Jimma University

College of Natural Sciences

Department of Biology

Anti-microbial Activities of two selected traditional medicinal plants used in Bero Wereda, Bench Maji Zone, Southwest Ethiopia

By: **Engida Mikre**

A Thesis Submitted to Department of Biology, College of Natural Sciences, Jimma University, in Partial Fulfilment of the Requirements for the Degree of Master of Science in Biology (Applied Microbiology).

July, 2015

Jimma, Ethiopia

JIMMA UNIVERSITY

COLLEGE OF NATURAL SCIENCES

DEPARTMENT OF BIOLOGY (APPLIED MICROBIOLOGY)

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A THESIS SUBMITTED TO DEPARTMENT OF BIOLOGY, COLLEGE OF NATURAL SCIENCES, JIMMA UNIVERSITY, IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN BIOLOGY (APPLIED MICROBIOLOGY).

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JULY, 2015

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DECLARATION

I, the undersigned, declare that this May original work with the exception of the citations contained herein being submitted to the University of Jimma for the degree of Master of Science in Biology (Applied Microbiology), College of Natural science. I also declare that this work has not been submitted to any other university in partial or entirety for the award of any degree.

ENGIDA MIKRE

Signature----------

Date of submission: ----------

The thesis has been submitted to the Department with May approval as a university advisor

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DR. Ketema Bacha (Associate professor, JU**)**

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LIST OF ABBREVIATIONS

DAEC Diffuse adhering *E. coli*

EAggEC enteroaggregative *E. coli*

EIEC enteroinvasive *E. coli*

EPEC enteropathogenic *E. coli*

ETEC enterotoxigenic *E. coli*

MBC Minimum Bactericidal Concentration

MIC Minimum Inhibitory Concentration

NA Nutrient agar

SNNPRS Southern Nations Nationalities and Peoples' Regional State

STEC Shiga toxin-producing *E. coli*

THP’s Traditional Health Practitioners

TMP's Traditional Medical Practitioners

ABSTRACT

The present study was conducted to investigate the antibacterial activities of roots of *Carissa spinarm* and *Cissampelos mucronata*. The plants were collected from Bench Maji zone in Bero woreda, southwest of Ethiopia. Root parts of the two plants were air dried under shade and the dehydrated roots were separately crushed into fine texture using mortar, and further grinded into powder using electronic grinder machine. Crude extracts were prepared using 100 g of powdered roots using methanol, acetone and petroleum ether. Microbial activities of different concentration of the extracts were evaluated against standard strains of *Staphylococcus aureus* (ATCC25923), *Escherichia coli* (ATCC 25922), *Pseudomona aeruginosa* (ATCC 27853) and *Salmonella typhimurium* ATCC 133110) following the disc diffusion method and micro broth dilution assay. Phytochemical screening of plant extracts was also conducted following standard methods. Result of the current study indicated that the methanol extract of root of *C.mucronata* had better inhibitory activity with maximum inhibition zone of 19 mm against *Staphylococcus aureus* (*ATCC25923*) and 17 mm against *Salmonella typhimurium* (ATCC 133110). In the case of *Carissa spinarm*, the methanol extract was more effective with inhibition zones diameter of 14 mm and 11 mm against *Staphylococcus aureus* (ATCC25923*)* and *Pseudomona aeruginosa* (ATCC 27853), respectively. Accordingly, methanol extracts of the root of *C. spinarm* was relatively more effective against Gram positive bacteria than Gram negative bacteria. The result of phytochemical screening revealed the presence of saponin, flavonoids, tannins and terpenoids although at different intensity. The minimum inhibitory concentrations (MIC) of the three extracts ranged from 6.5 mg/ml to 25 mg/ml. The root of *Cissampelos mucronata* extracts exhibited better antimicrobial activity than the root extract of *Carissa spinarm*.

Keywords: Antibacterial activities, Disc diffusion, Minimum inhibitory concentrations (MIC), Minimum Bactericidal Concentration (MBC), Phytochemical screening.

INTRODUCTION

Herbal medicine involves the use of plants for medicinal purposes. The use of plant such as herbs, shrubs or tree, in parts or whole in the treatment and management of diseases dated back to pre-historic times. Plants extracts have been used in folk medicinal practices for the treatment of different types of ailments since antiquity (Okanla *et al.,* 1990). Plants are used medicinally in different countries and are a source of many potent and powerful drugs (Srivastava *et al*., 1996). According to WHO (2010), from 119 plant derived pharmaceutical medicines, about 74% are used in modern medicine in ways that associated directly with their traditional uses as herbal medicines by cultural practice.

Medicinal plants are the most exclusive source of life saving drugs for majority of the world's population. The utilization of plant cells for the production of natural or recombinant compounds of commercial interest has gained increasing attention over past decades (Canter, *et al*., 2005). African traditional medicine is mainly based on herbal remedies. Bacteria that are causative agents for most infectious diseases are becoming increasingly resistant to some or most antibiotics. The cost of drugs in use today is too expensive or the majority of the population in the third world countries are poor and therefore the search for some cheap sources of antimicrobial substances in nature become inevitable (Olukemi *et al*., 1997). The development of medicinal chemistry, as a major route for the discovery of novel and more active therapeutic agents, further investigation into the chemical and biological activities of the plants have been carried out (Rao and Roja, 2002). The discovery of huge amount of therapeutic drugs from folkloric medicinal plants may solve problems arising from multidrug resistance food borne pathogenic microorganisms. Foods borne illness are global problem in developing and developed countries. Food spoilage or deterioration is predominantly caused by the growth of microorganisms on unhygienic food products and consuming it lead to illness. Pirbalouti *et al.* (2010) were studied the most common food borne bacterial pathogens, including *Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus, Salmonella* and *Shigella* spp. and each have great health complication. When ingested, bacterial toxins usually act locally within the human body, but may spread to other parts and damage cells, tissues, and the host immune system. *Bacillus cereus, Staphylococcus aureus*, and *Clostridium botulinum* are well-documented toxic food-borne agents (Kansas Department of Health and Environment Division of Health,

2008). *E. coli* O157:H7 and *Shigella spp.* also produce toxins that cause disease, which may lead to severe complications. *Staphylococcus aureus* is the most reported cause of food borne intoxications.

Each year in the United States, food- borne illnesses infect an estimated 76 million people. More than 300,000 people are hospitalized and 5,000 people will die from food-borne illnesses. The young, old and immunocompromised are more susceptible to complications (Delaware Health and Social service, 2009). In Ethiopia Health and health related indicators of the Ministry of Health published in 2004 shows that among the ten leading causes of outpatient visits to health institutions are all forms diarrhea diseases, intestinal parasites which are directly or indirectly related to food. However health institutions that compiles monthly morbidity statistic do not segregate if the cause for such illnesses is due to food or other.

The medicinal plant of Ethiopia (Debella *et al.,* 2001) and the other developing countries play major supplementary roles to the limited modern health care available (Constable, 1990).The various literature available show the significant role of medicinal plant in primary health care delivery in Ethiopia where 70% of human and 90% of livestock population depend on traditional medicine again similar to many developing countries particularly that of Sub-Saharan African countries (WHO, 2002).Those plants are part of the economic commodity for some members of the society which make their livelihood on their collection, trade and medicinal practices by practitioners or healers. Ethiopian plants have shown very effective remedy for some ailments of human and domestic animals (Aklilu Lemma, 1965).

Plants used for traditional medicine contain a wide range of substances that can be used to treat chronic as well as infectious diseases. A vast knowledge of how to use the plants against different illnesses may be expected to have accumulated in areas where the use of plants is still of great importance. The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive compounds of plants are alkaloids, flavanoids, tannins and phenolic compounds (Edeoga *et al*., 2005).

According to Dawit Abebe (2001), traditional remedies are the most important and sometimes the only source of therapeutics for nearly 80% of the population and 95% of traditional medicinal plants preparations in Ethiopia. The majority of population living

in rural areas and an increased number of the poor in urban centres rely mainly on traditional medicine and its practitioners to meet their primary health care needs (Berhane, 2001). Despite the use of traditional medicine over many centuries, only relatively small number of plant species has been studied for possible medical applications and the spread of this knowledge is mostly limited to indigenous societies (Cunningham, 1993).

Ethiopia is a country with rich tradition of the use of plant based drugs for curing or treating of many diseases. Report indicate that more than 35,000 plant species are being used around the world for medicinal purposes (Lewington, 1993) and, in Ethiopia an estimate of 800 plant species are employed as medicinal agents (Tesema *et al.,* 2002). In agreement with this, WHO estimates that majority of the population in developing countries (90% of the population in Africa) primarily rely on traditional medicinal plant for their healthcare (WHO, 2002). Bero woreda is found in Bench-Maji Zone of SNNPRS. Though this Woreda is widely covered with forests and has the potential traditional medicinal practitioners (TMP) yet, the reports are relatively few in comparison with the diversity of plants species being used traditionally for human medication.

In Ethiopia, ethno medicinal plant knowledge and use is under reported and most of the studies made so far are not focused on specific ethnic group or agro-ecological zone of the country. Therefore the present study was focused on the evaluation of biological activities of some medicinal plants and documentation of the indigenous knowledge of traditional healers. For this purpose two traditional medicinal plants namely, root of *Cissampelos mucronata* and root of *Carissa spinarum* were selected for evaluation of efficacy of their crude extracts against *E. coli*, *Salmonella typhimurium*, *Staphylococcus aureus,* and *Pseudomonas aurogenosa.* The selection of plants was based on their traditional medicinal value in the community especially in Bero Wereda, Bench Maji zone, South West Ethiopia.

1. 2. OBJECTIVES OF THE STUDIES

1.2.1 General Objective

The major purpose of this study was:

- To evaluate the *in-vitro* antibacterial activities of the root extracts of *Cissampelos mucronata* and *Carissa spinrum* plants against standard food-borne bacterial pathogens.

1.2.2 SPECIFIC OBJECTIVES

The specific objectives of the current work were:

- To extract the bioactive compounds from root of *Cissampelos mucronata and Carissa spinrum* plants
- To identify the phytochemical present in the root of *Cissampelos mucronata* , *Carissa spinrum* plants.
- To evaluate *In Vitro* antimicrobial activities of crude extracts and determine MIC and MBC of the extracts against the test strains (*S. aureus* ATCC25923, *E. coli* ATCC 25922 , *P. aeruginosa* ATCC 27853 and *S. typhimurium* ATCC 133110) using disk diffusion method and broth macro-dilution assay.

2. LITERATURE REVIEW

2.1. History of medicinal plants

Early knowledge and practices about medicinal plants opened door to the discovery of modern drugs. Langenheim *et al*. (1982) for example, noted that the poison hemlock containing the simplest of alkaloids, coniine, Nicotiana tabacum containing nicotine, painkillers and sleep bearers form atropine and opium and quinine that are used in the conquest of malaria, brain stimulants, heart stimulants, medicines, drugs and poisons from non-flowering plants and antibiotics are among such practices. Hence, it becomes important noting here the point emphasized by Soejarto *et al.* (2005) mass bio-prospecting (large-scale operation of bio prospecting) in future endeavors must take heed of the lessons learnt from past and present experiences in planning a successful mass bio-prospecting venture that may lead to more and more modern drug discovery.

Kim (2005) added that in ancient cultures, people methodologically and scientifically collect information on herbs and developed well defined herbal pharmacopoeias. Hence, much of the pharmacopoeias of scientific medicine of today were derived from the herbal lore of native people. Leonti *et al*. (2003) noted that the relevance of the historical depth of medicinal plant use from a variety of perspectives. Posey (2002) quoted in Leonti *et al.* (2003) emphasized that not only would it show unambiguously that indigenous cultures have an in depth knowledge of certain botanical taxa, which has been transmitted over centuries prior to it becoming important in the context of developing novel pharmaceuticals but also as importantly, such research would demonstrate the historical development of an intricate relationships between culture and its environment. The presence of various laboratories in academia, private institutions, and industry that are following leads from natural sources of plants and other organisms at global scale is also a good indication of the possibility to discover potential drugs for combating disease (Dawit *et al*., 2003).

2.2. Traditional Medicine in Ethiopia

Traditional medical practitioners mostly implement herbs, spiritual healing, bone-setting and minor surgical procedures in treating disease. Ethiopian traditional medicine is vastly complex and diverse and varies greatly among different ethnic groups. Most traditional medical practices in Ethiopia rely on an explanation of disease that draws on

both the “mystical” and “natural” causes of an illness and employ a holistic approach to treatment (Bishaw, 1991). Under the rule of Menelik (1865-1913) Western medicine became significantly more incorporated into the Ethiopian medical system. Numerous medical envoys from abroad, starting with the Italians and Russians, were influential in building hospitals, providing medical training and participating in vaccination campaigns. However, most medical establishments primarily served the urban elites and foreign missionaries and were concentrated in the major cities (Pankhurst, 1990).

Despite Western medicine becoming more widespread in Ethiopia, Ethiopians tend to rely more on traditional medicine. Conventional medical services remain concentrated in urban areas and have failed to keep pace with the growing population, keeping health care access out of reach for most Ethiopians living in Ethiopia. Because traditional medicine is culturally entrenched, accessible, and affordable, up to 80% of the Ethiopian population relies on traditional remedies as a primary source of health care (Kassaye *et al.,* 2006). Moreover, Western medicine has become more focused on preventative measures and people seeking curative practices still rely on indigenous medicine as the primary source for health care (Pankhurst, 1990). The influence of traditional medicine is also seen in Ethiopian migrant populations. In countries with substantial Ethiopian immigrant populations, traditional herbs, medical devices, and practitioners are readily available (Papadopoulos, 2002).

2.3. Medicinal plant diversity and distribution in Ethiopia

Vegetation types found in various agro ecological zones of Ethiopia accommodate various types of medicinal plants. The woodlands, Montana vegetation including grasslands and forests and the evergreen scrubs and rocky areas contain more medicinal plants with higher concentrations in the woodlands (Edwards, 2001). The microphylous vegetation of the wood lands listed more medicinal plants species followed by the Montana-grassland and riverine vegetation while the afro alpine vegetation ranked last.

The greater concentration of medicinal plants are found in the south and south western Ethiopian parts of the country following the concentration of biological and cultural diversity (Edwards, 2001).According to Dawit Abebe (2001), there is a large magnitude of use and interest in medicinal plants in Ethiopia due to acceptability, accessibility and biomedical benefits. Different vegetation types that are found in the various agro

ecological zones of Ethiopia accommodate various types of medicinal plants (Endeshaw Bekele, 2007).

Mirutse Giday (2007), studied the medicinal plants of Sheko, Bench and Meinit ethnic group of the south west Ethiopia. His study revealed a total of 124 medicinal plants belonging to 51 families. Of the total 65% were herbs, 15% were shrubs, 12% were trees and 9% were climbers. The great majority of the recorded medicinal plants in each ethnic group were used to treat human ailments. In this country, the long history of use of medicinal plants is reflected in various medico- religious manuscripts produced on parchments and believed to have originated several centuries ago (Fassile Kibebew, 2001).

2.4. Antibiotics and Resistance to Antibiotic

An antibiotic in a broader sense is defined as a chemotherapeutic agent that inhibits or abolishes the growth of microorganisms such as bacteria, fungi or protozoa. The classical definition of an antibiotic is a compound produced by a microorganism which inhibits the growth of another microorganism and over the years this definition has been expanded to include synthetic and semi-synthetic products (Kummerer, 2009). Antibiotics are used extensively in human and veterinary medicine as well as in aquaculture for the purpose of preventing (prophylaxis) or treating microbial infections (Kummerer, 2009) Antibiotics can be grouped by their chemical structure or mechanism of action into different classes such as beta-lactams (ß-lactams), quinolomes, tetracycline, macrolides, sulphonamides, amino glycosides, glycopeptides, sulphonamides, cyclic lipopeptides, oxazolidonones, metronidazole, streptogramins, ketolides, fluoroquinolones, lincosamides, trimethoprim, polymyxins and others (Alanis,2005; Tenover,2006).

2.5 Mechanisms of Antibiotic Resistance in Pathogenic Bacteria

Bacterial resistance to antibiotics has its foundation at the genetic level meaning that changes in the genetic make- up of the previously susceptible bacteria takes place either via a mutation or by introduction of new genetic information. The resistance can be natural (intrinsic) or acquired and can be transmitted horizontally or vertically (Alanis, 2005). The natural form of resistance is caused by a spontaneous gene mutation in the absence of selective pressure due to the presence of antibiotics. Once the genetic

mutation occurs and causes a change in the bacterial deoxyribonucleic acid (DNA), genetic material can be transferred among bacteria by several mechanisms of genetic transfer such as conjugation, transformation and transduction resulting in acquired resistance (Alanis, 2005; Tenover, 2006).

The expression of the resistance gene and the subsequent production of tangible biological effects results in loss of activity of the antibiotic. The expression of the resistance gene can occur via three general biological mechanisms which are antibiotic destruction or modification, antibiotic efflux from the cell and alteration of target site/receptor modification (Sibanda and Okoh, 2007). Prevention of interaction of the antibiotic with the target occurs when the intracellular target or receptor of the antibiotic is altered by the bacteria resulting in the lack of binding or reduced affinity of the antibiotic to its binding site and consequently the lack of antibacterial effect (Alanis, 2005; Lambert, 2005).

Antibiotic efflux from the bacterial cell takes place when the microorganism is capable of developing an active transport mechanism that pumps the antibiotic molecules that penetrated into the cell to the outside milieu until it reaches a concentration below that necessary for the antibiotic to have antibacterial activity (Alanis, 2005). This means that the efflux transport mechanism must be stronger than the influx mechanism in order to be effective (Hooper, 2005). Efflux is common in tetracyclines, macrolides and fluoroquinolones among others (Hooper, 2005). Multi antibiotic resistance efflux pumps are ubiquitous proteins present in both gram positive and gram negative bacteria as either chromosomally encoded or plasmid encoded (Akama *et al*., 2005). Although such proteins are present constitutively in bacteria, the continued presence of the substrate induces over-expression (Teran *et al*., 2003) whilst the increased transcription is responsible for the acquired resistance (Sibanda and Okoh, 2007).

Destruction or modification of the antibiotic is mainly through enzymatic inactivation and this affects the action of several antibiotics. Antibiotic hydrolysing enzymes and group tranferases production by bacteria are the main factors leading to antibiotic destruction or modification as they chemically degrade or modify the antibiotic rendering it inactive against the bacteria. Group transferases covalently modify antibiotics resulting in structural alterations that impair target binding. Antibiotic modification can be through acyltransfer, phosphorylation, glycosylation, nucleotidylation, ribosylation and thiol transfer (Wright, 2005). Resistance to

aminoglycosides in gram negative bacteria is most often mediated by a variety of enzymes that modify the antibiotic molecule by acetylation, adenylation or phosphorylation (Over *et al*., 2001). The production of beta-lactamases by bacteria confer resistance by hydrolysis of the amide bond of the four membered beta-lactam ring whose intergrity is central to the biological activity in beta lactam antibiotics (Jacoby and Munoz,2005).

2.6. Antimicrobial Agents of plant derivative

Traditionally used medicinal plants have recently attracted the attention of the biological scientific communities. This has involved the isolation and identification of secondary metabolites produced by plants and their use as active principles in medicinal preparations (Taylor *et al*., 2001). Antimicrobials of plant origin have enormous therapeutic potential. They are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials. The beneficial medicinal effects of plant materials typically result from the combinations of secondary products present in the plant. In plants, these compounds are mostly secondary metabolites such as alkaloids, steroids, tannins, and phenol compounds, flavonoids, resins fatty acids gums which are capable of producing definite physiological action on body. (Joshi *et al.*, 2009). Alkaloids are produced by large variety of organisms including bacteria, fungi, plants and animals; and are part of the group of natural products; some alkaloids have a bitter taste while many to toxic to other organisms (Gupta *et al.*, 2010).

Flavonoids are a group of polyphenolic compounds which influence the radical scavenging, inhibition of hydrolytic and oxidative enzymes and also act as anti-inflammatory agent (Frankel, 1995). The flavonoids show antioxidant activity and their effects on human nutrition and health is considerable. The mechanism of action of flavonoids are through scavenging or chelating process (Kessler *et al.,* 2003), they also inhibit microbes which are resistant to antibiotics (Linuma *et al.*, 1994). Flavonoids are free radical scavengers, super antioxidants and potent water soluble which prevent oxidative cell damage and have strong anti-cancer activity (Salah *et al.,* 1995). As antioxidants flavonoids provide anti-inflammatory actions (Okwu, 2001).

Anthraquinones are used better stomach-ache and in the treatment of diarrhoea and these are an important chemical raw material and organic intermediates that are broadly

applied in the field of dyestuff, papermaking, medicines, and agricultural chemicals. Glycoside compounds are containing a carbohydrate and non-carbohydrates residue (moiety) in the same molecule. In these compounds, the carbohydrate moiety is attached by an acetyl linkage carbon-I to the non-carbohydrate residue (Sabris and Daniel, 1990).

2.7. Herbal medicine against microorganisms

Enteric bacteria are major causes of food-borne illnesses and gastrointestinal problems in the developing countries and human beings around the world. The most common agents are *Escherichia coli* and *Salmonella typhimurium* but *Shigella* species and other species of *Salmonella* also have been implicated in a significant number of cases .Symptoms of food borne illnesses range from stomach upset to more serious symptoms including diarrhea, vomiting, abdominal cramps and fever. In some people, especially children, haemolytic uremic syndrome (HUS) can occur from infection by a particular strain of *E. coli* 0157:H7 and can lead to kidney failure and death (NIH, 2003). Vehicle of transmission of these etiologic agents are mainly food and water. Many disease-causing organisms of medical importance have developed resistance to antibiotics. Palombo and Sample (2001) suggests there is a distinct and constant need for safe and more efficient therapeutic agent. A way out of reducing antibiotic resistance and adverse effects on host is the employment of antibiotic resistance inhibitors of plant origin. Chariantly *et al*. (1999) stated that plant–derived medicines have been part of traditional health care in most parts of the world for thousands of years and there is increasing interest in them as sources of agents to fight microbial diseases. Aburjai *et al.* (2001) also confirms folkloric accounts in literatures on the use of variety of plant preparations for the treatment of infections. Clinically, antibiotics produced by soil microorganisms and higher plants have been known sources of antibiotics.

According to Bennish (2004) the use of medicinal plants also contributes significantly to primary health care in various parts of the world. Enteric infections remain a leading cause of childhood mortality in developing countries. It has been reported that enteric pathogens are the most frequent cause of diarrhea illnesses that account for an animal mortality rate of 5 million people worldwide and prominent pathogenic enterics include *Salmonella, Shigella* and strains of *E. coli* (Talaro and Talaro, 1996). *S. aureus* is present ubiquitously in the environment. Only those strains that produce enterotoxin can cause food poisoning. Food is usually contaminated from infected food handler. The food handler with an active lesion or carriage can contaminate food (Argudín, 2010) .

2.8. Food-borne Disease

The term food-borne disease is used for any disease that arises from the contamination of food by disease-producing agents that cannot multiply, or at any rate have not multiplied, on or in the incriminated food. Many diseases have the potential to be transferred through food from one human being or animal to another human being without the organism having grown on the food to increase its number. A clear distinction is difficult to get between this means of spread giving rise to a "food-borne disease" and the means of spread which requires growth on the food to occur giving rise to an "infection-type food poisoning." This is due to factors such as the nature of food, the amount of the initial contamination of the food and the sensitivity of the individual eating the food that may all affect the outcome (Harrigan and Park, 1991).Micro-organisms such as *Salmonella* spp., *Shigella* spp., *E. coli* and *Vibrio cholerae* are capable of growing on foods under normal conditions. However, if the food is initially heavily contaminated, these organisms cause disease so that further growth is not required, or the food would be of a type that protects the pathogens from the acid barrier of the stomach so that a smaller dose than usual is infective. Sometimes the pathogens such as verotoxin-producing *E. coli* O157:H7 and *Shigella* spp. naturally have a low infective dose (Samelis *et al*., 2002).

There are more than 250 known food-borne diseases which can be caused by bacteria, viruses, or parasites (CDC, 2005). Every individual is at risk of food-borne illness. Common symptoms of food-borne illness include diarrhea, headache, abdominal cramping, fever, vomiting, severe exhaustion, and sometimes blood or pus in the stools. The high risk populations for food-borne illness include infants, young children, elderly people, pregnant women, cancer patients, immune-compromised people such as organ transplant patients, or people with HIV/AIDS infection (Kendall *et al.,* 2003). While all individuals are susceptible to food-borne illness, these groups are much more likely to suffer serious illness or death from food-borne illness due to underdeveloped immune function. Most food-borne illness can be prevented if consumers possess sufficient food safety knowledge and take extra care when handling food.

2.8.1 Infection-type Food Poisoning

The term food poisoning is defined as any disease that results because microorganisms have grown on the incriminated food before it is ingested (Harrigan and Park, 1991).

Infection type-food poisoning is caused by the ingestion of live organisms that have grown on the food to produce a sufficiently large population to constitute an infective dose (Harrigan and Park, 1991). Multiplication of microorganisms in food to give an infective dose is linked to outbreaks of disease caused by *Salmonella* spp., *Listeria monocytogenes, Shigella* spp., *E. coli, Yersinia enterocolitica, Vibrio parahaemoliticus, Vibrio cholerae, Aeromonas* spp. and *Clostridium perfringens.*

2.8.2. Intoxication-type food poisoning

Intoxication-type food poisoning is caused by the growth of microorganisms in a food producing a metabolite that is toxic to the consumer. Examples of intoxication-type food poisoning include intoxications by *Staphylococcus aureus*, *Clostridium botulinum* and *Bacillus cereus.* Other diseases included in this class are scombrotoxicosis, diflagenolate poisoning, food-associated mycotoxicoses such as ergotism, and "yellow rice disease" (South African Department of Health, 2000).

2.9. Current updates on food-borne pathogens

2.9.1. *Staphylococcus aureus*

Staphylococcus aureus is a bacterium that causes staphylococcal food poisoning, a form of gastroenteritis with rapid onset of symptoms. It is commonly found in the environment (soil, water and air) and is also found in the nose and on the skin of humans. *S. aureus* is a Gram-positive, non-spore forming spherical bacterium that belongs to the *Staphylococcus* genus. The *Staphylococcus* genus is subdivided into 32 species and subspecies. *S. aureus* produces *staphylococcal-enterotoxin* (SE) and is responsible for almost all staphylococcal food poisoning (FDA, 2012). *S. intermedius*, a *Staphylococcus* species which is commonly associated with dogs and other animals, can also produce SE and has been rarely associated with staphylococcal food poisoning (Le Loir *et al.*, 2003). The growth and survival of *S. aureus* is dependent on a number of environmental factors such as temperature, water activity (aw), pH, the presence of oxygen and composition of the food. These physical growth parameters vary for different *S. aureus* strains .And the temperature range for growth of *S. aureus* is 7–48°C, with an optimum of 37°C. *S. aureus* is resistant to freezing and survives well in food stored below -20°C; however, viability is reduced at temperatures of -10 to 0°C. *S.*

aureus is readily killed during pasteurisation or cooking. Growth of *S. aureus* occurs over the pH range of 4.0–10.0, with an optimum of 6–7 (Stewart, 2003).

S. aureus is uniquely resistant to adverse conditions such as low aw, high salt content and osmotic stress. In response to low aw, several compounds accumulate in the bacterial cell, which lowers the intracellular aw to match the external aw (Montville and Matthews, 2008). As such, most *S. aureus* strains can grow over a aw range of 0.83 to >0.99 (FDA, 2012). *S. aureus* is a poor competitor, but its ability to grow under osmotic and pH stress means that it is capable of thriving in a wide variety of foods, including cured meats that do not support the growth of other food borne pathogens (Montville and Matthews 2008). *S. aureus* is a facultative anaerobe so can grow under both aerobic and anaerobic conditions. However, growth occurs at a much slower rate under anaerobic conditions (Stewart, 2003).

Symptoms of disease

Staphylococcal food poisoning symptoms generally have a rapid onset, appearing around 3 hours after ingestion (range 1–6 hours). Common symptoms include nausea, vomiting, abdominal cramps and diarrhoea. Individuals may not demonstrate all the symptoms associated with the illness. In severe cases, headache, muscle cramping and transient changes in blood pressure and pulse rate may occur. Recovery is usually between 1–3 days (FDA, 2012). Fatalities are rare (0.03% for the general public) but are occasionally reported in young children and the elderly (4.4% fatality rate). And *S. aureus* can cause various non-food related health issues such as skin inflammations (e.g. boils and styes), mastitis, respiratory infections, wound sepsis and toxic shock syndrome (Montville and Matthews, 2008).

Virulence and infectivity

Staphylococcal food poisoning is an intoxication that is caused by the ingestion of food containing pre-formed SE (Argudin *et al.,* 2010). There are several different types of SE; enterotoxin A is most commonly associated with staphylococcal food poisoning. Enterotoxins D, E and H, and to a lesser extent B, G and I, have also been associated with staphylococcal food poisoning (Pinchuk *et al.,* 2010). Staphylococcal-enterotoxin (SE) is produced during the exponential phase of *S. aureus* growth, with the quantity being strain dependent. Typically, doses of staphylococcal-enterotoxin (SE) that cause

illness result when at least 10^5 – 10^8 cfu/g of *S. aureus* are present (Montville and Matthews ,2008). Most genes for SEs are located on mobile elements, such as plasmids or prophages. As such, transfer between strains can occur, modifying the ability of *S. aureus* strains to cause disease and contributing to pathogen evolution (Pinchuk *et al.*, 2010).

S. aureus produces SEs within the temperature range of 10–48°C, with an optimum of 40–45°C As the temperature decreases, the level of SE production also decreases. However, SEs remains stable under frozen storage. SEs is extremely resistant to heating and can survive the process used to sterilise low acid canned foods. SE production can occur in a pH range of 4.5–9.6, with an optimum of 7–8. Production of SEcan occurs in both anaerobic and aerobic environments; however, toxin production is optimum in aerobic conditions and SEs is resistant to the heat and low pH conditions that easily destroy *S. aureus* bacteria (Stewart, 2003). The SEs are also resistant to proteolytic enzymes, hence SEs retain their activity in the gastrointestinal tract after ingestion. SEs range in size from 22–28 kDa and contain a highly flexible disulphide loop at the top of the N-terminal domain that is required for stable conformation and is associated with the ability of the SE to induce vomiting (Argudin *et al.,* 2010).

It has been suggested that SEs stimulate neuroreceptors in the intestinal tract which transmit stimuli to the vomiting centre of the brain via the vagus nerve (Argudin *et al.*, 2010). In addition, SEs is able to penetrate the lining of the gut and stimulate the host immune response. The release of inflammatory mediators, such as histamine, causes vomiting. The host immune response also appears to be responsible for the damage to the gastrointestinal tract associated with SE ingestion, with lesions occurring in the stomach and upper part of the small intestine. Diarrhoea that can be associated with staphylococcal food poisoning may be due to the inhibition of water and electrolyte re-absorption in the small intestine (Argudin *et al.*, 2010).

2.9.2. *Escherichia coli*

Escherichia coli are a versatile microorganism within the family *Enterobacteriaceae*, genus *Escherichia* (Nataro and Kaper, 1998). The German paediatrician and bacteriologist, Theodore Escherichia first described the bacterium in 1885 and named it bacterium coli commune. Later it was named *Escherichia coli* (Geyid, 1995). These organisms are gram negative, can be non motile or motile with peritrichous flagella. *E.*

coli is non-spore forming oxidase negative bacilli and predominantly facultative anaerobe (Boyed, 1995). It forms gas from glucose, ferments lactose, gives positive methyl red, a negative Voges-Proskauer reaction and does not utilize citrate. It is a consistent inhabitant of the human intestinal tract, which often remains harmlessly confined. However, in immunocompromised host, or when gastrointestinal barriers are violated, even normal 'nonpathogenic' strains of *E. coli* can cause infection (Nataro and Kaper, 1998). Illnesses due to pathogenic *E. coli* may be limited to mucosal surface or can be disseminated throughout the body. The regular presence of *E. coli* in the human intestine and feces has led to tracking the bacterium in nature as indicator of water contamination by feces (Power *et al*., 2005).

Most *E. coli* strains are harmless commensals but others are pathogenic. Differentiation of the pathogenic strains from the commensal ones was accomplished on the basis of virulence properties, mechanisms of pathogenicity, clinical syndromes and serotyping of distinct "O" (somatic), "H" (flagella) and "K" (capsule) antigens (Wilshaw *et al*., 2000). The pathogenic strains may further be classified into virotypes which include enteropathogenic *E. coli* (EPEC), enteroinvasive *E. coli* (EIEC), enterotoxigenic *E. coli* (ETEC), enteroaggregative *E. coli* (EAggEC), diffuse adhering *E. coli* (DAEC) and verocytotoxin producing *E. coli* (VTEC) also referred to as Shiga toxin-producing *E. coli* (STEC)(Bell, 2002).

Enteropathogenic *E. coli* cause a watery diarrhoea accompanied by vomiting and fever inchildren under the age of three (Wilshaw *et al.*, 2000). Enteroinvasive *E. coli* cause *Shigella* like dysentery bacillary diarrhoea (Harris, 2001) which is acute and watery at first accompanied by fever and abdominal cramps. The diarrhoea can worsen leading to bloody and mucoid stools (Wilshaw *et al*., 2000). Enterotoxigenic *E. coli* cause "traveller's diarrhoea" characterised by a watery stool, abdominal cramps, fever, and malaise and vomiting (Harris, 2001). Enteroaggregative *E. coli* cause persistent watery diarrhoea accompanied by vomiting, dehydration and abdominal pain in children (Wilshaw *et al*., 2000; Harris, 2001). Diffuse adhering *E. coli* cause childhood diarrhoea and they have been associated with diarrhoea in children in Mexico (Doyle *et al*., 1997). Verocytotoxin producing *E. coli* were first described in 1977 by Konowalchuk and his co-workers (Wilshaw *et al*., 2000). They were recognised as significant causative agents of haemorrhagic colitis (HC), haemolytic uremic syndrome (HUS) and thrombotic thrombocytopaenic purpura (TTP). Verocytotoxin producing *E. coli* illness can be fatal

especially to children, the elderly, the pregnant and the Immunocompromised (Wilshaw *et al*., 2000).

Enterohemorrhagic Escherichia coli (EHEC), in particular serotype O157:H7, is a highly pathogenic subset of Shiga toxin-producing E. coli (STEC) that causes gastrointestinal illnesses ranging from aqueous and bloody diarrhea to hemorrhagic colitis in humans (Karmali, 2009). Hemolytic-uremic syndrome (HUS) is a potentially life-threatening complication that can arise from STEC infection. The production of Shiga toxins (Stx) is a key factor contributing to the development of HUS (Griffin & Tauxe, 1991). In addition to Stx, a type III protein secretion system (T3SS), through which the pathogen translocates effector proteins into host cells, causes attaching and effacing (A/E) lesions (Karmali, 2004). The genes required for A/E lesions are encoded within a chromosomal pathogenicity island named the locus of enterocyte effacement (McDaniel *et al.,* 1995). The LEE encodes T3SS, an adhesin (the intimin Eae) and its receptor (Tir) required for intimate adherence to epithelial cells, and effector proteins translocated through the T3SS that are injected into the host cell (Naylor *et al.,* 2005).

The genome sequences of O157:H7 strains isolated from the major outbreaks share about 75% of a highly conserved sequence backbone of the E. coli chromosome (Perna et al., 2001). The remaining O157:H7-specific sequences are named O islands, most of which are horizontally transferred and include other virulence genes in addition to stx and LEE genes (Croxen and Finlay, 2010). Cattle are recognized as the main reservoir for E. coli O157:H7 resulting in zoonotic transmission by consumption of undercooked meat or dairy products inadequately pasteurized and contaminated with bovine feces (Jay *et al.,* 2004). Here, we review the established and putative environmental behaviors of E. coli O157:H7 and present potential reservoirs and ecological niches where EHEC may persist in the environment.

2.9.3. *Salmonella typhiymurium*

Salmonella typhimurium is a pathogenic Gram-negative bacteria predominately found in the human intestinal lumen. Not only in human but also found in the wild birds such as house sparrow and fiches (Une *et al.*, 2008). The outer membrane of *Shigella typhimurium* responsible for toxicity and consisting largely of lipopolysaccharides (LPS); which protect the bacteria from the environment. The LPS is made up of an O-antigen, a polysaccharide core, and lipid A, all are connected to the outer membrane.

The Lipid A component is made up of two phosphorylated glucosamines which are attached to fatty acids and determine bacterial toxicity. In contrast animals carry an enzyme that specifically removes these phosphate groups in an attempt to protect from these pathogens (Tuin *et al.,* 2005). In most cases *Salmonella typhimurium* has the ability to undergo acetylation of the O-antigen, which changes its conformation, and makes it difficult for antibodies to recognize (Slauch *et al*., 1995).

Salmonella typhimurium is rod shaped bacteria that contain peritrichous flagella and produce hydrogen sulfide. (Surette *et al.,* 1998) describe the cell structure and metabolism of *Salmonella typhimurium.* They stated that *Salmonella typhimurium* able to secrete small signaling molecules called autoinducers. The LuxS gene is responsible for initiating a series reaction that produce this molecule and allow for cell to cell communication. Sugar compounds, preferably glucose, activate LuxS and the resulting autoinducer concentration increases with the bacterial concentration until the substrate is depleted. At this point the autoinducer is degraded and can be recycled by the bacterial cell. This quorum sensing allows cells to determine the metabolic potential of the environment.

Salmonella typhimurium is an important pathogenic organism in both humans and animals (Guerin *et al.,* 2005). Animals Food is the primary reservoir for human pathogenic of *Salmonella typhimurium.* Poultry products like eggs are the major mode for transmission of the organisms from animals to human. *Salmonella typhimurium* causes gastroenteritis in humans and other mammals. When the bacterial cells enter to epithelial cells lining of the intestine they cause host cell ruffling which temporarily damages the microvilli on the surface of the cell. It harms the host by causing the levels of intracellular free calcium to increase and disorganize the cytoplasm of the cell. From the intestines, it is transferred to the liver or the spleen where it continues to grow. Then, it either goes back into the host's intestines, or is excreted in the organisms' feces. It can be spread from the feces by contaminated water, soil, or poor sanitary conditions (Susan *et al.,* 2004).

2.9.4. *Pseudomonas aeruginosa*

P. aeruginosa is a common bacterium that can cause disease in animals, including humans. 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 hypoxic atmospheres, and has, thus, 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 colonization occurs 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 implicated in hot-tub rash. It is also able to decompose hydrocarbons and has been used to break down tarballs and oil from oil spills (Itah and Essien, 2005).

It is a Gram-negative, aerobic, coccobacillus bacterium with unipolar motility.With An opportunistic human pathogen, *P. aeruginosa* is also an opportunistic pathogen of plants .*P. aeruginosa* is the type species of the genus *Pseudomonas* (Anzai *et al.*, 2000). Although classified as an aerobic organism *P. aeruginosa* is considered by many as a facultative anaerobe, as it is well adapted to proliferate in conditions of partial or total oxygen depletion. This organism can achieve anaerobic growth with nitrate as a terminal electron acceptor, and, in its absence, it is also able to ferment arginine by substrate-level phosphorylation (Palmer *et al.*, 2007). Adaptation to micro-aerobic or anaerobic environments is essential for certain lifestyles of *P. aeruginosa*, for example, during lung infection in cystic fibrosis patients, where thick layers of lung mucus and alginate surrounding mucoid bacterial cells can limit the diffusion of oxygen.

P. aeruginosa secretes a variety of pigments, including pyocyanin (blue-green), pyoverdine (yellow-green and fluorescent), and pyorubin (red-brown). King, Ward, and Raney developed *Pseudomonas* agar P (King A medium) for enhancing pyocyanin and pyorubin production, and *Pseudomonas* agar F (King B medium) for enhancing fluorescein production (King *et al.*, 1954).

Pathogenesis

Phagocytosis of *P. aeruginosa* by neutrophil in patient with bloodstream infection (Gram stain)**.**An opportunistic, nosocomial pathogen of immunocompromised individuals, *P. aeruginosa* typically infects the pulmonary tract, urinary tract, burns, wounds, and also causes other blood infections(Mathee *et al.*,2008).

It is the most common cause of infections of burn injuries and of the outer ear (otitis externa), and is the most frequent colonizer of medical devices (e.g., catheters). *Pseudomonas* can, in rare circumstances, cause community-acquired pneumonias,as well as ventilator -associated pneumonias, being one of the most common agents isolated in several studies(Diekema *et al.*,1999). Pyocyanin is a virulence factor of the bacteria and has been known to cause death in *C.elegans* by oxidative stress. However, research indicates salicylic acid can inhibit pyocyanin production (Prithiviraj *et al.*, 2005).one in ten hospital-acquired infections are from *Pseudomonas*. Cystic fibrosis patients are also predisposed to *P. aeruginosa* infection of the lungs. *P. aeruginosa* may also be a common cause of "hot-tub rash" (dermatitis), caused by lack of proper, periodic attention to water quality. The most common cause of burn infections is *P. aeruginosa*. *Pseudomonas* is also a common cause of postoperative infection in radial keratotomy surgery patients. The organism is also associated with the skin lesion ecthyma gangrenosum. *P. aeruginosa* is frequently associated with osteomyelitis involving puncture wounds of the foot, believed to result from direct inoculation with *P. aeruginosa* via the foam padding found in tennis shoes, with diabetic patients at a higher risk (Prithiviraj *et al.*, 2005).

Toxins

P. aeruginosa uses the virulence factor exotoxin A to inactivate ADP-ribosylate eukaryotic elongation factor 2 in the host cell, much as the diphtheria toxin does. Without elongation factor 2, eukaryotic cells cannot synthesize proteins and necrotise. The release of intracellular contents induces an immunologic response in immunocompetent patients. In addition *P. aeruginosa* uses an exoenzyme, ExoU, which degrades the plasma membrane of eukaryotic cells, leading to lysis. Triggers with low phosphate levels, *P. aeruginosa* has been found to activate from benign symbiont to express lethal toxins inside the intestinal tract and severely damage or kill the host, which can be mitigated by providing excess phosphate instead of antibiotics.(Pool,2004) *P. aeruginosa* is naturally resistant to a large range of antibiotics and may demonstrate additional resistance after unsuccessful treatment, in particular, through modification of a porin. It should usually be possible to guide treatment according to laboratory sensitivities, rather than choosing an antibiotic empirically. If antibiotics are started empirically, then every effort should be made to obtain cultures (before administering first dose of antibiotic), and the choice of antibiotic used should be reviewed when the culture results are available. Phage therapy against *P. aeruginosa* remains one of the

most effective treatments, which can be combined with antibiotics, has no contraindications and minimal adverse effects. Phages are produced as sterile liquid, suitable for intake, applications etc (Sulakvelidze *et al.,* 2001).

Antibiotic resistance

One of the most worrisome characteristics of *P. aeruginosa* is its low antibiotic susceptibility, which is attributable to a concerted action of multidrug efflux pumps with chromosomally encoded antibiotic resistance genes (e.g., *mexAB, mexXY* and the low permeability of the bacterial cellular envelopes (Poole,2004). In addition to this intrinsic resistance, *P. aeruginosa* easily develops acquired resistance either by mutation in chromosomally encoded genes or by the horizontal gene transfers of antibiotic resistance determinants. Development of multidrug resistance by *P. aeruginosa* isolates requires several different genetic events, including acquisition of different mutations and/or horizontal transfer of antibiotic resistance genes. Hyper mutation favours the selection of mutation-driven antibiotic resistance in *P. aeruginosa* strains producing chronic infections, whereas the clustering of several different antibiotic resistance genes in integrons favors the concerted acquisition of antibiotic resistance determinants. Some recent studies have shown phenotypic resistance associated to biofilm formation or to the emergence of small-colony variants may be important in the response of *P. aeruginosa* populations to antibiotics treatment (Matin *et al.,* 2007).

2.9.5 Control of food-borne diseases

General approaches needed for validation of the effectiveness of the HACCP system should be undertaken, with identification of the most relevant critical control points through hypothesis driven research (Callaway *et al.,* 2004). Methods for controlling contamination of produce are of greatest importance, requiring research to define routes of transmission and critical control points at all phases of the food production-consumption cycle.

Improving food safety in restaurants although all eating establishments are instructed by city and health departments to have an HACCP plan, the impact of these programs in preventing food-borne illness remains largely untested. In 1998, in Los Angeles County, a restaurant hygiene-grading program was implemented that used publicly posted grade cards (Simon *et al.,* 2005). The grading program was associated with a 13.1% decrease

in the number of food-borne-disease hospitalizations in the county during the 2 subsequent years of the study.

Improving food safety in homes with the reduction of cross-contamination and improvement in personal hygiene, an estimated 80,000 enteric infections could be prevented each year in US households, resulting in savings of $138 million (Duff *et al.*, 2003). Inn ranking behaviours in the home that would lead to reduced food-borne illness, use of a thermometer for cooking and hand washing in the kitchen were ranked as the 2 most important behaviour modifications (Hillers *et al.,* 2003). In a study of home food-handling practices, a significant association was found between inconsistent hand washing and food preparation and an increased risk of sporadic salmonellosis in adults (Kohl *et al.,* 2002). An economic model found benefit of a disinfection program for high-risk food preparation, particularly when the household contained elderly or immunocompromised persons (Simon *et al.,* 2005). In-home food-borne transmission of enteric pathogens is most important when persons at the extremes of age or others with underlying medical conditions and compromised immune systems live in the house hold. Such persons should not eat certain foods, including raw seed sprouts (Taormina *et al.,* 1999). Persons who are receiving long-term treatment with H2 antagonists and proton pump inhibitors are at increased risk for food-borne illness .The CDC (2006) has recommended that all consumers avoid consumption of unpasteurized milk and milk products and certain raw or undercooked products, including oysters, eggs, ground beef, and poultry; more can be found at the fight. .A mathematical model has been proposed to address individual hygiene practices during food preparation and consumption patterns in private homes that could be tested for an association with sporadic enteric illness (Christensen *et al*., 2005).

Food irradiation is a non-thermal method to reduce or eliminate pathogenic microbes in food. Cobalt 60 or Cesium137 emit high-energy photons called gamma rays, which yield neutrons that penetrate food up to a few feet in depth. E-beam irradiation directs a stream of high-energy electrons by opposing guns resembling the technology seen in televisions. X-ray irradiation releases electrons, which can pass through thick foods. In food irradiation, the energy from the rays is transferred to water and other microbial molecules, creating transient, reactive chemicals that cause defective microbial DNA, making organisms unable to propagate. Microbes differ in their susceptibility to irradiation and the rate at which they can repair damaged DNA. The larger the pathogen (and the more DNA), the more susceptible they are to the lethal effects of irradiation.

The methodologies are safe, and the World Health Organization has endorsed food irradiation, as have the CDC, the US Department of Agriculture, and the FDA. There are 60 commercial irradiation facilities available in the United States. In 1997, the FDA approved the use of ionizing radiation to inactivate pathogenic bacteria in red meat. The principal arguments against pursuing food irradiation are 2-fold. First, the technology has not been adequately studied for harmful effects caused when altered chemical bonds and potentially toxic ions or free radicals react with constituents in food to form "radiolytic products" (Ashley *et al.*,2004). Second, the specific foods for which the approach should be used and the specific methodology for decontamination have not been fully developed. A major issue in moving this concept forward is consumer acceptance of irradiated foods in the absence of public health laws and requirements (Frenzen *et al.*, 2001). With efforts to educate the public about irradiation of food at the grocery store level, consumer acceptability is likely to improve for the next years, the FDA proposed letting companies use the term "pasteurized" to describe irradiated foods (Hoefer *et al.*, 2005).

2.10. Description of the plants assessed for their antimicrobial activities

2.10.1. *Carissa spinarum*

Plants of the Apocynaceae are often poisonous and are rich in alkaloids or glycosides, especially in the seeds and latex. Some species are valuable sources of medicine, insecticides, fibers, and rubber. About 155 genera and 2000 species distributed primarily in the tropics and subtropics, poorly represented in the temperate regions (Bingtao *et. al.*, 2012). *Carissa spinarum* are shrubs that climbers, or small trees, mostly spiny, branches dichotomous. Leaves opposite; petiole 2-3 mm. Cymes terminal or auxiliary, dichotomous, pedunculate, usually many flowered. Flowers are 5- [or 4]-merous, Calyx without gland or rarely with many basal gland inside. Corolla salver form, tube cylindrical, dilated at stamina insertion, lobes overlapping to left or to right. Stamens included in throat; anthers lanceolate, obtuse or apiculate, base not appendage; disc absent. Ovary are 2-loculed; ovules 1-4 in each locule, rarely numerous, biseriate. Style are fili form; pistil head narrowly oblong or fusi form, apex shortly 2-cleft. Berries are 1- [or 2]-loculed. Seeds are 2 or more, peltate; endosperm fleshy; cotyledons ovate, radicle inferior. About 30 species: tropics and subtropics of Africa, Asia, and Australia; four species in China (Bingtao *et al.*, 2012).

Carissa spinarum is a thorny, evergreen shrub, widely distributed throughout the drier, sandy and rocky soils. The roots of this plant has long been prescribed in the indigenous system of medicine as purgative, for the treatment of inflammation-related disorders such as rheumatism and pain, cleaning worm infested wounds of animals and in snake bite (Kirtikar and Basu, 2003). In Chinese system of medicine the roots of the plant is known for the treatment of rheumatism and hepatitis. Previous phytochemical investigations revealed the presence of caffeic acid (Raina *et. al*., 1971), ursolic acid, naringin (Mathuram *et al.,* 1998) various cardiac glycosides (Rastogi *et al.,* 1969), germacrane sesquiterpene and lignans (Rao *et al.,* 2005). Earlier studies have shown that the extract of the plant possesses cardiotonic, antibacterial) and potent antioxidant activity.The roots of the plant are used by the tribal healers of Western Ghats region of Karnataka to treat intermittent fever and inflammatory conditions. (Mathuram *et al*., 1998).

Propagation of *Carissa spinarum*

Cultivation by seedlings has been reported (Dharani *et al.,* 2010). Seeds germinate in 4 to 10 days and pouring of hot water at a temperature of 80°C and allowing to cool overnight improves germination. Seeds should be grown in pots containing 1:1 ratio of sand and compost mixture in a warm and moist nursery conditions. Seedlings need only few months before planting out in 6 by 6 m spacing in a farmland. Seedlings develop a long taproot, and growth rate may be up to 70 cm per year.

2.10.2. *Cissampelos mucronata*

The plant *Cissampelos mucronata* belongs to the family *Menispermaceae;* it *is* a climbing shrub that is widespread in dry parts of Africa. The leaves are entire, thickly papery, alternate and about 8 cm long, while the root is fibrous in nature (Hutchinson and Dalzies, 1954). The family, *Menispermaceae* is a temperate to tropical family of around 70 genera and 450 species of dicotyledonous tropical flowering vines with twining stems and a few herbs, shrubs and trees. Leaves are alternate and simple, but may be palmately veined and often lobed. Floral parts of the unisexual flowers are in whorls of three. Male and female flowers are on separate plants, usually small and clustered in panicles or cymes. Fruits are drupes, the hard endocarp of which has features such as warts and ribs useful for identifying species.

Cissampelos mucronata is widely used as traditional herbal medicine .In the Coast region, Tanzania, the powdered roots of *C. mucronata* are mixed with coconut oil for treatment of fresh wounds after extraction of jigger (Tunga penetrans) while in other parts of Tanzania, *C. mucronata* is used for treatment of indigestion, fever due to malaria and wounds (Gessler *et al.*,1994). In South Africa, the root decoction is used for the treatment of schistosomiasis while in India roots are used as antisnake venom. Pharmacologically root extracts of *C. mucronata* are reported to be active against chloroquine - sensitive and chloroquine - resistant P. falciparum strains, and active against Trypanosoma cruzi and Trypanosoma rhodensiense (Tshibangu *et al.,* 2002). Other pharmacological properties of root extracts include sedative effect and antimicrobial activity (Akah *et al.,* 2002). Extracts from leaves are reported to have antibacterial, anti-ulcer and hypoglycaemic activities as well as uterine relaxant properties (Nwafor *et al.,* 2003).

The leave and root of *C.mucronata* are used by some communities to treat venereal diseases, wounds and gastro-intestinal complaints such as diarrhoea and dysentery (Tshibangu *et al.,* 2002).

In vitro propagation of *Cissampelos mucronata*

Development of plant tissue culture technology offers a great potential for rapid multiplication of plant germplasm. It serves as a powerful tool for short to medium term conservation of important plant species. Tissue culture technology provides an ideal way for large-scale propagation and the reintroduction of the plants in its natural habitats (Bhojwani *et al.,* 1998). *In vitro* propagation increases the efficiency and scales up plant production (Chaturvedi *et al.,* 1983). Moreover, plant cell and tissue culture, as well as genetic engineering may be an alternative to the conventional method for the improvement of medicinal plants. The *in vitro* cultures could be preserved overtime and multiplied as and when required. Tissue culture also facilitates the exchange of germplasm within and across the countries (Sehrawat *et al.,* 2002).

There are several literatures available on *in vitro* propagation of plant .In most of the work, nodal explants have been used in MS basal media with various combinations and concentration of plant growth hormones. A protocol of micropropagation *Cissampelos mucronata* of was reported where various explants (shoot tip, axillary bud and

cotyledonary node) were cultured on MS medium supplemented with different concentrations of plant growth hormone (Mridula *et al.*, 2001).

In vitro multiplication of *Cissampelos mucronata* via direct somatic embryogenesis using leaf explants of 15 days old plants on MS medium supplemented with 2,4-D (0.5mg/l) and glutamine (20mg/l) produced viable somatic embryos (Reddy et al.,2003). Another protocol was developed for rapid clonal propagation of *Cissampelos mucronata* through *in vitro* culture of mature nodal explants. Shoots were initiated on both MS medium and Woody Plant Medium (WPM) supplemented with 2.32 µm Kinetin. Of the two basal media tested, WPM was found to be superior to MS medium for the induction of multiple shoots. Among the cytokinins tested, Benzyl Adenine (BA) was more effective than Kinetin for axillary shoot proliferation. Nodal explants were reported as best explants for *in vitro* regeneration of *Cissampelos mucronata* (Gururaji *et al.*, 2007).

In *Cissampelos mucronata* tissue culture, callus formation was observed from nodal segments, leaf and inter-node explants when planted on different combinations of hormones in MS Medium. However only nodal explants showed better shoot growth in MS medium containing kinetin (1.5 mg/l). Roots were developed in the medium containing 1.0mg/l BAP (1.0mg) and 2.5mg/l Naphthaleneacetic Acid (NAA) .Induction of callus was also obtained from leaf explants while culture in MS medium with 2,4-D alone or in combination with kinetin. However such callus failed to differentiate. Direct shoot induction was achieved from nodal explants culture in MS medium supplemented with kinetin (8µM) or in combination of kinetin and BAP (12 and 2µM respectively). The microshoots developed roots in medium fortified with NAA (8µM) (Bhalerao *et al.,* 2013). Regeneration of multiple shoot was also obtained from nodal segments of *Cissampelos mucronata* in MS basal medium supplemented with the combination of BA (0.5 mg/l) and NAA (0.2mg/l). Regenerated shoots were rooted on half strength MS basal medium containing both BA (1.0mg/l) and IAA (0.2mg/l). Rooted plantlets were transferred to pots containing soil for acclimatization, for a period of three weeks and were successfully established in soil. The shoot proliferation was also observed in MS medium containing BA and kinetin. While rooting of the microshoots was obtained in half strength MS medium supplemented with 0.4mg/l NAA .Production of active principle through *in vitro* culture also draws attention of the scientific communities (Khanapurkar *et al.*,2012).

Berberine, an isoquinolene alkaloid, together with its related analogs protoberberine and palmatine were detected in cell suspension cultures derived from leaf explants of *Cissampelos mucronata* Berberine production was achieved in an optimized Linsmaier and Skoog's medium with specific pH, plant growth regulators and carbon sources. The yield of berberine in cell suspensions of *Cissampelos mucronata* was reported as 5-14-folds higher than that of intact plant (Rao *et al.,* 2008). In an attempt to up gradation of the content of berberine in *Cissampelos mucronata* through biotechnological interventions, four week old leaf, petiole and stem derived calli of the plant *was* sub-cultured on to MS medium, supplemented with various growth regulators. MS medium with NAA (2 mg l^{-1}) supplemented with BA or kinetin, each at 2 mg/l, was identified as the basal production medium for *in vitro* production of berberine, yielding 7.55 µg and 7.36 µg berberine respectively, per gram of calli. Calli produced from stem segments registered maximum amount of berberine compared to leaf and petiole derived callus cultures. Roots were sub-cultured on liquid MS medium containing B5 vitamins and 3% sucrose without hormone under an optimized growth condition (Kalimuthu *et al.*,2005).

3. Materials and Methods

3.1. Description of the study site

Bero woreda is one of the woredas in SNNP Regional State of Ethiopia bordered with Guraferad and South Bench Woreda in the North direction, Maji woreda to the North-East, and Surma woreda to South-West and East (almost surrounded) (Fig. 1). The altitude of the woreda ranges from 500-1670 meters above sea level. Total area of Bero woreda was 171,804 hectors from, 12,833 hectare area covered by forest. Woreda is divided in to 12 administrational kebeles. The climatic division of the same was desert (10%), kola (70%) and weyinadega(20%) With respect to soil type, 20% of the soil belongs to red soil followed by 60% black soil, 15% gray soil and the remaining 5% belongs to others (Bench-Maji Zone Statistics office).

Meteorological data sources from the woreda Meteorological Service Agency during the years 2000 to 2004 indicated that the annual average minimum and maximum temperature for four years data is $15°C$ and $27°C$ respectively. The annual average rainfall for the same years‟ data is 125.8 mm. Most of the lands of the study area are covered with mix of grass and acacia or other semi desert trees, while small portions are covered with wild coffee and forest. The SNNP Regional government considers Bero as "Gold producer district". The population of Bero woreda is estimated to be 47,532 of which 28,972 are men and 18,560 women. About 14% of its population are urban dwellers .The different ethnic groups reported from Bero are native Dizi (15%), Suri/Zilmamo (6%) and mix of the people (79.3%) from different parts of Ethiopia for Gold extraction and became permanent settlers.

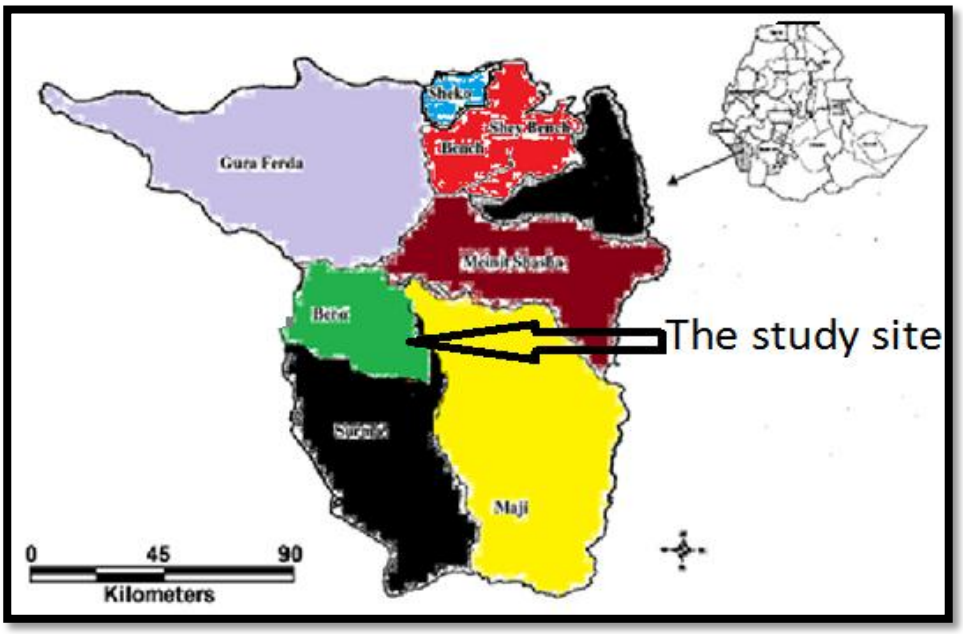

Figure 1 Map of Bench-Maji Zone showing the study site (the green shaded area). (Source: NTFP, 2006)

3.2. Study Design

A cross-sectional study was used to collect information from the respondents. Experimental design for bioassays involved varying experimental groups via test groups, positive and negative controls. Test group consisted of the standard ATCC strains and extract of different concentrations. The second group represents positive control these consisted of organism plus known antibiotics. This ensured that the utilized organism, were susceptible to common chemotherapeutics and were not resistant strains. The third group contained negative controls (Tween 80) to confirm that the solvents used for dissolution had no inhibitory action on their own. All determination involving quantitative data were carried out in triplicate.

Investigation of the antimicrobial activities and screening of photochemical constituents of the selected plants was carried out at Research laboratory of Postgraduate Studies, Jimma University.

3.3. Sampling Techniques

Purposive sampling techniques were utilized to select traditional healers and knowledgeable persons about traditional medicinal plant usage in the study area. The ethno-botanical survey of study was conducted in Bero Wereda, Bench Maji Zone

which is located about 700 Km Southwest of Addis Ababa. The remaining part of this work (i.e. investigation of the antimicrobial activity and phytochemical constituents of selected medicinal plant) was carried at Research laboratory of Postgraduate Studies, Department of Biology, Jimma University.

3.4. Preliminary Data Collection and the Study Population

Traditional Medical Practitioners (TMP's) were the main informants in the survey. Although the woreda has 12 kebeles, were purposively included in the study. For collection of traditional medicinal plants, 10 key informants were sampled in systematic way as recommended by Martin (1995). Sampling of key informants is most commonly systematic and their collections have been based on the recommendation and comments of elders, local traditional healer, local administrators and religious leaders from the community group.

The collected potential medicinal plants were identifying with the help of the traditional Practitioners, knowledgeable religious persons and the community members of the study area. Accordingly, 30 TMP's and 30 others community members were interviewed for collection of pertinent information. After secured their consent, the interview was carried out using semi-structured interview (Appendix I). Data on the local names of the plants, the plant parts used, and mode of administration were collected. The interviews and discussions were conducted in the local language to avoid language barrier.

The information was asked to compare the given medicinal plant based on their efficacy and to give the highest number(7) for the medicinal plant which they thought most effective in treating disease and the lowest number (1) for the least effective plant in treating food borne illness(Appendix II). Based on ethno-medicinal information, decision was made on two of the medicinal plant species most frequently used but not investigated by other researcher in the study areas. Accordingly, root of *Cissampelos mucronata* and *Carissa spinarum* were selected.The fresh root part of the *Cissampelos mucronata* and *Carissa spinarum* plants were collected using plastic bag and chopped by mortal in to pieces and dried under shade place.

3.5. Extraction of plant material by maceration (*soaking)* method

The clean shade dried root of the *Cissampelos mucronata* and *Carissa spinarum* were pre-chopped separately and mechanically broken down by electronic grinder machine (Dietz-motoren GmbH&co.KG D-7319 Dettingen U.Teck, Germany) to obtain powdered plant material. Then, 100 g of each plant powder were soaked in 500 ml petroleum ether, methanol and acetone solvent in conical flask and kept for 72 hours using shaker at speed of 400 rpm at room temperature. After three days, the extract was filtered by using folded Whatman no.1 filter paper and the collected extract was further separated by rotary evaporator at 40ºC. Finally, the crude extract was placed in desiccators containing calcium chloride ($CaCl_2$). The dried extract was stored at 4ºC in refrigerator for further uses.

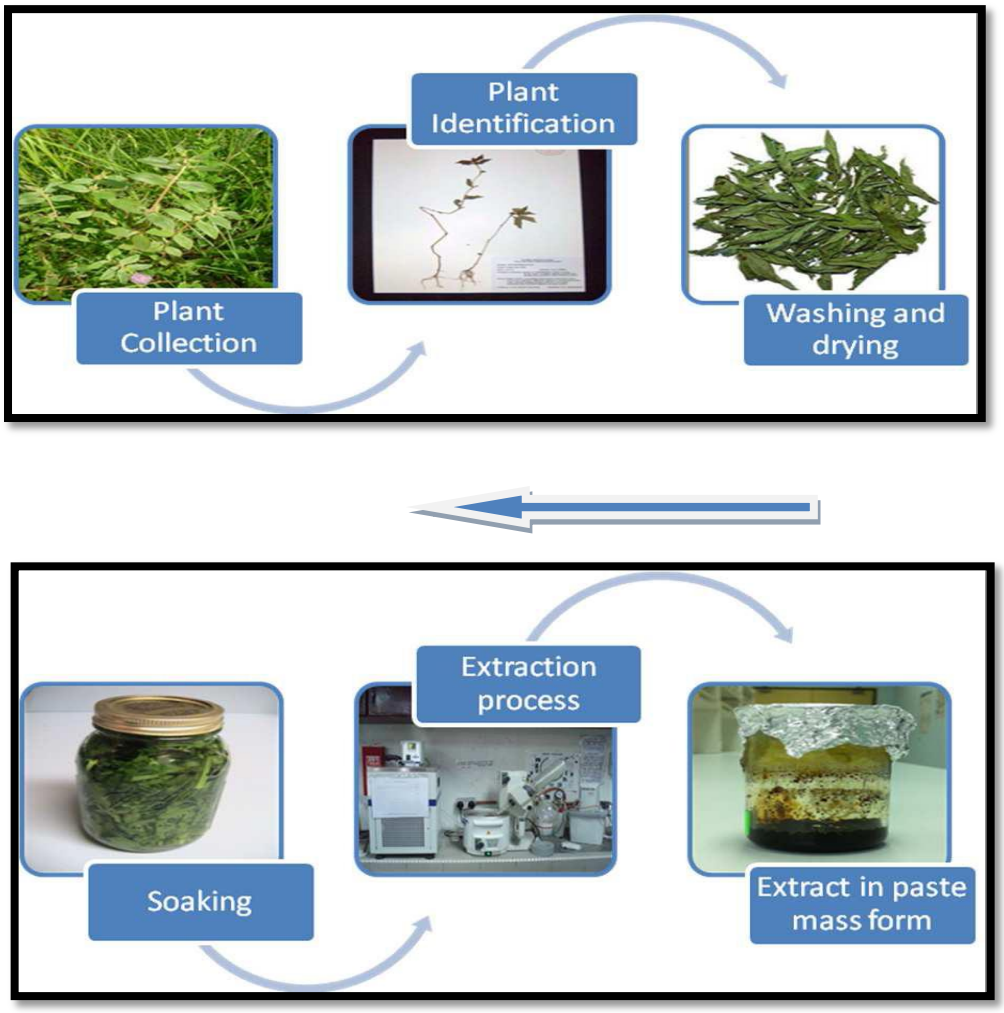

Figure 2 Pictorial presentation of procedure for maceration of traditional medicinal plants using solvent (Sasidharan , 2012).

3.6. Culture media and inoculums preparation

3.6.1. Bacterial strains

Accordingly, standard microbial strains were obtained from Research laboratory of Postgraduate Studies, Jimma University. Four bacterial strains namely: (*Escherichia coli* ATCC 25922, *Pseudomona auroginosa* ATCC 27853, *Salmonella typhimurium* ATCC 133110) and *Staphylococcus aureus* ATCC25923). These organisms were selected based on their disease burden and increasing trend of antibiotic resistance in the developing world.

The bacterial strain were re-activated by sub culturing in nutrient broth at 37 ºC and temporarily maintained on nutrient agar slant at 4ºC until used..

3.6.2. Standardization of Inoculum

The inoculums of each bacteria strain were prepared using the colony suspension method (EUCAST, 2000). Colonies picked from 24 hours old cultures grown on nutrient agar slant were used to make suspension of the test organisms in saline solution. The suspension was diluted 1:100 by transferring 0.1 ml (100 µl) of the bacteria suspension to 9.9 ml of sterile nutrient broth before use. The density of bacteria suspension for susceptibility test was finally determined by comparison with 0.5 McFarland standard of Barium sulphate solution (Cheesbrough, 1987). The turbidity of actively growing broth culture was adjusted with sterile saline or/ and broth to obtain turbidity comparable to the 0.5 McFarland standard. To perform this step properly, adequate light was needed to visually compare the inoculums tube and the 0.5 McFarland standards against card with a white background and contrasting black lines.

3.7. Antimicrobial Disc preparation

Disc of 6 mm diameter were made from Whatman no. 1 filter paper using a paper puncher. Batch of disc were transferred into beaker wrapped with aluminium sheet and sterilized in the hot air oven at 160ºC for 1 hour (Ayo *et al.*,2007).They were then impregnated with the varying concentrations of plant extract solutions after putting on the inoculated plates (Baris *et al.*, 2006).

3.8. Antibacterial activity test

3.8.1 Agar disc diffusion method

Pure culture of the test organisms were activated overnight before activity evaluation. The 24 hours cultures of cell density equivalent to 0.5 McFarland standards (1-2 x 10^8 cfu /ml) were uniformly spread onto the entire surface of a Mueller – Hinton Agar plate with sterile cotton swab. Test solutions were prepared by dissolving appropriate weight of plant extracts to achieve final stock concentrations of 100 and 50 mg/ml in Tween 80. Sterile filter paper discs (6 mm) were evenly placed on the agar plate surface previously inoculated with the 0.1 ml suspensions of each test microorganisms. Then 20 µl of the various plants extracts i.e. methanol, acetone and petroleum ether extract were aseptically transferred to each disc at all dilutions that were made in triplicate. Standard disc of tetracycline (30 µg/disc) and discs with 20 µl of Tween 80 were also included as controls. The plates were placed inverted and incubated for 24 hours at 37^{o}C. After 24 hrs the diameter of zone of inhibition was measured by the help of ruler and results was recorded in millimetres. The antibacterial activity results were expressed in term of the diameter of zone of inhibition and <9 mm zone was considered as inactive; 9-12 mm as partially active; while13-18 mm as active and >18 mm as very active (Junior and Zanil, 2000). The mean and standard deviation of the diameter of inhibition zones were calculated.

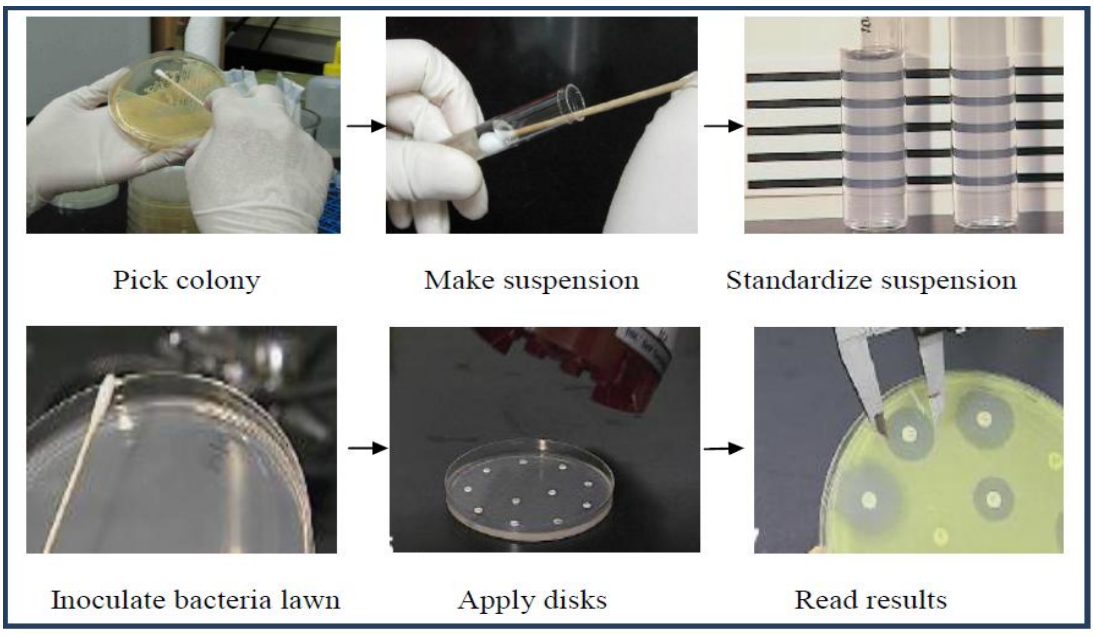

Figure 3. Pictorial presentation for Disk Diffusion Method procedures (Lin, 2011).

3.8.2 Determination of Minimum Inhibitory Concentration (MIC)

Minimum inhibitory concentration of the crude extracts was determined as described by Komuraiah *et al.* (2009). To obtain the minimum inhibitory concentrations; the crude extracts at the concentration of (50 mg/ml) was prepared by completely dissolving 50 mg of plant extract in 1ml of Tween 80 and added 9 ml of nutrient broth. Then two-fold serial dilution of the extract were done from 50 mg/ml stock plant extracts using nutrient broth to make 6 test concentrations ranging from 50 mg/ml ,25 mg/ml, 12.5 mg/ml, 6.25 mg/ml, 3.125 mg/ml and 1.56 mg/ml for each solvent extract in a test tube. Then 0.1 ml (100 µl) of standard inoculums (1 - 2 × 10^8 cfu/ml) was inoculated in each test tube and mixed thoroughly. Positive and negative Control test tubes containing 0.5 ml (500 µl) Gentamicin and 0.5 ml (500 µl) of Tween 80 are used for control test tubes respectively. The tubes were then incubated aerobically at 37°C for 24 h. The tube with lowest concentration of extract and with no detectable bacterial growth (no turbidity) was considered as the MIC.

3.8.3. Determination of the Minimum Bactericidal Concentration (MBC)

MBC was determined following the MIC broth microdilution assay approach by sub culturing 10 µl volumes from each test tube that did not exhibit growth after 24 hours of incubation and spot inoculated them separately into fresh nutrient agar plates (Sudjana *et al.*, 2009).The plates were incubated for 24 hours at 37°C & after which the numbers of colonies were counted. The MBC was defined as the lowest concentration killing more than or equal to 99.9% of the inoculums (Reuben *et al.*, 2008).

3.9. Photochemical Screening

A small portion of the powdered plant form or their extract was used for phytochemical tests (i.e. to test for the presence of plant secondary metabolites such as alkaloids, tannins, saponins, flavonoids, steroids and terpenoids) following methods described in Evans and Trease (1989) and Harbourne (1973). These methods are summarized as follows.

3.9.1. Test for Alkaloids

A 0.5 g of the plant extract was dissolved in 5 mL of 1% HCl on steam bath. A millilitre of the filtrate was treated with few drops of Dragendorff‘s reagent (a solution of

potassium bismuth iodide prepared from bismuth nitrate (Bi $(NO_3)_3$), tartaric acid and potassium iodide (KI) (Khatun *et al.,* 2014)). Turbidity or Orange red precipitation was taken as indicative of the presence of alkaloids.

3.9.2. Test for Flavonoids

A 0.2 g of the extract was dissolved in 2 ml of methanol and heated. A chip of magnesium metal was added to the mixture followed by the addition of a few drops of concentrated HCl. The appearance of a red or orange colouration was indicating the presence of flavonoids.

3.9.3. Test for Tannins

A 1.0 g of the plant extract was dissolved in 10 ml of distilled water and filtered (Acrodisc syringe filter, Pall USA). Two to three drops of 10% Ferric chloride (FeCl3) was added to 2 ml of the filtrate. A blackish-blue (A dark green solution) colouration indicate the presence of tannins. To another 2 ml of the filtrate was added 1 ml of bromine water. A precipitate was also taken as positive for tannins (Ayoola *et al*., 2008).

3.9.4. Test for Saponins

A 0.5 g of powdered plant materials in a test tube and 5 ml of distilled water was added and the mixture was vigorously shaken. Formation of froth persistent for 30 min confirms the presence of saponins (Ayoola *et al*., 2008).

3.9.5. Test for Terpenoids

A 0.2 g of the extract of the whole plant sample were mixed with 2 ml of chloroform ($CHCl_3$) and concentrated H_2S0_4 (3 ml) carefully added to form a layer. Reddish brown colouration of the interface was formed to indicate positive results for the presence of terpenoids.

3.10. Data analysis

The experiments were conducted in duplicate and the results are presented as mean ± SD. The statistical analysis was performed by one way analysis of variance (ANOVA) followed by multiple comparison test using statistical package for social sciences (SPSS) version 16:00 and P value < 0.05 were considered as significant.

4. Result

4.1. Socio-demographic characteristics of the respondent

A large number of the respondents 38 (63.3%) males and the rest 22 (36.7%) are females. And (45%) fall within the age group of 31-35 years and followers of traditional religion (Kalicha) (90%) (Table 1). A large number of the respondents were pastoralist (60%), while 15(25%) of them were business men. In addition, most of the respondents were either illiterate (81.7%) or have only (18.3%) elementary level education.

Table 1. Socio-demographic characteristics of the respondents, Southwest Ethiopia, 2014

Variables	Categories	Frequency	Percent (%)
Sex distribution	Male	38	63.3
	Female	22	36.7
Age distribution	25-30	3	5
	31-35	27	45
	36-40	20	33.3
	>40	10	16.6
Religion status	Muslims	1	1.6
	Orthodox	5	8.33
	Traditional religion (″kalich″)	54	90
Marital status	Married	43	71.7
	Single	12	20
	Divorced	5	8.3
Educational status	Illiterate	49	81.7
	Grade 1-4	9	15
	Grade 5-8	2	3.3
Occupation status	Farmers	9	15
	Business men	15	25
	Pastorals	36	60

4.2. Respondents Knowledge about sources and diversity of medicinal plants

Some of the respondents (35%) were familiar to more than seven medicinal plants in the study area. Furthermore, a large number of the respondents (60%) access medicinal plants mainly from nearby forests followed by home gardens (Table 2).

Table 2. Medicinal plant used by respondent for treatment of food borne disease, 2014

Parameter	**Number of respondents (N=60)**	**Percentage%**
No. of plant known by respondents:		
1-3	11	18.3
4-6	16	26.7
7-10	21	35
>10	12	20
Where do they obtain the medicinal plants		
Forest	36	60
Home gardens	15	25
On market	9	15

4.3. Selection of the study plants

Based on the respondent preference, the degree of importance or frequencies of use of the major traditional medicinal plants for the treatment of various ailments was: root of *Cissampelos mucronata* and *Carissa spinarum* was selected.

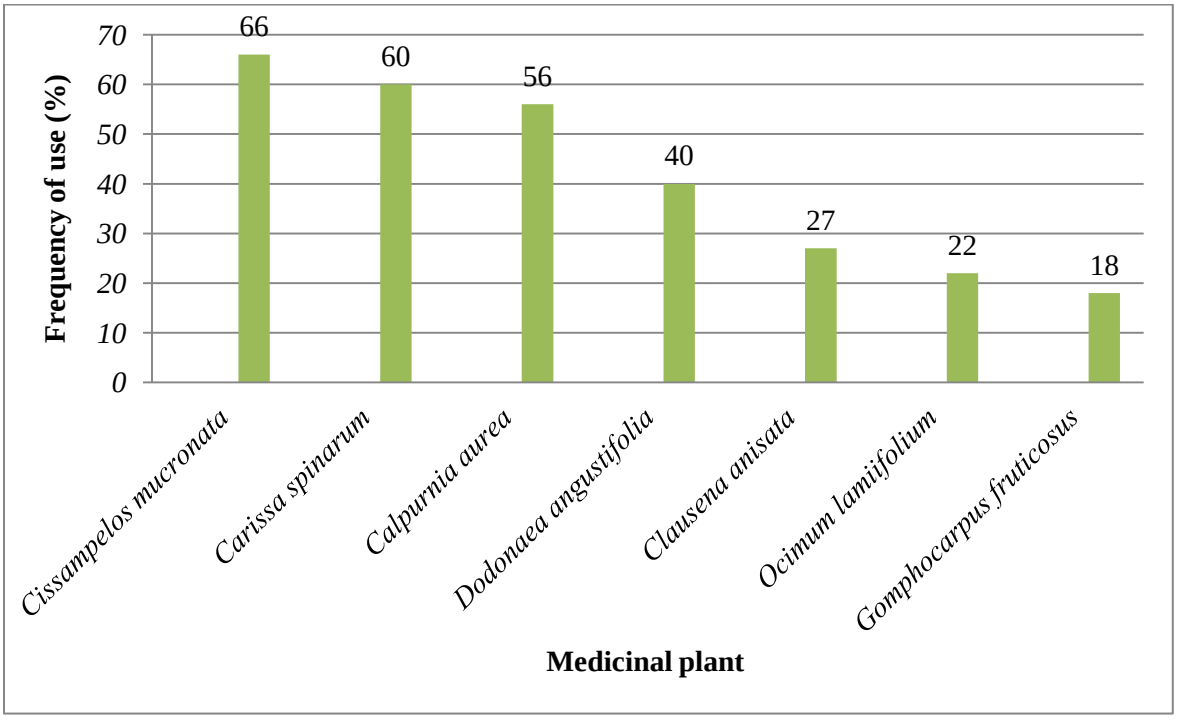

Figure 4. Traditional medicinal plant and frequencies of their use in Bero woreda, South west Ethiopia.

4.4. Methods of preparation of medicinal plant

The traditional healers prepared the medicinal plants in different ways and applied it differently. In the current study, the preparation processes of *C. spinarum* and *C. mucronata* were mainly chewing of fresh root and sucking of the juices (Table 3). Briefly, the root of *C.mucronata* is properly washed by water and the outer layer of the root scratched with knife or with sharp materials followed by chewing and sucking of juices. The length of the root part to be used for treatment of common food born disease depends on age difference. But, in many cases, amounts of plant part/parts to be processed and doses to be used were roughly estimated and therefore lacked precision. Likewise, for *C. spinarum*, a similar procedure was applied.

Table 3 Description of the two most frequently used traditional medicinal plants, Bero woreda, south west Ethiopia,

Scientific name	Amharic name	Plant part used	Preparation processes and application	Medicinal use
C. spinarum	Agam	Root	Chewing of fresh root and sucking of juices	Treatment of stomach ache
C. mucronata	Ingochit hareg	Root	Chewing of fresh root and sucking of juices	Treatment of stomach ache

4.5. Effect of Extraction solvents on percentage yields.

Plant materials extracted with methanol yielded more extract than those extracted with the other solvents (Table 4).

Table 4 Percentage yield of extract with three different solvent

Name of medicinal plant	Sample weight (g)	Solvent used for Extract	Yield (in gram)	% yield
C. mucronata	100	Petroleum ether	4.98	4.98
		Acetone	5.65	5.65
		Methanol	5.78	5.78
C.spinarum	100	Petroleum ether	4.29	4.29
		Acetone	5.23	5.23
		Methanol	5.55	5.55

(% yield= Extracted weight ÷ Sample weight X 100)

4.6. **Antimicrobial sensitivity testing by disc diffusion method**

All the metabolic extract of root of *C. mucronata* and *C. spinrum* used in study was effective against the test bacterial strains (Table 5). The best activity was shown at the concentration of 100 µg/ml by methanol extract of root of *C.mucronata* a maximum zone of inhibition 19 mm (18.67±0.56) against *S. aureus* ATCC25923 (Table5) .And by the same concentration, the acetone extract root of *C.mucronata* showed , a maximum zone of inhibition 16 mm (16.70±1.53) against *E. coli* ATCC 25922. Furthermore, the Acetone extracts of *C. spinrum* made wider zones as compared to petroleum ether extracts of root of *C. spinrum.* Thus, the acetone extracts of root of *C. spinurum* showed maximum zone of inhibition 12 mm (11.67±1.15) against *Pseudomona auroginosa* ATCC 27853 (Table 5) and 11 mm (8.67±2.08) against *S.aureus* (Table 5), while petroleum ether extracts of root of *C. spinrum* showed least results compared to the all extracts. Maximum zone of inhibition given by methanol extract root of *C.mucronata* at the concentration of 100 mg/ml was, 19 mm against *S. aureus* ATCC25923 and by the same concentration, (18 mm) against *E. coli* ATCC 25922 and *Salmonella typhimurium* ATCC 133110.

There was significant difference for agar disc diffusion assay among the various microorganisms used ($p<0.05$). Besides, antibacterial activities of the crude extracts were more effective ($p=0.000$) against gram positive bacterium (*S. aureus* ATCC25923)

Table 5.Inhibition zones resulting from the disk diffusion assay and effect of extract concentration and standard antimicrobial drugs against the test strains. (N=3; Mean ± S.D)

Extracts	Conc. (mg/ml)	Zone of inhibition (mm) against tested strains (Mean± SD)			
		E. coli ATCC 25922	*S. aureus* ATCC25923	*P. auroginosa* ATCC 27853	*S.typhimurium* ATCC 133110
RCMME	100	16.70±1.53^{a}	18.67±0.56^{a}	12.67±0.58 ^{cb}	17.33±0.56^{a}
	50	13.30±0.56^{b}	14.67±0.56^{b}	10.33±2.08^{c}	14.33±1.53^{b}
RCMAE	100	14.30±1.53^{b}	14.00±1.00^{b}	12.00±1.00^{cb}	14.33±0.58^{b}
	50	11.00±1.00^{cb}	12.67±1.15^{cb}	8.33±1.53^{c}	10.33±1.53^{c}
RCMPE	100	9.00±1.00^{c}	12.67±2.08^{cb}	11.00±1.00^{cb}	9.67±1.53^{c}
	50	NA	12.00±1.00^{cb}	8.67±1.53^{c}	8.67±0.58^{c}
RCSME	100	9.00±1.73^{c}	13.00±1.00^{cb}	11.67±1.15^{cb}	10.00±1.00^{c}
	50	7.67±1.15^{c}	11.00±1.00^{cb}	10.67±1.15 ^{c}	8.00±1.00^{c}
RCSAE	100	8.33±1.53^{c}	8.67±2.08^{c}	11.67±0.58^{cb}	8.00±1.00^{c}
	50	7.33±0.58^{c}	7.67±0.56^{c}	9.00±1.00^{c}	7.67±7.83^{c}
RCSPE	100	NA	8.33±1.53^{c}	NA	NA
	50	NA	7.67±1.15^{c}	NA	NA
Tetracycline	30µg/disc	29.67±0.58	30.00±0.00	29.67±0.58	29.33±1.15
Tween 80	20µg/disc	-	-	-	-

Where: N, Number of observation; SD, Standard Deviation; RCMME, Root of *C.mucronata* methanol extract; RCMAE, Root of *C.mucronata* acetone extract; RCMPE, Root of *C.mucronata* petroleum ether extract; RCSME, Root of *C.spinarum* methanol extracts; RCSAE,Root of *C.spinarum* acetone extracts; RCSPE, Root of *C .spinarum* petroleum ether extracts; NA, Not activity. Values are mean inhibition zone (mm) ± S.D of the triplicates. Statistical significance was considered at $p<0.05$.Means values followed by different letters within the same column differ significantly ($\alpha = 0.05$).

4.7. **Evaluation of Minimum inhibitory Concentration (MIC) of the crude Extracts**

The MIC values of all the extracts of *C. mucronata* plant were 12.5 mg/ml against *E. coli* ATCC 25922 (Table 6) whereas MIC values of all the extracts of *Carissa spinarum* plant were 25 mg/ml against *S. typhimurim* (ATCC 13311) and *S. aureus* (ATCC 25923) and the root *C. mucronata* methanol and acetone extract (RCMME and RCMAE) showed the lowest MIC value of 6.25 mg/ml against gram positive bacteria (S. aureus ATCC 25923).Whereas, RCSPE(Root of *C. spinarum* Petroleum ether Extract) the MIC value were 50 mg/ml against the two gram negative bacteria (*E, coli* ATCC 25922 and *P. aeruginosa* ATCC 27853)(Table 6).

Table 6 Minimum inhibitory concentration (MIC) values (mg/ml) of the two roots extract of *C.mucronata* and *C.spinarum* against the test strains

Root extract	Concentration mg/ml	Growth Inhibition in (mg/ml)on test strains			
		E. coli (ATCC 25922)	*S. typhimurim* (ATCC 13311)	*P. aeruginosa* (ATCC 27853)	*S.aureus* (ATCC 25923)
RCMME	50 mg/ml	12.5	12.5	12.5	6.25
RCMAE	50 mg/ml	12.5	12.5	12.5	6.25
RCMPE	50 mg/ml	12.5	25	25	12.5
RCSME	50 mg/ml	12.5	25	25	25
RCSAE	50 mg/ml	25	25	25	25
RCSPE	50 mg/ml	50	25	50	25

Where: RCMME, Root of *Cissampelos mucronata* Methanol Extract; RCMAE, Root of *Cissampelos mucronata* Acetone Extract; RCMPE, Root of *Cissampelos mucronata* petroleum ether Extract; RCSME, Root of *Carissa spinarum* Methanol Extract; RCSAE , Root of *Carissa spinarum* Acetone Extract ; RCSPE, Root of *Carissa spinarum* petroleum ether Extract.

4.8. **Evaluation of Minimum Bactericidal (MBC)**

The MBC value of RCMME and RCMAE were 12.5 mg/ml against *S.aureus* ATCC 25923 (Table 7). At the same way, the MBC values were 25 mg/ml against *E.coli*, *S.typhimurim* and *P.aeruginosa*. But, RCSAE and RCSME value were 50 mg/ml, against *S. typhimurim*, *P. aeruginosa* and *S. aureus* (Table 7).

Table 7. Minimum bactericidal concentration (MBC) values of the two roots extract of *C.mucronata* and *C.spinaru* against the test strains (mg/ml)

Root extract	Concentration mg/ml	No bacterial growth is observed (bactericidal concentration) on test strains (mg/ml)			
		E. coli ATCC 25922	*S. typhimurim* ATCC 13311	*P. aeruginosa* ATCC 27853	*S. aureus* ATCC 25923
RCMME	50 mg/ml	25	25	25	12.5
RCMAE	50 mg/ml	25	25	25	12.5
RCMPE	50 mg/ml	50	50	50	25
RCSME	50 mg/ml	25	50	50	50
RCSAE	50 mg/ml	50	50	50	50

4.9. **Phytochemical Screening**

The quantitative analysis of phytochemical screening of the root extracts of *C. mucronata* and *C. spinarum* plant (Table 8) revealed the presence of various chemical compounds such as, Saponins and Tannins were present on both traditional plant root extract. However, Alkaloids was absent in both root extract the medicinal plants.

Table 8 .Qualitative analysis of phytochemical constituent of the crude extract root of *C.mucronata* and *C. spinarum*

Class of phytochemical constituent	Name of medicinal plant	
	Cissampelos mucronata	*Carissa spinarum.*
Saponins	+	±
Terpenoids	±	-
Tannins	+	+
Flavonoids	+	-
Alkaloids	-	-

Where; + Indicates, present; - Indicates, absente, ± Indicates, variable result.

Phytochemical screening showed the presence of terpenoids, saponins, tannins and flavonoids in the root extract of *Cissampelose mucronata* and *Carissa spinarum* (Table 8). But the two plant root extract on reaction with Dragendorff‘s reagent were not formed turbidity or orange red precipitation in the reaction mixture which indicated the absent of alkaloids in both plant root extracts. Extract when shaken with water formed the froth which persisted for a few minutes in both root extract, indicating the presence of saponins but variable result show in the root of *C. spinarum.*

Likewise, extract formed a dark green (blackish-blue solution) with ferric chloride and distilled water indicating the presence of tannins in both root extracts. The extract also formed orange colouration dissolved with methanol, a chip of magnesium and concentrated hydrochloric acid indicating the presence of flavonoids in the root extract of. *C.mucronata*, But absent in *C. spinarum.*

5. DISCUSSION

Historically, medicinal plants have been used as traditional medicine to treat different human ailments by the local community from time immemorial. In Ethiopia, too, the majority of population that live in the rural area and some of the urban dwelling low income community usually rely on traditional medicines to meet their primary health care needs. These medicinal plants are estimated to be over 800 species and most of them are confined to the south-western regions of the country (Kebebew and Addis, 1996). It appears that there is a high degree of dependence on traditional knowledge and use of diverse traditional medicinal plants in Ethiopia due to several reasons: the existence of diverse cultures and beliefs besides accessibility and low cost of the traditional medicinal plants. However, since cultural systems are dynamic (Cunningham, 2001), the skills are fragile and easily forgettable as the transfer of indigenous knowledge was based on oral transmission (Abebe and Ayehu, 1993). This fact makes scientific documentation of the knowledge and practices necessity as was done in the current study. Traditional medicine practitioners mostly rely on herbs and spiritual healing in treating disease in the studies areas. As indicated in the result section (socio-demographic characteristics), larger proportion of the participants [54(90%)] were followers of traditional religion. Our findings also show that, individuals with good knowledge of traditional medicine were elders aged between 31-40 years. Similar observation was reported earlier by Bishaw (1991). Thus, the depths of knowledge on traditional medicinal plants are the reflections of longer exposure to the practice and inheritance of the exercise from elders in the family.

Most of the respondent who participated in the current study were either illiterate (81%) or have only elementary level education and pastoralist (60%). The poor or low academic status and their poor economic conditions could be among the major reasons to use traditional medicine in the study area. The utilization of medicinal plants in traditional medicine was found to be effective, cheap and practical (Belewu *et al*., 2009). The practice is fast developing due to poor economic situation, expensive and inadequate availability of drugs. The use of plants and plant products in health care are even much higher particularly in those areas with little or no access to modern health services (Saeed *et al*., 2004).

The status of phytomedicines, preparation of crude extracts and isolation of active compounds in Ethiopia is very minimal (Endeshaw, 2007). Therefore, biological

evaluation of plant extracts is vital to ensure their efficacy, safety and quality (Eloff, 1998). In the present preliminary study, phytochemical screening of such traditional medicinal plants was carried out, and qualitative phytochemical analysis of these plants confirm the presence of various secondary metabolites like, tannins, saponin, flavonoids, and terpenoids. These phytochemical compounds have been known to have antimicrobial activities. Likewise, related study elsewhere reported the presence of various chemical compounds such as saponins, flavonoids phenols, glycosides, proteins, anthocyanin, some of which have been previously associated with antibacterial activity against food born infections and others (Nweze *et al*., 2004). Moreover, previous reports have demonstrated the anti-diarrhoeal activity of plant extracts containing tannins (Shama *et al*., 2011), flavonoids (Galvez *et al.,* 1993), Saponins and Anthraquinones (Sabris and Daniel, 1990). The presence of these metabolites is considerably important for the antibacterial activities observed. The antimicrobial activities of the plant extract involves various mechanisms such as the inhibition of various cellular processes like, increase in plasma membrane permeability and impairment of energy or synthesis of structural components in microbial cells (Walsh *et al.,* 2003).

Flavonoids are a group of polyphenolic compounds which influence the radical scavenging, inhibition of hydrolytic and oxidative enzymes and also act as anti-inflammatory agent (Frankel, 1995). They are free radical scavengers, super antioxidants and potent water soluble which prevent oxidative cell damage and have strong anti-cancer activity (Salah *et al.,* 1995; Kessler *et al.,* 2003).The flavonoids show antioxidant activity and their effects on human nutrition and health is considerable. As antioxidants, flavonoids provide anti-inflammatory actions (Okwu, 2001B). They also inhibit microbes which are resistant to antibiotics (Linuma *et al.,* 1994). Other metabolites like, tannins, saponins, terpenes, phenolics and glycosides were also reported to have *in vitro* antimicrobial properties (Das *et al.,* 2010). Generally, phytochemical present in medicinal plant have been used for treatment of gastrointestinal infections (Ekundayo *et al*., 2011).Therefore, the presence of different phytochemical in root extracts it's the current plants supports the scientific basis of traditional healers practice of using the plants for treatment of stomach ache.

The result of disc diffusion methods indicated that methanol and acetone extracts of root of *C. mucronata* showed better activity against *S. aureus*. This result suggests that root of *C. mucronata* might contain more bioactive compounds than the root extract of *C.*

spinurum. According to Gonzalez *et al*. (1994), the optimal effectiveness of a medicinal plant may not be due to one main active constituent, but to the combined action of different compounds originally present in the plant.

The petroleum ether extract of root of *C. spinurum* did not show any activity against the tested Gram negative bacterial strain. Lack of antimicrobial activities observed in the present study does not necessarily mean the complete absence of bioactive compounds (Taylor *et al*., 2001). The bioactive compounds are present in small amounts or there could be other constituents that exert antagonistic effects on the existing low quantity bioactive compound (Jager *et al.*, 2006,). It is also important to note that the absence of activities could be due to photo-oxidation of the active compounds (Ambrose, 2011). In addition, they may act in other ways by stimulating the immune system of the patient, or by creating internal conditions unfavourable for the multiplication of the pathogen (Lisa, 2006).

The finding showed that except Petroleum ether extract of root *C. spinarum*, all the root extracts had better antimicrobial activities against Gram positive and Gram negative bacteria. In most cases, Gram-negative bacteria are less sensitive to antibiotics as compared to Gram positive bacteria. This fact was also found to be true for most plant extracts. A study conducted elsewhere revealed that Gram negative bacteria are less sensitive to most plant extracts as compared to Gram positive bacteria (Shelf, 1996). Different factors account for Gram negative bacteria to be less sensitive to antimicrobial agents such as modern antibiotics and plant extracts. One of the major factors is the low permeability of the outer membrane which reduces the drug diffusion across the cell envelope (Gao *et al.*, 1999). In some cases the permeability of the outer membrane can be further decreased by the loss of porins (Nikaido, 1994). However, once the drugs have entered the membrane it cannot prevent the drugs from exerting their toxic action. The active efflux of drugs is another mechanism in Gram negative bacteria, which is essential to ensure significant levels of drug resistance .This, is a mechanism which evades the toxic effects of antibiotics by the active extrusion of structurally unrelated drugs from the cell. Also, there are still unknown factors which help these bacteria to resist different toxic and antimicrobial agents. In agreement with these reports, the result of this study has shown that, generally the plant extracts and modern antibiotics used in this study have high zone of inhibition in case of *S. aureus*.

The two plant species evaluated in the current study have exhibited different antibacterial activities against the tested strain. The antimicrobial activities increased with the increase in the concentration of the extracts except for the root *C. spinarum* petroleum ether extracts as observed for activities against Gram-negative bacteria. Generally, the root of *C. mucronata* extract have shown higher activity as compared to the root extract of *C. spinarum* against both Gram positive and Gram negative bacterial strains, which may be explained to be due to the synergetic effect of the total compounds found in the root extract of *C. mucronata*. The methanol and acetone extracts of root of *C.mucronata* had shown highest activities with 100% growth inhibition at 6.25 mg/ml against *S. aureus* (ATCC 25923), and 12.5 mg/ml against *E. coli* (ATCC 25922), *S. typhimurim*(ATCC 13311) and *P. aeruginosa*(ATCC 27853). The least activity had been recorded for petroleum ether extracts of root of *C. spinarum* with growth inhibition at 50mg/ml against *E. coli* (ATCC 25922), and *P. aeruginosa* (ATCC 27853). The methanol extract of root of *C.mucronata* has also shown its best activity against *S. aureus* (ATCC 25923), where inhibition have been observed up to 6.25 mg/ml. Similarly, the acetone extract has shown highest activity against *S. aureus* (ATCC 25923) up 6.25 mg/ml while for concentration < 12.5mg/ml no inhibition was observed for both Gram-negative bacteria (*E, coli* ATCC 25922, *P. aeruginosa* ATCC 27853 and *S. typhimurim* (ATCC 13311). With further investigation into the active compounds associated with the antimicrobial activities of the two root extracts, optimization of the growth conditions and critical determination of the MIC values for the extracts, the antimicrobial activities of these traditional medicinal plants could be further exploited at large scale.

The efficacy of extracts varies with the solvent used for its extraction. From among the solvents used, the methanol extracts demonstrating the highest activity against all the test bacteria. Accordingly, the methanol extracts of the plant parts were more potent than the acetone and petroleum ether extracts, which showed less inhibitory activity. This is similar to the reports of El-Mahmood and Amey (2007) but contrary to observations made by Roy *et al.* (2006). It has been reported that different phyto-constituents have different degrees of solubility in different types of solvents depending on their polarity (El-Mahmood and Dougharia, 2008). The higher activity demonstrated by organic solvents in this study is therefore an indication that less bioactive substance principles are extracted when petroleum ether is used as a solvent. There can be several factors which can affect or reduce the efficacy of extracts of medicinal plants of their

antimicrobial activity. This begins from the time of plant collection (it's recommended that the collection of plant parts should be done after the flowering stage of the plant, if not always), the state of plant processing and the state of storage of plant (Grrigs *et al*., 2001). The method of plant extraction is another factor which affects the antimicrobial activity of the medicinal plant. In general, the results reported from different studies are difficult to compare because of the use of different test methods, bacterial strains and sources of antimicrobial samples used. The Overall antibacterial activities of the tested plants have, however, a comparable MIC values.

High MBC-values correlating with higher resistance were often seen when Gram-negative microorganisms were tested. Three strains of the tested Gram-negative bacteria [*E. coli (*ATCC 25922), *P. aeruginosa* (ATCC 27853) and *S.typhimurim* (ATCC 13311)] presented the highest MBC-values at 25 mg/ml of methanol and acetone extracts of root of *Cissampelos mucronata* and also with the petroleum ether extract of *Cissampelos mucronata* , demonstrating the highest MBC-value of 50 mg/ml. Higher resistance of Gram-negative bacteria to external substances had been reported (Negi, *et al*., 2005). It is attributed to the presence of lipopolysaccarides, making them naturally resistant to certain antibacterial agents, in their outer membrane (Nikaio and Vaara, 1985). On the other hand, Gram-positive test organisms showed higher sensitivity against the tested medicinal plants than the Gram-negative bacteria. Gram-positive bacteria contain an outer peptidoglycan layer, which is an ineffective permeability barrier (Scherrer and Gerhardt, 1971). In addition, Pasqua *et al*. (2006) have studied the changes in membrane fatty acids composition of microbial cells in the presence of a sub lethal concentration of antimicrobial compounds (e.g. thymol, carvacrol, limonene, cinnamaldehyde and eugenol) in response to a stress condition. It was found that Gram-negative bacteria did not show substantial changes in its fatty acid compositions. This is an indication of the high resistance of Gram negative bacteria to the tested compounds.

The bactericidal activities of medicinal plants could be the result of a mixture of several components. The methanol and acetone extract of *C. mucronata* showed high activity against Gram-positive and low effect against Gram-negative. The major bioactive compounds and their amount in each medicinal plant extract were varying and the bactericidal effect was a result of the mixture in the actual extracts.

Besides efficacy, the extraction yields from the studied plants were dependent upon the extraction solvent. The amount of yield obtained from the two plants root differs with

the solvent used. Moreover, the yields were minimum as the polarity of the organic solvents decreased. Thus, the result of the present study revealed that high amount of yield produced from methanol extracts with the highest yield being from *C.mucronata* (5.78%). The study result is in agreement with report from India by Prekh *et al*. (2006) where the maximum yield was obtained with methanol (12.62%), while the minimum was with petroleum ether (0.62%). In general, it is not only the presence or absence of active compound in a given medicinal plant that determines its antimicrobial activities but also the type of solvent used as it matters the yield to be obtained from those potential plants.

6. Conclusions

- Traditional medicinal plants are still playing significant role as an alternative medicine with large segments of the population relying on it for medication purpose.
- The present study scientifically proved the local community's believes of the role medicinal values of the two traditional medicinal plants (*Cissampelos mucronata* and *Carissa spinarum)* for curing of ailments as the root extracts of these two plants revealed antimicrobial activity against *Escherichia coli (*ATCC25922), *Staphylococcus aureus (*ATCC 25923), *Salmonella typhimurium* (ATCC 13311), *and Pseudomonas aurogenosa (*ATCC 27853).
- Over all, methanol was found better solvent for the extraction of roots of the studied traditional medicinal plants with the highest amount of crude extracts, followed by acetone.
- The methanol extract of root of *Cissampelos mucronata* exhibited the largest zone of inhibition at 100 mg/ml and 50 mg/ml concentration, respectively, against *Staphylococcus aureus* (ATCC25923. Likewise, extracts of *Carissa spinarum* showed better activity against *Staphylococcus aureus* (ATCC25923) in methanol extract both 100 mg/ml and 50 mg/ml concentration, respectively.

7. Recommendation

Based on the findings from this study, it is recommended that:

- ❖ The current study focused on the antibacterial activities of only the root extract of the studied plants. It needs further study on the bark, trunk and the leaf parts of the same plants.
- ❖ The root extracts of C*issampelos mucronata* and *Carissa spinarum* should also be tested on other pathogenic microorganisms associated with serious human infections.
- ❖ Further detailed work is needed to know the bioactive compounds of the studied plant that are responsible for their current antimicrobial properties
- ❖ *In-situ* and *ex-situ* conservation strategies of medicinal plants should be adopted and implemented in the district besides training (educating and awareness creation) of the practitioners.

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ANNEX I

COLLEGE OF NATURAL SCIENCES

POSTGRADUATE STUDIES, DEPARTMENT OF BIOLOGY

Data Collection tool for survey of traditional medicinal plants for the treatment of common food borne illness caused by pathogens bacterial in Bero wereda in Bench-Maji Zone, (SNNPRS).

Part I Questions on socio-demographic characteristics of the Community

1. Head of the house

Male: _____ Female: _____

2. Age: _____

3. Religion

Muslim: _____ Orthodox:_____ Protestant:_____ Other:_____

4. Marital status:

Married: _____ Single: _____ Divorced: _____

Widowed: _____ Never married: _____

5. Academic status:

Illiterate: _____ Diploma: _____

Educated: Grade 5 or below: _ Bachelor: _____

Grade 5 to 10:_____ Masters and above: _____

Preparatory: _____

6. Occupation/Economic activity:

Agriculture/farming: _____ Trade: _____ Civil servant: _____

- Pastoralist;----------

Part II local harvesting practices

1. Do you know plants used to treat stomach ache?

a) Yes b) No

11. If yes how many medicinal plants do you know for treatment of food related disease?

1.2. Please, list some medicinal plants and the plant part that you use for treatment of stomach ache, fever, vomiting, Diarrhoea, nausea, cramp?

1.3. Rank these plants according to your preference for effective treatment of stomach ach

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1.4. Are they effective in treating/ preventing food borne illness?

a) Yes b) No

1.5. Where do you obtain these plants?

THANK YOU VERY MUCH FOR YOUR UNDESERVED COLLABORATION IN TO THE QUESTIONNARES

ANNEX II

Preference ranking of seven selected medicinal plants on the degree of their use for treatment of illness as rated by key respondents

Respondent	Plant species						
	O.lamiiolium	C.anisata	D.angustifola	C.aurea	C.spinarum	C.mucronata	G.fruticosus
R1	3	2	4	5	6	7	1
R2	2	4	3	7	5	6	1
R3	1	3	5	4	6	7	2
R4	2	1	4	5	6	7	3
R5	1	3	4	7	6	5	2
R6	2	3	4	5	6	7	1
R7	2	3	4	5	6	7	1
R8	1	3	4	5	7	6	2
R9	3	2	4	5	6	7	1
R10	2	3	4	5	6	7	1
Total	18	27	40	56	60	66	15
Rank	6th	5th	4th	3rd	2nd	1st	7th

Where R= Respondent