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Antibacterial Activity of *Moringa stenopetala* against Some Human Pathogenic Bacterial Strains

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A Thesis Submitted to the School of Graduate Studies of Addis Ababa University and Presented in Partial Fulfillment of the Requirements for the Degree of Masters of Science in Applied Microbiology Stream

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A Thesis Presented to the School of Graduate Studies of the Addis Ababa University in Partial Fulfillment of the Requirements for the Degree of Master of Science in Biology (Applied Microbiology)

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ABBREVIATIONS

AAU-Addis Ababa University

ATCC-American Type Culture Collection

EHNRI-Ethiopian Health and Nutrition Research Institute

MHA-Mueller Hinton Agar

MIC-Minimum Inhibitory Concentration

SE-Standard Error of Mean

SPSS-Statistical Package for Social Sciences

TM-Traditional Medicine

WHO-World Health Organization

ABSTRACT*: An emerging of antibiotic resistance brings most serious public health problems. It is therefore, important to look for more effective, safer and less toxic alternate options of treatment. The aim of the present study was to investigate antibacterial activity of Moringa stenopetala against some human pathogenic bacteria using disk diffusion method and agar dilution for minimum inhibitory concentration. The result revealed that, most of the plant extracts had antibacterial activity. Staphylococcus aureus was found to be the most susceptible bacteria to crude 80% methanol extract of seeds and ethyl acetate extract of root barks with inhibition zones of 18.66±0.88mm and 16.00±1.15mm and minimum inhibitory concentration of 1.25mg/ml and 2.5mg/ml respectively, whereas Pseudomonas aeruginosa was the most resistant bacteria to all of crude extracts. Similarly, Staphylococcus aureus was the most susceptible bacterial strain to chloroform fraction with inhibition diameter of 28.00±0.57mm and minimum inhibitory concentration of 0.31mg/ml, while Pseudomonas aeruginosa was the most resistant strain with inhibition zone of 9.66±0.33mm and minimum inhibitory concentration of10mg/ml respectively. In conclusion, this study is not only proves antibacterial activity of Moringa stenopetala, also provides a scientific basis for their traditional use. Pure chemical compounds and antimicrobial activity against many fungi and bacteria should be studied to use them as sources and templates for synthesis of drugs to control infectious diseases.*

Key words and phrases: Antibacterial activity, *Moringa stenopetala*, traditional medicine, extracts, fractionation, MIC, solvents

1. INTRODUCTION

Traditional medicine (TM) is the sum total of all the knowledge, beliefs and practices that are used in prevention, diagnosis and elimination of physical, mental and social imbalance that exclusively rely on practical experiences and observation (WHO, 2002). Whereas, a medicinal plant is any plant which contains substance that can be used for therapeutic purpose or which is a precursor for synthesis of useful drugs. It has been established that the plants which naturally synthesize some secondary metabolites, like alkaloids, glycosides, tannins, volatile oils and containing minerals and vitamins, possess medicinal properties (Sofowora, 1982).

Medicinal plants are important sources of TM for millions of people and additional inputs to modern medicine in terms of exploring and producing new drugs to meet the need for the overgrowing population of the world (Abad *et al.*, 2007). It is reported that more than 3.5 billion people rely on plants for the treatment of both human and livestock diseases (Newman *et al*., 2000). As elsewhere in Africa, indigenous people in Ethiopia, by large employed plant based TM to get cured from diseases arising from worms, fungi, bacteria, viruses and protozoa (Abebe, 2001). Although, herbal medicine represents one of the most important fields of TM all over the world, the search for the active ingredients for the synthesis of new drugs has not been extremely undertaken (Kumaraswamy *et al.*, 2008).

The wide use and misuse of antibiotics in the treatment of bacterial infections and using it in agriculture, livestock and poultry has led to the emergence and spread of resistant strains. As a result, society is facing one of the most serious public health problems over the emergence of infectious bacteria displaying resistance to even some effective antibiotics (Gibbons, 2005). In addition to increasing the cost of drug regimes, this situation has paved way for the re-emergency of the high frequency of opportunity and chronic infection cases in developing countries (Ako-nai *et al.*, 2003). It is therefore, important to look for more effective, safer and less toxic alternate options of treatment. Medicinal plants have many biologically active compounds as secondary metabolites that had a capability of overcoming the problems of drug resistance (Douglas, 1987). In addition, they are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials (Iwu *et*

al., 1999). So, the examination of medical properties of crude medicinal plants is needed (Ranpal, 2009).

Therefore, the studies of TM should have to continue which had different significance such as to develop local initiatives for pharmaceutical industry and to create possible motives for conserving biodiversity and to provide convenient access to modern medicines more easily that is available and acceptable by rural communities (Caceres *et al*., 1990).

About 65-85% of the populations in every country of the world rely on TM. In developed countries, this may be partly due to dissatisfaction with the conventional medicine while in the developing countries this is due to inaccessibility of modern medical system and TM are relatively safe. Easy accessibility, efficacy on treatment and affordable cost in getting health services are main reasons in preferring TM than costly synthetic drugs that have adverse effects (Sofowora, 1982). However, the majority of TM used in developing country has not been evaluated for their quality, safety and efficacy to some standards, while those in developed countries there are some remarkable claims made for their effectiveness (Yineger, 2005).

Ethiopia is rich of plant biodiversity. It is therefore, not surprising that some of these plants have chemical compounds of therapeutic value that may be used in the treatment of major diseases such as malaria, cancer and pathogenic microorganisms (Yirga, 2010). However, only few studies were geared towards indigenous medicine with an objective to improve their usage. Consequently, the overall use of these plants remained within the domain of local healers as they resort to them for the treatment of different health problems (Abbiw, 1996). However, in the recent years some studies have been undertaken regarding medicinal plants that have been screened for their antimicrobial activities (Mekonnen and Dräger, 2003; Bruck *et al.,* 2004; Tesfaye *et al.,* 2006; Asmamaw *et al.*, 2007). So, Ethiopian flora offers great possibilities for the discovery of new compounds with antimicrobial activities (Fernandez *et al*., 2008).

Moringa stenopetala (*M.stenoetala*) belongs to the family Moringaceae with one genus *Moringa* and with about fourteen species. It is a deciduous tropical plant widely distributed in the Southern parts of Ethiopia at an altitude range of about 1100-1600m. *M. stenopetala* is a

traditional medicinal and nutritional plant in Ethiopia (Mekonnen, 1999). It is commonly used in folk medicines as antimalarial, antihypertensive, against stomach pain, antidiabetic, anticholesterol and to expel retained placentae during birth (Mekonnen and Gessesse, 1998; Mekonnen, 1999).

Although, there were some studies made on the leaves and seeds of *M. stenopetala* (Mekonnen and Gessesse, 1998; Mekonnen *et al*., 1999; Mekonnen and Dräger, 2003; Sahilu, 2010), there was little information about different solvent extract antimicrobial property of its leaves, seeds, stem barks and root barks. The present study, thus, aims to evaluate the antibacterial activity of a widely used medicinal plant *M. stenopetala* against four pathogenic bacteria namely *Staphylococcus aureus (S. aureus), Escherichia coli (E. coli), Pseudomonas aeruginosa (P. aeruginosa)* and *Shigella boydii (S. boydii)* by using different solvents.

2. LITERATURE REVIEW

2.1 Herbal drugs in medical health practices

2.1.1 Historical perspectives

The history of herbal medicine is rather old and dates back to the time when the early man became conscious of his environment. Medicinal plants have been used in virtually all cultures as a source of medicine (Lanfranco, 1999). The earliest record of human civilization and culture of China, Egypt, Assyria, and Indies valley reveals that the elders and wise men of those times used herbal medicines to treat various diseases. Information regarding these medicinal herbs is available in the old literature, epic poems and copper plates which are preserved even today. The excavation of Shanidar cave in Iraq in 1963 revealed the grave of Neanderthal man buried sixty thousand years ago along with many flowers of his time. The plants found in the grave were later identified having various medicinal properties (WHO, 2002).

One of the earliest records of the use of herbal medicine is that of Chaulmoogra oil from *Hydnocarpus gaertn*, which was known to be effective in the treatment of leprosy. Such a use was recorded in the pharmacopoeia of the Emperor of China between 2730 and 3000 B.C. Similarly, the seeds of the opium poppy (*Papaver somniferum*) and castor seeds (*Ricinus communis*) were excavated from some ancient Egyptian tombs, which indicated their use in that part of Africa as far back as 1500 B.C. The records available in "Ebers papyrus" also confirm that medicinal plants were used at that time in Egypt (Baquar, 1995).

The ancient use of plants for healing purposes forms the origin of much of modern medicine. Many conventional drugs originate from plant sources, a century ago. Examples include aspirin (from willow bark), digoxin (from foxglove), quinine (from cinchona bark), and morphine (from opium poppy). The development of drugs from plants continues, with drug companies engaged in large-scale pharmaceutical screening of herbs (Tyler *et al*., 1976).

2.1.2 Current status of herbal drugs: Global perspectives

For centuries, right up to the 19th century, herbs were the major sources of drugs. With upswing consumer interest since 1960s, high demand has been seen for modern alternative medicine, to the extent that health foods and herbals have become a several billion dollars per year business (Ming *et al*., 2003).

The practice of TM is widespread throughout Asia including China, India, Japan, Pakistan, Srilanka and Thailand. In Japan, herbal medicinal preparations are more in demand than normal pharmaceutical products (Baquar, 1995). In Malaysia, traditional forms of Malay, Chinese and Indian medicine are used extensively. China is the leading country for incorporating traditional herbal medicine into a modern health care system. In this country, TM accounts for around 40% of all health care delivered and are used to treat roughly 200 million patients annually. According to a recent survey, almost 7,300 plants have been used in traditional Chinese medicine (Der *et al.,* 2000).

Many people in the US are turning to herbal medicine to treat their ills. It has been estimated that up to 50% of the prescription presently dispensed in the US may contain one or more natural product drugs (Silver, 1993). Over 20,000 herbal and other natural products are available in the United States. In Europe, some 1,500 species of medicinal and aromatic plants are widely used. Germany and France together represent 39% of the global retail markets (Lanfranco, 1999).

According to Der *et al.* (2000), majority of African population relies on TM for the treatment of both human and animal diseases. He showed that in Ghana, Mali, Nigeria and Zambia, more than 60% of children with high fever are treated at home with herbal medicine. One of the key reasons cited for this was the readily accessibility of herbal medicine in the rural areas.

2.2 Traditional herbal medicine in Ethiopia

The introduction of medicine to Ethiopia dates back to the 16th century during the regime of Emperor Libne Dingel (1508-1540) restricted to introducing drugs. The first government that ran modern health care was established in 1906 with the opening of Menelik II Hospital in Addis Ababa. Since then the government has taken the formal responsibility of delivering health care to the population and health institutions were established in the different regions of the country. However, the growth and development of modern health care in Ethiopia as a whole has been very slow and to date, its coverage is less than 50% of the population. The vast majority of the rural population, therefore, still depends on TM and its practitioners (Shiferaw, 1996).

The beginning of Ethiopian TM could not be established with certainty due to lack of adequate written sources. The early report on the Ethiopian TM practices was the one provided by Francisco Alvares in the early 16th century in which he mentioned that, Ethiopians knew about the use of bleeding and cupping and about the use of various herbs as purgatives (Desta *et al*., 1996). However, Pankhrust (1976) noted that, it would seem reasonable to assume that the country's medical lore was then already well established. He also added that, despite the probable long established nature of Ethiopian traditional remedies, the earliest known texts are the Geez "Matshafa Faws" of mid-seventeenth century and "Matshafa Madhanit" of the early 18th century. These medical texts contain several references to plants, animal products and minerals.

Ethiopian flora is estimated to contain between 6,500 and 7,000 species of higher plants of which about 12% are endemic. Ethiopia is also a home for many languages, cultures and beliefs that have in turn contributed to the high diversity of traditional knowledge and practice of the people, among others include the use of medicinal plants. More than 95% of traditional preparations in the country are of plant origin (Cowan, 1999). Despite its significant contribution to society, TM has received very little attention in modern research and development and less effort has been paid to upgrade the traditional health practices in the country. But, the long history in the use of medicinal plants in Ethiopia and its huge biotic riches can be of paramount importance in future research and drug discovery (Gidey, 2001).

Traditional remedies represent not only part of the struggle of the people to fulfill their essential drug needs but also they are integral components of the cultural beliefs and attitudes. It is customary to find medicinal plants in markets where food items and spices are sold. Fresh and dried leaves, flowers, roots, barks and seeds of medicinal plants are displayed for sale in most markets in Ethiopia. Antimicrobial and wound healing plants are among some of the major medicinal plants that are commonly available in markets (Abebe, 1996).

2.3. Application of traditional medicinal plants

2.3.1 Potential of herbal remedies as sources of new drugs

Historically, plants have provided a source of inspiration for novel drug compounds, as plant derived medicine have made large contribution to human health and well-being. Their role in the development of new drugs could be either by serving as a natural blueprint for the development of new drugs, or as a phytomedicine to be used for the treatment of disease. It is estimated that, plant materials have provided 50% of the western drugs. Many commercially proven drugs used in modern medicine were initially used in crude form in traditional healing practice (Dagne, 1996).

Since the beginning of the 19th century, a large number of biologically active secondary metabolites of plant origin have been found to have commercial application as drugs. Recently, there has been an upsurge of interest in the use of plants with folkloric reputations as sources of potentially useful compounds (Mourice *et al*., 1999). Analysis of the number and sources of anticancer and anti infective agents, reported from 1984 to 1995, indicates that over 60% of the approved drugs are of natural origin. A recent review reported that at least 119 compounds derived from 90 plant species could be considered as important drugs currently in use in one or more countries, with 77% of these being derived from plants used in traditional medicine. Further evidence of the importance of natural products is provided by the fact that close to half of the best selling pharmaceuticals in 1999 were either natural products or their derivatives (Douglas, 1987).

Several new small molecules of natural product-derived drugs have been introduced into therapy in western countries in recent years, including acarbose, artemether, capsaicin, galanthamine,

irinotecan, and topotecan. This trend is likely to continue in the future, at least for the treatment of disease states such as cancer and infectious diseases. In a recent statistical survey, it was pointed out that the origin of 30,000 bioactive natural products could be divided between animals (13%), bacteria (33%), fungi (26%), and higher plants (27%) (Tyler, *et al.*, 1976).

It is estimated that there are 250,000 to 500,000 species of higher plants on earth. Only small proportion (1-10%) of these is used as foods by both humans and other animal species. It is possible that even more are used for medicinal purposes. But, only a relatively small percentage (5-15%) has been systematically investigated for the presence of bioactive compounds. Plants offer the scientist searching for novel bioactive compounds. It has been estimated that nearly 75% of about 120 biologically active plant derived substances used throughout the world were discovered by following up on leads from TM (Ates and Erogrul, 2000).

Besides their importance in health care, medicinal plants, have high socio cultural and socio-economic values, providing off-farm income and employment opportunities to local people. The incorporation of medicinal herbs into health foods, dietary supplements, herbal teas, cosmetics, massage oils, fragrances and dyeing agents have dramatically increased the international demand of medicinal plants (Ranpal, 2009).

2.3.2 Application of traditional medicinal plants as antimicrobial agents

Worldwide, infectious diseases are the leading causes of death accounting for approximately one-half of all deaths in tropical countries. They are also becoming a significant problem in developed nations. It is estimated that infectious diseases are the underlying causes of death in 8% of the deaths occurring in the US (Demissew and Dagne, 2001). Development of new antimicrobials is among the proposed solutions to curb this problem. In this regard, plants could provide a good alternative in search for new chemical agents with a wide-ranging antimicrobial activity (Pinner *et al*., 1996).

The use of higher plants and preparations taken from them for the treatment of infections predates written records (Fauci, 1998). The isoquinoline alkaloid emetine obtained from *Cephaelis ipecacuanha* and related species, has been used for many years as amoebicidal drug as

well as for the treatment of abscesses due to *Escherichia histolytica* infections. Another important drug of plant origin with a long history of use is quinine, which occurs naturally in the bark of cinchona tree. The bacteriostatic and fungicidal properties of lichens, the antibiotic action of allicine in *Allium sativum*, the antimicrobial action of berberine in *Hydrastis canadensis* are also examples of medicinal plants that have been used as sources of antibiotics (Dagne, 1996).

Many medicinal plants of Africa have been investigated for their chemical components and some of the isolated compounds have been shown to possess interesting biological activities. *Garicinia cola, Aframomum melegueta, Xylopia aethiopica, Cryptolepis sanguinolent* and *Chasmanthera dependens* are among the most widely used species that are found to possess different groups of compounds with wide ranging anti-inflammatory and antimicrobial activities (Dagne, 1996).

All these are indicative of the fact that plant based antimicrobials represent a vast untapped source for medicines. Plant based antimicrobials have enormous therapeutic potential. They are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials. Phytomedicines usually have multiple effects on the body. Their actions often act beyond the symptomatic treatment of disease. For example, *Hydrastis canadensis* not only has antimicrobial activity, but also increases blood supply to the spleen promoting optimal activity of the spleen to release mediating compounds (Dagne, 1996).

2.4 Human pathogenic bacteria used in this study

2.4.1 *Staphylococcus aureus*

S. aureus is facultatively anaerobic, Gram-positive, non-motile cocci, ferment glucose and has large, round, golden-yellow colonies. The golden appearance is the etymological root of the bacterium's name; *aureus* means "golden" in Latin. Cell division occurs in more than one plane; so that cells form irregular clumps resembling bunches of grapes (Ryan and Ray, 2004).

S. aureus is catalase and coagulase-posative which are used as distinguishing characteristics. It is mesophile with a growth temperature range between 7 and 48^0C (Ebrahimi and Akhavan, 2009).

Growth occurs optimally at pH values of 6-7, with minimum and maximum limits of 4.0 and 9.8-10.0 respectively (Addis *et al*., 2011).

Though normally a harmless parasite of human body surfaces where it plays a useful role metabolizing skin products and possibly preventing skin colonization by pathogens, *S. aureus* can cause variety of diseases, ranging from mild skin infections to fatal forms of bacteraemia as an opportunistic pathogen when the skin barrier is breached or host resistance is low (Adams and Moss, 2000). The most common infection caused by *S. aureus* is superficial skin inflammation with a furuncle (boil). Other skin and subcutaneous infections caused by *S. aureus* include folliculitis, cellulitis, mastitis and impetigo. The more severe infections caused *S. aureus* include osteomyelitis, pneumonia, arthritis, endocarditis, pericarditis and bacteraemia (Tenover and Gorwitz, 2006).

Several diseases caused by *S. aureus* are toxin-mediated, including food poisoning, impetigo, toxic shock syndrome and necrotising pneumonia. *Staphylococcal* enterotoxins ingested via contaminated food cause self-limiting *Staphylococcal* food poisoning. The symptoms of *Staphylococcal* food poisoning include nausea, vomiting, headache, and less commonly diarrhoea (Tenover and Gorwitz, 2006). Toxic shock syndrome is caused by toxic shock syndrome toxin 1, which is a potent superantigen (Schlievert *et al*., 1981). Some of its symptoms include high fever, hypotension, rash and the involvement of multiple organ systems (Davis *et al*., 1980).

S. aureus colonises the skin and mucosal surfaces of humans. Although multiple sites in the body can be colonised by *S. aureus*, the anterior nares of the nose are the most consistent carriage site in humans. Other typical sites for *S. aureus* colonization include the pharynx, and less frequently the gastrointestinal tract, vagina and maxillae (Wertheim *et al*., 2005).

It can withstand harsh environments for extended periods allowing susceptible individuals to become infected through contact with contaminated objects, but direct contact with colonized people is the more important route of transmission. Such characteristics have made *S. aureus* the

most common hospital acquired (nosocomial) pathogen with a formidable array of virulence and resistance strategies (Goldmann *et al*., 1996).

When penicillin was introduced to the general public following World War II, almost all strains of *S. aureus* were highly susceptible to this antibiotic. Since then, 80 to 90% of isolates are now resistant it. This is due to the production of penicillinase encoded by plasmid genes and acts by opening the β-lactam ring, making the drug unable to bind with its target (Bashir *et al*., 2007). Alterations in the β-lactam target, the peptidoglycan transpeptidases (often called penicillin-binding proteins, PBPs), is the basis for resistance to methicillin. These methicillin-resistant *S. aureus* (MRSA) strains are also resistant to penicillins such as oxacillin. (Abera *et al*., 2010).

2.4.2 *Escherichia coli*

E. coli is Gram-negative, non-spore forming and oxidase negative bacilli that exist singly or in pairs. It is facultative anaerobes, can undergo both fermentative and respiratory metabolisms. It forms gas from glucose, ferments lactose and does not utilize citrate. They are motile by peritrichous flagella (Hudault *et al*., 2001).

E. coli is a typical mesophile growing from 7-10^{0}C up to 50^0**C** with an optimum around 37^{0}C, although there have been reports of some ETEC strains growing at temperatures as low as 4^{0}C. A near-neutral pH is optimal for growth but growth is possible down to pH 4.4 under otherwise optimal conditions (Ryan and Ray, 2004).

Because of its prominence as a normal intestinal bacterium in most humans, *E. coli* is currently one of the indicator bacteria to monitor fecal contamination in water, food and dairy products. According to this rationale, if *E. coli* is present in a water sample, fecal pathogens such as *Salmonella*, viruses, or even pathogenic protozoa may also be present. Coliforms such as *E. coli* are used because they are present in larger numbers, can survive in the environment and are easier and faster to detect than true pathogens. If a certain number of coliforms are detected in a sample, the water is judged unsafe to drink (Geissler *et al.*, 2000).

It is inhabitant of the human intestinal tract, which often remains harmlessly confined. However, in immunocompromised host, when gastrointestinal barriers are violated and through plasmid transfer even nonpathogenic strains can cause infection (Nataro and Kaper, 1998). So, it is responsible for three types of infections in humans.

Uropathogenic *E. coli* causes 90 % of urinary tract infections in atomically normal and unobstructed urinary tracts. Complicated UTI caused by *E. coli* are observed in elderly patients with structural abnormalities such as prostatic hypertrophy, neurogenic bladders or in patients with urinary catheters (Srinivasan *et al*., 2003).

Neonatal meningitis another infection caused by *E. coli*, which is a life-threatening disease mainly affect infants. The disease is transmitted from mothers who are colonized with the K1 strain of *E. coli* during pregnancy to their infants (Rodriguez-Bano *et al*., 2004).

The third infection caused by these bacteria is gastrointestinal infection. As a cause of enteric infections, 6 different mechanisms of action of 6 different varieties of *E. coli* have been reported. Enterotoxigenic *E. coli* (ETEC) causes traveller‟s diarrhea. Enteropathogenic *E. coli* (EPEC) is responsible for watery diarrhea in children. Enteroinvasive *E. coli* (EIEC) cause *Shigell*a-like dysentery. Enterohemorrhagic *E. coli* (EHEC) causes hemorrhagic colitis or hemolytic-uremic syndrome (HUS). Enteroaggregative *E. coli* (EAggEC) is primarily associated with persistent diarrhea in children in developing countries and Enteroadherent *E. coli* (EAEC) is the cause of childhood diarrhea and traveller‟s diarrhea in Mexico and North Africa. All the different varieties colonize the small bowel, except EIEC and EHEC which preferentially colonize the large bowel prior to causing diarrhea (Hudault *et al*., 2001).

In some hospitals, *E. coli* ranked first and second as the most common cause of community and hospital-acquired infections respectively. A strain of the bacterium was implicated in a diarrheal outbreak among infants in 1935. It is the most commonly reported nosocomial pathogen in surveillance at some hospitals in the United States (Tortora *et al*., 2010).

It is resistant to penicillin and cephalosporin, primarily due to hydrolysis of these antibiotics by the enzyme, β-Lactamase and by modify their penicillin binding proteins (PBPs) (Tenover, 2006). Resistant to the Aminoglycosides is by a decreased in permeability of the cell wall due to alteration in the aminoglycosides transport system and modification in the lipopolysaccharides phenotype (Shaw *et al*., 1993).

2.4.3 *Pseudomonas aeruginosa*

P. aeruginosa is a small, non-sporulating, aerobic, Gram-negative rod belonging to the family Pseudomonadaceae. It is motile by virtue of its single polar flagellum. More than half of all clinical isolates produce the blue-green pigment pyocyanin; this pigment is helpful in the identification of the organism and accounts for the species name „*aeruginosa*" (Harris, 1999).

Cultures of *P. aeruginosa* give off a distinctive grape-like odor due to one of their pigments, 2-aminoacetophenone (Ohl and Pollack, 2004). *P. aeruginosa* grows well at 37-42^{0}C. Its growth at 42^{0}C helps differentiate it from other *Pseudomonas* species. It is oxidase positive. It does not ferment carbohydrates, but many strains oxidize glucose (Friendland *et al*., 2004). *P. aereginosa* can produce arginine dihydrolase and gelatinase and can use glucose but not trehalose as sole carbon and energy source (Chen *et al.,* 1995).

P. aeruginosa has very simple nutritional requirements. It does not require special organic growth factors as it can use over 75 organic compounds for growth (Lambert, 2002). It is resistant to high concentrations of salts and dyes, weak antiseptics and many commonly used antibiotics. *P. aeruginosa* has a strong linking for growth in moist environments, which is probably a reflection of its natural existence in soil and water. These natural properties of the bacterium contribute to its ecological success as an opportunistic pathogen. These also help to explain the ubiquitous nature of the organism and its prominence as a nosocomial pathogen. These infections are difficult to manage, in part because of the natural resistance of the bacterium to antibiotics and ultimately lead to pulmonary failure and death (Stover *et al*., 2000).

Although *P. aeruginosa* is not a significant cause of diseases in healthy individuals outside of a hospital setting, it is the causes of many disease mainly superficial and localized infections

(Murray, 1999). Common infections from this organism include hair follicle and ear canal infections from swimming in contaminated water. Infections of a more serious nature can still be caused by the bacterium, such as endocarditis in injection drug users, or eye infections in contact lens users with preexisting cornea damage (Madigan *et al*., 2009).

P. aeruginosa can cause disease in any part of the gastrointestinal tract (GIT) from the oropharynx to the rectum. As in other forms of *P. seudomonas* disease, those involving the GIT occur primarily in immunocompromised individuals. The organism has been implicated in pediatric diarrhea, typical gastroenteritis and necrotizing enterocolitis (Tortora *et al*., 2010).

P. aeruginosa infections acquired in a hospital setting are a major concern to medical practitioners and public health officials. The bacterium is the leading cause of nosocomial respiratory infections in intensive care unit (ICU) patients and cystic fibrosis (CF) patients (Rello *et al*., 2003). Other common *P. aeruginosa* nosocomial infection sites include urinary tract infections from contaminated catheters and life threatening wound or burn infections which can lead to bacteremia. These types of infections occur more frequently in immunocompromised patients, especially those with AIDS (Murray, 1999).

Most *Pseudomonas* species are naturally resistant to penicillin and the majority of related beta-lactam antibiotics, but a number are sensitive to piperacillin, imipenem, tobramycin, or ciprofloxacin (Gailienė *et al.*, 2007). Low susceptibility to antibiotic is one of the most striking characteristics of *P. aeruginosa*. This is due to the low permeability of the bacterial cellular envelopes, mutation in chromosomal genes which regulate resistance and acquisition of additional resistance genes from other organisms via plasmids and bacteriophages (Srikumar *et al.*, 1998).

2.4.4 *Shigella boydii*

The genus *Shigella* is found in the family Entrobacteriacea and the tribe Escherichia. It is Gram negative, facultative anaerobic, nonsporulating, non-motile and rod shaped bacteria. With few exceptions, *Shigella* species are nonproductive gas from fermentable substances. Lactose is not fermented except *Shigella* sonnei (Edward and Ewing, 1972).

S. boydii inhabits the intestine and rectum of humans and other primates. It can survive in faeces and soil or food and water contaminated with faecal matter. This indicates, poor sanitary methods of food processing may led to raw sewage exposure. In 1998 an outbreak of Shigellosis occurred in Chicago due to *Shigella boydii* type 18 found on the cilantro and parsley in bean salad (Roberts and Greenwood, 2003).

Shigella bacteria cause diarrhea and shigellosis (bacillary dysentery) through oral-faecal transmission. *Shigella* is a highly infective agent able to infect a host with less than 20 cells with an onset of about 12-48 hours, in favorable conditions. Once ingested, the *Shigella* makes its way through the gastrointestinal tract until it reaches the epithelial cells of the intestinal mucosa, there it infects, causing inflammation and necrosis (swelling and breaking of infected cells, which spreads infection) (Black *et al*., 1984). Some strains produce enterotoxin and shiga toxin, which are associated with causing hemolytic uremic syndrome (Lee *et al*., 2006).

The clinical syndrome of shigellosis is characterized by frequent, but small volume, loose stools, consisting largely of blood and mucus, fever and pain, which are frequently present. It invades distal small bowel or colon by bacteria (Bennish, 1991). In most cases these symptoms are mild and resolve in about a week but other cases can become severe enough to be fatal without proper medical care. The elderly, very young and those weakened by disease are much more sensitive to the bacteria. In very young children very high fever may also be accompanied with seizures (Ansaruzzaman *et al*., 2005).

The severity of symptoms and the length of time the stool contains *Shigella* can be reduced with antibiotics. However, recently widespread outbreak of shigellosis due to multiple antibiotic resistant *Shigella* to tetracycline, streptomycin, ampicillin, tetracycline, chloramphinicol has been documented all over the world. This is due to resistance genes enquired from other bacteria by plasmids (Mache, 2001).

2.5 *Moringa stenopetala*

M. stenopetala belongs to the family Moringaceae with one genus *Moringa* and with about fourteen species which follow the distribution pathway from Rajasthan through to South West Africa. There are nine species endemic to the Somali-Masai floristic region, one of which extends through Arabia to Israel, one species confined to South West Africa (Angola), two species to Madagascar and two in India including the widely cultivated *M. oleifera* (Edward *et al*., 2002).

M. stenopetala tree is 6-10 m tall; bark white to pale gray and smooth; wood soft; branches with leaf; pods elongate reddish with grayish bloom; seeds cream and brown (Edward *et al*., 2002). It is a deciduous plant widely distributed in the Southern parts of Ethiopia at an altitude range of about 1100-1600m. The major growing regions are Arbaminch and surrounding areas, Negelle and Wollayeta Sodo area which are about 400-550 km South of Addis Ababa (Mekonnen and Gessesse, 1998).

M. stenopetala is commonly called shiferaw in Amharic, cabbage tree in English and it has different names like shelagda, tallakata, haleko, alako in the various areas of Southern Ethiopia (Mekonnen and Gessesse, 1998). It is cultivated in field and backyard within the villages; almost every household has at least one or two *Moringa* plant in its compound. This might be taken as an indication of a wide use of the plant in the community (Mekonnen and Gessesse, 1998).

As well as being eaten, *M. stenopetala* has a variety of uses, many of them medicinal value. Traditionally, the fresh leaves of the tree are cooked and eaten as vegetable. The leaves are also boiled as tea and used in the treatment of various diseases like malaria, hypertension, stomach problem, asthma, diabetes and common cold. It is also used to expulsion of retained placenta (Mekonnen and Gessesse, 1998; Mekonnen, 2005).

It is reported that the edible parts of *M. stenopetala* are exceptionally nutritious; the leaves of which have strong, mustard like taste, contain calcium, iron and other trace minerals, and are eaten as a supplement to the major staple foods in the Southern parts of Ethiopia (Mekonnen,

2005). *Moringa* is especially promising as a food source in the tropics because tree is in full leaf at the end of the dry season when other foods are typically scarce (Fahey, 2005). The leaves are good as a sources of vitamin A and, when raw, vitamin C. They are a good source of B vitamins and among the best plant sources of minerals. They are rich in protein and a very low source of fat and carbohydrates. The leaves are incomparable as a source of the sulfur-containing amino acids methionine and cystine, which are often in short supply (Anjorin *et al.*, 2010).

In addition to these importance, *M. stenopetala* have been used for water clarification in some parts of Africa (Jahn, 1981), in Ethiopia (Hundie and Abebe, 1991; Mekonnen, 2005) especially in Southern part of the country where it is grown widely. It is also used to expel retained placenta during birth and antileshimanial effect (Mekonnen and Gessesse, 1998). There are also reports on antimicrobial properties (Mekonnen and Dräger, 2003) and antitrypanosomal activity (Mekonnen *et al*., 1999).

3. OBJECTIVES OF THE STUDY

3.1 General objective

- The overall objective of the present study is to investigate the antibacterial activity of *M. stenopetala* on some human pathogenic bacteria

3.2 Specific objectives

- To test antibacterial property of the leaves, stem barks, root barks and seeds on selected pathogens
- To compare antibacterial activity of semi-purified fractions with crude extracts
- To compare the efficacy of *M. stenopetala* extracts with selected standard antibiotics against some pathogenic bacteria
- To determine the minimum inhibitory concentrations (MICs) of the extracts as a prediction tool for the therapeutic potential of the plant

4. MATERIALS AND METHODS

4.1 Plant sample collection and identification

The fresh and matured seeds separated from its pods, leaves, stem barks and root barks of *M. stenopetala* were collected from Southern parts of Ethiopia, the compound of Arbaminch University, Arbaminch in October 2011. The identity of plant specimen was confirmed and its voucher specimen was deposited at the National Herbarium, Department of Plant Biology and Management, College of Natural Science, Addis Ababa University (AAU).

4.2 Preparation of crude extract

All plant samples were dried under shade in Biomedical Research Laboratory, Department of Microbial, Cellular and Molecular Biology, AAU. The kernels of seeds after shelled from its husk, the leaves, stem barks and root barks were grounded by mortal and pistil, then by electronic mill (IKA).

Each 100g powder of the leaves, stem barks and root barks were separately macerated in 600ml of chloroform, ethyl acetate and 80% methanol. Similarly, 100g powder of the seeds was dissolved only in 600ml of 80% methanol. This maceration process was done in 1000ml Erlenmeyer flask on a rotary shaker (120rpmin) at room temperature for 72 hours. The extracts were filtered using filter paper (whatman No.1, Whatman Ltd., England) and the solvent was evaporated on the rotary evaporator (BÜCHI Rota-vapor R-205, Switzerland) under reduced pressure at 45^0c. The waxy residues of chloroform and ethyl acetate extracts were further dried at room temperature, where as 80% methanol extract was dried by lyophilizer (CHRiST). The resulting solidified plant extracts were kept in deep freeze (-20^0C) for future use after weighted (Devendra *et al*., 2011).

4.3 Solvent fractionation of *M. stenopitala* seeds

As described by Tadeg (2004) and Unasho (2005), part of crude 80% methanol extract (60g) was taken and packed in a thimble. Successive and exhaustive extractions were undertaken using petroleum ether (80-100^{o}C) (400ml), chloroform (400ml) and methanol (400ml) as fraction **I**, **II**

and **III** respectively by using soxhlet apparatus (BÜCHI E-816 Sox, Switzerland). Each fraction was collected separately and concentrated at reduced pressure using rota-vapor at 45^0C. The semisolid mass was then dried in room temperature. The residue that was left in a thimble was dissolved in distilled water (200ml), then filtered by whatman No.1 filter paper and taken as fraction **IV**. Finally, it was dried by lyophilizer and weighted. These dried mass was powdered and kept in a deep freeze (-20^0C) for future use.

4.4 Tested bacteria

The bacterial test organisms used in this investigation were *S. aureus* ATCC25923, *E. coli* ATCC25922, *P. aeruginosa* ATCC 27053 and *S. boydii* ATCC 9289 which were taken from Ethiopian Health and Nutrition Research Institute (EHNRI), Addis Ababa.

4.5 Standard antibiotics

Standard disks impregnated with amoxicillin (30µg), erythromycin (15µg), gentamicin (10µg) and kanamicin (30µg) were used as positive controls for the antibacterial susceptibility test; whereas 3% tween 80 served as a negative control.

4. 6 Preparation of inocula and culture media

To obtain pure culture and to avoid contamination the standard test organisms were streaked on differential or selective media that is S-S Agar (OXOID) for *S. boydii*, Mannitol Salt Agar (OXOID) for *S. aureus*, MacConkey Agar (TECHNO PHARMCHEM) for *E. coli* and Pseudomonas Isolating Agar (OXOID) for *Pseudomonas aeruginosa*.

After purity of the cultures was checked, each bacterium was activated on Nutrient Agar (OXOID) for preparation of inocula. Inoculums were standardized by inoculating 18-24hr old culture bacteria in saline solution and compared with 0.5 McFarland turbidity standard that was prepared by adding a 0.5ml aliquot of 0.48 mol/L $BaCl_2$ (1.175% w/v $BaCl_2$. $2H_2O$) added to 99.5 ml of 0.18 mol/L H_2SO_4 (1%v/v) (McFarland, 1979).

4.7 Antibacterial assay

The crude and semi-purified extracts were tested against the test organisms using the disc diffusion method as described by Bauer *et al*., (2006) and Lalitha (2008). Inoculums were prepared by mixing a few bacterial colonies from 18-24hr old culture in 5ml sterile saline solution and comparing the turbidity with that of the standard 0.5 McFarland solution which is equivalent to1.5 x 10^6-10^8cfu/ml. The sterile cotton swab was dipped into the properly adjusted inoculums and the excess was removed by gentle rotation of the cotton swab against the inner wall of the tube. The test bacteria were uniformly swabbed on the Mueller Hinton Agar (MHA) (OXOID) using the cotton swab. The inoculated plates were left at room temperature for 3-5 minutes to allow for any surface moisture to be absorbed before applying the extract. Each extract was dissolved in 3% of tween 80 with 300 mg/ml concentration. Ten micro litres of each plant extract was transferred onto a sterile filter paper disc (Whatman No.1; 6 mm in diameter) and allowed to dry for 15minute. Using aseptic conditions all the selected antibiotics and the plant extracts were applied on the MHA and left for 15 minutes to allow the extract to diffuse. The plates were then incubated at 37^0c for 18-24 hours in an incubator. All tests were performed in triplicate and zone of inhibition were measured after the incubation period by using ruler.

4.8 Determination of minimum inhibitory concentrations

The determinations of minimum inhibitory concentrations (MIC) of the extracts were carried out using the agar dilution method described by Aniel and Naidu (2007). This was done for all extracts that had produced an inhibition zone of greater than 7 mm in the disk diffusion test. The dried extracts were dissolved in 3% tween 80 at a concentration of 200mg/ml and then further serially diluted with 3% tween 80. Two milliliters of the extracts from each dilution was mixed with 18ml of molten MHA and poured into sterile petri-dishes allowing the agar to set. The final concentrations of the extracts in the culture medium ranged from 20 to 0.07mg/ml. The agar was streaked with fresh colonies of bacteria, of which its turbidity was adjusted to 0.5 McFarland standards (1.5 x10^6-10^8 cfu/ml). One petri-dish with 2ml of 3% tween 80 and 18ml MHA was used as negative control, while other petri-dish with only 20ml MHA was used as positive control. Then, it was incubated at 37 ºC and examined after 18-24hr. The lowest concentration of each extract that did not allow any colony growth in the solid medium after the incubation period was regarded as the MIC. All tests were performed in triplicates.

5. DATA ANALYSIS

The data were presented as the mean ± SE for the group of the experiments. Zone of inhibition was analyzed using windows SPSS version 16.0 to compare results. Descriptive statistics was used to calculate the mean and standard error of the mean. All data were analyzed at a 95% confidence interval (α=0.05).

6. RESULTS

6.1 Yields of the crude extracts and semi-purified fractions

Table 1 showed that, the amount of extracts ranges from 2.56% to 14% on dried mass basis. Methanol extract of the leaves afforded maximum yield (14%), followed by methanol extract of the seeds (11%) and chloroform extract of the leaves (8.59%). Minimum yield was obtained from ethyl acetate extract of root barks (2.56 %), which is almost comparable to the yield obtained from chloroform extract of root barks (2.73%), ethyl acetate (2.6%) and chloroform (3.32%) extracts of stem barks.

Table 1. Percentage yield of crude extracts in g

Extracts	SM	LM	LE	LC	BM	BE	BC	RM	RE	RC
Percentage yield (w/w)	11	14	7	8.59	4.7	2.6	3.32	7.34	2.56	2.73

Notes:-SM: seeds 80% methanol extract, LM: Leaves 80% methanol extract, LE: Leaves ethyl acetate extract, LC: Leaves chloroform extract, BM: Stem barks 80% methanol extract, BE: Stem barks ethyl acetate extract, BC: Stem barks chloroform extract, RM: Root barks 80% methanol extract, RE: Root barks ethyl acetate extract, RC: Root barks chloroform extract

Table 2 indicated that, the percentage yields obtained from successive fractionations of the seeds are relatively higher than crude extracts. The maximum yield was obtained from methanol fraction (30.83%) followed by water fraction (23.4%) and chloroform fraction (12.31%) and the minimum yield from petroleum ether fraction (9.86%).

Table 2. Percentage yield of semi-purified fractions in g

Solvents	Petroleum ether	Chloroform	Methanol	Water
Percentage yield (w/w)	9.86	12.31	30.83	23.4

6.2 Antibacterial assay

Activities of crude extracts

Crude extracts of the leaves, seeds, stem barks and root barks of *M. stenopetala* were tested for their antibacterial activity against *S. aureus, E. coli, P.aeruginosa* and *S. boydii*. Antibacterial activity was tested at 300gm/ml for both crude extracts and semi-purified fractions. Among all crude extracts 80% methanol extract of the seeds (18.66±0.88mm) showed highest antibacterial

activity, while ethyl acetate extract of stem barks (11.00±0.57mm) showed lowest activity against *S. aureus*. All extracts of the leaves were inactive against tested bacterial strains. *S.aureus* was highly susceptible bacterial strain, while *P. aeruginosa* was the most resistant to all of the crude extracts (Table 3).

Table 3. Antibacterial activity of crude extracts of the plant parts at 300mg/ml concentration.

		Zone of inhibition (mm)			
Plant parts	Extracts	*S. aureus*	*E. coli*	*P. aeruginosa*	*S. boydii*
Seeds	SM	18.66±0.88	11.66±0.88	-	14.66±0.33
Leaves	LM	-	-	-	-
	LE	-	-	-	-
	LC	-	-	-	-
Stem barks	BM	13.33±0.88	-	-	-
	BE	11.00±0.57	-	-	-
	BC	-	-	-	-
Root barks	RM	-	-	-	-
	RE	16.00±1.15	13.00±0.57	-	15.33±0.33
	RC	15.00±1.15	11.66±0.66	-	13.00±0.57

Note:- SM: seeds 80% methanol extract, LM: Leaves 80% methanol extract, LE: Leaves ethyl acetate extract, LC: Leaves chloroform extract, BM: Stem barks 80% methanol extract, BE: Stem barks ethyl acetate extract, BC: Stem barks chloroform extract, RM: Root barks 80% methanol extract, RE: Root barks ethyl acetate extract, RC: Root barks chloroform extract, (-): No inhibitory activity

Activities of semi-purified fractions

Table 4 indicated that, chloroform fraction showed best antibacterial activity against all tested bacterial strains, especially against *S. aureus* (28.00±0.57mm) followed by *S. boydii* (23.00±0.57mm). *P. aeruginosa* was the most resistant to this fraction, followed by *E. coli* with inhibition zone of 9.66±0.33mm and 14.66±0.33mm respectively. The second fraction that exhibited antibacterial activity was petroleum ether fraction. It was active only on *S. aureus* with

inhibition zone of 14.33±0.88mm. Water fraction also showed antibacterial activity only on *S. aureus*, while methanol fraction was inactive against all selected bacterial strains.

Table 4. Antibacterial activity for semi-purified fractions of seeds at 300mg/ml concentration.

	Zone of inhibition (mm)				
Plant part	Solvents	*S. aureus*	*E. coli*	*P. aeruginosa*	*S. boydii*
Seeds	Petroleum ether	14.33±0.88	-	-	-
	Chloroform	28.00±0.57	14.66±0.33	9.66±0.33	23.00±0.57
	Methanol	-	-	-	-
	Water	9.00±0.57	-	-	-

Note: - (-): no inhibitory activity

6.3 Comparisons with controls

The activity of the standard antibiotics and the negative control are presented in Table 5. In this case, *S. aureus* was highly susceptible to all standard drugs, while *E. coli and S. boydii* were resistant to amoxicillin and erythromycin and susceptible to gentamicin and kanamicin. *P. aeruginosa* was also resistant to amoxicillin and erythromycin and intermediately succebtable to kanamicin, but susceptible to gentamicin.

Table 5. Inhibition zone of standard antibiotics and tween 80 in mm.

Controls	Concentration	Inhibition zone against tested bacteria (mm)			
		S. aureus	*E. coli*	*P. aeruginosa*	*S. boydii*
Amoxicillin	30µg	23.33±0.88	-	-	-
Erythromycin	15µg	28.00±1.15	9.66±0.88	8.66±0.33	11.33±0.88
Gentamicin	10µg	27.66±0.66	25.33±0.33	24.00±0.57	21.66±0.88
Kanamicin	30µg	26.33±0.33	25.66±0.88	13.66±0.33	21.33±0.88
Tween 80	3%v/v	-	-	-	-

Note: (-): no inhibitory activity

6.4 Minimum inhibitory concentrations

Crude extracts

In agar dilution, MIC value was determined only for extracts that had antibacterial activity against tested bacteria. The MIC value of crude extracts of plant parts against the tested bacteria ranged from 1.25 to 20 mg/ml. The most frequent MIC value of the extracts were 5 mg/ml and 10 mg/ml. Among crude extracts, 80% methanol extract of seeds had lowest MIC value of 1.25 mg/ml, followed by ethyl acetate extract of root barks with MIC of 2.5mg/ml against *S. aureus*. Both 80% methanol extract of seeds and ethyl acetate extract of root barks had same MIC value of 10 mg/ml against *E. coli* and same MIC value of 5mg/ml against *S. boydii*. Crude 80% methanol extract of stem barks showed lower MIC value of 5mg/ml than ethyl acetate extract of stem barks (10 mg/ml) against *S. aureus*. The crude extract which had highest MIC value was chloroform extract of root barks with MIC value of 20 mg/ml against *E. coli*. This extract also showed MIC value of 5mg/ml and 10mg/ml against *S. aureus* and *S. boydii* respectively (Fig. 1).

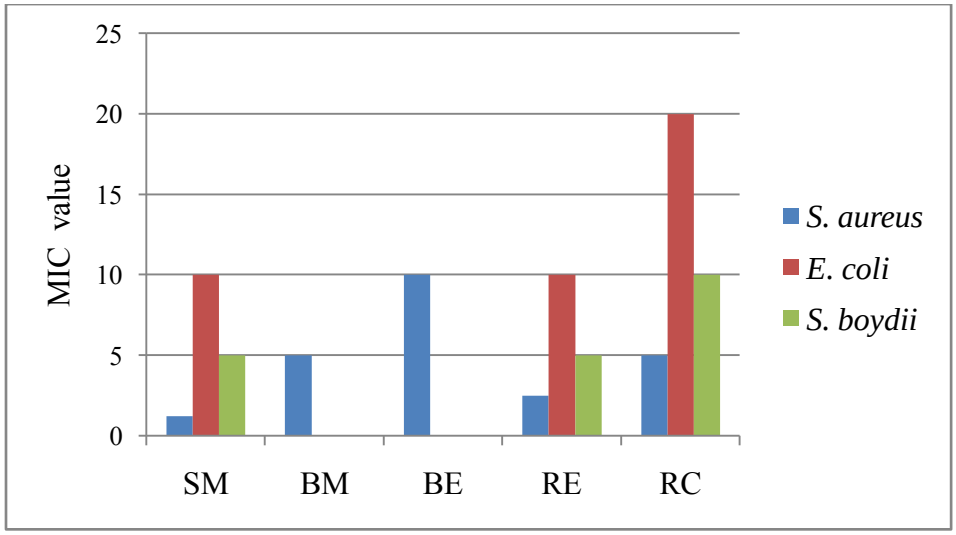

Fig.1 MIC value of crude extracts against selected bacterial strains

Note:- SM: seeds 80% methanol extract, BM: Stem barks 80% methanol extract, BE: Stem barks ethyl acetate extract, RE: Root barks ethyl acetate extract, RC: Root barks chloroform extract.

Semi-purified fractions

Among solvent used, chloroform fraction showed best activity against tested bacteria which is followed by petroleum ether fraction. Chloroform fraction had lowest MIC value against *S. aureus* with MIC of 0.31 mg/ml and highest MIC against *P. aeruginosa* with MIC value of 10mg/ml. Chloroform fraction also showed an MIC of 0.62 and 2.5 mg/ml against *S. boydii* and *E. coli* respectively. Petroleum ether fraction was also inhibited *S. aureus* at 1.25mg/ml, while the MIC for water fraction against *S. aureus* was not within the concentration range tested (Fig. 2).

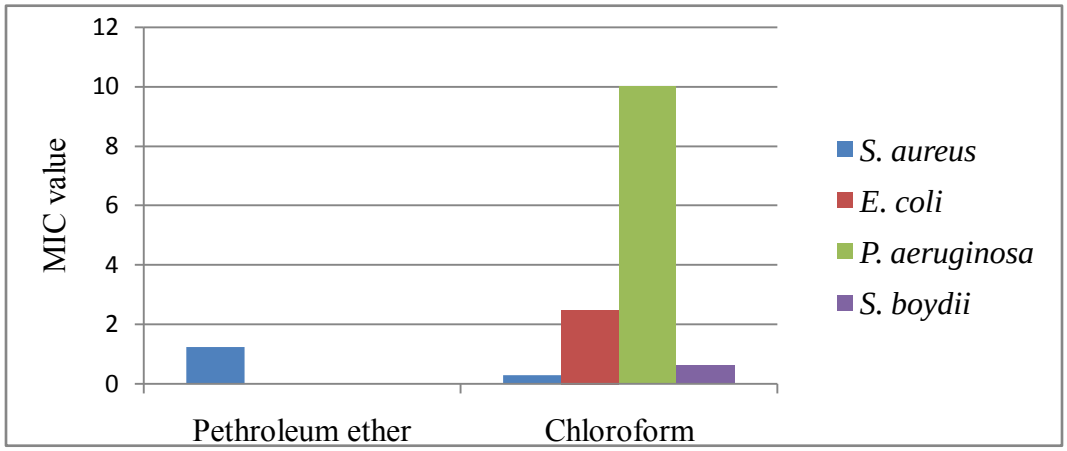

Fig. 2 MIC value of semi-purified fractions against selected bacterial strains

7. DISCUSSION

Medicinal plants must be tested for their claimed activity and safety and efficacy profiles have to be assessed including the proposed dosage form of the extract before it is made available for use (Dahanukar *et al*., 2000). The traditional healers use primarily water as the solvent, but in the present study the plant extracts by petroleum ether, chloroform, ethyl acetate and methanol provided more consistent antibacterial activity compared to those extracted by water.

7.1 Yields of the crude extracts and semi-purified fractions

It has showed in Table 1 that, methanol extract of the leaves afforded maximum yield (14%), while ethyl acetate extract of root barks (2.56 %) gives minimum yield. Such a high yield is an indication of the extracting ability of the solvent used with respect to both polar and non-polar components. Table 2 also indicated that, the percentage yields obtained from successive fractionations of the seeds are relatively higher than crude extracts. The maximum yield was obtained from methanol fraction (30.83%) followed by water fraction (23.4%) and minimum yield obtained from petroleum ether fraction (9.86%). In general, some of the yields obtained from these plant parts are quite adequate, thereby making further development of these herbal drugs economically feasible. Because formulation of phytopharmaceuticals is commonly based on the total extracts which do not incur cost of fractionation and other processing (Girma, 2005).

7.2 Antibacterial assay

Activities of the crude extracts

The antimicrobial effect of many medicinal plants is well documented (Valero and Salmeron, 2003). The results of different studies provide evidence that some medicinal plants might indeed be potential sources of new antibacterial agents even against some antibiotic-resistant strains (Kone *et al*., 2004). In this study, using the disk diffusion method, it was observed that extracts of seeds, stem barks and root barks of *M. stenopetala* produce antibacterial activity against both Gram negative and Gram positive pathogens.

The result from Table 3 showed that, 80% methanol extract of the seeds showed maximum antibacterial activity against *S. aureus* (18.66±0.88mm) followed by *S. boydii* (14.66±0.33mm).

But, it exhibit less activity against *E. coli* (11.66±0.88mm) and inactive against *P. aeruginosa*. This activity strengthens the report of Mekonnen and Dräger (2003), in which crude water extract of the seeds of *M. stenopetala* showed strong antibacterial effect against *S. aureus, Salmonella typhi* and *Shigella* species.

These results are also in agreement with the study conducted by Sahilu (2010), in which antibacterial activity for crude water extract of the seeds of *M. stenoptala* was analyzed against five bacteria strains. The maximum antibacterial activities were observed against *S. aureus* (28.2mm) and *S. boydii* (26.4mm) at a concentration of 200mg/ml, which closely agreed with this study. Among the five bacterial organisms the growth of *E. coli* was not inhibited by the crude water extract of the seed. But, in this study crude 80% methanol extract showed inhibitory activity against *E. coli* (11.66±0.88mm). This indicates that methanol extract was better than water extract against *E. coli*. In other word, methanol had the potential to extract active compounds than water which inhibit the growth of *E. coli*. The anti-bacterial activity of methanol extracts of *M. oleifera* and *M. stenopetala* seeds was also conducted by Walter *et al.* (2011), on three bacterial strains, which closely agreed with this study, that both methanol extracts of *M. oleifera* and *M. stenopetala* seeds showed antibacterial activity against *E. coli*.

Saadabi and Abu-Zaid (2011) evaluated aqueous and methanol extracts of *M. oleifera* seeds for their antibacterial activity against four types of bacteria namely, *S. aureus, B. subtilis, E. coli and P. aeruginosa*. Its aqueous extract showed superior antibacterial activity against all bacterial strains than its methanol. Both of the seeds extracts inhibited strongly the growth of Gram positive bacteria (*S. aureus* and *B. subtilis*) than Gram negative bacteria (*E. coli* and *P. aeruginosa*). It is common observation that Gram-negative bacteria are more resistant to many compounds than Gram-positive ones. This is might be because of morphological differences that Gram-negative bacteria have an outer membrane (Nikaido and Varra, 1985). Particularly, *E. coli* and *P. aeroginosa* are incriminated in several infections for their insensitivity to antibiotics (Morse *et al*., 1986). Hence, this is in congruency with the observation in this experiment that most of the extracts have shown lesser activity against *P. aeruginosa* and *E. coli* than that of *S. aureus*.

The Gram-positive bacteria are more susceptible due to the fact that their outer peptidoglycan layer which is not an effective permeability barrier (Nikaido and Varra, 1985). In spite of the permeability differences, some of the tested extracts demonstrated reasonable activities against Gram-negative bacteria; especially ethyl acetate extract of root barks and chloroform fraction which showed better activity on *E. coli* and *S. boydii* strains than the other plant extracts.

The extracts of the leaves of *M. stenopetala* did not shown any antibacterial activity against all tested bacteria. This may indicate that, the active compounds are not extracted by these solvents or this plant part may not contain bioactive compounds against these bacteria. Similar to this study, Mekonnen and Dräger (2003) reported that aqueous extract of *M. stenopetala* leaves did not shown any antibacterial activity against *S. aureus*, *Salmonella typhi* and *Shigella* species.

In this study, *S. aureus* was the only bacterium which was inhibited by extracts of stem barks. The antibacterial activity assay of these three solvent extracts of stem barks revealed that methanol extract showed higher antibacterial activity (13.33±0.88mm) than ethyl acetate extract (11.00±0.57mm), suggesting that the bioactive compounds are better extracted with methanol than ethyl acetate and immiscible with chloroform (Table 3).

In the study of Chetia and Gogoi (2011), the methanol extract of *M. oleifera* stem barks was studied for their antibacterial activities against *E. coli*, *P. aeruginosa* and *S. aureus* using disc diffusion method. The results suggest that, this extract had significant antibacterial activities against *E coli,* but inactive against *P. aeruginosa* and *S. aureus.* However, Sarin *et al*. (2010) reported that methanol extract of *M. oleifera* stem barks inhibited the growth of both *S. aureus* and *E. coli* with inhibition zone of 15.0±0.0 and 7.0± 0.0 mm respectively. This report was confirmed by the present work, in which methanol extract of stem barks showed best activity against *S. aureus*.

The results from Table 3 also show that, all extracts of root barks were active, except methanol extract against all tested bacteria except *P. aeruginosa*. Among these strains, *S. aureus* was highly sensitive to ethyl acetate extract (16.00±1.15mm) and chloroform extract (15.00±1.15mm), followed by *S. boydii* with antibacterial activity of (15.33±0.33mm) and

(13.00±0.57mm) respectively. Chloroform extract (11.66±0.66mm) of this plant part showed less activity, followed by ethyl acetate extract (13.00±0.57mm) against *E. coli*.

In the study conducted by Raj *et al*. (2011), the antibacterial activity of root barks of *M. oleifera* was analyzed against four bacterial strains. Its ethyl acetate extracts showed higher antibacterial activity than its chloroform extract against *P. aeruginosa* with inhibition zone of 18.2 ± 0.2 mm and 12.2±0.2mm respectively. Ethyl acetate extract also showed higher antibacterial activity than chloroform extract against *S. aureus* with inhibition zone of 11.10±0.1 and 8.2±0.2mm respectively and lower activity against *E. coli* (9.9±0.1mm), but chloroform extract was inactive against same bacteria. Dewangan *et al*. (2010) also reported that, ethyl acetate and chloroform extracts of root barks of *M. oleifera* inhibited the growth of four bacteria. The ethyl acetate and chloroform extracts of *M. oleifera* root barks were found to have strong antibacterial activity against *S. aureus* with inhibition zones of 19.66±0.88mm and 16.66±0.33mm, followed by *P. aeruginosa* with inhibition zone of 19±0.57mm and 15±0.57mm respectively. But, *E. coli* was the least susceptible test organism to ethyl acetate and chloroform extracts with inhibition zone of 13.66±0.33mm and 13.00±0.57mm respectively. In agreement with this study, *S. aureus* was highly susceptible than *E. coli* and ethyl acetate extract showed maximum antibacterial activity than chloroform extract. This indicates that the active ingredient which inhibits the growth of these bacteria might be better extracted by ethyl acetate than in chloroform and immiscible in methanol.

The results from Table 3 also showed that, the four tested *M. stenopetala* parts have varied antibacterial activity against the tested organisms. Among these, seeds and root barks were most effective against the selected strains. Stem barks extracts were slightly active against these strains, while the leaves extracts were inactive totally. When comparing tested bacteria, *S. aureus* was highly susceptible bacterial strain, while *E. coli* was less sensitive and *P. aeruginosa* was totally resistant bacterial strains. The difference in efficacy of these different extracts may be due to the distribution of the active ingredient in plant parts, potency of extracting solvent and inherent resistance of the tested bacteria species (Nayan *et al*., 2011). The present phyotochemical study of *M. stenopetala* are similar to those plant extracts that have been

reported by possessing strong antibacterial activity against both Gram positive and Gram negative bacteria.

Activities of the semi-purified fractions

Isolating active compounds from crude plant extracts needs extracting this component from the complex matrix of the crude extracts to get the pure form. One of such attempt is solvent fractionation that involves extracting components depending on their polarities (Nostro *et al.*, 2000).

Table 4 indicated that, the entire fractions showed antibacterial activity against *S. aureus*, except methanol fraction, but chloroform fraction (28.00±0.57mm) showed highly significant activity. Methanol fraction was inactive against all selected bacteria. *P. aeruginosa* was the most resistant to this fraction, followed by *E. coli* with inhibition zone of 9.66±0.33mm and 14.66±0.33mm respectively. The results from Table 4 also illustrated that the non-polar fractions (petroleum ether and chloroform) were stronger in their activity compared to polar fractions (methanol and water). Antibacterial activities were found to be decreased with increasing polarity, indicating that the active compounds responsible for antibacterial activity of the extract reside in the non-polar fractions in relatively higher concentrations. This result strongly supported the report of Taddese (2004), who analyzed antibacterial activity of selected medicinal plants topically applied in Ethiopia by using petroleum ether, chloroform, methanol and acetone. Among these, non polar fractions (petroleum ether, chloroform) showed maximum antibacterial activity than polar fractions (methanol and water).

The antibacterial effect was noticeably higher by chloroform fraction than activity in other fractions. This difference might be due to a) the active compounds are concentrated by using chloroform as a solvent; b) the concentration of inhibiting components are decreased by selecting the solvent (Taddese, 2004). Hence, the active ingredient is most likely non-polar and further activity-guided fractionation work on the non-polar fraction seems to be beneficial.

The results in Table 3 and Table 4 indicated that, semi-purified (chloroform fraction) showed significantly higher antibacterial activity than all crude extracts against all selected bacterial

strains. The reason behind is that the active compounds in semi-purified fractions are relatively concentrated to particular solvent (chloroform in this case) than in crude extracts against tested bacteria.

7.3 Comparison with controls

According to WHO (2012) the relationship between susceptibility of bacterial strains against standard antibiotics applied and the nearest inhibition zone diameter can be classified as follows: For amoxicillin (30µg), erythromycin (15µg) and kanamicin (30µg), if the diameter is ≤13mm: resistant; between 14 to 17mm: intermediate and ≥ 18mm: the bacteria is susceptible and for gentimicin (10µg) ≤12mm: resistant, between13-14mm: intermediate and ≥15mm: the bacteria is susceptible. From this it can be deduced that, *S. aureus* was susceptible to all standard antibiotics, while *P. aeruginosa* was susceptible to gentamicin, intermediate to kanamicin and resistant to amoxicillin and erythromycin. *E. coli* and *S. boydii* were resistant to amoxicillin and erythromycin and susceptible to genthamicin and kanamicin (Table 5).

When the activities of *M. stenopetala* were compared with these standard antibiotics, all crude extracts showed lower antibacterial activity than all standard antibiotics against *S. aureus*, but better activity than amoxicillin and erythromycin and lower activity than gentamicin and kanamicin against *E. coli*, *P. aeruginosa* and *S. boydii* (Table 3 and 5). Among semi-purified fractions, chloroform fraction was as potent as erythromycin and slightly more potent than amoxicillin, gentamicin and kanamicin against *S. aureus.* Similarly, it showed more antibacterial activity than amoxicillin and erythromycin and lower activity than gentamicin and kanamicin against *E. coli* and *P. aeruginosa.* It is also more active than all selected standard antibiotics against *S. boydii* (Table 4 and 5). Generally, chloroform fraction and some crude extracts showed relatively better antibacterial activity, when compared with standard antibiotics. This might be due to the appearance of drug resistant bacterial strains to these standard antibiotics. Therefore, if active ingredients of these extracts are purified, the extracts of this plant parts will show best antibacterial activity at low concentration.

7.4 Minimum inhibitory concentration

Crude extracts

The MIC assay was also employed to evaluate the effectiveness of the extracts to inhibit the growth of test bacteria. Among crude extracts, 80% methanol extract of seeds had lowest MIC value of 1.25 mg/ml, followed by ethyl acetate extract of root barks with MIC of 2.5mg/ml against *S. aureus*. This indicated that 80% methanol extract of seeds was more effective against this bacterium. The crude extract which had highest MIC value was chloroform extract of root barks with MIC value of 20 mg/ml against *E. coli* (Fig. 1).

In Ethiopia, Sahilu (2010) reported that crude water extract of *M. stenopetala* seeds inhibited *S. aureus* and *E. coli* at MIC of 1.25 and 10 mg/ml respectively. This report agreed with the present work in which, water extract of *M. stenopetala* seeds was equivalent in effectiveness to crude 80% methanol extract of the seeds against *S. aureus* with MIC value of 1.25mg/ml and it is also equivalent in effectiveness with crude 80% methanol extract of the seeds and ethyl acetate extract of root barks against *E. coli,* ethyl acetate extract of stem barks against *S. aureus* and chloroform extract of root barks against *S. boydii* at MIC value of 10mg/ml.

Lalas *et al*. (2012) analyzed antibacterial activity of n-hexane extract of *Moringa peregrina* seeds with MIC value of 4.5mg/ml against *S. aureus*. Busani *et al*. (2012) also reported that, acetone extract of *M. oleifera* leaves inhibited the growth of *S. aureus* at MIC value of 5mg/ml. According to Bukar *et al*. (2010), chloroform extract of *M. oleifera* seeds and chloroform extract of *M. oleifera* leaves inhibited the growth of *S. aureus* at MIC value of >4mg/ml, while methanol extract of *M. oleifera* seeds and chloroform extract of *M. oleifera* leaves inhibited *S. boydii* at same concentration. When these reports were compared with this study, they were relatively equivalent in effectiveness to 80% methanol extract of stem barks and chloroform extract of root barks of against *S. aureus* and crude 80% methanol extract of the seeds and ethyl acetate extract of root barks against *S. boydii* at 5mg/ml MIC value. Ethyl acetate extract of root barks was showed MIC value at 2.5mg/ml against *S. aureus*, which relatively agreed with the report of Bukar *et al*. (2010) in which methanol extract of *M oleifera* leaves inhibited *S. aureus* with MIC value of 2mg/ml.

Although the MIC result showed that *E. coli* was the highest resistant bacterial strain to have been inhibited at 20 mg/ml concentration of the chloroform extract of root barks, Sarin *et al*. (2010) reported that, this test organism can be inhibited at a concentration as low as 0.62mg/ml of the hexane and methanol extracts of *M. oleifera* stem barks. Sarin *et al*. (2010) also reported that, hexane and methanol extracts of *M. oleifera* stem barks gave MIC values of 0.312 and 0.078 mg/ml on *S. aurues* respectively. Sahilu (2010) reported, crude water extract of *M. stenopetala* seeds inhibited *S. boydii* with MIC of 0.63mg/ml. Comparing these results with the present study, solvent extracts of *M. stenopetala* parts were less effective in inhibiting the tested bacteria than the extracts of *M. oleifera* stem barks and water extract of *M. stenopetala.* Generally, among crude extracts 80% methanol extract of the seed highly potent against *S. aureus*, while chloroform extract of root bark was less efficient against *E. coli*.

Semi-purified fractions

MIC for semi-purified fraction of the seeds was done for petroleum ether, chloroform and water fractions. Chloroform fraction had lowest (0.31mg/ml) and highest (10mg/ml) MIC value against *S. aureus* and *P. aeruginosa* respectively. Petroleum ether fraction exhibited MIC value of 1.25mg/ml against *S. aureus*. Water fraction was inactive at these concentrations range (Fig. 2).

Nikkon *et al*. (2003) conducted a research work to investigate antibacterial activity of the compound N-benzyl, S-ethly thioformate isolated from *M. oleifera* against fourteen bacterial strains. This compound-1 isolated from chloroform fraction of ethanol extract showed lowest MIC value of 0.032mg/ml against *S. aureus* and *S. boydii*. Although fraction of *M. oleifera* was more potent than *M. stenopetala* fraction, this report agreed with the present study in which chloroform fraction had lowest MIC value against *S. aureus* and *S. boydii*. Generally, semi-purified fraction (chloroform fraction) had lowest MIC value than all crude extracts, which indicated that semi-purified fractions are more effective against tested bacteria than crude extracts, because bioactive compounds in semi-purified fractions were concentrated to specific solvent (chloroform) than in crude extracts.

8. CONCLUSIONS AND RECOMMENDATIONS

- From the above results, it can be concluded that traditional medicinal plant, *M. stenopetala* possess diverse antibacterial activity in its different parts against the selected bacterial strains.

- The chance to find active compounds was more apparent in crude 80% methanol extract of the seeds, followed by ethyl acetate extracts and chloroform extracts of root barks.

- The pattern of inhibition also showed that, *S. auseus* was the most susceptible bacterial strain followed by *S. boydii* to both crude and semi-purified fractions, while *P. aeruginosa* was the most resistant bacteria followed by *E. coli.*

- Much of the antibacterial activities of semi-purified fractions were because of the non-polar fractions than the polar one. Among these, chloroform fraction had highest inhibition zone and lowest MIC value. This means, chloroform fraction is more effective against test bacteria than both crude extracts and other fractions. This indicated that, active compounds in semi-purified fraction were concentrated to specific solvent (chloroform).

- Thus, *M. stenopetala* could become promising natural antibacterial agents with potential applications in pharmaceutical industry as sources and templates for the synthesis of new drugs to control infectious diseases. However, if plant extracts are to be used for medicinal purposes, issues of safety and toxicity will need to be considered and determined in near future for further application.

Based on these findings the following are recommended;

- The antibacterial testing was conducted on limited number of bacteria. So, the same work should be carried out on large variety of fungal and bacterial strains so as to have a clear picture of the spectrum of antimicrobial activity of the herbal drugs.

- Further work is needed to know and characterize the pure chemical compounds of the plant that are responsible for antimicrobial properties and to increase the efficiency of these extracts.

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10. ANNEXES

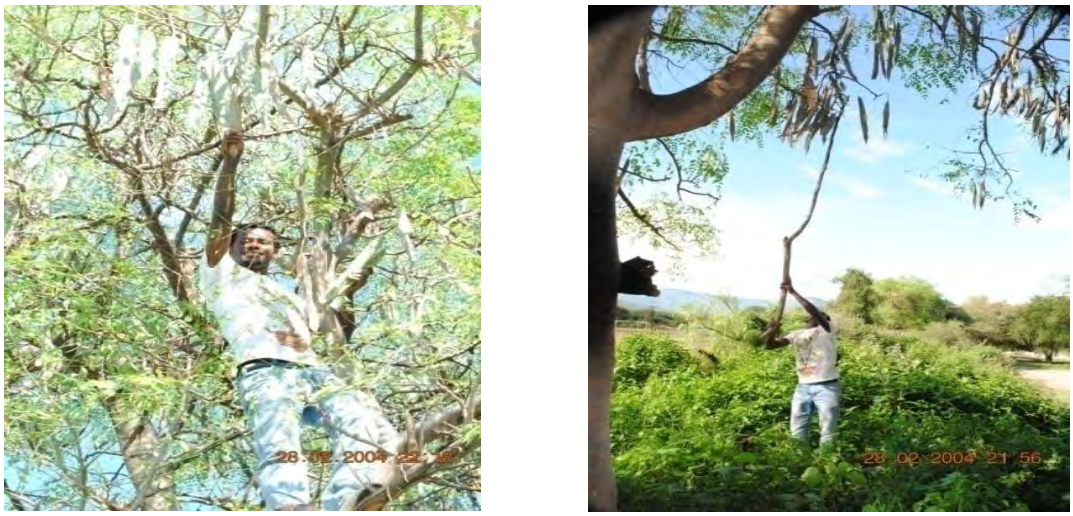

Fig. 3 Plant sample collection

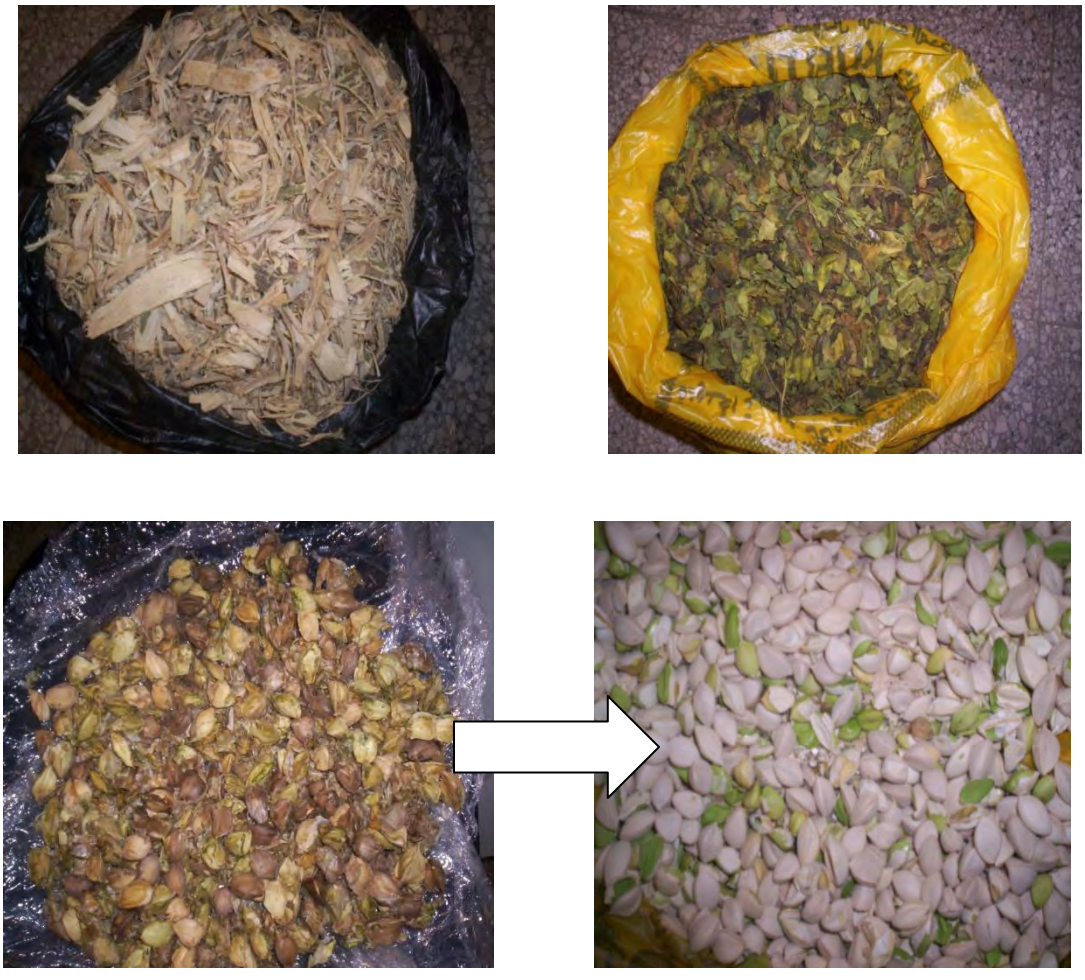

Fig. 4 Dried form of samples

Fig. 5 Filtration

Fig. 6 Extraction and fractionation

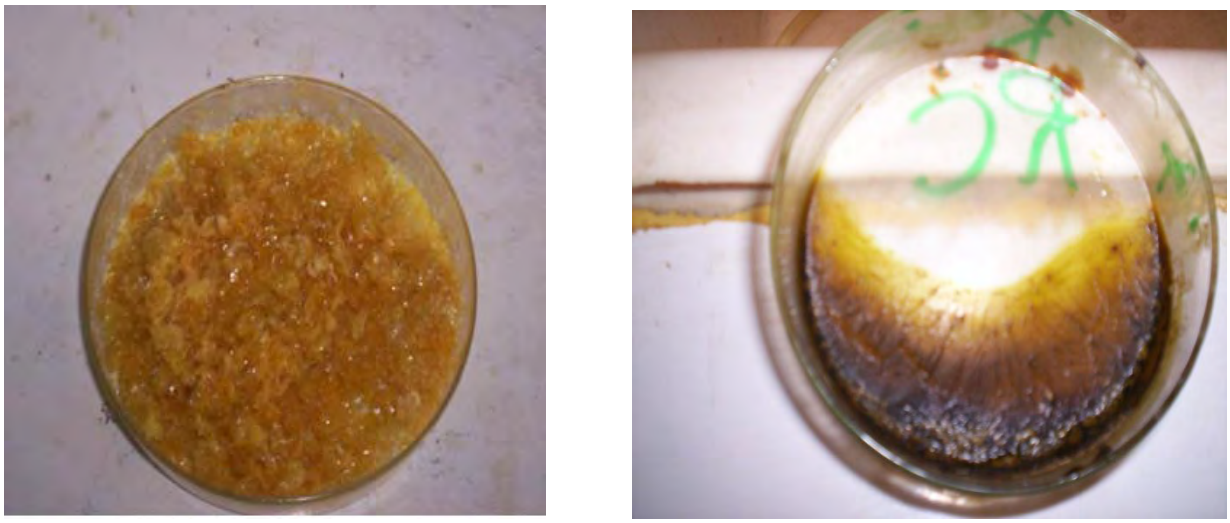

Fig. 7 Dried form of extracts

Fig. 8 Observation of the results

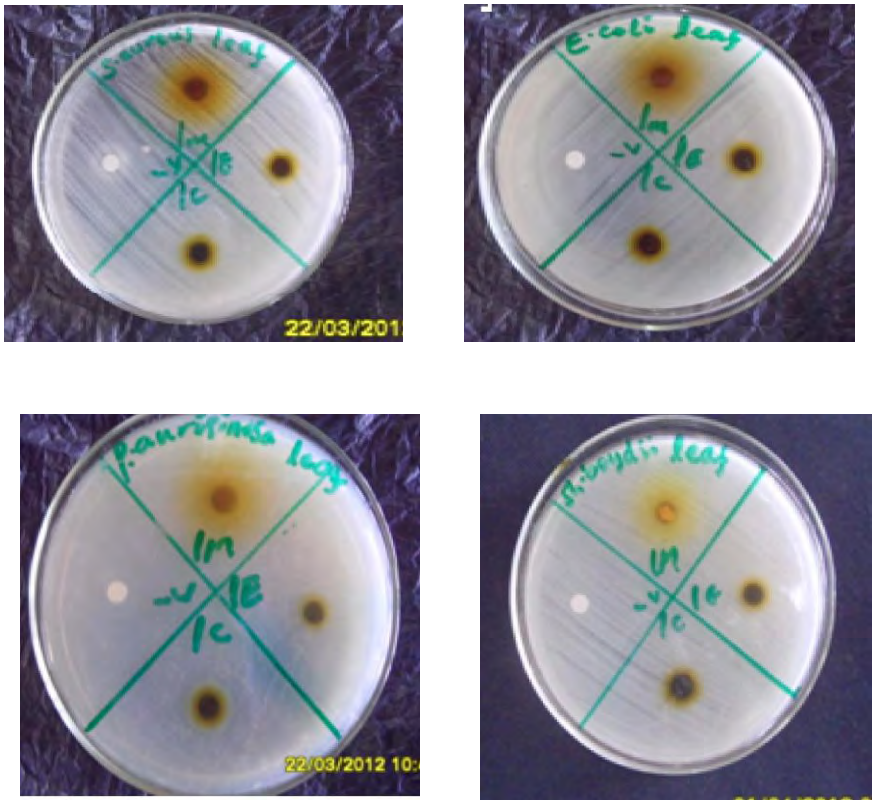

Fig. 9 Antibacterial activity of leaves against tested bacteria

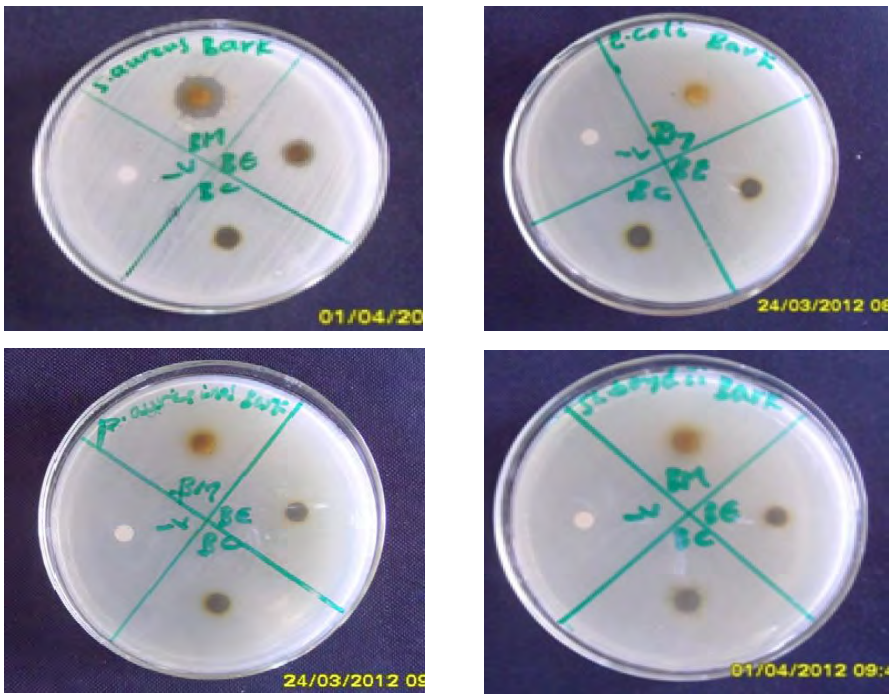

Fig. 10 Antibacterial activity of stem barks against tested bacteria

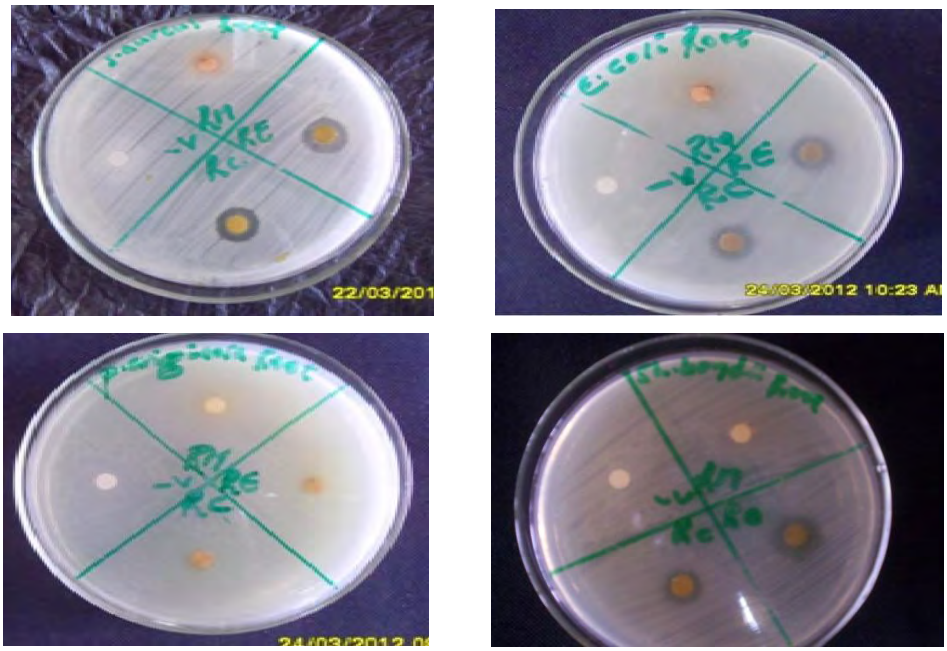

Fig. 11 Antibacterial activity of root barks against tested bacteria

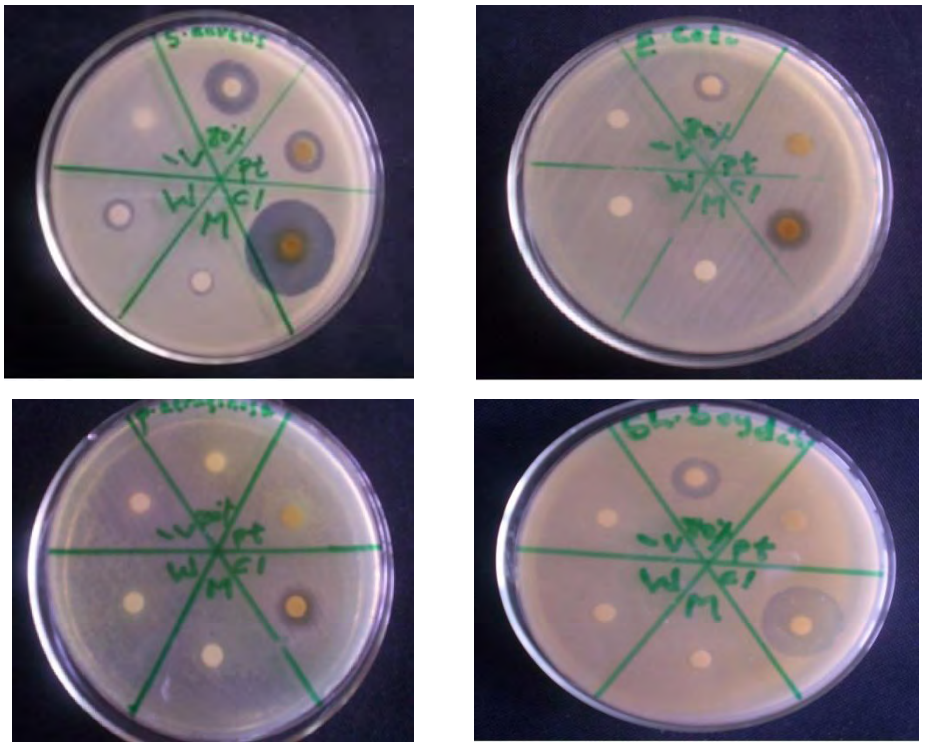

Fig. 12 Antibacterial activity of semi-purified fractions of seed against tested bacteria

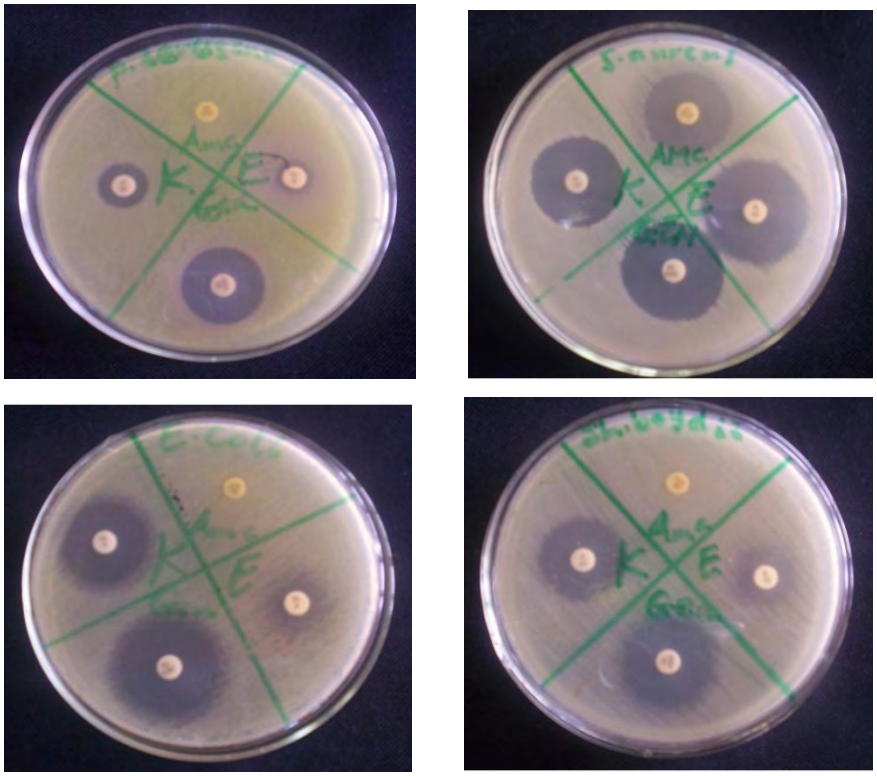

Fig. 13 Inhibition zone of standard antibiotics against tested bacteria

Notes:- Amc: Amoxicillin, E: Erythromycin, Gen: Gentamicin and K: Kanamicin