MADDA WALABU UNIVERSITY

COLLEGE OF NATURAL AND COMPUTITIONAL SCIENCE

DEPARTMENT OF BIOLOGY

ANTIBACTERIAL ACTIVITIES OF EXTRACTS OF BERRIES OF *Juniperus procera, Olea europeae* AND *Podocarpus falcatus* COLLECTED FROM ADABA-DODOLAFOREST AGAINST SELECTED HUMAN BACTERIAL PATHOGENS

A Master Thesis Submitted to School of Graduate Studies of MaddaWalabu University In Partial Fulfillment of Requirements for Master of Science in Applied

Biology (Plant and Environmental Microbiology specialization)

BY:

AHMED ESMAEL

ADVISOR: ADDISU ASSEFA (PhD)

Bale Robe

January,2020

ETHIOPIA

DECLARATION

I, the undersigned, declare that this is my own original work, has not been presented fora degree to any other university and that all sources of materials used for the thesis havebeen duly acknowledge.

Ahmed Esmael

Signature:_____

Date:_____

The work has been done under my supervision

Advisor: Addisu Assefa , (Ph.D)

Signature:_____

Date:_____

ACKNOWLEDGMENTS

First of all I would like to thank the Almighty and the greatest God who allowed me to live and enabledme to achieve this level. My deepest thanks go to my advisors Dr. Addisu Assefa who provided me all possible supports including reading materials, comments during review paper development. I also thank my staff members for sharing me reading materials. Last, but not the least, my heartfelt appreciation and special thanks goes to my family and my wife for moral and financial support.

ABBREVIATIONS AND ACRONOMS

DF	Degree of freedome
DMSO	Dimethyl sulfoxide
MHA	Muller hinton agar
MIC	Minimum inhibitory concentration
MS	Mean of square
NC	Negative control
OD	Optical density
PC	Positive control
SS	Sum of squire

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

The use of plants for treatment of various diseases is universal and has been practiced for many years even up to now where many people are treated by modern drugs. Plants produce a wide variety of secondary metabolites which are used either directly as precursors or as lead compounds in the pharmaceutical industry.Numerous studies have aimed to describe the chemical composition of these plants' antibacterial and the mechanisms involved in bacterial growth inhibition either separately or associated with conventional antibacterialmechanisms.In the present study, berries of Juniperus procera, Olea europeae subsp. cuspidata and Podocarpus falcatus were extracted by using different solvents such as methanol, petroleum ether, ethanol, chloroform and hexane.The solvent extracts were evaluated against six human pathogenic bacterial strains by usingagar well diffusion and agar dilution method (for MIC) assay methodswere employed. The test pathogenic organisms such as E. coli, S. typhi, S. dysenteriae, P. aerugenosa, S.aureus and K.pneumonia and were used.Among the solvents used to extract the biologically active substances from three medicinal plants, methanol was the best solvents followed by ethanol,chloroform, petroleum ether and hexane. The highest antibacterial activity was exhibited by methanol extract of Olea europeaeagainst S. dysentriae (18.33 ± 0.57mm) and the lowest againstK.pneumonia (10.66 ± 1.52mm) by hexane extract.The highestzone of inhibitionby methanol and ethanolextract ofPodocarpus falcatusagainstE. coli andS.aureus(16.33 ± 1.52mm)and the lowestagainst K. pneumonia (9.0 ± 1.0mm) by hexaneextractandthe highestzone of inhibitionbyethanolextract of Juniperus proceraagainstS. typhi(14.66 ± 1.52mm) and the lowestagainst K.pneumonia (3.0 ± 1.0mm) by petroleum ether;The lowesttMIC of crude extract ofOlea europeae,Podocarpus falcatus andJuniperus procera wereexhibited againstS.aureus,E.coli and S. dysentriae at the concentration of 1.56 mg/ml 3.13 mg/ml and 6.25 mg/ml bymethanol andethanolextract.Two dissimilar antibiotics such as ciprofloxacin and gentamicin were used as standard for testing antibacterial activity against six different human pathogens. The indicated the presence of some secondary metabolites existed in the tested pathogens which have related with antibacterial activities. The extracts of all Olea europeae plant should be translated into clinical practice.

***Key words**: Antibacterial activity, berries, human pathogens, medicinal plants, solvents extraction*

TABLE OF CONTENTS

1. INTRODUCTION

1.1.Background of the study

Plants have been used for thousands of years as medicines for treating a variety of diseases and medical complaints by most, if not all civilizations (Turner, 1999; Clatchey *et al*., 2009). Relatively 1-10 % of plants are used by humans out of estimated 250,000 to 500,000 species of plants on Earth (Kunzelman *et al*., 2005).The natural products derived from medicinal plants have proven to be an abundant source of biologically active compounds, many of which have been the basis for the development of new lead chemicals for pharmaceuticals (Palombo, 2009), as well as being useful therapeutic agents in their own right, an understanding of these traditional medicines has provided new plant derived drug leads for allopathic medicine.

Phyto-chemicals have provided modern medicine with new analgesics, anti-inflammatory, anti-cancer, cardiovascular and immune-modulator drugs (Cock, 2015). Furthermore, with the development of increasing numbers of antibiotic resistant bacterial strains, much recent attention has also focused on screening traditional medicines for bacterial growth inhibitory activity and the isolation of bioactive components from promising species (Cock, 2015). Traditional plant medicines have also provided modern medicine with laxatives, anti-depressants and with new leads for the treatment of diabetes mellitus (Petlevski *et al*., 2001; Sanchez *et al*., 1994). A large number of indigenous plant species are being used as a source of herbal therapies in Ethiopia. Numbers of medicinal plants and their purified constituents have shown beneficial therapeutic potentials. Medicinal plants are the richest bio-resource of drugs of traditional medicines, modern medicines, food supplements, folk medicines, pharmaceutical remediates and chemical entities for synthetic drugs(Abdillahi *et al*., 2008).Plants such as *Juniperus procera, Podocarpus falcatus and Olea europeae* which are indigenous to Ethiopia are used traditionally for healing many diseases (Kibebew, 2001; Mesfin *et al*., 2014).

Juniperus procera(Tsid)or junipers are well known for their pleasant smell as well as their resistance to both termite attack and microbial decay.The berries are used to flavor meats and sauces and as the flavoring agent for the alcoholic beverage gin. They have also been used in several traditional medicine systems. *J. procera*is used as chewing stick in Ethiopia (Seshathri

and Thiyagarajan, 2011). Antimicrobial screening also supports the use of *J. procera* which offer protection against strep throat caused by *Streptococcus pyogenes* (Seshathri and Thiyagarajan, 2011). Tafesse and Mekonnen(2012) recorded anti-diarrheal activity of leaf extract of *J. procera* and its effect on intestinal motility in albino mice. In the northern parts of the country people are known to use this plant to treat menorrhagia, emmenagogue, constipation, toothache, gum pain, and biliousness (Abebe and Ayehu, 1993). In traditional medicine the plant was documented to cure intestinal worms, fever, used as stimulant, birth control agent, sudorific, emmenagogue and rheumatism, bladder infections, chronic arthritis, gout, fluid retention, kidney disease, menstrual irregularities, heartburn, and dyspepsia (Wurges, 2005).

The leaves of *Olea europaea* (Weira) are used in folk medicine as a remedy for eye infections, sore throat, urinary tract infections, kidney problems and backaches or headaches. It is also used as a hypotensive, emollient, febrifuge and styptic (Somova *et al*., 2003). The leaves of the tree were reported to be potent for the treatment of malaria in 1854 (Altinyay, *et al*., 2011).Today, the olive plant is most well-known for its fruit crop and oil. As a folk remedy, olives have been used to reduce the incidence of heart diseases(Covas, 2008). Experimental studies on the fruits and leaf extracts from olives show that they possess antithrombotic, antihypertensive, anticancer, hypoglycemic, antiinflammatory, antimicrobial and antiatherogenic properties(Caponio *et al*., 1999; Rosignoli *et al*., 2013). The major active components of olives are phenolic compounds including oleuropein, hydroxytyrosol, tyrosol, 4-hydroxyphenyl acetic acid, protocatechuic acid, caffeic acid and p-coumaric acid. Several other biologically active constituents are also present(Caponio *et al*., 1999; Rosignoli *et al*.,2013).

Extracts and compounds isolated from *Podocarpus falcatus*(Zigba)have shown a range of pharmacological activities such as in treating gonorrhoea, antitumor, antimicrobial, plant growth regulatory, insect growth regulatory and herbivorous mammalian antifeedant activities(Ying *et al*., 1990; Kubo *et al.,* 1992). Biological activity has been associated with nor- and bisnorditerpene dilactones, with over 50 compounds already isolated (Kubo and Ying, 1991).In traditional medicine, a decoction of the fruit serves as a tonic for cleaning the kidneys, lungs and stomach. In Ethiopia *Podocarpus falcatus* oils are used to cure gonorrhea(Abdillahi*et al*., 2008). *Podocarpus species* are used in the treatment of fevers, asthma, coughs, cholera, distemper, chest complaints and venereal diseases (Abdillahi*et al*., 2010). Although extensive research has been

carried out on species of *Podocarpus* over the last decade, relatively little is known about their oils compared to other secondary metabolites.

1.2.Statement of the problem

Medicinal plantshave been used to cure intestinal worms, fever, stimulant, birth control agent, sudorific, emmenagogue and rheumatism, bladder infections, chronic arthritis, gout, fluid retention, kidney disease, menstrual irregularities, heartburn, and dyspepsia (Wurges, 2005).Traditional plant medicines have also provided modern medicine with laxatives, anti-depressants and with new leads for the treatment of diabetes mellitus (Sanchez *et al*., 1994; Petlevski *et al*., 2001). The emergence of pathogenic microorganisms resistant to commonly used antibiotics is a worldwide concern of the 21st century. Antibiotic resistance seems to be increasing, and multiple antibiotic resistant strains have started to emerge (Otter and French, 2010).With the development of increasing numbers of antibiotic resistant bacterial strains, much recent attention has focused on screening traditional medicines for bacterial growth inhibitory activity and the isolation of bioactive components from promising species (Cock, 2015). These plant species (*Juniperusprocera* (Tsid),*Olea europeae* (Weira)and *Podocarpus falcatus*(Zigba)) are endemic plants to Ethiopia and also in some countries in East and South East Africa. In Ethiopia, there is a long history of using medicinal plants to treat a variety of diseases (Kibebew, 2001; Mesfin *et al*., 2014). Eighty percent of the human population and 90% of livestock in Ethiopia rely on traditional medicine, as many plants species have displayed medicinal value for some diseases of human and livestock (Abebe, 2001). According toBekele (2007), the major reasons why medicinal plants are demanded in Ethiopia are due to culturally linked traditions, the trust the communities have in traditional medicine, and relatively low cost in using them.Despite the documented antimicrobial activities of *Juniperus procera*, *Olea europeae* and *Podocarpus falcatus*plants, little attention has been given to the antibacterial activities of berries of those plants against selected human bacterial pathogens. The exact picture of antibacterial activities of berries of those plants in the areas is not well documented. To my knowledge, there is no scientific study conducted and no published data on antibacterial activities of berries of those plants from Adaba-Dodola forest. Therefore, this research is aimed to evaluate the antibacterial activities of berries of*Juniperusprocera* (Tsid),*Olea europeae* (Weira)and *Podocarpus falcatus*(Zigba) against human bacterial pathogens.

1.3.Significance of the study

Herbal medicine is still the mainstay of about 75-80% of the whole population, mainly in developing countries for primary health care because of better cultural acceptability and better compatibility with the human body and fewer side effects. Use of plant extracts to control human disease causing organism has the advantage of low production cost, minimal environmental damage and higher accessibility to rural communities. Medicinal plants are also expected to be the future alternative source of new antimicrobial products. Further a focused phyto-chemical screening of medicinal plants often leads to the discovery of new lead compounds that can play a role in the global efforts against pathogens.This analysis may also offer a source of informationto identify effective medicinal plants against many infectious organisms and facilitate selection of plants for further phyto-chemical investigation.

1.4.Objectives

The study was executed based on the following objectives:

1.4.1. General objective

- To investigate the antibacterial activities of extract of berries of *Juniperusprocera* (Tsid),*Olea europeae*(Weira)and *Podocarpus falcatus*(Zigba) from Adaba-Dodola forestagainst human bacterial pathogens.

1.4.2. Specific objectives

- To determine the yield of the crude antibacterial compounds from a given mass of medicinal plants by using different solvents
- To evaluate theactivity of crude extracts of*Juniperusprocera* (Tsid),*Olea europeae* (Weira)and *Podocarpus falcatus*(Zigba)extracts against human bacterial pathogens.
- To determine the minimum inhibitory concentration of the plant extracts by using broth dilution method.

1.5.The scope of the study

This study was delimited to an investigation of the antibacterial activity of extracts of *Juniperus procera, Olea europeae* and *Podocarpus falcatus* against human bacterial pathogens (*Staphylococcus aureus, Escherichia coli, Pseudomonas aerugunosa, Klebsiella pneumonia, Salmonella typhi, Shigella dysentriae*). The medicinal plants were collected from Adaba-Dodola forest.

1.6.Limitations of the study

The major limitation of the study includes problems encountered such as the difficulty to get chemicals and solvents at the appropriate time. Besides, inaccessibility of relevant materials,shortage of time and finance to extract data were considered as a limitation of this study.

2. REVIEW OF RELATED LITERATURE

2.1.Juniperus procera (Tsid)

2.1.1. Common names and Taxonomic Classification

Amharic (tid,tedh); Arabic (arar); English (pencil cedar, East African pencil cedar, East African cedar,cedar,African pencil cedar); Swahili (mwangati); Afan Oromo (hindhessa); Trade name (cedar, African pencil cedar).

Domain: Eukaryota, Kingdom:Plantae, Phylum:Tracheophyta, Class:Pinopsida, Order:Pinales, Family:Cupressaceae

Scientific Name:*Juniperus procera* Hochst. ex Endl(Farjon,. 2010).

2.1.2. Botanical description

Bole: Straight/fluted. Large. To 40 m, Bark: Pale brown; thin; fibrous; cracking and peeling in long narrow strips, Slash: Pale yellow. Fibrous. Resinous smell, Leaf: Simple. Opposite/whorled, Petiole: Sessile, Lamina: Small. Juvenile: 0.8 - 1 × 0.1 cm. Adult: 0.1 × 0.1 cm. Lanceolate when, young; becoming scales. Cuneate. Acute. Entire. Glabrous, Domatia: Absent, Glands: Present. Juvenile leaves with a linear gland on the back and adult leaves with an elliptic oil gland on the back near the base, Stipules: Absent, Thorns & Spines: Absent, Flower: Absent, Cones. Dioecious, Fruit**:** Brown/black berry-like cone; 0.4 - 0.8 cm in diameter with 1 - 4 seeds (Earle 2009).

2.1.3. Distribution

Juniperus procera is native to the Arabian Peninsula (in Saudi Arabia and Yemen), and northeastern, eastern, west-central, and south tropical Africa (in the Democratic Republic of the Congo; the Republic of the Congo; Djibouti; Eritrea; Ethiopia; Kenya; Malawi; Mozambique; Somalia; Sudan; Tanzania; Uganda; Zambia and Zimbabwe) (Earle, 2009). It is the only juniper to occur south of the equator, and is thought to be a relatively recent colonist of Africa; the species shows very little of the variability associated with a long period of evolution(Farjon, A. 2005). It is closely related to *Juniperus excelsa* from southwestern Asia, probably deriving from a common ancestor with that species in southwestern Asia (Farjon, 2005).

2.1.4. Ethno medicinal uses

Junipers are used as culinary components by a variety of cultures in the regions in which they occur. The berries are used to flavor meats and sauces and as the flavoring agent for the alcoholic beverage gin. They have also been used in several traditional medicine systems. In Ethiopia,*J. procera*is used as chewing stick (Seshathri and Thiyagarajan, 2011).*Juniperus procera* has a wide range of traditional uses including charcoal and timber productions, fire wood, fencing etc. Its leaves are smoked to deter insects (personal observation) in rural areas of the country. In the northern parts of the country people are known to use this plant to treat menorrhagia, emmenagogue, constipation, toothache, gum pain, and biliousness (Abebe and Ayehu, 1993). In traditional medicine the plant was documented to cure intestinal worms, fever, stimulant, birth control agent, sudorific, emmenagogue and rheumatism, bladder infections, chronic arthritis, gout, fluid retention, kidney disease, menstrual irregularities, heartburn, and dyspepsia (Wurges, 2005).

2.1.5. Antimicrobial Activities

Juniperus procera resulted in the isolation of different classes of diterpenes and sesquiterpenes. The two lignans β-peltatin ether and deoxypodophyllotoxin were isolated from the bark of *J. procera* (Muhammad *et al*., 1995). Several antimicrobial diterpenes were isolated from the barkand leaves of *J. procera* including isocupressic acid, (+)-Z-communic acid, (+)-totarol and sugiol (Muhammad *et al*., 1995; Muhammad *et al*., 1996).Antimicrobial screening also supports the use of *J. procera* which offer protection against strep throat caused by *Streptococcus pyogenes*(Seshathri and Thiyagarajan, 2011). Tafesse and Mekonnen, 2012 recorded anti-diarrheal activity of leaf extract of *J.procera* and its effect on intestinal motility in albino mice. A bioassay-guided fractionation of *J. procera* berries yielded ant parasitic, nematicidal and antifouling constituents, including a wide range of known abietane, pimarane and labdane diterpenes (Samoylenko *et al*., 2008).The phenolic diterpene totarol, isolated from *J. procera* showed synergistic effect against four species of *Mycobacterium* (Mossa *et al*., 2004). The essential oil of *J. procera* was effective as antioxidant and OH-radical-scavenging agents when assessed in the deoxyribose degradation assay (Burits, 2001). The petroleum ether fraction of *Juniperus procera* showed significant activity as hepatoprotective when investigated against carbon tetrachloride induced liver injury(Abdel-Kader *et al*., 2009).

2.2.*Olea europeae* (Weira)

2.2.1. Common names and taxonomic classification

Afrikaans (olyfboom,olienhout,swartolienhout); Amharic (weira); Arabic (zeitun bari); English (brown olive, wildolive, Indianolive, African wild olive,olive); German (Ölbaum); Hindi (bair banj,zaitoon,kan,kau,kahu,kao); Ndebele (umnquma); Shona (mupfungo); Somali (wera); Tigrigna (awliie); Afan Oromo (Ejersa); Trade name (kao,brown olive,kau); Zulu (umNqumo) (Jones,1991).

Domain: Eukaryota, Kingdom: Plantae, Phylum: Spermatophyta, Subphylum: Angiospermae, Class: Dicotyledonae, Order: Oleales, Family:Oleaceae, Genus: Olea, Species: *Olea europaea*, subsp. Cuspidate. The cultivated olive, *Olea europaea*, belongs to *Oleaceae*, a medium-sized family of approximately 25 genera and 688 species distributed throughout temperate and tropical regions of the world (The Plant List, 2013). Plants in this family are primarily trees and bushes alongwith a number of vines, many of which produce essential oils in their flowers or fruits (Janick and Paull, 2008).

2.2.2. Morphology

Olea europaea ssp. cuspidata is a shrub or a small to medium sized tree 5-10 m in height, occasionally reaching 18 m. Bark is grey to brownish-blackish, smooth to rough when old.Leaves narrowly oblong-elliptic, 2-10 cm x 7-17 mm, grey-green to shiny dark green above, grayish or yellowish with a dense covering of silvery, golden or brown scales on the under surface; apex and base narrowly tapering, apex sharp tipped; margin entire, rolled under and curved back from the midrib, petiole slender, up to 10 mm long, so the leaves tend to droop.

Flowers greenish-white or cream, 6-10 mm long, sweetly scented, in loose auxiliary or occasionally terminal heads, 5-6 cm long. Fruit ovoid, thinly fleshy, about 10 x 8 mm tapering to a sharp tip, dark brown or black when mature (Zohary, (1995).

2.2.3. Distribution and Habitat

The major area of natural distribution for *Olea europaea* ssp.cuspidatais eastern Africa, where it extends throughout the eastern African states from the southern tip of Africa to the north-east regions. It is commonly recorded for the countries of Tanzania, Kenya, Ethiopia, Mozambique,

Sudan, Namibia, Somalia and South Africa. From eastern Africa, the distribution extends into the Middle East region with occurrences in Yemen and Saudi Arabia. A significant Asian centre of *Olea europaea* species distribution is northern India, Afghanistan, Pakistan and Kashmir. The most easterly natural distribution is China, particularly the drier parts ofYunan and Sichuan (Green, 2002).

2.2.4. Botanical description

African Olive is part of the *Olea europaea*complex, which includes the edible European Olive, long-cultivated throughout the Mediterranean region. The *Olea europaea*complex extends from the Canary Islands and Madeira westwards across the Mediterranean, and south-west Asia to the Sino-Himalayan region, and south through eastern Africa to southern Africa (Green and Wickens, 1989).

African Olive is part of a tropical wild olive group, geographically isolated from their Mediterranean relatives and adapted to totally different climates (Zohary, 1995). Thegenus Olea occurs across a very wide geographic range with 33 species and nine subspecies described, the name *Olea europaea* subsp.cuspidatais proposed for the wild olive occurring in South to North-East Africa and southwest Asia, and naturalized in Hawaii, Australia, New Zealand and Norfolk Island. In Australia both the European Olive (*Olea europaea* subsp.europaea) and African Olive (*Olea europaea* subsp.cuspidata) have naturalized. In southwest African Olive was previously known as Olea Africana and more recently *Olea europaea* subsp. Africana. It is readily distinguished from European Olive by the presence of a hooked leaf apex and a lower leaf surface which is green or yellowish brown (Harden, 1992).

2.2.5. Ethno Medicinal uses

The *O. europaea* subspecies *africana* plant leaves are used in folk medicine as a remedy for eye infections, sore throat, urinary tract infections, kidney problems and backaches or headaches. It is also used as a hypotensive, emollient, febrifuge and styptic (Somova, *et al*., 2003). The leaves of the tree were reported to be potent for the treatment of malaria.

2.2.6. Antimicrobial Activities

Of the four major polyphenol compounds identified, oleuropein and vabascoside were tested for their antimicrobial activities against the three food borne pathogens. Compared to the crude extract, oleuropein, and vabascoside were more potent for the inactivation of the studied food borne pathogens(Soler-Rivas *et al*., 2000). Although present in olive seeds, oleuropein is present predominantly in olive leaves (Le Tutour and Guedon, 1992). Oleuropein has been used in pharmacology to treat inflammation and in the control of obesity (Vogel *et al*., 2014). Its application in the food industry is limited due mainly to its bitter taste. The antimicrobial mechanism of oleuropein in *S. aureus* has been studied using a system biology approach. The genome model predicted the changes in gene expression, which correlated very well with experimental data (Li *et al*., 2016). Vabascoside is another abundant polyphenol present in olive leaves. It is more effective than oleuropein in inhibition of bacterial growth. The Gram-negative bacteria are less sensitive to polyphenols than Gram positive bacteria (Seow *et al*., 2014). The Gram-positive bacteria are sensitive to polyphenols since the bacterial membranes interact with hydrophobic components of the polyphenols. On the other hand, Gram-negative bacteria are more resistant to polyphenols because they possess a hydrophilic cell wall (Calo *et al*., 2015).

2.3.*Podocarpus falcatus*(Zigba)

2.3.1. Common names and taxonomic classification

Afrikaans (outeniekwageelhout); Amharic (zigba); Afan Oromo (Bribrsa); English (smooth-barked yellow wood,podo,oteninqua yellow wood,East African yellow wood); is one of theindigenous conifers in Ethiopia(Breitenbach 1963; Negash, 1995). Trade name (podo); Zulu (umSonti)(Tesemma,.*et al*, 1993).

Kingdom: Plantae, Phylum: pinophyta, Class: pinopsida, Order: pinales, Family: podocarpaceae, Genus: podocarpus, Species *Podocarpus falcatus Podocarpus* (poʊdəˈkɑːrpəs)(*Sunset Western Garden Book* 1995) is a genus of conifers, the most numerous and widely distributed of the podocarp family, Podocarpaceae. There are approximately 97 to 107 species in the genus depending on the circumscription of the speciesBarker*et al*. 2004; Chris, 2013; Farjon, 2010; Ornelas.,*et al*. 2010).

2.3.2. Morphology

Podocarpus falcatus is a dioecious species with male and female cones (or fruits). The fruit consists of a fleshyouter covering (epimatium), and a hard woody seed coat (or sclera testa) covering the mega gametophyte containingthe embryo (Geldenhuys, 1993; Negash, 1995, 2003).

Podocarpus are ever greenshrubs or trees, usually from 1 to 25 meters (3 to 82 ft) tall, known to reach 40 meters (130 ft) at times. The cones have two to five fused cone scales which form a fleshy, berry-like, brightly colored receptacle at maturity. The fleshy cones attract birdswhich then eat the cones and disperse the seeds in their droppings. Due to predation by insects and pathogens, *P. falcatus* has no persistent seed banks (Teketay, 1995;Granstrom, 1997) which further challenges regeneration of the species. Infection of seed by pathogenic fungi can result in the death of seed or seedlings', placing large con-straints on recruitment, as has been shown for several other plant species (Bell *et al*., 2006; Assefa *et al*., 2014a, b).

2.3.4. Botanical description

Podocarpus falcatus is an evergreen tree up to 46 m in nature but quite smaller if planted, with a long clean and cylindrical trunk. The crown is slender with a light branching system or sub-opposite or verticellate spreading limbs or small, with crowded branches. The bark is thin, rather smooth and grayish-brown to dark brown in color. The genus name is derived from 'podos', a Greek word for foot and 'karpos' meaning fruit, in reference to the swollen seed stalk. Falcatus means sickle-shaped, referring to the shape of the leaves(Hong *et al*.,1996).

2.3.5. Distribution

In the early 1940s and 1950s, the biogeographical information on Podocarpaceae was published (Florin, 1940; Buchholz and Gray, 1948; Li, 1953). However, due to great changes in the taxonomy of Podocarpaceae (De Laubenfels, 1969; Quinn, 1982; Molloy, 1995; Kelch, 1997, 1998), the earlier biogeographical statement is rendered invalid. For example the genus *Dacrydium* is not present in both western and eastern hemispheres (Sinclair *et al*., 2002). The conventional view was that the Podocarpaceae had a Gondwanan origin and migrated northwards to reach the present northerly limits in the Caribbean, Ethiopia and eastern Asia. The only limitation to this view is that it does not consider the numerous Laurasian fossils that have

beenassigned to Podocarpaceae (Mill, 2003). The greatest generic diversity of Podocarpaceae is in Malesia and Australasia where 17 of the 19 living genera are found. Malesia, New Caledonia and New Zealand each have eight genera, seven genera in Australia, four genera in South America and Africa, and Asia two genera each. With respect to living endemism, Australia has three endemic genera (*Lagarostrobos*, *Microcachrys* and *Pherosphaera*), two for New Zealand (*Halocarpus* and *Manoao*), New Caledonia, South America and Africa each have one (*Parasitaxus*, *Saxegothaea* and *Afrocarpus*, respectively) (Mill, 2003).

2.3.6. Ethno Medicinal Uses

Unspecified communities in East Africa use *P. latifolius* and *P. falcatus* to treat stomach ache and cattle diseases (Sindiga, 1995). In Ethiopia *P. falcatus* oils are said to have medicinal properties in curing gonorrhea and the powder from the bark is used for curing headaches (Pankhurst, 2000).

2.3.7. Antimicrobial activity

Crude extracts of four South African *Podocarpus* species viz; *P. elongatus*, *P. falcatus*, *P. henkelii* and *P. latifolius* exhibited broad spectrum antimicrobial activity against *B. subtilis* (98 µg/ml), *S. aureus* (98 µg/ml), *E. coli* (390 µg/ml), *Klebsiella pneumoniae* (330 µg/ml) and *C. albicans*(30 µg/ml). The extracts were assayed using the micro dilution bioassay described by Eloff (1998) and plates were incubated for 24 hours. Neomycin was used as a positive control and it gave an MIC value of 0.07 µg/ml against *B. subtilis*, and 0.26 µg/ml against the other three bacteria (Abdillahi *et al*., 2008).These antimicrobial activities provides rational for the traditional uses of some of the species in treating microbial infections such as the use of oils from *P. falcatus* in treating gonorrhoea, sap from *P. falcatus*, *P. henkelii* and *P. latifolius* as a remedy for chest infections (Hutchings *et al*., 1996; Pankhurst, 2000).

2.4. Characteristics of Test Pathogens

2.4.1. Escherichia coli

(Oxford University Press. September 2005) also known as *E. coli*) is a Gram-negative, facultatively anaerobic, rod-shaped, coliform bacterium of the genus *Escherichia* that is commonly found in the lower intestine of warm-blooded organisms (endotherms).(Tenaillon *et*

al., 1 March 2010; Singleton, 1999). Most *E. coli* strains are harmless, but some serotypes can cause serious food poisoning in their hosts, and are occasionally responsible for product recalls due to food contamination (Vogt and Dippold 2005).

The harmless strains are part of the normal microbiota of the gut, and can benefit their hosts by producing vitamin K_2, and preventing colonization of the intestine with pathogenic bacteria, having a symbiotic relationship. *E. coli* is expelled into the environment within fecal matter. The bacterium grows massively in fresh fecal matter under aerobic conditions for 3 days, but its numbers decline slowly afterwards(Russell JB. and Jarvis GN. 2001).

2.4.1.1. Type and morphology

Escherichia coli is a Gram-negative, facultative anaerobic and non sporulating bacterium. Cells are typically rod-shaped, and are about 2.0 μm long and 0.25–1.0 μm in diameter, with a cell volume of 0.6–0.7 μm.*E. coli* stains Gram-negative because its cell wall is composed of a thin peptidoglycan layer and an outer membrane. During the staining process, *E. coli* picks up the color of the counterstain safranin and stains pink. The outer membrane surrounding the cell wall provides a barrier to certain antibiotics such that *E. coli* is not damaged by penicillin(Tortora, 2010). Strains that possess flagella are motile. The flagella have a peritric hous arrangement. It also attaches and effaces to the microvilli of the intestines via an adhesion molecule known as intimin (Madigan and Martinko 2006).

2.4.1.2. Culture growth

Optimum growth of *E. coli* occurs at 37 °C (98.6 °F), but some laboratory strains can multiply at temperatures up to 49 °C (120 °F)(Fotadar.*et al*.,2005). *E. coli* grows in a variety of defined laboratory media, such as lysogeny broth, or any medium that contains glucose, ammonium phosphate, monobasic, sodium chloride, magnesium sulfate, potassium phosphate, dibasic, and water. Growth can be driven by aerobic or anaerobic respiration, using a large variety of redox pairs, including the oxidation of pyruvic acid, formic acid, hydrogen, and amino acids, and the reduction of substrates such as oxygen, nitrate, fumarate, dimethyl sulfoxide, and trimethylamine N-oxide (Ingledew. *et al*., 1984). The ability to continue growing in the absence of oxygen is an advantage to bacteria because their survival is increased in environments where water predominates(Tortora, 2010).

2.4.2. Klebsiella pneumoniae

2.4.2.1. Description and significance

Klebsiella pneumoniae is a gram negative bacterium. It is facultative anaerobic. It is rod-shaped and measures 2 µm by 0.5 µm. In 1882, Uber first discovered *Klebsiella* to be a pathogen that caused pneumonia (Cortés *et al*., 2002). Many hospital cases around the world have been linked to *K. pneumoniae*. Therefore, more studies of the strains were important and performed. The bacterium was isolated and sequenced from a patient in 2004. *K. pneumoniae* is commonly found in the gastrointestinal tract and hands of hospital personnel(Podschun and Ullmann. 1998).

The reason for its pathogenicity is the thick capsule layer surrounding the bacterium. It is 160 nm thick of fine fibers that protrudes out from the outer membrane at right angles (Amako *et al*., 1988; Lawlor *et al*., 2005).

Another site on the human body that this bacteria can be found is the nasopharynx. Its habitat is not limited to humans but is ubiquitous to the ecological environment. This includes surface water, sewage, and soil (Brisse and Verhoef 2001). The frequent occurrence of resistant K. pneumoniae infections continues to spark great interest in research on how to control these resistant infections, possibly by preventing them with vaccines (Lee. *et al*., 2015).

2.4.2.2. Ecology

Klebsiella pneumoniae is ubiquitous as it is found in mammals and ecological environment. It has pathogenic effects worldwide. There is evidence of community-acquired and hospital-acquired infections in countries such as Taiwan and South Africa. Community-acquired *K. pneumoniae* has been found, in some places, to be associated with alcoholism. There are a large number of infections acquired when it affects different organs of the body. It can affect the liver, urinary tract, lungs, to name a few (Mohammed.*et al*., 2007).

2.4.2.3. Pathology

Klebsiella pneumoniae is pathogenic and is responsible for a large number of infections every year. The most common pathogenic strains of *K. pneumoniae* are the K1 and K2 capsular serotype (Amako,*et al*., 1988). In addition, a study done on the K1 and K2 serotypes discovered that the mucoid phenotype was present in greater than 94% of *K. pneumoniae* infections (Amako,*et al*., 1988). The main virulence factor in pathogenic *K. pneumoniae* strains is aerobactin (produced by K1 and K2 serotypes), which is also the main virulence factor in pathogenic E. coliStrains(Amako,*et al*., 1988).

In a recent study, hypervirulent strains were responsible for the deaths of five patients in China in 2013 (Amako.*et al*., 1988). Most cases of *K. pneumoniae* infections were the result of patient to patient transfer and were hospital acquired. Common symptoms of *K. pneumoniae* infections in the blood include fever, chills, rash, and light-headedness (Lee.*et al*., 2015).Infections in the lungs can lead to meningitis and can result in breathing problems. The most common treatment for *K. pneumoniae* infections are antibiotics but recent strains have been found to resist many common antibiotics including carbapenem.

2.4.3. Shigella dysentriae

2.4.3.1. Description and significance

Morphological characteristics: *S. dysenteriae* is a small, uncapsulated, non-motile, Gram-negative bacillus (rod-shaped). It does not form spores(Niyogi.2005).

Habitat: *S. dysenteriae* is found worldwide but concentrates in areas where the population suffer from overcrowding and malnutrition and do not possess adequate waste management and safe drinking water supplies. *S. dysenteriae* has caused endemic dysentery in Africa, Southeast Asia, and the Indian subcontinent. Humans are the only natural hosts for *S. dysenteriae*, though houseflies may serve as vectors for the transmission of *S. dysenteriae*. Although *S. dysenteriae* has been found in contaminated food and water, this organism generally survives poorly outside the human body(Niyogi.2005).

Significance: *S. dysenteriae* causes a significant threat to public health by causing shigellosis, especially in developing countries. Shigellosis is associated with 5-15% of cases of diarrhea and

30-50% of cases of dysentery worldwide. In malnourished children, shigellosis can cause a vicious cycle of "further impaired nutrition absoprtion, recurrent infection, and growth retardation." Without proper care, shigellosis can become life-threatening(Niyogi.2005).

Discovery: *S. dysenteriae* was first isolated by Kiyoshi Shiga in 1896 from stool of dysentery patients. When a culture of the organism was fed to dogs, diarrhea ensued. He confirmed *S.dysenteriae* as the cause of the dysentery in these patients by a simple agglutination technique. Shiga demonstrated that the organism repeatedly coalesced when exposed to the serum of dysentery patients. In the years immediately following Shiga's discovery, similar organisms were reported by other investigators, and over the next 40 years three additional relatedorganisms were taxonomically placed in the genus Shigella and named *S. flexneri, S. boydii*, and *S. sonnei* (Niyogi. 2005).

2.4.4. Pseudomonas aeruginosa

Pseudomonas aeruginosa is a common Gram-negative, rod-shapedbacterium that can cause disease in plants and animals, including humans. A species of considerable medical importance, *P. aeruginosa* is a multidrug resistantpathogen recognized for its ubiquity, its intrinsically advanced antibiotic resistance mechanisms, and its association with serious illnesses – hospital-acquired infections such as ventilator-associated pneumonia and various sepsis syndromes. The organism is considered opportunistic insofar as serious infection often occurs during existing diseases or conditions – most notably cystic fibrosis and traumatic burns. It is also found generally in the immunecompromised but can infect the immune competent as in hot tub folliculitis. Treatment of *P. aeruginosa* infections can be difficult due to its natural resistance to antibiotics. When more advanced antibiotic drug regimens are needed adverse effects may result. It is citrate, catalase, and oxidase positive. It is found in soil, water, skin flora, and most man-made environments throughout the world. It thrives not only in normal atmospheres, but also in low-oxygen atmospheres, thus has colonized many natural and artificial environments. It uses a wide range of organic material for food; in animals, its versatility enables the organism to infect damaged tissues or those with reduced immunity. The symptoms of such infections are generalized inflammation and sepsis. If such colorizations occur in critical body organs, such as the lungs, the urinary tract, and kidneys, the results can be fatal (Balcht and Smith, 1994).

Because it thrives on moist surfaces, this bacterium is also found on and in medical equipment,Including catheters, causing cross-infections in hospitals and clinics. It is also able to decompose hydrocarbons and has been used to break down tar balls and oil from oil spills (Itah and Essien 2005).

P. aeruginosa is not extremely virulent in comparison with other major pathogenic bacteria species – for example *Staphylococcus aureus* and *Streptococcus pyogenes* – though *P. aeruginosa* is capable of extensive colonization, and can aggregate into enduring bio films (Høiby, *et al*., 2010).

2.4.5. ***Staphylococcus aureus***

Staphylococcus aureus: is a Gram-positive, round-shapedbacterium that is a member of the Firmicutes, and it is a member of the normal flora of the body, frequently found in the nose, respiratory tract, and on the skin. It is often positive for catalase and nitrate reduction and is a facultative anaerobe that can grow without the need for oxygenMasalha, *et al*., 2001).

Although *S. aureus* is not always pathogenic, it is a common cause of skin infections including abscesses, respiratory infections such as sinusitis, and food poisoning. Pathogenic strains often promote infections by producing virulence factors such as potent proteintoxins, and the expression of a cell-surface protein that binds and inactivates antibodies. The emergence of antibiotic-resistant strains of *S. aureus* such as methicillin-resistant *S. aureus* (MRSA) is a worldwide problem in clinical medicine. Despite much research and development, no vaccine for *S. aureus* has been approved. *Staphylococcus* was first identified in 1880 in Aberdeen, Scotland, by surgeon Sir Alexander Ogston in pus from a surgical abscess in a knee joint(Ogston.1984).This name was later amended to *Staphylococcus aureus* by Friedrich Julius Rosenbach (Kluytmans, *et al*., July 1997).

2.4.6. Salmonella typhi

Salmonellosis is considered one of the most important food borne zoonoses contributing to public health. *Salmonella enterica* subsp. *enterica* serovar (*S*.) Typhimurium is among the most prevalent *Salmonella* serotypes worldwide and is of serious public and animal health concern. Several different *S*. Typhimurium phage types have arisen during the last decades. Most of them

have been found in a wide variety of animal hosts and were characterized by particular resistance patterns (Daly, *et al*., 2000).

In the early 1990s the emergence of a new clone was observed and shortly after *S*. Typhimurium DT104 gained an epidemiological importance in different animal reservoirs and subsequently in humans. Unlike the previous *S*. Typhimurium phage types with plasmid-mediated resistance, DT104 resistance genes for pentaresistant profile (ampicillin, chloramphenicol, streptomycin, sulphonamides and tetracycline) were located in a genome-integrated gene cluster containing also class I integrons (Pooley. 1993); Liebana,*et al*., 2002).

Recently *S*. Typhimurium DT104 have been reported from a variety of countries throughout the world Gebreyes, and Altier .2002; Daly, *et al*., 2000) and the high level of genetic homogeneity within strains of this lineage indicated a clonal spread of the pathogen (Liebana, *et al*., 2002).

3. MATERIALS AND METHOD

3.1.Location of the Study Area

The study was conducted on berries of *Juniperus procera,Olea europeae* subsp. *Cuspidata* and *Podocarpus falcatus* collected from Adaba-Dodola forest, West Arsi zone of Oromia regional state, south east Ethiopia. It is located at a distance of 325km from the capital city of Ethiopia, Addis Ababa. The area is situated at a latitude and longitude of 6°50′–7°00′N and 39°07′–39°22′E, respectively and had altitude in the range of 2400-3100 meters above sea level (Assefa *et al*., 2014 a, b; Assefa and Abate, 2018). The maximum annual temperature and rainfall of the area is 7-24^{0}cand 1200mm, respectively. The major and minor rainy season of the study district is from July-September and March-April, respectively (Assefa *et al*., 2014 a, b; Assefa and Abate, 2018).

3.2.Treatments and Experimental Design

The experiment were arranged in factorial randomized complete design (CRD) combination of 3 plant materials (berries of *Juniperus procera, Olea europeae* subsp. *Cuspidata* and *Podocarpus falcatus)* x 5 organic solvents (chloroform, ethanol, hexane, petroleum ether, and methanol) x 6 test pathogens (*Staphylococcus aureus, Escherichia coli, Pseudomonas aerugunosa, Klebsiella pneumoniae, Salmonella typhi* and *Shigella dysentriae*) with three replications. Accordingly, a total of 91treatments including one control (without extract plant) were investigated.

3.3. Collection and Identification of Plant Materials

Samples of berries of *Juniperus procera,Olea europeae* subsp. *Cuspidata* and *Podocarpus falcatus*were collected in a large quantity from the study areas. The taxonomic position of the plants was identified and authenticated by plant experts from National Herbarium in Addis Ababa and published species descriptions (Bekele, 2007). Prior to use, the plant materials were repeatedly washed under tap water to removeany debris and were air-dried for twenty one days.

3.4.Preparation of Plant's Crude Extracts

The preparation of crude extracts of plants under this study was conducted followed the methods described by (Tadeg *et al*., 2005) who used different solvents. Accordingly, five hundred grams

of berries from each plant was taken for extraction procedure and ground using a mortar and pestle separately under aseptic condition. 100grams of each powdered plant materials were extracted using 250ml of chloroform, ethanol, hexane, petroleum ether, and methanol separately by maceration for 48hrs with frequent agitation on orbital shaker for continuous two days and the extractedliquid wasfiltered (Whatman No. 3 filter paper, Whatman Ltd., England). Extraction was repeated three times and the filtrates of all portions were combined in one vessel. The organic solvent was removed by evaporation using Rota vapor (BUˇCHI Rota-vapor R-205, Switzerland) at 40°C. The extracted dehydrated mass was then crushed, packed into a glass vial until used. Finally, the gram yield of dried residue of each plant extracts were calculated. The concentrated extracts were stored at 4°C for the next antimicrobial study. Dried residues were dissolved in 100% dimethyl sulfoxide (DMSO) to obtain a stock concentration of 200 mg/ml, which were kept at 4°C until used.Finally, the gram yield of dried residue of each plant extracts were calculated as

$$\text{Percentage of yield} = \frac{\text{Weight of extracts obtained}}{\text{Weight of plant material}} \times 100\%$$

3.5.Preparation of Test Organisms

The test pathogenssuch as *Escherichia coli, Salmonella typhi, Shigelladysenteriae, Pseudomonas aerugenosa, Staphylococcus aureus*and*Klebsiellapneumoniae*were used for the study.These test pathogenswere obtained from Ethiopian Public Health Institute (EPHI), Addis Ababa, Ethiopia. These bacterial specieswere suspended in nutrient broth and sub cultured into fresh nutrient agarmedium and kept at 4°C until use. The inoculums preparations were standardized by inoculating bacterial strains from the exponential phase and standardized with 0.5 McFarland turbidity standard prepared by adding a 0.5 ml aliquot of 1.175% w/v $BaCl_2.2H_2O$, added to 99.5 ml of 0.18 mol/l H_2SO4 (1% v/v).

3.6.Antibacterial Sensitivity Testing Using Agar Well Diffusion Method

A sterile cotton swab (on a wooden applicator stick) was dipped into the standardized bacterial suspension of the test organism. Broth were reduced from the swabs by pressing and rotating the swabs firmly against the inside of the tube above the fluid level. The swab was then evenly streaked in three directions over the entire surface of the Muller Hinton Agar (MHA) (Oxoid,

England) plates to obtain uniform inocula in a form that lawn growth has been observed; a final sweep of the agar rim wasmade with the cotton swabs. Six equidistant wells were made on Muller Hinton Agar(MHA) plates using a sterile cork borer of 5mm in diameter after which 100µl of 200mg/ml of plant extract was dispensed into each of the five wells while the sixth well was dispensed with dimethyl sulfoxide (DMSO) (100 µg ml^{-1}) used as negative control (Shahidi, 2004). The inoculated plates were kept in the refrigerator for 1 hour to allow the extracts to diffuse into the agar. The Mueller Hinton Agar (MHA) plates were incubated at 37^{o}C for 24 hours. Bacteria inhibition zones were measured in millimeters which determined antibacterial activity of the extract (NCCLS, 2000). Inhibition zones with diameter less than 12 mm was considered as having low antibacterial activity. Diameters between 12 and 16 mm was considered as moderately active, and >16mm was considered as highly active (Indu *et al*., 2006). The test organisms were also tested for their sensitivity against the standard antibiotics, Gentamicin and Ciprofloxacin (35 µg), as positive control by the disc diffusion method (Bauer *et al*., 1966).

3.7.Antibacterial Activity by Agar Dilution (MIC) Assay Methods

The minimum inhibitory concentration (MIC) was determined by comparing the various concentrations of plant extracts which have different inhibitory effect and selecting the lowest concentration of extract showing inhibition (Agatemor, 2009). The test was performed by using agar dilutionmethod onMuller Hinton Agar.This was done by incorporation of varying desired concentrations of the antimicrobial agent into an agar medium (molten agar medium), habitually using serial two-fold dilutions, followed by the inoculation of a 10^4 CFU/ml in-oculum onto the agar plate surface (Balouiri et al. 2016). Accordingly, the plant extract was prepared by double serial dilution from 200 mg/ml (NCCLS, 2000) to obtain1:2, 1:4, 1:8, 1:16, 1:32, and 1:64in order to get 100, 50, 25, 12.5, 6.25, 3.125, 1.56 and 0.78mg/ml concentration of extract respectively using 50% DMSO. A sterile Muller-Hinton agar medium (when molten at 40-45 ^{o}C)was mixed well with a 50 µl of a known concentration of the crude extract of each plant in three directions of the inoculated surface of the Muller Hinton Agar (with an approximate equal distance that did not collide). The poured MHA medium was allowed to dry under laminar hood. Then, the plate's surfaces were inoculated with test pathogen. Minimum inhibitory

concentrations (MICs) were determined as the lowest concentration of antimicrobial agents that were inhibited the visible growth of a microorganism after incubation at 37°C for 24 h.

3.8.Data Analysis

Data on mean inhibition zone formed by each plant extract and the MIC of individual plant extract on each test bacterial pathogen were entered into Microsoft excels spreadsheet. The data was cleaned and exported to SPSS (Statistical Package Software for Social Science version 16) for testing statistical significance at $P < 0.05$ was used. Values were given as mean±standard deviation (SD).

4. RESULTS

4.1. Yield of the Plant Extracts

The crude extracts of berries of *Juniperus procera,Olea europeae* subsp. *cuspidata* and *Podocarpus falcatus*were tested for antibacterial activity on six human pathogens. The solvents that were used in this study produced an overall yield of plant crude extracts that were ranging from 2.8 to 1.5 gm from different plants (Table1). In all of the evaluated plants, the highest yield was obtained from chloroform extract followed the methanol extract. The hexane produced the smallest yield in all of the study plants evaluated in this study.

Table1. The yield of plant crude extracts by using different solvents.

Plant species	**Parts used**	**Dry weight (gm)**	**Extraction type**	**Yield in grams**	**Percent yield**
Olea europeae subsp. *cuspidata*	Berries	20	chloroform methanol ethanol hexane petroleum ether	2.6 2.4 1.8 1.5 1.8	13% 12% 9% 7.5% 9%
Podocarpus falcatus	Berries	20	chloroform methanol ethanol hexane petroleum ether	2.8 2.6 2.2 1.8 2.1	14% 13% 11% 9% 10.5%
Juniperus procera	Berries	20	chloroform methanol ethanol hexane petroleum ether	2.3 2.2 1.9 1.6 1.7	11.5% 11% 9.5% 8% 8.5%

4.2. Antimicrobial activity of the Plant Extracts

In-vitro antimicrobial activity of crude extracts of plants under this study was evaluated against human pathogenic bacteria of *E.coli*, *S.Typhi*, *S.dysenteriae*, *P.aeruginosa*, *S. aureus* and *K.pneumoniae*. The results obtained in the present study revealed that the tested three medicinal plants extracts possess a potential antibacterial activity.

4.2.1. Antibacterial assay of crude extracts of Berries of Olea europeae subsp. cuspidata

Crude extracts was assayed by agar well diffusion method using five solvents and 6 test pathogens. The mean zone of inhibition varied significantly among solvents of extraction (ANOVA, $F = 15.38$, $df = 4,60$, $p < 0.0001$), the test pathogens ($F = 24.67$, $df = 5.60$, $p < 0.0001$), and among the interaction of solvents of extractions and test pathogens ($F = 1.75$, $df = 20,60$, $p = 0.048$) using ANOVA test (Table 2). The methanol followed by the ethanol crude extracts of berries of *Olea europeae* subsp. *Cuspidata* showed a significantly higher mean antibacterial activity as compared to other solvents. The highest antibacterial activity was exhibited against *S. dysenteriae* (18.33 ± 0.57mm) followed by *S.Typhi* (18 ± 1mm), and *E.coli* (17.66 ± 0.57mm) by methanol extract. The methanol extract exhibited amoderate rate of inhibition against *S. aureus* (15.66 ± 1.52mm) and the least activity against *P.aeruginosa* (14.33 ± 0.57mm) and *K. pneumoniae* (12.33 ± 2.08mm). The ethanol extracts on the other showed an appreciably high inhibitory activity against *S.Typhi* (17.33 ± 0.57mm)followed by *S.dysenteriae* (16.66 ± 0.57mm), *S. aureus* (16.33 ± 1.52mm) and *E.coli* (16 ± 1mm) and a moderate inhibition against *P. aeruginosa* (14.33 ± 1.52mm). With ethanol extract of *Olea europeae* subsp. *cuspidata* , the minimum zone of inhibition (10 ± 2mm) was exhibited against *K. pneumoniae*.

The chloroform extracts of *Olea europeae* subsp. *cuspidata*were exhibitedthe maximum zone of inhibition against *S.Typhi* (16.66 ± 1.15mm) followed by *E.coli* (15.66 ± 1.52mm) and *S. dysenteriae* (15.33 ± 0.57mm), and a moderate inhibition against *S. aureus* (14.33 ± 3.51mm) and the minimum activity against *P.aeruginosa* (10.33 ± 3.0mm) and *K. pneumoniae* (11.33 ± 1.57mm). The petroleum ether extracts showed maximum inhibitory activity against on *E.coli* (16 ± 2mm) followed by *S.aureus* (13.66 ± 1.52mm),and *S.Typhi* and *S. dysenteriae* (13.66 ± 0.57mm). The least activity was exhibited against *K. pneumoniae* (12.66 ± 1.52mm). The hexaneextract generally exhibited the least inhibitory activity against all the test pathogens as compared to the other solvents extracts of berries of *O.europae* (Table 3).

Table 2. Univariate analysis of Crude extracts of *O. europeae* against test bacterial pathogen

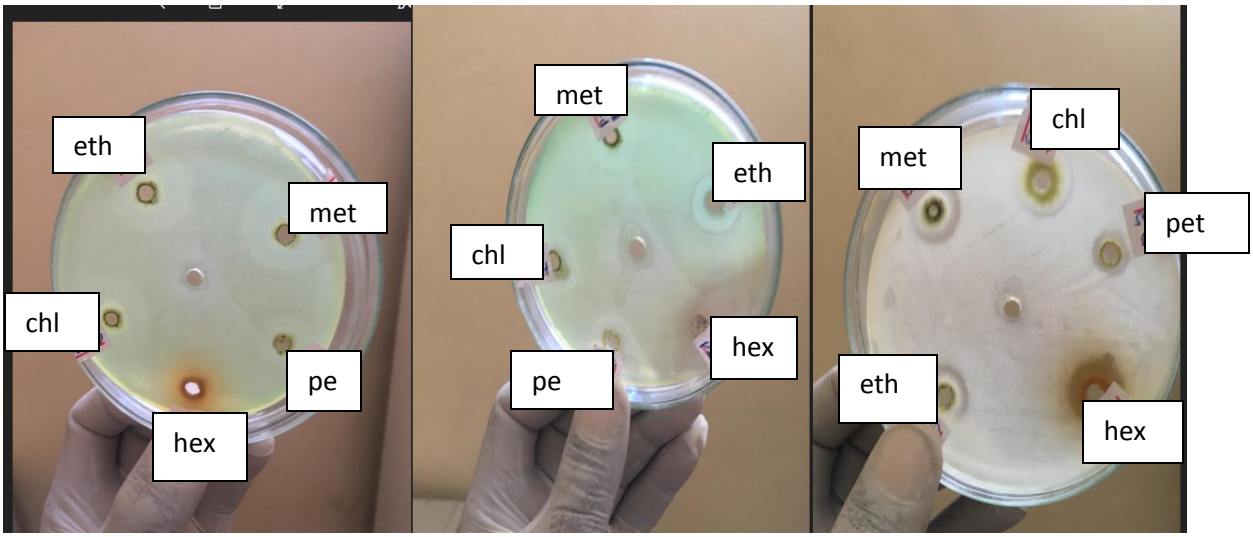
Effects	SS	DF	MS	F-ANOVA	P-value
Solvent	158.60	4	39.65	15.381	0.000000
Pathogen	318.09	5	63.62	24.679	0.000000
Solvent * Pathogen	90.47	20	4.52	1.755	0.048840
Error	154.67	60	2.58		

SS: sum of squire, DF: degree of freedom, MS: means square

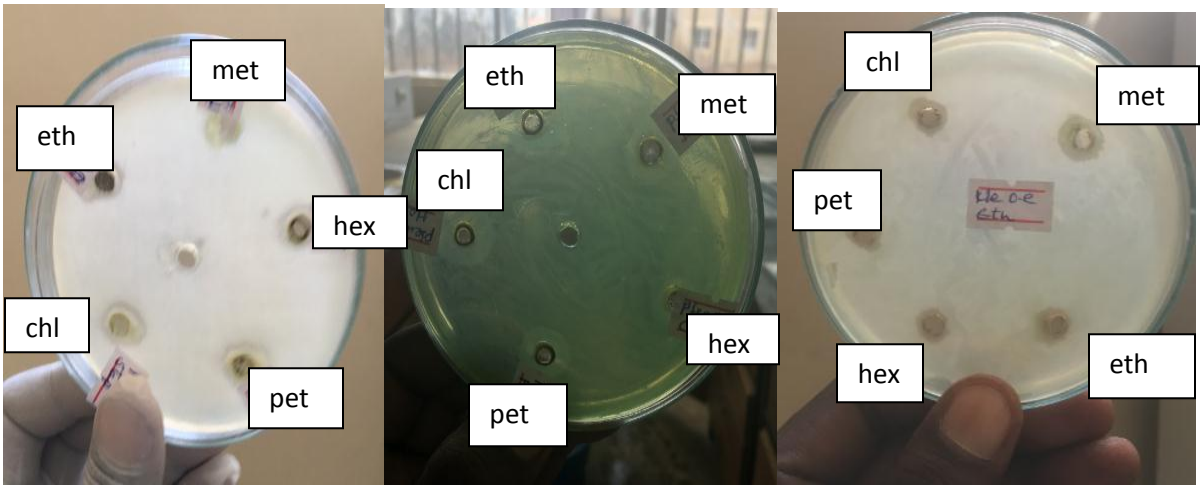
Table 3.The effect of the different crude extracts of berries of *Olea europeae* subsp. *cuspidata*against tested pathogenic bacteria (Zones of inhibition in mm; mean±SD).

Test organism	control		Mean Inhibition zone of berries extract of *Olea europeae* subsp. *Cuspidata*in mm(Mean± SD)				
	ciprofloxacin	DMSO	methanol	ethanol	chloroform	P.ether	hexane
Escherichia coli	33	0.0	17.66 ± 0.57^{hi}	$16.0 \pm 1.0^{e-i}$	$15.66 \pm 1.52^{d-i}$	$16.0 \pm 2.0^{e-i}$	$13.33 \pm 1.52^{a-i}$
Salmonella typhi	32	0.0	18.0 ± 1.0^{i}	$17.33 \pm 0.57^{g-i}$	$16.66 \pm 1.15^{g-i}$	$13.66 \pm 0.57^{b-i}$	$13.33 \pm 1.15^{a-i}$
Staphylococcus aureus	38	0.0	$15.66 \pm 1.52^{d-i}$	$16.33 \pm 1.52^{f-i}$	$14.33 \pm 3.51^{b-i}$	$13.66 \pm 1.52^{b-i}$	$14.33 \pm 1.52^{b-i}$
Shigella dysenteriae	30	0.0	18.33 ± 0.57^{i}	$16.66 \pm 0.57^{g-i}$	$15.33 \pm 0.57^{c-i}$	$13.66 \pm 0.57^{b-i}$	$13.33 \pm 1.15^{a-i}$
Pseudomonas aerugenosa	30	0.0	$14.33 \pm 0.57^{b-i}$	$14.33 \pm 1.52^{b-i}$	$10.33 \pm 3.05^{a-c}$	$11.0 \pm 3.0^{a-e}$	8.33 ± 1.52^{a}
Klebsiella pneumoniae	24	0.0	$12.33 \pm 2.08^{a-h}$	10.0 ± 2.0^{ab}	$11.33 \pm 1.52^{a-f}$	$12.66 \pm 1.52^{a-h}$	$10.66 \pm 1.52^{a-d}$

∗ Ciprofloxacin = positive control; DMSO® = negative control



E. coliS. aureusS. dysenteriae



S.TyphP.aeruginosaK. pneumoniae

met: methanol, eth: ethanol, chl: chloroform, pet: petroleum ether, hex: hexane

Figure 1. *In vitro* inhibition of crude extracts of berries of *Olea europeae* subsp. *Cuspidata* against six test bacterial pathogens(at concentration of 200 mg/ml)..

4.2.2. .Antibacterial Activityof Crude Extracts ofBerries ofPodocarpus falcatus

Berries of*Podocarpus falcatus* were similarly extracted with five solvents and evaluated against six bacterial test pathogens. The mean zone of inhibition significantly varied among solvents of extraction (ANOVA, $F = 5.64$, $df = 4,60$, $p < 0.001$), and the test pathogens ($F = 24.19$, $df = 5,60$, $p < 0.0001$). However, the mean zone of inhibition did not vary among the interaction of solvents of extractions and test pathogens ($F = 1.15$, $df = 20,60$, $p= 0.32$) using ANOVA test.Crude Extracts with methanol solventshowed considerably a higher mean antibacterial activity followed by ethanol and chloroform solvents and the least inhibitory activity was exhibited by the hexane extracts.The highest antibacterial activity was exhibitedagainst*E.coli* (16.33 ±1.52mm) by methanol extract, followed by *S.Typhi* and *S. aureus* (15.33 ± 1.15mm each), and a moderate inhibition was exhibited against *S. dysenteriae* (13.0 ± 1.73mm) and), *P.aeruginosa* (13.0 ± 1.0mm) and the least activity against *K. pneumoniae* (11.66 ± 0.57mm).

The ethanol extracts showed a strong inhibitory activity against *S. aureus* (16.33 ± 1.53mm) followed by *E.coli* (15.66 ± 2.0mm) and *S.Typhi* (15.0 ± 2.0mm), and a moderate inhibition against *S. dysenteriae* (13.33 ± 1.52 mm) and the least activity against *K. pneumoniae* (9.66 ± 1.52 mm).

The chloroform berries extract of .*P. falcatus*were exhibited a maximum zone of inhibition against *E.coli* (15.66 ± 0.57mm*S. dysenteriae* (13.66 ± 1.53mm), *S.Typhi* (13.0 ± 1.73mm), and *S.aureus* (13.33 ± 0.57mm) were moderately inhibited whereas the minimum activity against *K. pneumoniae* (8.33 ± 1.52mm).

The Petroleum ether extracts were exhibited a maximum zone of inhibition against *E.coli* (14.66 ± 2.08mm) followed by *S.aureus* (13.66 ± 2.51mm) and *P.aeruginosa* (13.33 ± 1.15mm). The minimum activity was displayed against and *K. pneumoniae* (9.0 ± 1.73mm).

The hexane extracts showed maximum inhibitory activity against*S. aureus* (13.33 ± 2.0mm) followed by *E.coli* (13.0 ± 1.73mm) and *P.aeruginosa* (12.66 ± 1.52mm) and *S.Typhi* (12.33 ± 0.57mm) and the least activity against *K. pneumoniae* (9.0 ± 1.0mm).

Table 4. Univariate analysis of Crude extracts of *Podocarpus falcatus* against test bacterial pathogen.

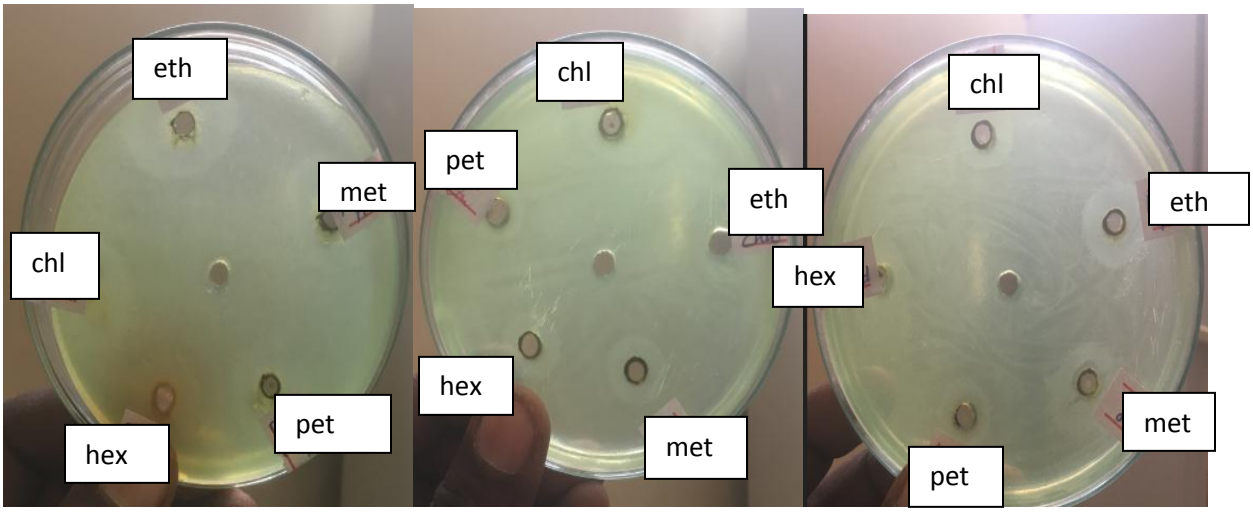
Effects	DF	SS	MS	F	P-value
Solvent	4	52.62	13.16	5.638	0.000640
Pathogen	5	282.23	56.45	24.191	0.000000
Solvent*Pathogen	20	54.04	2.70	1.158	0.320963
Error	60	140.00	2.33		

SS: sum of squire, DF: degree of freedom, MS: means square

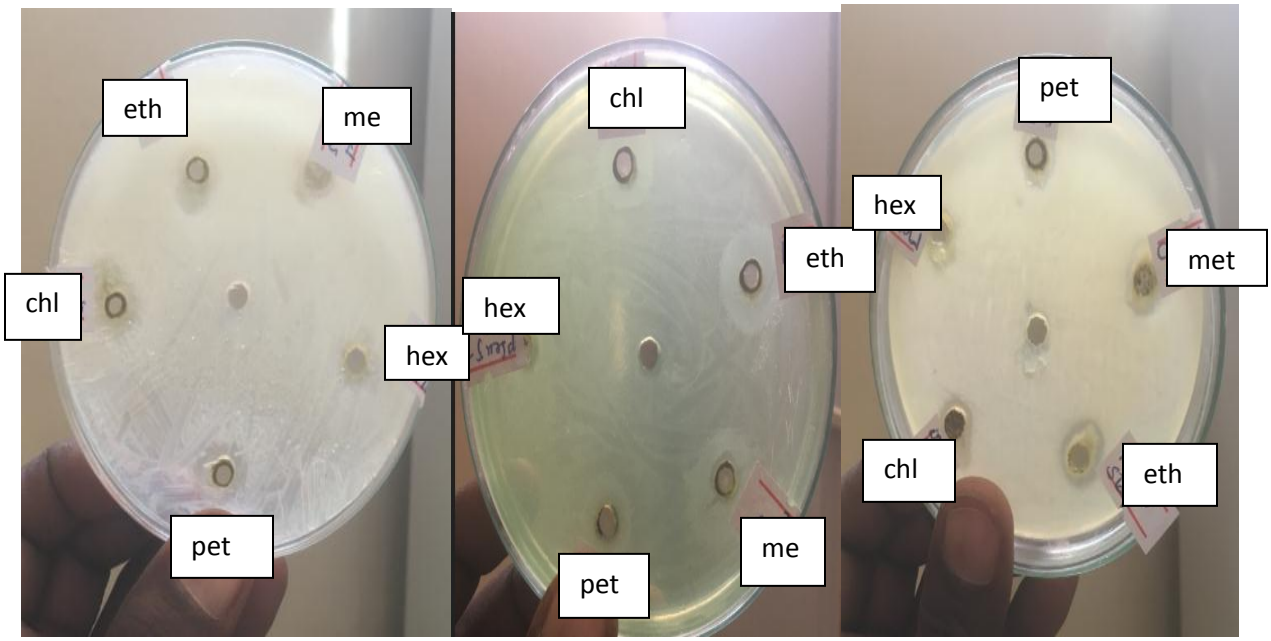
Table 5.The effect of the different extracts of the berries of*Podocarpus falcatus*against tested pathogenic bacteria (Zones of inhibition in mm; mean±SD).

Test organism	control		Mean Inhibition zone of berries extract of * *Podocarpus falcatus*in mm(Mean± SD)				
	ciprofloxacin	DMSO	methanol	ethanol	chloroform	P.ther	hexane
Escherichia coli	33	0.0	16.33 ± 1.52^{d}	$15.66 \pm 2.0^{c-d}$	$15.66 \pm 0.57^{c-d}$	$14.66 \pm 2.08^{c-d}$	$13.0 \pm 1.73^{a-d}$
Salmonella typhi	32	0.0	$15.33 \pm 1.15^{c-d}$	$15 .0 \pm 2.0^{c-d}$	$13 \pm 1.73^{a-d}$	$12 .0 \pm 2.0^{a-d}$	$12.33 \pm 0.57^{a-d}$
Staphylococcus aureus	38	0.0	$15.33 \pm 1.15^{c-d}$	16.33 ± 1.52^{d}	$13.33 \pm 0.57^{b-d}$	$13.66 \pm 2.51^{b-d}$	$13.33 \pm 2.0^{b-d}$
Shigella dysenteriae	30	0.0	$13.0 \pm 1.73^{a-d}$	$13.33 \pm 1.52^{b-d}$	$13.66 \pm 1.52^{b-d}$	$12.0 \pm 1.0^{a-d}$	$11.66 \pm 0.57^{a-d}$
Pseudomonas aerugenosa	30	0.0	$13.0 \pm 1.0^{a-d}$	$11.3 \pm 1.52^{a-c}$	$12.33 \pm 1.52^{a-d}$	$13.33 \pm 1.15^{b-d}$	$12.66 \pm 1.52^{a-d}$
Klebsiella pneumonia	24	0.0	$11.66 \pm 0.57^{a-d}$	9.67 ± 1.52^{ab}	8.33 ± 1.52^{a}	9.0 ± 1.73^{ab}	9.0 ± 1.0^{ab}

∗ Ciprofloxacin = positive control; DMSO® = negative control



E. coliS. aureusS. dysenteriae



S.TyphP.aeruginosaK. pneumoniae

met: methanol, eth: ethanol, chl: chloroform, pet: petroleum ether, hex: hexane

Figure 2. *In vitro* inhibition ofcrude extracts of berries of *Podocarpus falcatus*against six testbacterial pathogens(at concentration of 200 mg/ml),

4.2.3. Antibacterial Activity of Crude Extracts of Berries of Juniperus procera

Berries of*Juniperus procera* were also extracted with five solvents and evaluated against six bacterial test pathogens. Crude Extracts with methanol solventshowed considerably a higher mean antibacterial activity followedby ethanol and chloroform solvents. The mean zone of inhibition significantly varied among solvents of extraction (ANOVA, $F = 5.66$, $df = 4,60$, $p < 0.001$), and the test pathogens ($F = 137.40$, $df = 5,60$, $p < 0.0001$). However, the mean zone of inhibition did not vary among the interaction of solvents of extractions and test pathogens ($F = 1.24$, $df = 20,60$, $p= 0.25$) using ANOVA test (Table 6).The methanol extract of berries of*Juniperusprocera*exhibited a highest zone of inhibition against *S.Typhi* (15.33± 1.52mm) followed by *P.aeruginosa*(14.33± 1.52mm) and*S. aureus*(14.0± 2.0mm)a moderate inhibition against*E. coli*(13.66 ± 1.52mm) and*S. dysenteriae*(13.66 ± 0.57mm) as showed in (Table 7). The methanol extracts exhibited the least inhibitory activityagainst*K. pneumoniae*(5.33± 1.15mm). Theethanolextractof berries of *Juniperus procera*showed a highest zone of inhibition against *S. aureus*(15.33± 1.52mm)followed by *S.Typhi*(14.66 ± 1.52mm), *S. dysenteriae*(14.33± 2,08mm)and*P.aeruginosa*(14.33 ± 1.52mm)a moderate inhibition against*E. coli*(11.33± 1.52mm) and the least inhibitory activityagainst*K. pneumoniae*(4.0± 1.0mm)(Table 7). Chloroform extract of *Juniperus procera*inhibited *P.aeruginosa*(14.0± 1.0mm)with a highest zone of inhibitionfollowed by *S.Typhi*(13.33 ± 1.15mm)and*E. coli*(13.0± 1.0mm) and a moderate inhibition against*S. aureus*12.33± 1.15mm) and*S. dysenteriae*(12.33± 0.57mm)and the least inhibitory activityagainst*K. pneumoniae*(4.66± 0.57mm) (Table 7).Petroleum ether extract of *Juniperus procera*inhibited *P.aeruginosa*(13.0± 1.0mm) and *S. aureus*(13.0± 1.0mm)with a highest zone of inhibitionfollowed by*S.Typhi*(12.33 ± 1.52 mm), *S. dysenteriae*(12.66± 0.57mm)and*E. coli*(12.33 ± 0.57mm)and the least inhibitory activityagainst*K. pneumoniae*(3.0± 1.0mm) (Table 7).The hexaneextracts showedmaximum inhibitory activity against on*P.aeruginosa*(14.0± 1.0mm)followed by*S.Typhi*(13.0± 2.0mm) and*E. coli*(13.0± 1.0mm) a moderate inhibition against*S. dysenteriae*(12.66 ± 1.52mm)and*S. aureus*(11.66 ± 0.57mm)and least activity against on *K. pneumoniae*(3.33± 0.57mm)(Table 7).

Table 6. Univariate analysis of Crude extracts of *Juniperus procera*against test bacterial pathogen.

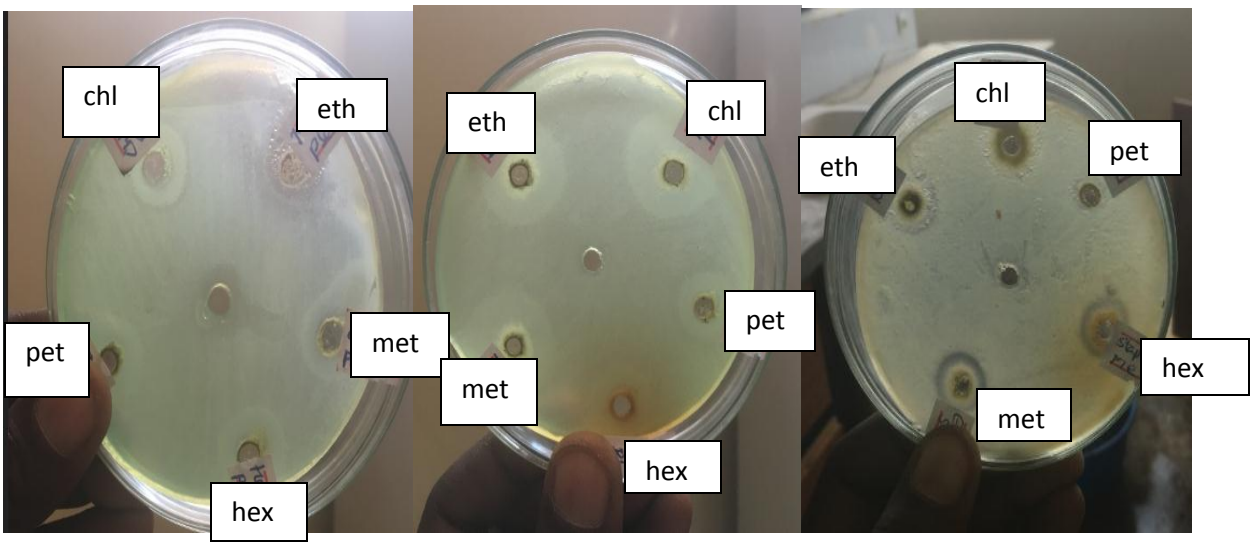
Effects	SS	DF	MS	F-value	p-value
Solve	35.96	4	8.99	5.657	0.000624
pathogen	1091.60	5	218.32	137.404	0.000000
Solvent* pathogen	39.51	20	1.98	1.243	0.253399
Error	95.33	60	1.59		

SS: sum of squire, DF: degree of freedom, MS: means square

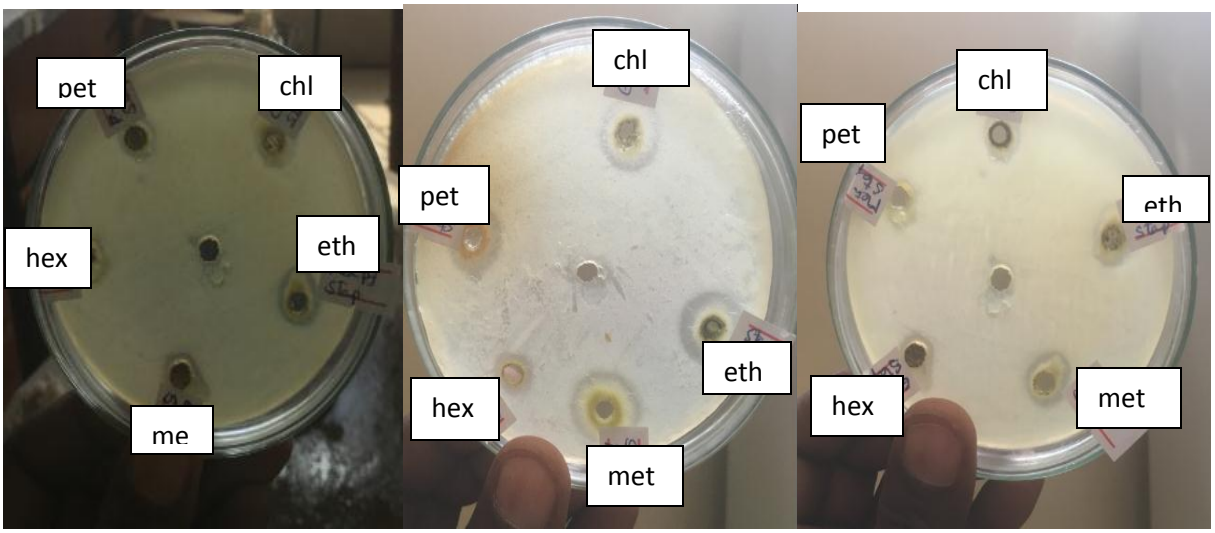
Table 7. The effect of the different extracts of the berries of *Juniperus procera*against tested pathogenic bacteria (Zones of inhibition in mm; mean±SD).

Test organism	control		Mean Inhibition zone of berries extractof * *Juniperus procera*in mm(Mean±SD)				
	ciproflo xactn	DM SO	methanol	ethanol	chloroform	Petroleum ether	hexane
Escherichia coli	33	0.0	13.66 ± 1.52^{b}	11.33 ± 1.52^{b}	13.0 ± 1.0^{b}	12.33 ± 0.57^{b}	13.0 ± 1.0^{b}
Salmonella typhi	32	0.0	15.33 ± 1.52^{b}	14.66 ± 1.52^{b}	13.33 ± 1.15^{b}	12.33 ± 1.52^{b}	13.0 ± 2.0^{b}
Staphylococcus aureus	38	0.0	14.0 ± 2.0^{b}	15.33 ± 1.52^{b}	12.33 ± 1.15^{b}	13.0 ± 1.0b	11.66 ± 0.57^{b}
Shigella dysenteriae	30	0.0	13.66 ± 0.57^{b}	14.33 ± 2.08^{b}	12.33 ± 0.57^{b}	12.66 ± 0.57^{b}	12.66 ± 1.52^{b}
Pseudomonas aerugenosa	30	0.0	14.33 ± 1.52^{b}	14.33 ± 1.52^{b}	14.0 ± 1.0^{b}	13.0 ± 1.0^{b}	14.0 ± 1.0^{b}
Klebsiella pneumonia	24	0.0	5.33 ± 1.15^{a}	4.0 ± 1.0^{a}	4.66 ± 0.57^{a}	3.0 ± 1.0^{a}	3.33 ± 0.57^{a}

∗ Ciprofloxacin = positive control; DMSO® = negative control



E. coliS. aureusS. dysenteriae



S.TyphP.aeruginosaK. pneumoniae

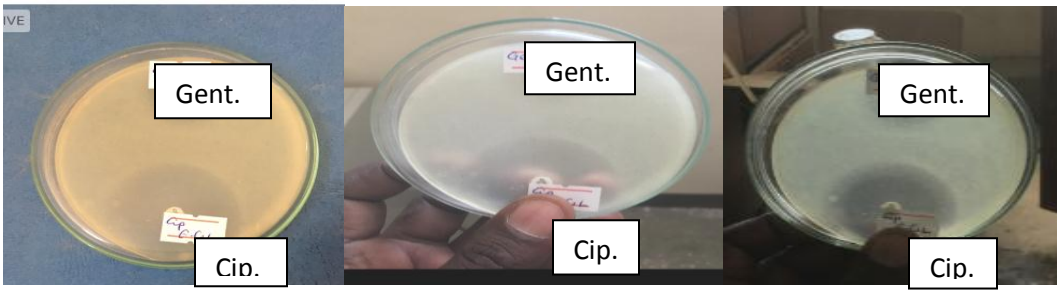
met: methanol, eth: ethanol, chl: chloroform, pet: petroleum ether, hex: hexane

Figure 3. *In vitro* inhibition ofcrude extracts of berries of *Juniperus procera*against six testbacterial pathogens(at concentration of 200 mg/ml).

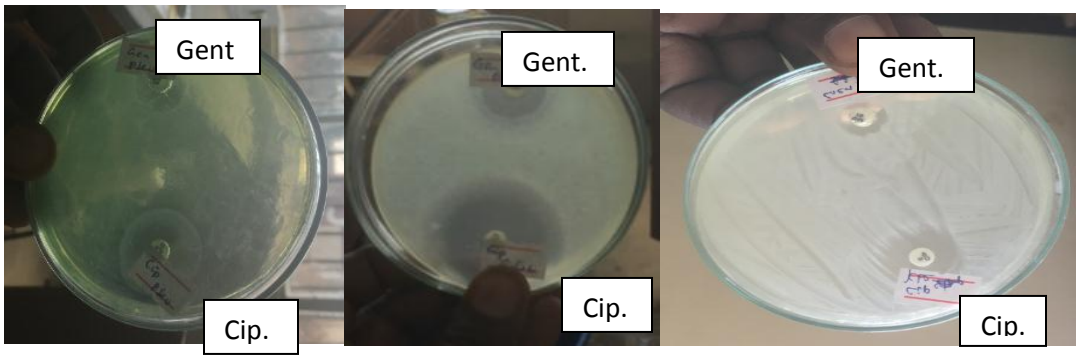
4.3. Inhibitory Zones of Test Pathogens with Standard Antibiotics (positive control)

Table. 8 Inhibitory Zones of Test Pathogens with Standard Antibiotics

Test organism	Positive control
	ciprofloxactn
Escherichia coli	33
Salmonella typhi	32
Staphylococcus aureus	38
Shigella dysenteriae	30
Pseudomonas aerugenosa	30
Klebsiella pneumonia	24



E. coliS. aureusS. dysenteriae



*S.TyphP.aeruginosaK. pneumoniae*Gent:Gentamicin, Cip:Ciprofloxacin

Figure4. Inhibitory Zones of Test Pathogens with Standard Antibiotics..

4.4. MIC of Plant Extracts

MIC assay was employed to evaluate the effectiveness of the plant extracts to inhibit the growth of bacterial tested microorganisms. The extracts of the three medicinal plants were exposed to the concentrations rangedfrom 0.78 to 100mg/ml. In the antibacterial activity tested, five different solventsmethanol, ethanol, chloroform and hexane, petroleum ether had selected for MIC test (Table 9).

4.4.1. MIC of Olea europeae berries extracts against Tested pathogenic Bacteria (in mg/ml)

The methanol extract of *Olea europeae* subsp. *cuspidata*exhibited the lowest MIC at 1.56 mg/ml against *S. aureus*. The methanol extract exhibited MIC at 3.12mg/ml against*E. coli, S. typhi* and at a concentration of 6.25 mg/ml against *P.aeruginosa.*And at concentration of 12.5mg/ml *againstS. dysenteriae*and*K. pneumoniae* (Table 9).

Theethanol extract exhibited MIC at 1.56mg/ml concentration against *E. coli* and *S. Typhi*and at concentration of 3.12mg/ml against.*S. dysenteriae* and *S. aureus*. The ethanol extract exhibited MIC at 6.25mg/ml concentration against *P.aeruginosa* and at concentration of 25mg/ml against *K. pneumonia*(Table 9).

The chloroform extract exhibited MIC at 3.12 mg/ml concentration against *E. coli, S. Typhi,S. dysenteriae* and *S. aureus*and at concentration of 6.25 mg/ml against *P.aeruginosa*. The chloroform extract exhibited MIC at 25mg/ml concentration against *K. pneumonia*(Table 9).

The Petroleum extract exhibited MIC at 1.56mg/ml concentration against *E. coli* and at a concentration of 25mg/ml against *K. pneumonia*(Table 9).

The Hexane extract exhibited MIC at 25mg/ml concentration against *E. coli* and at a concentration of 50mg/ml against *K. pneumonia.*The MIC of Petroleum ether and Hexane extract *Olea europeae* subsp. *Cuspidata* was 6.25 mg/ml against the *S. dysenteriae* and *S. aureus*followed by*S. Typhi* and *P.aeruginosa*at 12.5mg/ml (Table 9).

4.4.2. MIC of Podocarpus falcatus Berries Extracts against Pathogenic Bacteria (in mg/ml)

The methanol extract of *Podocarpus falcatus*crudeshowedMIC activity at 3.12mg/ml concentration against *E. coli*,*S. aureus* and*S. dysenteriae*and at concentration of 12.5 mg/ml against*S. Typhi*and *P.aeruginosa*followed by *K. pneumoniae*at 25mg/ml concentration as depicted in(Table 9).

The ethanolextract exhibited MIC at 6.25mg/ml concentration against *S. aureus*, *S. dysenteriae* and *E. coli* andat concentration of 25mg/mlagainst*S. Typhi*and *P.aeruginosa*.The ethanol extract exhibited MIC at 100mg/ml concentration against *K. pneumonia*(Table 9).

The chloroform extracts showed MIC activity at 6.25mg/ml against*E. coli*andagainst*S. aureus,S. dysenteriae*and*S. Typhi*at 12.5 mg/ml concentration followed by*P. aeruginosa* at 25 mg/ml (Table 9). ThePetroleum ether extract of*Podocarpus falcatus*crudeexhibited a MIC at 12.5 mg/ml against*E. coli*,*S. dysenteriae*and *P.aeruginosa* followed by*S. aureus*and *S. Typhi*at 25 mg/ml(Table 9).TheHexaneextract of*Podocarpus falcatus*crudeexhibited a MIC at 25mg/ml against*E. coli*,*S. dysenteriae*and *P.aeruginosa* followed by*S. aureus*and *S. Typhi*at 50mg/ml. (Table 9).

4.4.3.MIC of Juniperus procera Berries Extracts against Pathogenic Bacteria (in mg/ml)

The methanolextract exhibited MIC at 12.5mg/ml concentration against*S. dysenteriae*. The methanoland ethanolextract exhibited MIC at 25mg/ml concentration against*S. Typhi,S. aureus*and *E. coli*and*P.aeruginosa*at concentration of 6.25 mg/ml. (Table 9).

The ethanol extract exhibited MIC at 6.25mg/ml concentration against*S. dysenteriae*.The chloroform and Petroleum etherextract exhibited MIC at 12.5mg/ml concentration against*E. coli* and*S. dysenteriae*at 25 mg/ml concentration followed by *P.aeruginosa*and*S. aureus*. The chloroformextract exhibited MIC at 50mg/ml concentration against*S. Typhi*,andthe Petroleum ether extract exhibited MIC at 25mg/ml concentration against*S. Typhi*.(Table 9).
The MIC of Hexaneextract of *Juniperus procera*was 25 mg/ml against the *E. coli*and *S. aureus* followed by*S. dysenteriae,S. Typhi*and*P.aeruginosa*at 50mg/ml.(Table 9).

Table 9. Minimum Inhibitory Concentration (MIC) of crude extracts of berries ofthree plantsextracts against bacterial tested microorganism in mg/ml.

Plant species	Crude extracts	*E. coli*	*S.aureus*	*S.dysenteriae*	*S. typhi*	*P.aeruginosa*	*K.pneumoniae*
O.euroe	Methanol	3.125	1.56	12.5	3.125	6.25	12.5
	Ethanol	1.56	3.125	3.125	3.125	6.25	25
	Chloroform	3.125	3.125	3.125	3.125	6.25	25
	P.ether	1.56	6.25	6.25	12.5	12.5	25
	Hexane	25	6.25	6.25	12.5	12.5	50
P.falcatus	Methanol	3.125	3.125	3.125	12.5	12.5	25
	Ethanol	6.25	6.25	6.25	25	25	100
	Chloroform	6.25	12.5	12.5	12.5	25	_
	P.ether	12.5	25	12.5	25	12.5	_
	Hexane	25	50	25	50	25	_
J. procera	Methanol	25	25	12.5	25	6.25	_
	Ethanol	25	25	6.25	25	6.25	_
	Chloroform	25	12.5	25	50	12.5	_
	P.ether	25	12.5	25	25	12.5	_
	Hexane	25	25	50	50	50	_

4. DISCUSSION

Plants have been consumed in medicine to treat infectious diseases and to improve human's health. Traditionally, many plants with medicinal features are used to treat bacterial pathogens (Cowan, 1999). In both developed and underdevelopment countries, plant materials, which are the main sources of natural products, have a variety of antibiotic resistant bacteria and fewer negative impacts(Ozoula *et al*., 2010).The first step towards this goal was the in vitro antibacterial activity assay (Samy and Ignacimuthu, 2000). Many reports were available on the antiviral, antibacterial, antifungal, anthelminthic, and antiinflammatory properties of plants (Palombo and Semple, 2001; Kumarasamy *et al*., 2002).

In the present study, berries of *Juniperus procera,Olea europeae* subsp. *Cuspidata* and *Podocarpus falcatus*was extracted byuseddifferent solvents such as methanol, petroleum ether, ethanol, chloroform and hexane. The results of current study were an indication of such understandings. The yield of the extract that was obtained by different solvents considerably differs in three of the medicinal plants (Table 1).Among the solvents used in the present study to extract the biologically active substances from three medicinal plants, ethanol and methanol were the best solvents, followed by chloroform and least by petroleum ether and hexane (Tables2 to 4). This specifiedthat the extraction of medicinal plants with different solvents may produce different *in vitro* inhibitory result which is based on the potential of the solvents used to extract the biologically active constituents **(**George *et al*., 2010**).**

The methanolberries extracts of *Olea europeae* showed significant antibacterial activity against most of bacterial human pathogens evaluated in the present study. The highest antibacterial activity exhibited was against *S. dysentriae*(18.3 ± 0.57mm) by methanol extract, followed by*S. typhi*(18 ± 1mm) and a moderate inhibition against *E.coli* (17.6 ± 0.57mm)and MIC of 3.125 mg/ml against *E. coli* and *S. typhi* bymethanol extract In the present study.

Theethanol extract exhibited the second with inhibition zone of (17.3 ± 0.57mm) against *S. Typhi*, followed by *S. dysentriae*with a zone of inhibition of (16.6 ± 0.57mm)and MIC of 3.125 mg/ml against *S. typhi* and *S. dysenteriae* by ethanol extract of berries of *Olea europeae*. A different study reported that the products of olive tree that can live for centuries are known for many years with their beneficial effects on health (Soler-Rivas *et al*., 2000). It's reported by

some researchers that the *Olea europein* which is included in these products has a lot of pharmacological properties including antioxidant, antimicrobial, anti-inflammatory, antiatherogenic anticarcinogenic and antiviral activities (Owen *et al*., 2003; Visioli *et al*., 2002; Micol *et al*., 2005; Sanchez *et al*., 2007). According to this, the *Olea europeae*extracts were moderately active against*S. Typhi*and highly active against the other bacteria that used in this study.

The second result of the present study showed that the plant extracts of *Podocarpus falcatus*exhibited antibacterial activity against some of the common pathogenic bacteria. The highest antibacterial activity exhibited was against *E.coli*(16.3 ± 1.5mm) by methanol extract, followed by *S. aureus* (15.3 ± 1.1mm) and *S. Typhi* (15 ± 1.1mm) andshowedMIC activity at 3.12mg/ml concentration against *E. coli*,*S. aureus* and*S. dysenteriae*.

In the present study, the ethanol extract exhibited the second with inhibition zone of (16.3 ± 1.5mm) against *S. aureus*, followed with a zone of inhibition against *E.coli* (15.6 ± 2.3mm) and *S. Typhi* (15 ± 2mm) andexhibited MIC at 6.25mg/ml concentration against *S. aureus*, *S. dysenteriae* and *E. coli*. Previous study showed that the antibacterial activity in the disc diffusion assay against *S. aureus* and *Pseudomonas. sp.* and antifungal activity against *Aspergillus. sp*. and *Fusarium. sp*. Inhibited stronger antimicrobial activity of 7-18mm in diameter of inhibition. (Becerra *et al*., 2002).

The last result of the present study showed that the plant extracts of *Juniperus procera*exhibited antibacterial activity against some of the common pathogenic bacteria.The highest antibacterial activity exhibited was against *S. typhi*(15.3 ± 1.5mm) by methanol extract, followed by*S. aureus*(14.3 ± 1.5mm) and *P.aeruginosa*(14 ± 2mm) andexhibited MIC at 12.5mg/ml concentration against*S. dysenteriae*.

In the present study, the ethanol extract exhibited the second with inhibition zone of (17.3 ± 0.57mm) against *S. aureus*, followedwith a zone of inhibition against*P.aeruginosa*(14 ± 2mm) and *S. dysentriae*(14 ± 2mm) andexhibited MIC at 6.25mg/ml concentration against*S. dysenteriae*. The antimicrobial activity assayed for juniper extract showed the greatest inhibition zones were noted for two strains of *C. albicans*(37mm and 29.3 mm), *A. baumani* (24.3 mm) and two *S. aureus* strains: MRSA (24 mm) and ATCC (28 mm)(Natalia*et al*., 2003).

Higher potential to produce broad spectral antibacterial activity with minimal concentration against a wide range of human pathogens. The extracts were good in inhibited *E. coli, S. Typhi*, S. dysenteriae, *S. aureus*and in some instances *P.aeruginosa*.

The results of this study provided an insight into the antimicrobial properties of the extracts of *Juniperus procera, Olea europeae* subsp. *Cuspidata* and *Podocarpus falcatus*. As well as it formed an opportunity for selection of bioactive extracts for initial fractionation and further studies of these two medicinal plants in the antibacterial assays. This *in vitro* study demonstrated that these three folklore medicinal plants have good potential.

This study gives a suggestion of the efficacy of the plants acquired from the traditional healers. The results of study initiate basis for further studies of the powerful plants so as to segregate the compounds responsible for the antimicrobial activity. Numerous modern drugs were extracted from traditional therapeutic floras through the use of plant material succeeding the ethno botanical leads from indigenous cures used by traditional remedial systems.

5. CONCLUSION AND RECOMMENDATIONS

The tested extracts of all *Olea* sp. plant was found to be active against pathogenic bacteria used in this study and consequently led to serious health issues. Due to quick growth of resistance and high cost of new generation antibiotics, lots of efforts were made to discover new antimicrobial agents from various sources.Different solvents such as ethanol, methanol, chloroform, etc can enhance the anti-microbial efficacy.Anti-bacterial activity of this medicinal plant, if translated into clinical practice would result in the development of indigenous, chemical free, cost-effective products, which can be integrated into various pathogenic bacteria treatments.

Based on the above conclusion, the following recommendations were forwarded:

- The extracts of all *Olea* sp. plant should be translated into clinical practice as it is chemical free, cost-effective products, which can be integrated into various pathogenic bacteria treatment.
- It is important to note that future studies on this type of plant should be carried out in order to identify antimicrobial activities of other parts of the plant such as leaves, roots, etc.
- Toxicity should be studied, further chemical analysis.

6. REFERENCE

Abdillahi.H.S., G.I. Stafford, J.F. Finnie, J. (2008). Van StadenAntimicrobial activity of South African *Podocarpus* species Journal of Ethnopharmacology, 119, pp. 191-194.

Abdillahi, H.S.; Stafford, G.I.; Finnie, J.F. and Van Staden J. (2010), ―Ethnobotany, phytochemistry and pharmacology of Podocarpus sensulatissimo (s.l.)‖, South African Journal of Botany, 76: 1–24.

Abebe, D. (2001). The role of medicinal plants in health care coverage of Ethiopia, the possible benefits of integration. Pp. 6–21.

Abebe, D., and A. Ayehu (1993). Medicinal Plants and Enigmatic Health Practices in Northern Ethiopia. In World Health Organization, Ethiopian Ministry of Health Project No. AF/ ETH/TRM/001RB and the United Nations Development Programme.

Agatemor, C. (2009). Antimicrobial activity of aqueous and ethanol extracts of nine Nigerian spices against four food borne bacteria. *Elec J Environ Agric food chem*; 8(3): 195-200.

Altinyay C, Güvenç A, Altun ML. (2011). Antioxidant activities of oleuropein and the aqueous extracts of *Olea europaea* L. varieties growing in Turkey. Turk J Pharm Sci.; 8(1):23–30.

Amako, K., Meno, Y., and Takade, A. (1988) "Fine Structures of the Capsules of *Klebsiella pneumoniae* and *Escherichia coli* K1". *Journal of Bacteriology*. Volume 170, No. 10. p. 4960-4962

Assefa A, Abate D, Stenlid J. (2014a). Characterization of Corynelia uberata Fr., a putative fungal pathogen of Podocarpus falcatus in Ethiopian forests. Forest Pathology 44: 45-55.

Assefa A, Abate D, Stenlid, J. (2014b). Corynelia uberata as a threat to regeneration of Podocarpus falcatus in Ethiopian Forests: Spatial pattern and temporal progress of the disease and germination studies. Plant Pathology. 64, 617-626

AssefaA & Abate D. (2018). Assessment of wounding factors (natural and anthropogenic) of *Juniperus procera* and their relation to disease occurrence of *Pyrofomes demidofffii* in some Afromontane forests of Ethiopia. Forest Ecology and Management 409: 361-371.

Balcht A, Smith R (1994). Pseudomonas aeruginosa: Infections and Treatment. Informa Health Care. pp. 83–84.

Bauer, A.W., Kirby, W.M.M., Sherris, J.C. and Turk, M. (1966). Antibiotic susceptibility testing by a standard single disc diffusion method. *AM. J.Clin. Pathol*; 45: 493-496.

Barker. (2004). A yellowwood by any other name: molecular systematics and the taxonomy of *Podocarpus* and the Podocarpaceae in southern Africa.*South African Journal of Science* 100(11 & 12), 629-32.

Becerra,J., Flores, C., Mena, J., Aqueveque, P., Alarcen, J., Bittner, M., Hernandez, H.M., Ruiz,E., Silva, M., (2002). Antifungal and antibacterial activity of diterpenes isolated from wood extractable of Chilean podocarpaceae. Boletindela socieded chilena quimica.

Bell T, Freckleton RP, Lewis OT, 2006. Plant pathogens drive density dependent seedling mortality in a tropical tree.Ecology Letters 9,569–74.

Bekele-Tesemma A, Birnie A, Tengnas B. 1993. Useful trees and shrubs for Ethiopia. Regional Soil Conservation Unit (RSCU), Swedish International Development Authority (SIDA).

Bekele E. (2007) Study on Actual Situation of Medicinal Plants in Ethiopia. Addis Ababa: Prepared for Japan Association for International Collaboration of AAgriculture and Forestr.

Breitenbach, V. F., (1963). The Indigenous Trees of Ethiopia, 2nd edn. Addis Ababa, Ethiopia:

Brisse, S. and Verhoef, J. (2001). "Phylogenetic diversity of *Klebsiella pneumoniae* and *Klebsiella oxytoca* clinical isolates revealed by randomly amplified polymorphic DNA, gyrA and parC genes sequencing and automated ribotyping". *International Journal of Systematic and Evolutionary Microbiology*. Volume 51. p. 915–924.

Ethiopian Forestry Association.

Buchholz J.T., N.E. Gray (1948).A taxonomic revision of*Podocarpus*l. The sections of the genus and their subdivisions with special reference to leaf anatomy Journal of the Arnold Arboretum, 29 (1948), pp. 49-63.

Burits M, Asres K and Bucar F (2001). The antioxidant activity of the essential oils of Artemisia afra,Artemisia abyssinica and Juniperus procera. Phytother. Res., 15: 103-108.

Calo J. R., Crandall P. G., O'Bryan C. A., Ricke S. C. (2015). Essential oils as antimicrobials in food systems-A review.*Food Control*.54 111–119.

Caponio F, Alloggio V, Gomes T(1999). Phenolic compounds of virgin olive oil: Influence of paste preparation techniques. Food Chem.;64:203–9.

CDC National Center for Emerging and Zoonotic Infectious Diseases. Retrieved 2 October 2012.

Cherla, R.; Lee, S.; Vernon, T. (2003) "Shiga toxins and apoptosis." FEMS microbiology letters. Volume 228. P.159 –66.

Chris J. (2013). *Pod carpus*. The Gymnosperm Database.

Cock IE (2015). The safe usage of herbal medicines: counter indications, crossreactivity and toxicity. Pharmacognosy Communications. 5(1):250.

Cortés, G., Borrell, N., de Astorza, B., Gómez, C., Sauleda, J., and Albertí, S. (2002) "Molecular Analysis of the Contribution of the Capsular Polysaccharide and the Lipopolysaccharide O Side Chain to the Virulence of *Klebsiella pneumoniae* in a Murine Model of Pneumonia". *Infection and Immunity*. Volume 70, No. 5. p. 2583-2590.

Covas MI (2008). Bioactive effects of olive oil phenolic compounds in humans: Reduction of heart disease factors and oxidative damage. Inflammo pharmacology; 16:216–8.

Cowan MM. Plant products as antimicrobial agents. Clin Microbiol Rev 1999; 12: 564-582

Daly M, Buckley J, Power E et al., (2000). Molecular characterization of Irish *Salmonella enterica* serotype Typhimurium: detection of class I integrons and assessment of genetic relationships by DNA amplification fingerprinting. Appl Environ Microbiol.; 66:614–619.

De. D.J. (1969). Laubenfels**A** revision of the Malesian and Pacific rainforest conifers, I. Podocarpaceae, in part Journal of the Arnold Arboretum, 50 (1969), pp. 274-369.

Dold. A., M.L. Cocks(2001).Traditional veterinary medicine in the Alice District of the Eastern Cape Province, South Africa South African Journal of Sciences, 97 (2001), pp. 375-379.

Eloff, (1998) A sensitive and quick microplate method to determine the minimal inhibitory

Espin G, Alvarez-Morales A, Merrick M. (1981) "Complementation analysis of glnA-linked mutations which affect nitrogen fixation in *Klebsiella pneumoniae*". *Mol Gen Genet*. Volume 184, No. 2. p. 213-7.

Concentration of plant extracts for bacteria Planta Medica, 64, pp. 711-713.

Farjon, A. 2010. *A Handbook of the World's Conifers*. Koninklijke Brill, Leiden.

Florin. R. (1940).T) he tertiary fossils conifers of South Chile and their phytogeographical significance (with a review of the fossil conifers of southern lands).

Friedlander C. (1882) Uber die scizomyceten bei der acuten fibrosen pneumonie. Arch Pathol Anat Physiol Klin Med. 87:319-24.

Fotadar U, Zaveloff P, Terracio L (2005). "Growth of Escherichia coli at elevated temperatures".

Gebreyes WA, Altier C. (2002).Molecular characterization of multidrug-resistant *Salmonella enterica* subsp. *enterica* serovar Typhimurium isolates from swine. J Clin Microbiol.; 40:2813–2822.Journal of Basic Microbiology. 45 (5): 403–4.

Geldenhuys CJ, (1993). Reproductive biology and population structures of Podocarpus falcatus and P. latifolius in southern Cape forests. Botanical Journal of the Linnean Society 112, 59–74.

Green, P. S. (2002) A revision of Olea L. (Oleaceae). Kew Bulletin 57: 91–140.

Green, P. S. & Wickens, G. E. (1989) The Olea europaea complex. In Tan.K. (ed.), The Davis & Hedge Festschrift : pp 287–299. (Edinburgh University Press, Edinburgh).

George FOA, Ephraim RN, Obasa SO, BankoleMO (2010).Antimicrobial properties of some plant extracts on organisms associated with fish spoilage.University of Agriculture, Abeokuta (UNAAB) Nigeria.

Harden, G. J. (1992) (Ed.). Flora of New South Wales, Volume 3, p472 (New South Wales University Press: Kensington).

Høiby N, Ciofu O, Bjarnsholt T (November 2010). "Pseudomonas aeruginosa biofilms in cystic fibrosis". Future Microbiology. 5 (11): 1663–74.

Hsueh CT, Chin JC, Yu YY, Chen HC, Li WC, Shen MC, Chiang CY, Shen SC. (1977) "Genetic analysis of the nitrogen fixation system in *Klebsiella pneumoniae*". *Sci Sin.*. Volume 20, No. 6. p. 807-17.

Hutchings. A., A.H. Scott, A.B., (1996). CunninghamZulu Medicinal Plants — an InventoryNatal University Press, Pietermaritzburg.

Hong TD, Linington S, Ellis RH. (1996). Seed storage behaviour: a compendium. Handbooks for Genebanks: No. 4. IPGRI.

Indu M.N., Hatha A.A.M., Abirosh C., Harsha U. and Vivekanandan G. (2006). Antimicrobial Activity of Some of the South-Indian Spices against Serotypes of *Escherichia Coli, Salmonella, Listeria monocytogenes*and *Aeromonashydrophila*. *Brazilian Journal of Microbiology;* 37:153-158.

Ingledew WJ, Poole RK (Sep 1984). "The respiratory chains of Escherichia coli". Microbiological Reviews. 48 (3): 222–71.

Itah A, Essien J (2005). "Growth Profile and Hydrocarbonoclastic Potential of Microorganisms Isolated from Tarballs in the Bight of Bonny, Nigeria". World Journal of Microbiology and Biotechnology. 21 (6–7): 1317–22.

Janick, J., Paull, R. E., (2008). The encyclopedia of fruit &nuts. The encyclopedia of fruit & nuts:xviii + 954 pp.

Kelch. D.G. (1997). The phylogeny of the podocarpaceae based on morphological evidence Systematic Botany, 22 (1997), pp. 113-131.

Kibebew, F. 2001. The status and availability of oral and written knowledge on traditional health care on traditional health care in Ethiopia. Pp.107–119.

Kluytmans J, van Belkum A, Verbrugh H (July 1997). "Nasal carriage of Staphylococcus aureus: epidemiology, underlying mechanisms, and associated risks". Clinical Microbiology Reviews. 10 (3): 505–20.

Kubo, I., Ying, B-P., (1991). A bisnorditerpene dilactone from Podocarpus nagi Phytochemistry 30, 3476-3477.

Kubo. I., Y.M. Lee, M. Pettei, F. Pilkiewicz, K. Nakanishi (1976). Potent army worm antifeedants from the East African *Warburgia* plantsJ.C.S. Chemical Communication, pp. 1013-1014.

Kumarasamy Y, Cox PJ, Jaspars M, Nahar L, Sarker DS (2002). Screening seeds of Scottish plants for antibacterial activity. Journal of Ethnopharmacology 83:73-77.

KunzelmanJ.,Durako M.,Ken-WorthyW., StapletonA. And WrightJ. (2005). Irradianceinduced changes in the photobiology of *Halophilajohnsonii*. *Journal of Marine Biology*; 148:241.

Lawlor, M., Hsu, J., Rick, P., Miller, V. (2005). "Identification of *Klebsiella pneumoniae* virulence determinants using an intranasal infection model". *Molecular Microbiology*. Volume 58, Issue 4. p. 1054–1073

Le Tutour B., Guedon D. (1992). Antioxidative activities of Olea Europea leaves and related phenolic compounds.*Phytochemistry*31 1173–1178. 10.1016/0031-9422(92)80255-D.

Lee, W.H., H.I. Choi, S.W. Hong, K.S. Kim, Y.S. Gho, and S.G Jeon. (2015). Vaccination with Klebsiella pneumoniae-derived extracellular vesicles protects against bacteria-induced lethality via both humoral and cellular immunity. Experimental & Molecular Medicine 47(9):183.

Liebana E, Garcia-Migura L, Clouting C et al. (2002)Multiple genetic typing of *Salmonella enterica* serotype Typhimurium isolates of different phage types (DT104, U302, DT204b, and DT49) from animals and humans in England, Wales, and Northern Ireland. J Clin Microbiol.40:4450–4456.

Li H.L. (1953).Present distribution and habitats of the conifers and taxads Evolution, 7 (1953), pp. 245-261.

Li X., Liu Y., Jia Q., Lamacchia V., O'Donoghue K., Huang Z. (2016). A system biology approach to investigate the antimicrobial activity of oleuropein.*J. Ind. Microbiol. Biotechnol.*43 1705–1717.

Madigan MT, Martinko JM (2006). Brock Biology of microorganisms (11th Ed.). Pearson.

Masalha M, Borovok I, Schreiber R, Aharonowitz Y, Cohen G (December 2001).

McClelland, M., Florea, L., Sanderson, K., Clifton, S., Parkhill, J., Churcher, C., Dougan, G., Wilson, R., Miller, W. (2000)"Comparison of the *Escherichia coli* K-12 genome with sampled genomes of a *Klebsiella pneumoniae* and three *Salmonella enterica* serovars, Typhimurium, Typhi and Paratyphi". *Nucleic Acids Res.* olume 28(24). p. 4974–4986

Mesfin, F., T. Seta & A. Assefa. 2014. An ethnobotanical study of medicinal plants in Amaro Woreda, Ethiopia. Ethnobotany Research & Applications12:341–354.

Microbiologyonline. Retrieved 27 February 2014.

Micol, V., N. Caturla, L. Perez-Fons, V. Mas and L. Perez et al., 2005. The olive leaf extract exhibits antiviral activity against Viral Haemorrhagic Septicaemia rhabdovirus (VHSV). Antiviral Res., 66: 129-136. DOI: 10.1016/j.antiviral.2005.02.005

Mill R.R. (2003). Towards a biogeography of Podocarpaceae Acta Horticulturae, 615 (2003), pp. 137-147.

Molloy. B.P.J. (1995).*Manoao***(**Podocarpaceae), a new monotypic conifer genus endemic toNew ZealandNew Zealand Journal of Botany, 33 (1995), pp. 183-201.

Mohammed Benghezal, Eric Adam, Aurore Lucas, Christine Burn, Michael G. Orchard, Christine Deuschel, Emilio Valentino, Stéphanie Braillard, Jean-Pierre Paccaud, Pierre Cosson. (2007)."Inhibitors of bacterial virulence identified in a surrogate host model". Volume 9, Issue 5. p. 1336-1342.

Mossa JS, El-Feraly FS and Muhammad I (2004). Antimycobacterial constituents from Juniperus procera, Ferula communis and Plumbago zeylanica and their in vitro synergistic activity with isonicotinic acid hydrazide. Phytother. Res., 18: 934-937.

Muhammad I, Mossa JS, Al-Yahya MA, Ramadan AF and El-Feraly FS (1995). Further antibacterial diterpenes from the bark and leaves of Juniperus procera Hochst. Ex Endl. Phytother. Res., 9: 584-588.

Muhammad I, Mossa JS and El-Feraly FS (1996). Additional antibacterial diterpenes from the bark of Juniperus procera. Phytother. Res., 10: 604-607.

Natalia Filipowicz 1, Marcin Kamin´ski2,Julianna Kurlenda3, Monika Asztemborska4,J. Renata Ochock (2003) Department of Biology and Pharmaceutical Botany, Medical University of Gdan ´sk, Gdan ´sk, Poland

Negash, L. (1995). Indigenous Trees of Ethiopia: Biology, Uses and Propagation Techniques. Umea, Sweden: SLU Reprocentralen, pp. 285.

Niyogi, S. (2005) "Shigellosis" The journal of microbiology. Volume 43. p. 133 –43.

Ogston A (1984). "Classics in infectious diseases."On abscesses". Alexander Ogston (1844-1929)". Reviews of Infectious Diseases. 6 (1): 122–8

Ornelas, et al. (2010). *"Phylogeography of Podocarpus matudae (Podocarpaceae): pre-Quaternary relicts in northern Mesoamerican cloud forests"* (PDF). Journal of Biogeography. 37: 2384–96.

Otter A, French L (2010). Molecular epidemiology of community associated meticillin-resistant *Staphylococcus aureus* in Europe.Lancet Infect. Dis. 10(4):227-239.

Owen, R.W., R. Haubner, W. Mier, A. Giacosa and W.E. Hull et al., 2003. Food Chem. Taxicol., 41: 703-717. DOI: 10.1016/S0278-6915(03)00200-X

Oxford English Dictionary (3rd Ed.). Oxford University Press. September 2005.

Ozoula IR, Idogun SE, Tafamel GE. Acute and sub-acute toxicological assessment of aqueous leaf extract of Bryophyllum pinnatum (Lam) in Sprague-Dawley rats. Am J Pharmacol Toxical 2010; 5: 145-151?

Palombo EA, Sampled SJ (2001). Antibacterial activity of traditional Australian medicinal plants." Journal of Ethnopharmacology 77:151157

Palombo EA (2009) Traditional medicinal plant extracts and natural products with activity against oral bacteria: potential application in the prevention and treatment of oral diseases. eCAM DOI: 10.1093/ecam/nep067.

Pankhurst, A., (2000). Awliyaw: the largest and oldest tree in Ethiopia? Ethiopia Online, copyright.

Petlevski R, Hadzija M, Slijepcevic M (2001). Effect of 'antidiabetis' herbal preparation on serum glucose and fructosamine in NOD mice. Journal of Ethnopharmacology. 75(2):1815.

Podschun, R. and Ullmann U. (1998)"*Klebsiella* spp. as Nosocomial Pathogens: Epidemiology, Taxonomy, Typing Methods, and Pathogenicity Factors". *Clinical Microbiology Reviews*. Volume 11, No. 4. p. 589-603.

Pooley, E. (1993) The Complete Field Guide to the Trees of Natal, Zululand and Transkei. (Natal Flora Publications. Durban, South Africa).

Poppe C, Ziebell K, Martin L, Allen K. Diversity in antimicrobial resistance and other characteristics among *Salmonella* Typhimurium DT104 isolates. Microb Drug Resist. 2002; 8:107–122.

Quinn. C.J. **(**1982).Taxonomy of*Dacrydium*Sol. Ex Lamb. Emend. De Laub. (Podocarpaceae)Australian Journal of Botany, 30 (1982), pp. 311-320.

Rosignoli P, Fuccelli R, Fabiani R, Servili M, Morozzi G.(2013) Effect of olive oil phenols on the production of inflammatory mediators in freshly isolated human monocytes. J Nutr Biochem. ;24:1513–9.

Russell JB, Jarvis GN (2001). "Practical mechanisms for interrupting the oral-fecal lifecycle of Escherichia coli". Journal of Molecular Microbiology and Biotechnology. **3** (2): 265–72.

Samoylenko V, Dunbar DC, Gafur MdA, Khan SI, Ross SA, Mossa JS, El-Feraly FS, Tekwani BL, Bosselaers J and Muhammad I (2008). Antiparasitic, nematicidal and antifouling constituents from Juniperus berries. Phytother. Res., 22: 1570-1576.

Samy RP, Ignacimuthu, S (2000). Antibacterial activity of some folklore medicinal plants used by tribals in Western Ghats in India. Journal of Ethnopharmacology 69:63-71.

Sanchez de MF, Gamez MJ, Jimenez I (1994). Hypoglycemic activity of juniper "berries". Planta Medica. 60(3):197-200.

Sanchez, J.C., M.A. Alsina, M.K. Herrleinand and C. Mestres, 2007. Interaction between the antibacterial compound, oleuropein and model membranes. Colloid Polym. Sci., 285: 1351-1360. DOI: 10.1007/s00396-007-1693-x

Scott Jones P.(1991). Restoration of Juniperus excelsa and Olea europaea subsp. africana woodlands in Eritrea. A thesis presented for the degree of Doctor of Philosophy at the University of Stirling.

Seshathri K and Thiyagarajan T (2011). Antimicrobial Activity of Chewing Sticks of Jimma – Ethiopia against Streptococcus pyogens. ournal of Phytology 2011, 3(8): 34-37.

Soler-Rivas C., Espın J. C., Wichers H. J. (2000). Oleuropein and related compounds.*J. Sci. Food Agric.*80 1013–1023.

Sinclair. W.T., R.R. Mill, M.F. Gardner, P. Woltz, T. Jaffré, J. Preston, M.L. Hollingsworth, A. Ponge, M. Müller**(** 2002).Evolutionary relationships of the New Caledonian heterotrophic conifer,*Parasitaxus usta*(Podocarpaceae), inferred from chloroplast *trn*L-F intron/spacer and nuclear rDNA ITS2 sequencesPlant Systematics and Evolution, 233 (2002), pp. 79-104.

Sindiga. I. (1995).African ethnomedicine and other medical systems**I**. Sindiga, C. Nyaigototti-Chache, M.P. Kanuna (Eds.), Traditional Medicine in Africa, 9966-46-548-0, East Africa Educational Publishers Ltd, Nairobi.

Singleton P (1999). Bacteria in Biology, Biotechnology and Medicine (5th Ed.). Wiley. pp. 444–454.

Soler-Rivas, C., J.C. Espin and H.J. Wichers, 2000. Oleuropein and related compounds. J. Sci. Food Agric., 80: 1013-1023. DOI: 10.1002/(SICI)10970010(20000515)80:7<1013::AIDJSFA571>3.0.CO;2-C

Somova LI, Shode FO, Ramnanan P, Nadar A. (2003). Antihypertensive, antiatherosclerotic and antioxidant activity of triterpenoids isolated from *Olea europaea*, subspecies *africana* leaves. J Ethno pharm.; 84:299–305. doi: 10.1016/S0378-8741(02)00332-X.

Sunset Western Garden Book, (1995):606–607

Tadeg H, Mohamme E, Asres K,Gebre-Mariam T (2005). Antimicrobial activities of some selected traditional Ethiopian medicinal plants used in the treatment of skin disorders. Journal of Ethnopharmacology 100:168-175.

Tafesse G. and Mekonnen Y. (2012). Journal of Medical and Biomedical Sciences 1(4): 7-12.

Teketay D, Granstrom A, (1995, 1997). Soil seed banks in dry afromontane forests of Ethiopia. Journal of Vegetation Science 6, 777– 86.

The Plant List, (2013). http://www.theplantlist.org/

Tenaillon, Olivier; Skurnik, David; Picard, Bertrand; Denamur, Erick (1 March 2010). "The population genetics of commensal Escherichia coli". Nature Reviews Microbiology. 8 (3): 207–217.

Tortora, Gerard (2010). Microbiology: An Introduction. San Francisco, CA: Benjamin Cummings. pp. 85–87, 161, 165,.

Turner, N.J. 1999. Plant Technology of the First People of British Columbia: Including neighboring groups in Washington, Alberta and Alaska. Victoria, BC. Royal British Columbia Museum Handbook Series;, II, 181-201.

Visioli, F., A. Poli and C. Gall, 2002. Antioxidant and other biological activities of phenols from olives and olive oil. Med. Res. Rev., 22: 65-75. DOI: 10.1002/med.1028

Vogt RL, Dippold L (2005). "Escherichia coli O157:H7 outbreak associated with consumption of ground beef, June-July 2002".

Vogel P., Kasper Machado I., Garavaglia J., Zani V. T., de Souza D., Morelo Dal Bosco S. (2014). Polyphenols benefits of olive leaf (*Olea europaea* L) to human health.*Nutr. Hosp.*31 1427–1433. 10.3305/nh.2015.31.3.8400.

Watt. J.M., M.G. Breyer-Brandwijk(1962).The Medicinal Plants and Poisonous Plants of Southern and Eastern Africa(2nd ed.), Livingstone, London.

Wurges, J. (2005). The Gale Encyclopedia of Alternative Medicine, Farmington Hills, Mich. Farmington Hills, Mich: Thomson/Gale.

Ying, B.-P., Kubo, I., Chairul, Matsumoto, Y., Hayashi, Y.,(1990). Congeners of norditerpene dilactones from Podocarpus nagi. Phytochemistry 29, 3953}3955.

Zohary, D. (1995). Olive, Olea europaea (Oleaceae). Evolution of crop plants.Smartt, J. & Simmonds, N.W. (Eds) (Longman Scientific & Technical, Harlow, England).

7. APPENDIX

7.1. Collection and Identification of Plant Materials

- Samples of berries of *Juniperus procera,Olea europeae* subsp. *Cuspidata* and *Podocarpus falcatus* were collected in a large quantity from the study areas.
- The taxonomic position of the plants was identified and authenticated by plant experts from National Herbarium in Addis Ababa and published species descriptions (Bekele, 2007).
- Prior to use, the plant materials were repeatedly washed under tap water to remove any debris and were air-dried for twenty one days.

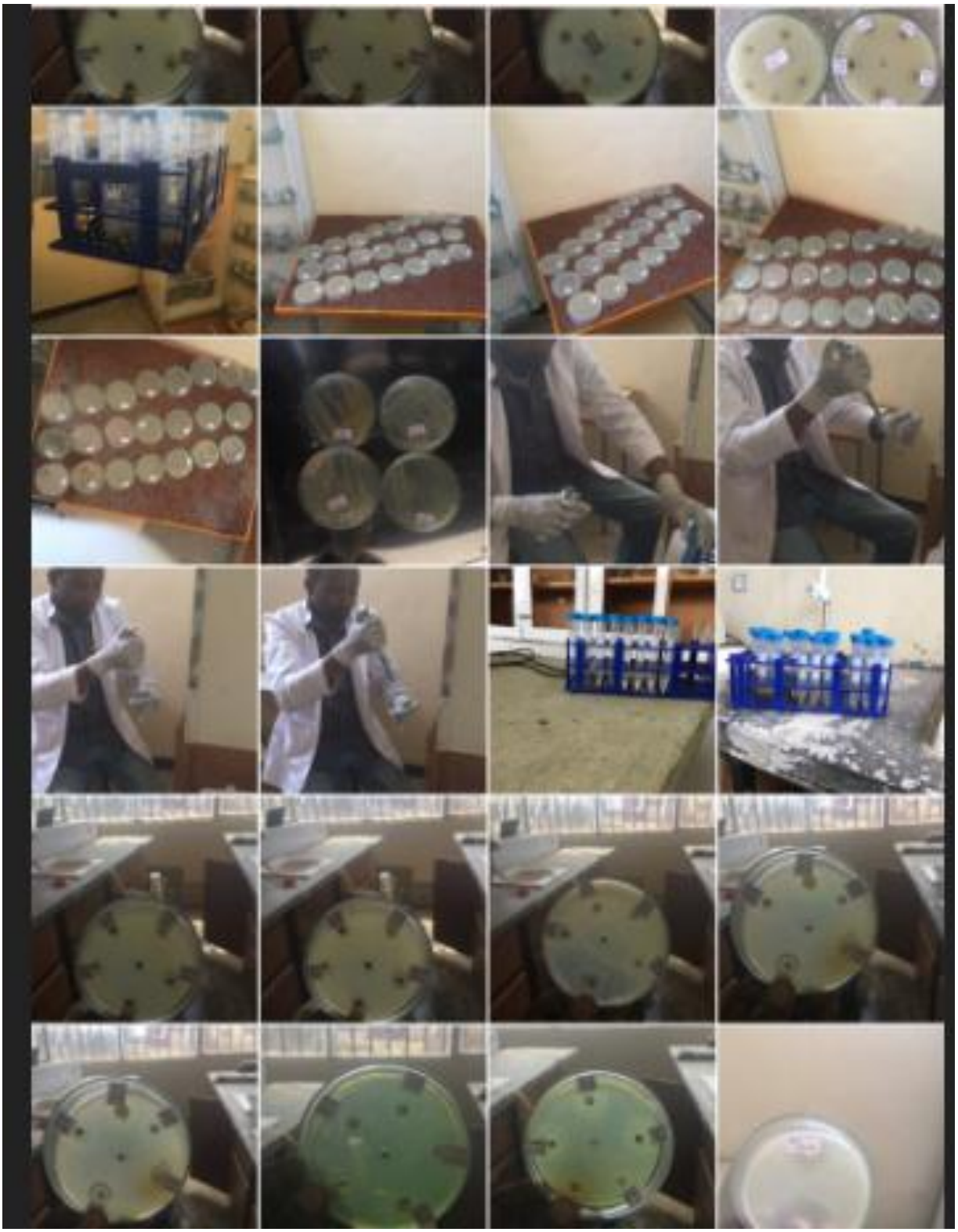
7.2.Preparation of Plant's Crude Extracts

- The preparation of crude extracts of plants under this study was conducted followed the methods described by (Tadeg *et al*. 2005).
- used different solvents. Accordingly, five hundred grams of berries from each plant was taken for extraction procedure and ground in a mortar and pestle separately under aseptic condition.
- 50grams of each powdered plant materials were extracted using apparatus with 250ml of chloroform, ethanol, hexane, petroleum ether, and methanol separately by maceration for 48hrs with frequent agitation on orbital shaker for continuous two days and the extracted liquid was filtered (Whatman No. 3 filter paper, Whatman Ltd., England).
- Extraction was repeated three times and the filtrates of all portions were combined in one vessel. The organic solvent was removed by evaporation using Rota vapor (BU˙CHI Rota-vapor R-205, Switzerland) at 40°C.
- The extracted dehydrated mass was then crushed, packed into a glass vial until used. Finally, the gram yield of dried residue of each plant extracts were calculated.
- The concentrated extracts were stored at 4°C for the next antimicrobial study.
- Dried residues were dissolved in 100% dimethyl sulfoxide (DMSO) to obtain a stock concentration of 200 mg/ml, which were kept at 4°C until used.


Samples collection and preparation


Media preparation

Testing



Collected sample and preparation of crude extract