (Verdcourt, 1975).

Natural habitat and geographic distribution: *Tife* grows on rocky places at altitude range 1680-3000 m (Verdcourt, 1975) from Ethiopia to the South Africa (EOL, 2012).

Traditional use: The leaves of *Tife* are used to treat stabbing pain (Lulekal *et al.*, 2008).

Antimicrobial activity: Tadeg *et al.* (2005) mention MICs of leaf methanol extract on *S. aureus* 5 mg/ml; *E. coli* 10 mg/ml; *P. aeruginosa* 2.5 mg/ml and *C. albicans* 10 mg/ml.

2.1.6.13 ***Rubus steudneri*** **Schweinf (Rosaceae)**

Vernacular name: *Injori*

Botanical description: A shrub up to 4 m in height. Leaves simple or trifoliolate, varying from thinly pilose to glabrous above and from thickly grey-white or greenish-white tomentose to glabrous except for the midrib and primary veins beneath. Leaflets obovate or ovate-elliptic or sometimes nearly round. The terminal leaflet often larger than the others is 9-15 cm long and 5.6-10 cm wide. Petiole is prickly, those of the terminal leaflets is 3-5.6 cm long, and of the laterals not exceeding 1 cm long. Inflorescence is usually a rather large pyramidal panicle. Calyx is 6-9 mm long, deeply divided into lanceolate lobes basally fused. Petals are pink, obovate-oblanceolate, exceeding the calyx, apically rounded in outline and often notched. Size of petals is 7-10 x 5-8 mm. Color of fruit is orange to dark red (Graham, 1960).

Natural habitat and geographic distribution: *Injori* grows in area such moist bamboo thicket, edges and clearings in upland rainforest, secondary bushland and scrub, forest and ravines with *Podocarpus latifolius* at altitude 1500-3000 m (CJB, 2012; Graham, 1960). This shrub occurs in Ethiopia, Tanzania and the United States of America (DL, 2011).

Traditional use: From stem of *Injori* is prepared remedy to headache. Leaves are used to treat rheumatism. *R. steudneri* is also used in treatment of stomachache with diarrhea (Giday *et al*., 2009b; Lulekal *et al.*, 2008; Yineger *et al*., 2008a).

Antimicrobial activity: According to our best knowledge, there is no report on antimicrobial activity of *R. steudneri.*

2.1.6.14 *Rumex nepalensis* Spreng. (Polygonaceae)

Vernacular name: *Lut*

Botanical description: Perennial herb, up to 1-2 m tall, glabrous or almost glabrous. Stem is green or pale brown. Leaves are alternate, simple, oblong-lanceolate, acute to obtuse at the apex, cuneate to cordate at the base. Size of blade is up to 30 x 6 cm. Lower leaves are long-petiolate, upper leaves shortly petiolate. Unisexual flowers in clusters are arranged in an open panicle with long branches. Fruit is a sharply trigonous, glossy brown ovoid nut with size 3-5 mm x 2-2.5 mm.

Natural habitat and geographic distribution: *R. nepalensis* occurs as a weed in disturbed habitats, in moorland, grassland, bushland at of cultivated fields and near dwelling areas (Getahun, 1976). This herb is widespread in Africa and the Mediterranean to eastern Asia at altitude 700-4000 m (Grubben and Denton 2004; Thulin, 1993).

Traditional use: Root remedy of *Lut* treat mainly gastro-intestinal complaints like abdominal cramps, stomachache, diarrhea, amoebiasis and it has also anthelmintic properties. The same part of the plant can be used in treatment liver diseases, eye infection, toothache, rabies, leshmania and cancer. Wounds and tonsillitis can be treated with extracts from the root and a stem. Leaves are in remedy to stop bleeding, treat amoebiasis and ear infection. Seeds of *Lut* are contained in medicine for eczema. This plant is also used as antidote and laxative (Abebe, 1986; Awas and Demissew, 2008; Bekalo *et al*., 2009; Bussmann *et al*., 2011; Gedif and Hahn, 2003; Getahun, 1976; Giday *et al.*, 2007; 2009a; 2009b; 2010; Lulekal *et al.*, 2008; Mesfin *et al*., 2009; Teklehaymanot, 2009; Teklehaymanot and Giday, 2007).

Antimicrobial activity: Ghosh *et al.* (2003) investigated antimicrobial activity of root methanol extract on *S. aureus*, *E coli* and other microorganisms by diffusion method. The zone of inhibition was determined and *S. aureus* was more susceptible to extract than *E. coli*. Hussain *et al.* (2010) mention that the root methanol extract at a concentration 10 mg/ml shows low antibacterial activity against *P. aeruginosa* (44 %) and higher activity against *E. coli* (49 %) and *S. aureus* (45 %).

2.1.6.15 *Thalictrum rhynchocarpum* Quart.-Dill. & A.Rich. (Ranunculaceae)

Vernacular name: *Sire bizu*

Botanical description: Somewhat scrambling perennial herb, up to 4 m tall. Leaves are up to 40 cm long, 3-4 times divided into elliptic to ovate, 3-lobed leaflets. Closely resembling the fronds of ferns in the genus *Adiantum*. Flowers are in lax many-flowered branched heads, small, green to purplish, petals absent. Fruit is asymmetrically spindle-shaped with a long beaked apex, pendent on long hair-like pedicels (Burkill, 1985; Hyde *et al.*, 2013).

Natural habitat and geographic distribution: This herb occurs along clearings and margins of evergreen forest at altitude range 1100-2350 m in tropical Africa and South Africa (Burkill, 1985; Hyde *et al.*, 2013).

Traditional use: The most used part of *Sire bizu* is a root. It is used to treat stabbing pain, ear infection, diarrhea and urinary tract retention. It is supposed that the root has also anthelmintic properties (Bekalo *et al*., 2009; Desta, 1995; Giday *et al.*, 2010; Lulekal *et al.*, 2008; Yineger *et al*., 2008a).

Antimicrobial activity: According to our best knowledge, there are no reports of antimicrobial activity of this plant in literature.

2.1.6.16 *Verbascum sinaiticum* Benth. (Scrophulariaceae)

Vernacular name: *Yeahya joro*

Botanical description: This plant is biennial herb, 1-2 m or more high. Stems are simple, densely stellate-pubescent, with a whitish or pale rusty color. Juvenile plants with a rosette of leaves oblanceolate to oblong, up to 30 cm long and 7-11 cm wide, broadly petiolate, coarsely serrate, acute to acuminate. Lower stem leaves are similar, smaller, upper ones becoming ovate and sessile. Both types of leaves are densely stellate-pubescent on both surfaces. Inflorescence is well-branched, rarely simple. Lower bracts are ovate, acuminate and are circa 2 cm long, upper bracts are inconspicuous. Pedicels are 3-5 mm long. Calyx is deeply lobed and it is 4-9 mm long, densely stellate-pubescent. Corolla is yellow and has 20–28 mm in diameter. Filaments and anthers are orange, filaments bearded with white and purple hairs. Ovoid pubescent capsule is 6-7 mm long and 4-5 mm broad (Ghazanfar *et al*., 2008).

Natural habitat and geographic distribution: *V. sinaiticum* grows at altitude 1500-2400 m in Sinai, Palestine, Syria, Iraq, Iran, Afghanistan, Pakistan and tropical East Africa (EOL, 2012; Ghazanfar *et al*., 2008).

Traditional use: Root of *Yeahya joro* is used in treatment of diarrhea, stomachache, vomiting, fever and evil eye. Leaves are applied against diarrhea, viral infections, anthrax, wounds and stop bleeding (Abebe, 1986; Parvez and Yadav, 2010; Teklehaymanot and Giday, 2007; Teklehaymanot *et al.*, 2007; Wilson and Mariam, 1979; Wondimu *et al*., 2007; Yirga, 2010).

Antimicrobial activity: MICs of aqueous extract from flowers on *B. cereus* and *S. aureus* is according Talib and Mahasneh (2010) 1 mg/ml and MIC of leaf methanol extract is >2 mg/ml (Geiyd *et al*., 2005). Tadeg *et al.* (2005) report MIC of leaf methanol extract on *S. aureus* 25 mg/ml. Geiyd *et al*. (2005) mention MIC of leaf methanol extract on *S. pyogenes* >2 mg/ml. MICs of aqueous extract from flowers on *E. coli* is 1 mg/ml (Talib and Mahasneh, 2010), leaf methanol extracts >2 mg/ml (Geiyd *et al*., 2005) and >25 mg/ml (Tadeg *et al.*, 2005). *P. aeruginosa* has MICs of aqueous, butanol, ethanol, n-hexane and methanol extracts from flowers >1 mg/ml (Talib and Mahasneh, 2010) and leaf methanol extract 25 mg/ml (Tadeg *et al.*, 2005). According to Talib and Mahasneh (2010) MICs of all tested extracts from flowers on *C. albicans* is >1 mg/ml and leaf methanol extract showed MIC at a concentration >25 mg/ml (Tadeg *et al.*, 2005).

2.1.6.17 *Vernonia amygdalina* Delile (Asteraceae)

Vernacular name: *Girawa*

Botanical description: A small much branched shrub or tree growing up to 2-5 m high. Sometimes it can reach a height of 10 m. Trunk is up to 40 cm in diameter. Bark grey to brown, smooth, becoming fissured. Young branches are densely pubescent. Leaves are alternate, simple, stipules with 0.2-4 cm long petiole. Blade is ovate-elliptical to lanceolate, 4-15 (28) cm x 1-4 (15) cm. Margin is entire or finely toothed. Leaves have a characteristic odor and bitter taste. The shrub is very conspicuous in full bloom and it is sweet-scented, particularly at night (Getahun, 1976). Flowering time is from May to September. Flowers are bisexual, regular and 5-numerous. Capitulum forming clusters are up to 15 cm long, creamy

white, occasionally tinged with violet. Fruit is a 10-ribbed brown to black achene 1.5-3.5 mm long, pubescent and glandular (Grubben and Denton, 2004; Hyde *et al*., 2013; Yeap *et al*., 2010).

Natural habitat and geographic distribution: *Girawa* usually grows in overgrazed areas, forest edges and in secondary scrub, at altitudes between 1700 and 3000 m. This shrub occurs wild in most countries of tropical Africa, from Guinea east to Somalia and south to north-eastern South Africa, and in Yemen. It is commonly grown as a vegetable in Benin, Cameroon, the Democratic Republic of Congo, Gabon, Nigeria and to a lesser extent in their neighboring countries (Erasto *et al.*, 2006; Hyde *et al*., 2013).

Traditional use: The most used part of *V. amygdalina* is a leaf. It is used in treatment wounds, allergies, rashes, tonsillitis, dysentery, stomachache, disease *Mich*, hepatitis, goiter and headache. Leaves are commonly used for anthelmintic properties. Root is applied on snake bite. Root powder is sprinkled on burning charcoal and inhaled to heal epidemic disease *Tesbo Beshita*. Bark is used against skin disease, sap is a purgative and dried flowers treat stomach disorders (Awas and Demissew, 2008; Bekalo *et al*., 2009; Gedif and Hahn, 2003; Getahun, 1976; Giday *et al.*, 2003; 2007; 2009a; 2009b; 2010; Mesfin *et al*., 2009; Teklehaymanot, 2009; Teklehaymanot and Giday, 2007; Teklehaymanot *et al.*, 2007; Wabel *et al*., 2011; Wondimu *et al*., 2007; Yineger and Yewhalaw, 2007; Yineger *et al*., 2008b; Yirga, 2010).

Antimicrobial activity: MICs of leaf ethanol extract on *B. cereus* is > 0.5 mg/ml (Cos *et al.*, 2002) and MICs of root acetone and methanol extract is 5 mg/ml (Kambizi and Afolayan, 2001). MIC 0.5 mg/ml of root aceton extract is on *S. aureus* (Kambizi and Afolayan, 2001), MICs of leaf ethanol extract on the same bacteria is > 0.5 mg/ml (Cos *et al.*, 2002), leaf methanol extract MIC 12.5 mg/ml (Akinpelu, 1999) and root methanol extract has MIC at a concentration >20 mg/ml (Taiwo *et al.*, 1999). The same author determined MIC of root methanol extract on *E. faecalis* >20 mg/ml. According Cos *et al.* (2002) MIC of leaf ethanol extract on *S. pyogenes* is >0.5 mg/ml. MIC of leaf ethanol extract on *E. coli* is >0.5 mg/ml (Cos *et al.*, 2002), root aceton and methanol extracts have MICs 5 mg/ml (Kambizi and Afolayan, 2001), root methanol extract >20 mg/ml (Taiwo *et al.*, 1999) and leaf methanol extract >25 mg/ml (Akinpelu, 1999). *P. aeruginosa* was inhibited by leaf ethanol extract, MIC >0.5 mg/ml (Cos *et al.*, 2002); leaf methanol extract, MIC 6.25 mg/ml (Akinpelu, 1999); root

methanol extract, MIC 20 mg/ml (Taiwo *et al.*, 1999) and root aqueous extract 40 mg/ml (Bolou *et al.*, 2011). MICs of leaf ethanol extract on *C. albicans* is >0.5 mg/ml (Cos *et al.*, 2002) and leaf methanol extract >25 mg/ml (Akinpelu, 1999). Desta (1993) notes that leaf dichlormethane fraction and methanol extracts of *V. amygdalina* at a concentration 1 mg/ml had equal activity to standard antibiotic neomycin at concentration 0.5 mg/ml against *P. aeruginosa.*

2.1.6.18 *Zehneria scabra* Sond. (Cucurbitaceae)

Vernacular name: *Hareg iresa*

Botanical description: Prostrate or climbing herb with annual stems is up to 6 m long. Leaves are more or less broadly ovate, usually unlobed but occasionally 3-5 lobed. Deep green leaves are covered with rough hairs above, paler hairs below. Leaves are cordate at the base and margin is prominently toothed. White small unisexual flowers are on different plants. Male flowers are in axillary clusters, female flowers solitary or in few-flowered clusters. Fruits are in spherical clusters. Fruit has 8-13 mm in diameter, it is hairless, bright red when ripe.

Natural habitat and geographic distribution: *Hareg iresa* grows in forest, on forest margins, near rivers and plantations at altitude range 900-2100 m. This species is widespread in tropical Africa, South Africa, Arabia, India, Java and Philippines (Hyde *et al*., 2013).

Traditional use: The most often medicinal use of *Z. scabra* is to treat disease *Mich* with leaf and stem remedy. Stem is used to cure warts and febrile illness. Swellings, fever, headache, rashes and gonorrhea are treated by leafs. The medicine used to treat malaria, anemia and stomachache is prepared from root (Abebe, 1986; Desta, 1995; Gedif and Hahn, 2003; Getahun, 1976; Giday *et al.*, 2007; 2010; Teklehaymanot, 2009; Teklehaymanot and Giday, 2007; Teklehaymanot *et al.*, 2007).

Antimicrobial activity: MICs of ethanol extract from whole plant on *B. cereus*, *S. aureus*, *S. pyogenes* and *E. coli* are >2 mg/ml (Geiyd *et al*., 2005). Desta (1993) notes that leaf aqueous extract of *Z. scabra* at a concentration 1 mg/ml had higher activity than standard antibiotic neomycin at concentration 0.5 mg/ml against *S. aureus.*

Hypothesis

Despite the fact that antimicrobial activity of plants used in traditional medicine in various regions of Ethiopia for treatment of infectious diseases have previously been described in several reports, the antimicrobial effects of species used in folk medicine in the Ankober district are still poorly known. Based on ethnobotanical and pharmacological data summarized above, it is possible expect, that some of species used in this region for treatment of infectious diseases can produce significant antimicrobial effect.

3 Objectives

The aim of this work is to determine *in vitro* antimicrobial activity of ethanol extracts obtained from various parts of eighteen medicinal Ethiopian plants, namely *Bersama abyssinica*, *Calpurnia aurea*, *Carissa spinarum*, *Clematis hirsuta*, *Clutia abyssinica*, *Croton macrostachyus*, *Dodonaea angustifolia*, *Embelia schimperi*, *Jasminum abyssinicum*, *Maesa lanceolata*, *Ocimum lamiifolium*, *Olinia rochetiana*, *Rubus steudneri*, *Rumex nepalensis*, *Thalictrum rhynchocarpum*, *Verbascum sinaiticum*, *Vernonia amygdalina* and *Zehneria scabra* against potentially human and animal pathogenic microorganisms by the broth microdilution method.

4 Material and Methods

4.1 Plant Material

Plant specimens were collected by Mr Ermias Lulekal in Ankober district (172 km northeast of the capital Addis Ababa), North Shoa Zone, Amhara region, Ethiopia in following periods: 25 June and 26 September 2009, 9 January and 20 February 2010, 22 May and 27 August 2010 and 14 February and 7 May 2011. Identification of specimens was performed both in the field and at the National Herbarium of Ethiopia using taxonomic keys, floras and by comparison with voucher reference herbarium specimens. Plant species were selected for screening based on local use reports from the study area and related to ethnobotanical reports from other parts of the country. The identified voucher specimens were deposited at the National Herbarium of Ethiopia.

4.2 Preparation of Extract

15g of air-dried plant material of each species was finely grounded using Grindomix (GM100 Retsch, Germany) and extracted at room temperature in 80% ethanol solution using a laboratory shaker for 24 hours. Extracts were subsequently filtered and concentrated to dry state using a rotary evaporator Rotavapor R-200 (Buchi, Switzerland) *in vacuo* at 40 °C. Dry residues were then dissolved in 100% Dimethyl Sulfoxide (DMSO) to create a concentration of 51.2 mg/ml stock solution of each extract and stored at -20 °C until tested. Yield (%) or amount of dry residues of each extract used as a starting material is shown in Table 2.

4.3 Microorganisms

Antimicrobial activity was evaluated for one yeast and eleven bacterial strains obtained from American Type Culture Collection (ATCC) (Oxoid, Basingstoke, United Kingdom) and the German Resource Centre for Biological Material (DSM) (Braunschweig, Germany). Bacterial strains were selected as representatives of both classes of Gram-positive and Gram-negative bacteria. Microbial strains used were *Bacillus cereus* (ATCC 11778), *Bacteroides fragilis*

(ATCC 25285), *Candida albicans* (ATCC 10231), *Clostridium perfringens* (DSM 11778), *Enterococcus faecalis* (ATCC 29212), *Escherichia coli* (ATCC 25922), *Listeria monocytogenes* (ATCC 7644), *Pseudomonas aeruginosa* (ATCC 27853), *Salmonella enteritidis* (ATCC 13076), *Staphylococcus aureus* (ATCC 29213), *Staphylococcus epidermidis* (ATCC 12228) and *Streptococcus pyogenes* (ATCC 19615).

S. pyogenes was grown in brain-heart infusion broth (Oxoid, Basingstoke, United Kingdom). *B. fragilis* and *C. perfringens* as representatives of anaerobic bacteria were grown in Wilkins-Chalgren anaerobic broth (Oxoid, Basingstoke, United Kingdom) under anaerobic conditions using anaerobic jar HP11 (Oxoid, Basingstoke, United Kingdom). Anaerobiosis was achieved in anaerobic chamber Bugbox (BioTrace, Bridgend, United Kingdom). The other microorganisms were grown on Mueller-Hinton broth (Oxoid, Basingstoke, United Kingdom). For *E. faecalis* Mueller-Hinton broth was enriched with glucose.

The sensitivity of tested aerobic bacteria and *B. fragilis* to a standard antibiotic Ciprofloxacin (Sigma-Aldrich, Prague, Czech Republic) was checked as a positive control. In the case of yeast, Tioconazole (Sigma-Aldrich, Prague, Czech Republic) was checked as a positive control and Penicillin G (Sigma-Aldrich, Prague, Czech Republic) was used for *C. perfringens*. Deionized water was used as dissolvent for all antibiotics following Andrews (2001).

4.4 Antimicrobial Assay

In vitro antimicrobial activity of extract was measured by the broth microdilution method described by Jorgensen *et al*. (1999) using 96-well microtiter plates, with slight modifications according to Cos *et al.* (2006). Ten two-fold serial dilutions of each extract were prepared. The broth concentrations ranged from 4 to 512 μg/ml. Each well was inoculated with 5 μl of bacterial suspension at a density of 10^7 CFU/ml. The microtiter plates were incubated at 37 °C for 24 hours (or for 48 hours for the yeast, *B. fragilis* and *C. perfringens*) and then checked for the minimum inhibitory concentrations (MICs).

The growth of microorganisms was observed as turbidity determined with Multiscan Ascent Microplate Reader (Thermo Fisher Scientific, Waltham, USA) at 405 nm. MICs were then

calculated based on the density of the growth control and were expressed as the lowest extract concentrations that resulted in $\geq$ 80% reduction in bacterial growth compared to that of the extract-free growth control. All samples were tested in triplicate in three independent experiments.

5 Results and Discussion

Seventeen from twenty-two ethanol extracts from plants selected according to their traditional use in Ankober district for treatment of infection-associated diseases such as diarrhea, otorrhoea and common cold (Table 1) showed antimicrobial activity at least against one of twelve microorganisms at concentration ranging from 64 to 512 μg/ml (Table 2).

Extracts of *E. schimperi*, *O. lamiifolium* and *R. steudneri* had the broadest spectrum of antimicrobial action. *E. schimperi* and *O. lamiifolium* inhibited growth of all Gram-positive bacteria and *C. albicans* with MICs ranging from 64 to 512 μg/ml. *E. schimperi* has the strongest antibacterial effect of all tested plants when showed MICs 64 μg/ml against three Gram-positive bacteria, namely *B. cereus*, *L. monocytogenes* and *S. pyogenes*. The leaf and twigs extract of this plant were more effective than the extract of seeds and fruit. The extract from leaves of *O. lamiifolium* inhibit eight microorganisms at MICs ranging from 128 to 512 μg/ml. The third most effective plant was *R. steudneri*. Two extracts of this plant inhibit twelve microorganisms at MICs ranging from 128 to 512 μg/ml. *R. steudneri* was the only one species, which showed antimicrobial effect against Gram-negative bacteria *P. aeruginosa* and *S. enteritidis* at MICs from 256 to 512 μg/ml.

B. abyssinica, J. abyssinicum and *R. nepalensis* inhibit at least three microorganisms with MICs 256 or 512 μg/ml. Both extracts of *V. amygdalina* inhibit growth of *E. faecalis* at the highest concentration 512 μg/ml and each extract suppressed growth of two different Gram-positive bacteria. The extract from flowers of this plant showed activity against *S. aureus* and *S. epidermidis* at the highest concentration tested. The extract from leaves and twigs of *V. amygdalina* inhibit *B. cereus* at the highest concentration and *S. pyogenes* at the concentration 256 μg/ml.

Six plant extracts inhibited the only one microorganism. *C. abyssinica, C. macrostachyus, D. angustifolia, M. lanceolata, O. rochetiana* showed MICs ranging from 256 to 512 μg/ml on *S. pyogenes* and *Z. scabra* inhibit growth of *C. albicans* at MIC 512 μg/ml.

No antimicrobial activity was observed from extracts of *C. aurea, C. spinarum, C. hirsuta, T. rhynchocarpum* and *V. sinaiticum*. These extracts were not effective even at the highest concentration tested (512 μg/ml).

Table 1. Medicinal use of selected plants from Ankober district, North Shoa Zone, Amhara region, Ethiopia

Species	Collection number (ErmiasL x)	Part	Medicinal use in the study area to treat
B. abyssinica	173	LT	diarrhea and constipation
C. aurea	76	R	diarrhea, toothache, epiglottitis and wounds
C. spinarum	16	R	constipation, rheumatism, headache, evil spirit
C. hirsuta	18	L	eczema, mumps, ringworm, diarrhea
C. abyssinica	10	R	bloody diarrhea, gastritis, constipation
C. macrostachyus	17	LT	anthelmintic, diarrhea eczema, allergic rushes
D. angustifolia	20	L	skin lesion, ringworm, diarrhea, constipation
E. schimperi	505	LT, SF	anthelmintic, diarrhea, constipation
J. abyssinicum	577	L	bloody diarrhea, gastritis and constipation
M. lanceolata	42	LT	diarrhea and constipation
O. lamiifolium	828	L	malaise, otorrhoea and eye infection
O. rochetiana	102	L	eczema, diarrhea
R. steudneri	242	LT, R	gastritis, diarrhea and constipation
R. nepalensis	24	LT, R	constipation, bloody diarrhea

Species	Collection number (ErmiasL x)	Part	Medicinal use in the study area to treat
T. rhynchocarpum	535	R	mumps, ottorhoea, ascariasis
V. sinaiticum	829	L	diarrhea and constipation
V. amygdalina	22	F, LT	anthelmintic, constipation
Z. scabra	830	LT	malaise, common cold, coughing

L, leaves; LT, leaves and twigs; R, root; F, flowers; SF, seeds, fruit.

Two of the Gram-negative bacteria *B. fragilis* and *E. coli* were found to be resistant to all extracts tested in this study. Similarly, *S. enteritidis* was found to be less sensitive to extracts. This bacteria was inhibited only by root extract of *R. steudneri* (MIC 512 μg/ml). *P. aeruginosa* was inhibited by root extract of *R. steudneri* (MIC 256 μg/ml) and leaf and twig extract of *R. steudneri* (512 μg/ml). The Gram-positive bacteria *C. perfringens* was found to be less sensitive to extracts. *C. perfringens* was inhibited only by extracts of two species *E. schimperi* and *O. lamiifolium*. Whereas *S. pyogenes* was shown to be the most sensitive bacterium which was inhibited by thirteen extracts with MIC value ranging from 64 to 512 μg/ml. *L. monocytogenes* was inhibited by seven extracts (MIC ranging from 64 to 512 μg/ml). Seven extracts inhibited *S. aureus* (MIC value ranging from 256 to 512 μg/ml). Eight extracts showed inhibition of *B. cereus* (MIC from 64 to 512 μg/ml). *E. faecalis* was inhibited at the highest concentration (512 μg/ml) by eight extract and *S. epidermidis* was inhibited by nine extract (MIC value ranging from 128 to 512 μg/ml). *C. albicans* was inhibited by seven extracts at the highest MIC concentration (512 μg/ml).

Table 2. Minimum inhibitory concentrations (µg/ml) of ethanol extracts from medicinal plants of Ankober district, Ethiopia

Species	Part	Extract yield (%)	Gram-positive						Gram-negative					Yeast
			B.c.	C.p.	E.f.	L.m.	S.a.	S.epi.	S.p.	B.f.	E.c.	P.a.	S.ent.	C.a.
B. abyssinica	LT	33.50	-	-	-	512	-	512	-	-	-	-	-	512
C. aurea	R	19.50	-	-	-	-	-	-	-	-	-	-	-	-
C. spinarum	R	14.50	-	-	-	-	-	-	-	-	-	-	-	-
C. hirsuta	L	30.00	-	-	-	-	-	-	-	-	-	-	-	-
C. abyssinica	R	9.50	-	-	-	-	-	-	256	-	-	-	-	-
C. macrostachyus	LT	21.00	-	-	-	-	-	-	256	-	-	-	-	-
D. angustifolia	L	28.50	-	-	-	-	-	-	512	-	-	-	-	-
E. schimperi	SF	25.50	128	512	512	256	512	512	128	-	-	-	-	512
	LT	38.50	64	512	512	64	512	256	64	-	-	-	-	512
J. abyssinicum	L	7.00	512	-	-	512	-	512	256	-	-	-	-	-
M. lanceolata	LT	27.15	-	-	-	-	-	-	256	-	-	-	-	-
O. lamiifolium	L	11.50	256	512	512	256	512	512	128	-	-	-	-	512
O. rochetiana	L	38.00	-	-	-	-	-	-	512	-	-	-	-	-
R. steudneri	R	32.82	256	-	512	512	256	128	512	-	-	256	512	-
	LT	23.00	512	-	512	256	512	256	512	-	-	512	-	512
R. nepalensis	R	19.25	512	-	512	-	-	256	-	-	-	-	-	-
	LT	8.11	-	-	-	-	512	-	512	-	-	-	-	512

Species	Part	Extract yield (%)	Gram-positive						Gram-negative					Yeast
			B.c.	C.p.	E.f.	L.m.	S.a.	S.epi.	S.p.	B.f.	E.c.	P.a.	S.ent.	C.a.
T .rhynchocarpum	R	17.50	-	-	-	-	-	-	-	-	-	-	-	-
V. sinaiticum	L	44.50	-	-	-	-	-	-	-	-	-	-	-	-
V. amygdalina	F	18.75	-	-	512	-	512	512	-	-	-	-	-	-
	LT	17.50	512	-	512	-	-	-	256	-	-	-	-	-
Z. scabra	LT	16.90	-	-	-	-	-	-	-	-	-	-	-	512
Positive antibiotic control			0.12^{c}	0.12^{p}	0.50^{c}	1^{c}	0.25^{c}	0.12^{c}	0.50^{c}	8^{c}	0.01^{c}	0.06^{c}	0.03^{c}	0.12^{t}

L, leaves; LT, leaves and twigs; R, root; F, flowers; SF, seeds, fruit;

B.c., *Bacillus cereus*; C.p., *Clostridium perfringens*; E.f., *Enterococcus faecalis*; L.m., *Listeria monocytogenes*; S.a., *Staphylococcus aureus*; S.epi., *Staphylococcus epidermidis*; S.p., *Streptococcus pyogenes*; B.f., *Bacteroides fragilis*; E.c., *Escherichia coli*; P.a., *Pseudomonas aeruginosa*; S.ent., *Salmonella enteritidis*; C.a., *Candida albicans*;

^{c}, ciprofloxacin;^{p}, penicilin G ;^{t}, tioconazole;

-, not active (>512 μg/ml).

Significant antimicrobial activity of detected for several tested plant species such as *B. abyssinica, E. schimperi, J. abyssinicum, O. lamiifolium, R. steudneri, R. nepalensis* and *V. amygdalina* supports wide traditional use reports from Ankober district (North Shewa Zone, Amhara Region) and in different part of Ethiopia.

The most interesting plant tested in our study seems to be *E. schimperi*, because their extracts showed the strongest antimicrobial effect of all plants tested. The lowest MIC (64 µg/ml) showed extract from leaves and twigs, which was never tested before. According to Rios and Recio (2005) and Cos *et al*. (2006) is MIC value below 100 µg/ml significant indicator of antimicrobial activity. Extract from leaves and twigs of *E. schimperi* showed MIC value below 100 µg/ml on three Gram-positive bacteria: *B. cereus*, *L. monocytogenes* and *S. pyogenes*. This indicates significant antibacterial effect of *E. schimperi*. The antimicrobial activity of extract from fruit of *E. schimperi* was determined only once before by Desta (1993). Results from previous study are partially in accordance with our results. Also in our study extracts showed inhibition of *S. aureus*. Our results (MIC 512 µg/ml) are more accurately because Desta (1993) determined the activity only at concentration 1000 µg/ml. In previous study the extract inhibited also *P. aeruginosa*, but in our study did not because this Gram-negative bacteria was resistent to extracts. In contrasty to previous study, in our study both extracts inhibited *C. albicans* (MIC 512 µg/ml) but in previous study the activity has not been demonstrated. According to the available literature, some of the reported medicinal plant species were found to have some compounds with antimicrobial properties. Awino *et al*. (2008) have reported antimicrobial activity of extracts from stem bark of *E. schimperi* against *S. aureus*, which corresponds with results of the present work. Report from the same authors on antimicrobial activity of a pure compound, 2,5- dihydroxy-3-methyl-1,4-benzoquinone from *E. schimperi* against two of the Gram-negative bacteria i.e. *P. aeruginosa* and *E. coli*, which were shown not to be susceptible in the present work, suggests presence of other antibacterial constituents in the extract tested in this study. Reports on chemical composition of this species show that *E. schimperi* leaves possess contain quinones (Midiwo *et al*., 2005); flavonol glycosides (Manguro *et al*., 2004; Manguro *et al.*, 2006; Manguro and Williams, 1997); oleanane-type triterpenes (Manguro *et al*., 2006) and schimperinone (Machocho *et al*., 2003). Among these constituents, embelin has been reported to possess significant

antibacterial properties at the concentration 100 µg/disc against *S. aureus, S. pyogenes* and *P. aeruginosa* (Chitra *et al*., 2003). Hence, the broadest spectrum of action and the strong antimicrobial activity shown by both leaf and twig extracts of *E. schimperi* against *B. cereus, L. monocytogenes* and *S. pyogenes* could be attributed to existence of such active chemicals.

Second interesting plant is *O. lamiifolium*. Our results are not in conflict with literature. In previous studies (Gebrehiwot and Unakal, 2013; Runyoro *et al.*, 2010) the leaf extract showed MIC range from 1.95 to 200 mg/ml on *S. aureus* and MIC value 1.75 mg/ml on *S. epidermidis*. In our study the extract had MIC 512 µg/ml on both bacteria. Authors used different method in previous study (Gebrehiwot and Unakal, 2013) that can cause higher value of MICs. They used agar-diffusion method which is according to Cos *et al*. (2002) not suitable for testing non-polar samples because of solubility, volatility and diffusion characteristics in agar. Presence of chemical compounds including bornyl acetate, p-cymene, camphene, α-pinene and sabinene was also reported from essential oil analysis of *O. lamiifolium* (Runyoro *et al*., 2010; Tchoumbougnang *et al.*, 2006). Since in previous studies, bornyl acetate (Ruynoro *et al*., 2010) and sabinene (Tchoumbougnang *et al*., 2006) showed antimicrobial properties, the observed broad spectrum activity for extracts of *O. lamiifolium* could relate to these active components.

The third promising result showed extract of *R. steudneri*. According our best knowledge no report on antimicrobial properties and chemical constituents of this plant could be obtained from the literature. Hence, this new observation calls for investigation on *R. steudneri* to isolate and characterize its antimicrobially active constituents.

B. abyssinica belongs among other promising plants. Our results of antimicrobial properties of this extract are in accordance with Geiyd *et al*. (2005) and Bolou *et al*. (2011). We did not determinate MICs of *B. cereus, S. aureus, S. pyogenes, E. coli* and *P. aeruginosa* because the extract had low activity at our maximum concentration. Both authors mention MICs higher than our maximum concentration which we tested. Only our value of MIC of *S. aureus* is in contrasty with Geiyd *et al*. (2005). In previous study they used for preparation of the extract root bark and stem bark of *B. abyssinica* and we used leaves and twigs. The reason why our extract had low activity on *S. aureus* can be that the antimicrobial compounds inhibiting *S.*

aureus were not present in such high quantities as in root and stem bark. No information of presence antimicrobial compounds in aerial part of *B. abyssinica* is available.

Inhibitions of growth of *B. cereus* and *S. pyogenes* by extract from *J. abyssinicum* are in accordance with literature. A report by Gallo *et al.* (2006) on chemical constituents of root bark of *J. abyssinicum* stated the presence of esters of a cyclopentanoid monoterpene but no information on their antimicrobial effect was available.

Both extracts of *R. nepalensis* inhibit three microorganisms but each suppress different microorganisms. Root extract showed MIC range from 256 to 512 µg/ml on *B. cereus, E. faecalis* and *S. epidermidis*. Extract from leaves and twigs inhibit at the MIC 512 µg/ml *S. aureus, S. pyogenes* and *C. albicans*. Comparison our results with two previous studies is difficult because authors did not determinate MICs. Ghosh *et al.* (2003) determined zone of inhibition of root extract on *S. aureus* and *E. coli*. In this study was *S. aureus* more susceptible to extract than *E. coli*. In our study we did not determinate MIC of root extract on these two bacteria. Hence we can not compare if *S. aureus* was more susceptible than *E. coli*. Hussain *et al.* (2010) expressed antimicrobial effect in percentage of inhibition of bacteria. Also these results we can not compare with ours because we did not determinate MICs of *E. coli* and *P. aeruginosa*. Ghosh *et al.* (2003) and Liang *et al.* (2010) isolated from the roots of *R. nepalensis* aloe-emodin and torachrysone. These compounds have been reported to have noticeable antibacterial effects on *S. aureus* (Hatano *et al*., 1999). Therefore, the antibacterial activity of *R. nepalensis* may be due to the presence of these compounds.

V. amygdalina is other promising plant. Our results are in accordance with literature. We used extracts from flowers and from twigs with leaves. Kambizi and Afolayan (2001) and Taiwo *et al.* (1999) used root extract, which has lower inhibition activity on *B. cereus, E. faecalis and S. aureus* than ours extracts. Aerial parts of *V. amygdalina* seem to have higher antimicrobial properties than root. Chemical constituents of *V. amygdalina* include flavonoids, steroidal alcohols and sesquiterpene lactones that have been reported as chemically active (Erasto *et al.*, 2006; Luo *et al*., 2010). The latter may be responsible for the relatively broad spectrum of actions of this species observed in the present work.

Gram-positive bacteria are generally found more susceptible to the extracts than the Gram-negative ones because of differences in cell wall morphology of the two groups. Also in our study were Gram-positive bacteria more susceptible than Gram-negative bacteria.

Among the Gram-positive bacteria *C. perfringens* was found to be less sensitive as inhibited by extracts of only two species *E. schimperi* and *O. lamiifolium*. Whereas *S. pyogenes* was shown to be the most sensitive bacterium which was inhibited by thirteen extracts with MIC value ranging from 64 to 512 μg/ml.

The growth of yeast strain *C. albicans* was inhibited by extracts from *B. abyssinica*, *E. schimperi*, *O. lamiifolium*, *R. steudneri*, *R. nepalensis* and *Z. scabra*. No growth inhibition was observed in the negative controls.

The antimicrobial activity of some Ethiopian plants tested in this study against some microorganisms have been reported before, but extracts of some plants, i.e. *D. angustifolia*, *R. steudneri* and *T. rhynchocarpum* were never tested for antimicrobial properties before. *R. steudneri* is the only ones from all tested plants inhibiting growth of two Gram-negative bacteria and all Gram-positive bacteria except *C. perfringens*. This indicates a significant antimicrobial properties and a favourable potential of this plant.

According to our best knowledge, the antimicrobial activity of none of selected plant was tested against *C. perfringens*, *B. fragilis* and *S. enteritidis* before. Antibacterial properties on these microorganisms showed three plants. *E. schimperi* and *O. lamiifolium* had MIC 512 μg/ml on *C. perfringens* and *R. steudneri* showed the same value of MIC on *S. enteritidis*. None of plant extract established inhibition of *B. fragilis*. This bacteria normally responded to standard antibiotic in our study, so the resistance of this bacteria is eliminated.

Only one of eighteen plants was tested against *E. faecalis* and one against *L. monocytogenes* before. Taiwo *et al.* (1999) mention that MIC of *V. amygdalina* on *E. faecalis* is higher than 20 mg/ml. This is in contrast to our results. We determined MIC 512 μg/ml of both extracts of this plant. In previous study was used different method for screening and the main difference is using various plant part for extract preparation. We used flowers and twigs with leaves and Taiwo *et al.* (1999) used root of *V. amygdalina*. In study of Belay *et al.* (2011) extract from berries of *C. macrostachyus* showed MIC 0.1 μg/ml on *L. monocytogenes*. In our study the

extract from leaves and twigs did not inhibit growth of *L. monocytogenes*. Berries of *C. macrostachyus* seem to have higher antimicrobial effect than leaves and twigs.

6 Conclusions

In our study, the antibacterial activities of twenty-two extract from eighteen Ethiopian medicinal plant species were assayed. The antimicrobial screening has been carried out with aim to verify traditional uses of these species in the Ankober district (North Shewa Zone, Amhara Region).

Seventeen from twenty-two ethanol extracts showed antimicrobial activity at least against one of twelve microorganisms tested. *E. schimperi, O. lamiifolium* and *R. steudneri* are the most promising plants for a potential discovery of leading antimicrobial compounds. *E. schimperi* had the strongest antimicrobial effect of all tested plants. MICs of this plant extract ranged from 64 to 512 μg/ml. The antimicrobial activity of extracts from *B. abyssinica, J. abyssinicum, R. nepalensis* and *V. amygdalina* are also promising and call for further investigation. Antimicrobial activity observed for tested plant species such as *B. abyssinica, E. schimperi, J. abyssinicum, O. lamiifolium, R. steudneri, R. nepalensis* and *V. amygdalina* supports wide traditional use reports from Ankober district.

Extracts of *R. steudneri* had one of the strongest antimicrobial effect and we have no information about compound responsible for antimicrobial activity. This species is the most promising for further investigation.

Tested plants possess active chemicals with pharmacological properties against microbes, hence there is a need for further investigation to isolate and characterise chemicals responsible for the observed strong antimicrobial activity. More scientific studies on medicinal plant species with traditional use reports will also bring more useful results that contribute significant role in the fight against pathogens.

7 References

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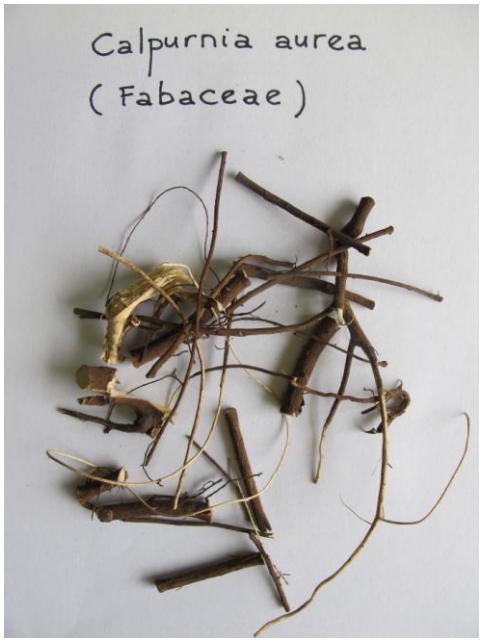
Appendix A. Photographic Illustrations of Plant Samples

Fig. 1. ***Bersama abyssinica***

Original photo by Jindřiška Čepková, 2011

Fig. 2. ***Calpurnia aurea***

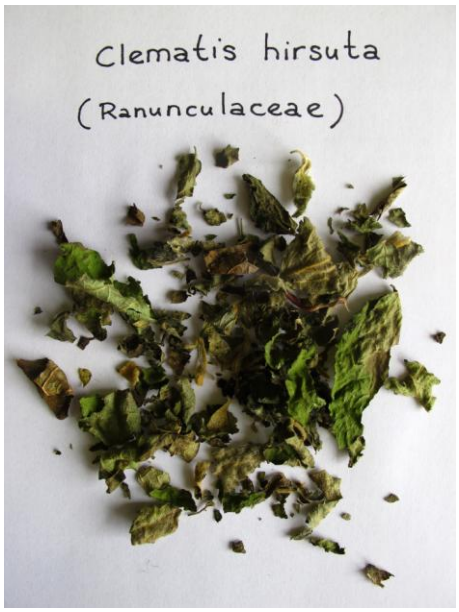


Original photo by Jindřiška Čepková, 2011

Fig. 3. ***Carissa spinarum***

Original photo by Jindřiška Čepková, 2011

Fig. 4. ***Clematis hirsuta***

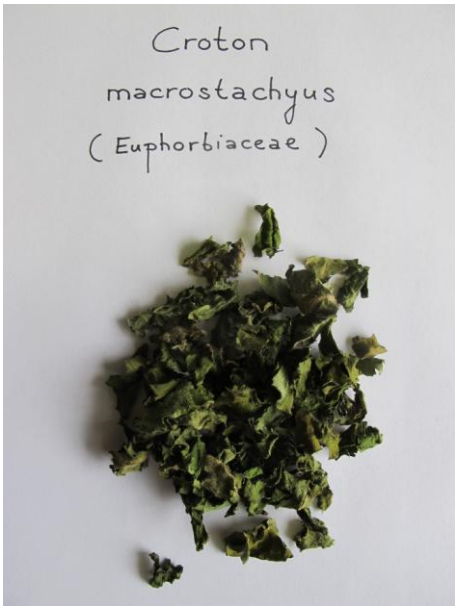


Original photo by Jindřiška Čepková, 2011

Fig. 5. ***Clutia abyssinica***

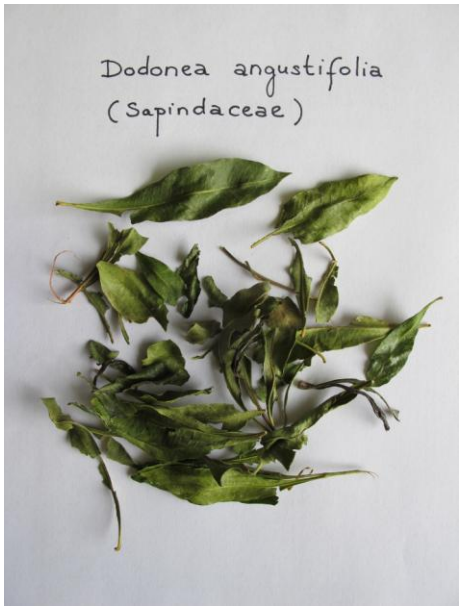
Original photo by Jindřiška Čepková, 2011

Fig. 6. ***Croton macrostachyus***



Original photo by Jindřiška Čepková, 2011

Fig. 7. ***Dodonaea angustifolia***



Original photo by Jindřiška Čepková, 2011

Fig. 8. ***Embelia schimperi***

Original photo by Jindřiška Čepková, 2011

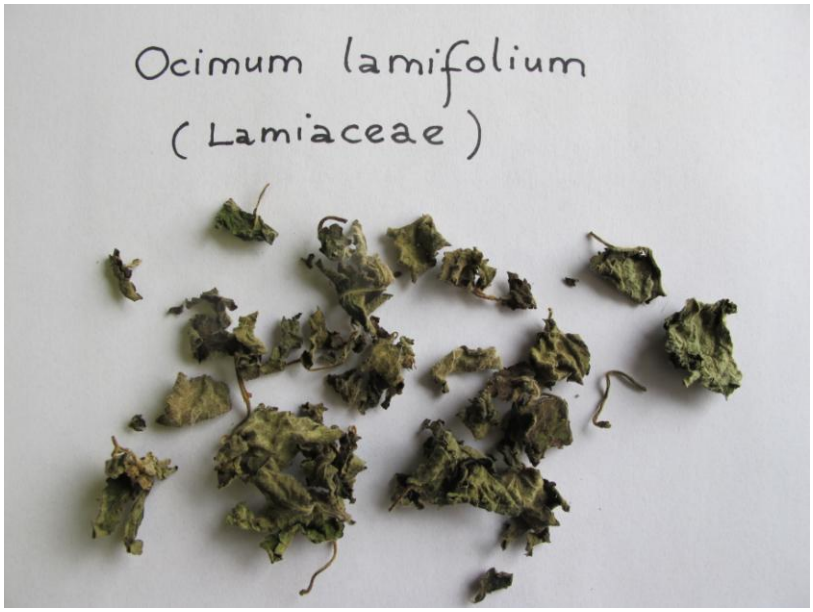
Fig. 9. ***Jasminum abyssinicum***

Original photo by Jindřiška Čepková, 2011

Fig. 10. ***Maesa lanceolata***

Original photo by Jindřiška Čepková, 2012

Fig. 11. ***Ocimum lamiifolium***

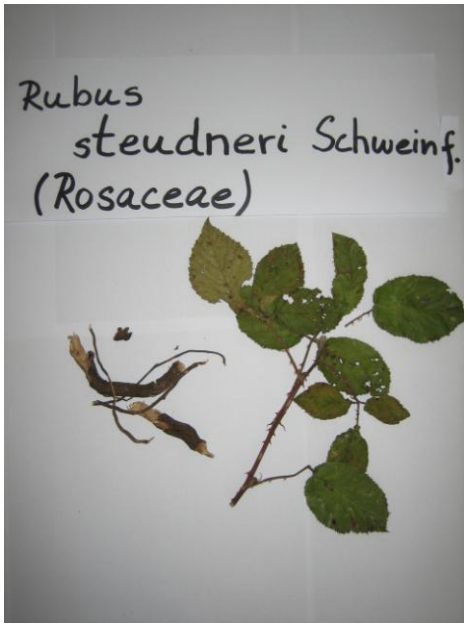


Original photo by Jindřiška Čepková, 2011

Fig. 12. *Olinia rochetiana*

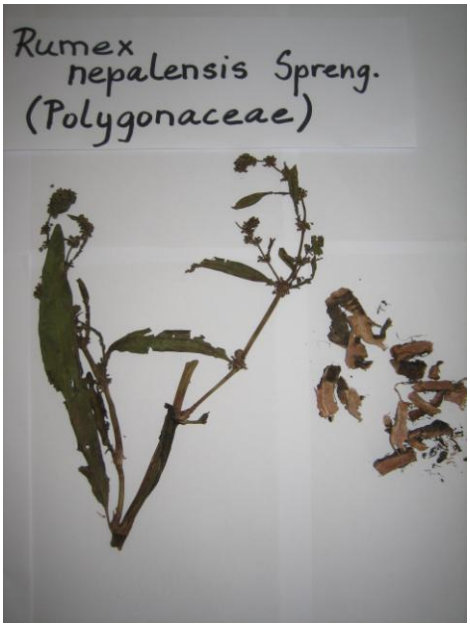
Original photo by Jindřiška Čepková, 2011

Fig. 13. *Rubus steudneri*



Original photo by Jindřiška Čepková, 2012

Fig. 14. *Rumex nepalensis*

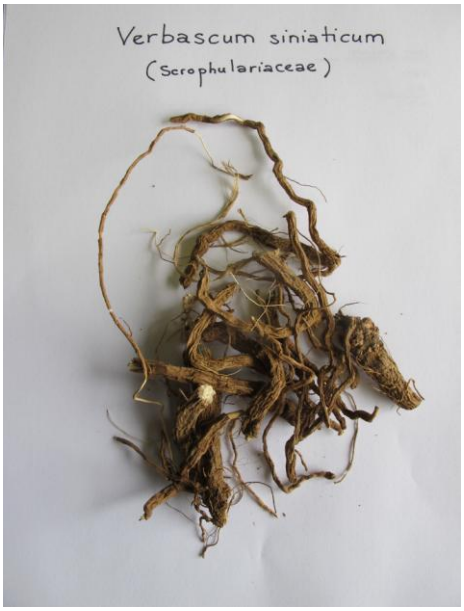


Original photo by Jindřiška Čepková, 2012

Fig. 15. ***Thalictrum rhynchocarpum***

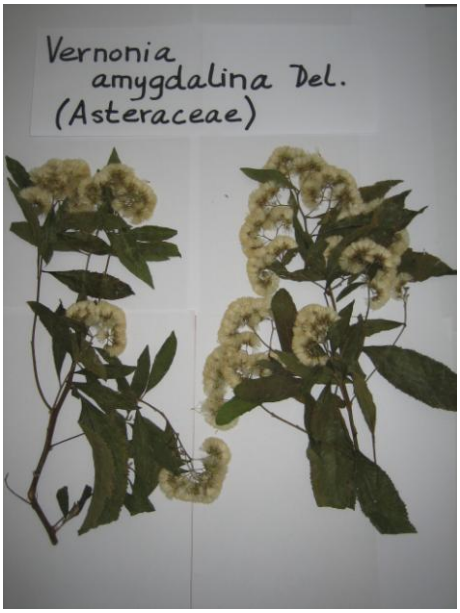
Original photo by Jindřiška Čepková, 2011

Fig. 16. ***Verbascum sinaiticum***



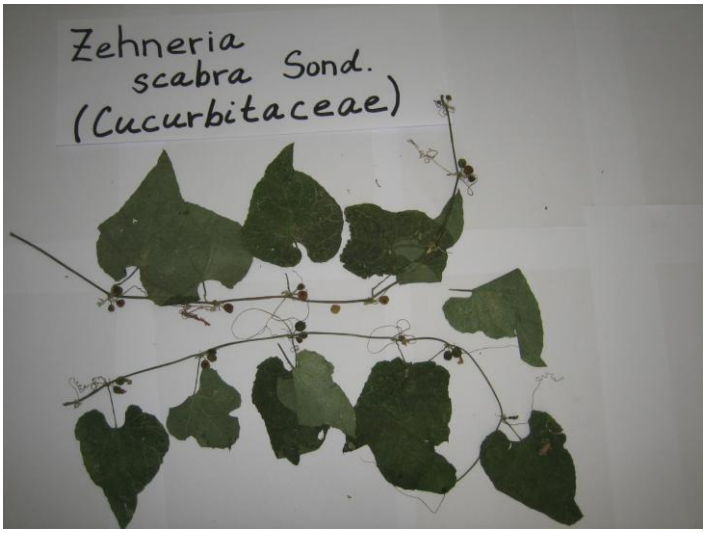
Original photo by Jindřiška Čepková, 2011

Fig. 17. ***Vernonia amygdalina***



Original photo by Jindřiška Čepková, 2012

Fig. 18. ***Zehneria scabra***



Original photo by Jindřiška Čepková, 2012

Appendix B. List of Author's Publications

Lulekal E., Rondevaldová J., Bernášková E., Asfaw Z., Kelbessa E., Kokoška L., Van Damme P. 2012. Antimicrobial activity of medicinal plants from Ankober district, Amhara region, Ethiopia. Ethiopia. 13th Congress of the International Society for Ethnopharmacology. Graz, Austria, 2.-6.9.2012. Book of Abstracts: p.31, abstr. SL 33.