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ANTIMICROBIAL ACTIVITY OF SOLVENT-EXTRACTS OF CUCUMIS FICIFOLIUS AND ZEHNERIA SCABRA ON SOME TEST MICROORGANISMS

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Abbreviations

AAU- Addis Ababa University

ANOVA-Analysis of Variance

ATCC American Type Culture Collection

EHNRI - Ethiopian Health and Nutrition Research Institute

MHA-Mueller Hinton Agar

MIC-Minimum Inhibitory Concentration

NCCLS- National Committee for Clinical Laboratory Standards

OD-Optical Density

PDA-Potato Dextrose Agar

SEM-Standard Error of Mean

SPSS-Statistical Package for the Social Sciences

WHO-World Health Organization

Abstract

The crude chloroform, ethyl acetate, acetone, methanol and ethanol extract of the leaves of *Cucumis ficifolius* and *Zehneria scabra* were tested against in vitro antibacterial activity of five human pathogenic bacteria using disc diffusion method and agar dilution method for Minimum Inhibitory Concentration (MIC). *Pseudomonas aeruginosa* and *Shigella boydii w*ere susceptible test organism for the ethyl acetate and acetone extracts of the leaves of *C. ficifolius* with inhibition zones of 14±0.577mm, 12±0.577mm and an MIC of 0.781mg/ml, 3.125mg/ml respectively, but *Escherichia coli* and *Salmonella typhi* were the most resistant bacteria to all extracts of the leaves of this plant species with no inhibition zones. *Staphylococcus aureus* and *E. coli* were found to be the most susceptible bacteria for the ethyl acetate and acetone extracts of the leaves of *Z. scabra* with inhibition zones of 22.6±0.33mm, 14±0.577mm and MIC of 0.781mg/ml, 1.56mg/ml respectively. However *S. typhi* was the most resistant to all extracts of the leaves of this plant with no inhibition zone. The acetone extracts of *C. ficifolius* and the ethyl acetate extracts of *Z. scabra* were most potent but the chloroform extracts of both medicinal plants were ineffective against all bacterial and fungal test organisms. *C. ficifolius* and *Z. scabra* didn't show any inhibitory activity against *Botrytis* and Fusarium. It can be concluded that *C. ficifolius* and *Z. scabra* had shown antibacterial activity having potential for further study to serve as source of antibacterial agents.

Key words and phrases: Antibacterial activity, antifungal activity, minimum inhibitory concentration, *Cucumis ficifolius, Zehneria scabra.*

1. Introduction

Traditional medicine is the sum total of knowledge, skills and practices based on the theories, beliefs and experiences indigenous to different cultures, that used to maintain health, as well as to prevent, diagnose, improve or treat physical and mental illnesses (WHO, 2010).

According to WHO (2010) report nearly 70-80% of the world population depends on herbal medicine. Especially in developing countries like Asia and Africa where about 80% of the human population and 90% of livestock rely on traditional medication. However, the traditional medical systems are characterized by variations in ecological difference of the countries' biodiversity, socio cultural backgrounds of different ethnic groups and historical developments that related to migration, introduction of foreign culture and religion (Balick and Cox, 1997).

In Ethiopia traditional medical practitioners use different types of medicine such as minerals, plants and animal products. The drugs are prepared in various dosage forms such as liquid, powder and prescribed in a non-formulated form (Tena, 2008).

The current demand for herbal remedies in both developed and developing countries is increasing (Bussamann, and Sharon, 2006). In developed countries, this may be partly due to dissatisfaction with the conventional medicine while in the developing countries this is due to the lack of medical doctors, shortage of pharmaceutical products and their unaffordable prices (Ermias, 2005). Traditional medicine in Ethiopia is reported to account 32-35% of medicinal staff (Haile, 2005). The majority of traditional medicines used in developing country have not been evaluated for quality, safety and efficacy to some standards while those in developed countries there are some remarkable claims made for their effectiveness (Haile, 2005).

In Ethiopia modern drugs are in short supply and are in accessible and unaffordable to the vast majority of the populations. The provision of essential drugs, their equitable distribution and rational uses are still serious problems (Mesfin, 2006; Tekalign *et al*., 2010).

The studies of traditional medicine have different significance such as to see important cure for the society and to develop local initiatives for pharmaceutical industry (Endashaw, 2007). And also it used to create possible motives for conserving biodiversity, to preserve indigenous botanical and medicinal knowledge and to provide convenient access to modern medicines more easily that is available and acceptable by rural communities (Caceres *et al*., 1990).

Higher plants produce hundreds and thousands of different chemical compounds with different biological activities (Cos *et al.,* 2006). These anti-microbial compounds produced by plants are active against plant and human pathogenic micro-organisms.

Ethiopia is a home land of many languages, cultures and believes that in turn have contributed to the high diversity of traditional knowledge which includes the use of medicinal plants (Desta, 1993). The Ethiopian flora offer great possibilities for the discovery of new compounds with antimicrobial activities (Fernandez *et al.,* 2008).

Pharmacological and phytochemical studies that are undertaken with medicinal plants in different countries have led to the isolation of novel structure for the manufacturing of new drugs or templates that served for the production of synthetically improved therapeutic agents (Reuben *et al.,* 2008)

Modern drugs discovered from natural products have a huge potential that still exists in plants for the production of many more novel pharmaceuticals (Nita *et al*., 2002). Scientists throughout the world have discovered thousands of phytochemicals which have inhibitory effect on all types of microorganisms in vitro (Agnese *et al*., 2001). However,

their effectiveness on all types of pathogens, their toxicity assays and possible effects on beneficial microorganisms have not yet been fully studied (Ali *et al.,* 2008).

Further more the expensive cost of modern medicine, to treat various infections and the acquisition of drug resistant by pathogens, particularly in third world countries necessitates the search for an alternative anti-infective agent from natural products (Nychas, 1995). Many previous studies conducted in Ethiopia have shown the anti-microbial activities of many indigenous plants used in traditional medicine (Teferi and Hahn, 2003; Tesfaye *et al.,* 2006).

2. LITERATURE REVIEW

2. 1 Application of Traditional Medicinal Plants

The use of medicinal plants as a source for relief from illness can be traced back over five millennia to written documents of the early civilization in China, India, and the Near east, but it is doubtless on an art as old as mankind (Mohsenzadeh, 2007).Natural products perform various functions and many of them have interesting and useful biological activities. There are more than 35,000 plant species being used in various human cultures around the world for medicinal purpose (Philip *et al*., 2009).

The potential of higher plants as source for new drugs is still largely an explored. Among the estimated 250,000 - 500,000 plant species only a small percentages has been investigated. Phytochemicals and the fractionation submitted to biological and pharmacological screening is even small (Ramor and Ponnampulam, 2008). Historically pharmacological screening of natural compounds or synthetic origin has been the source of enumerable therapeutic agents (Semere, 2006).Random screening as tool in discovering new biological active molecules has been most productive in the area of antibiotics (Penna *et al.,* 2001). Even now, contrary to common believes drugs from higher plants continue to occupy an important niche in modern medicine (Kokoska *et al*., 2002).

Medicinal plants represent a rich source of antimicrobial agents on a global basis; at least 130 drugs, all single chemical entities extracted from higher plants are modified further synthetically and currently in use, but some of them are not being made synthetically for economic reasons (Ramor and Ponnampulam, 2008). Plants are used medicinally in different countries and are a source of many potent and powerful drugs (Borchardt *et al*., 2008). Because of the large chemical diversity among natural products many research groups screen plant extracts in their search for new promising therapeutic candidates for infectious disease (Rios, 1989).

Bacterial and fungal infections were some of the most serious global heath issue of the present century (Vonshak *et al*., 2003). Numerous biologically active plants have been discovered by evaluation of ethno pharmacological data and these plants may offer the local population immediate and accessible therapeutic products (Bruck *et al*., 2004). Medicinal plants are important sources of traditional medicine for millions of people and additional inputs to modern medicine in terms of exploring and producing new drugs to meet the need for the over grown populations of the planet (Celikel and Kavas, 2008).
Several herbs were known to possess medicinal value including anti microbial properties (Jaturapronchai, 2003). The extensive use of synthetic drugs, excessive unwanted medication will cause increasing side effects that produced by the administration of drugs is much more serious problem than that of the disease itself (Babu *et al*., 2010).

In addition to the increasing demand for new drugs, over use and miss use of antibiotics have contributed to the development and spread of the resistant micro organisms to the treatment (Elgayyar *et al*., 2001). Due to this factor researchers are increasingly turning their attention to natural products looking for new leads to develop better drugs against cancer, as well as viral and microbial infection (Philip *et al*., 2009). For centuries, most of the population in Ethiopia, as else where in many other developing countries have relied on a system of traditional medicine which consists of both empirco- rational and magico-religious elements and a combination of both (Dawit *et al*., 2005).The pharmacological and phytochemical studies help for the discovery of modern drugs from medicinal plants, and also it signify the huge potential that still exists for production of many more novel pharmaceuticals (Farnsworth, 1994).

The search for new antimicrobial agents from medicinal plants is even more urgent in the context of developing countries like Ethiopia where infectious diseases or bacterial and fungal origin are not only rampant (Mirgessa, 1996). But, the causative agents are also developing an increasing resistance against many of the commonly used antibiotics (Abebe *et al*., 2003). Considering the high cost of the synthetic drugs and their various side effects the search for alternative products from plants used in folklore medicine is further justified (Ashebir and Ashenafi, 1999). It is believed that plants which are rich in a wide variety of secondary metabolites belonging to chemical classes such as tannins, terpenodis, alkaloids, polyphenols are generally superior in their antimicrobial activities (Cowan, 1999).This suggests that the strength of biological activities of a natural product is dependent on the diversity and quantity of a natural product.

In Ethiopia about 80% of human population and 90% of livestock rely on traditional medicine (Mesfin , 2006) due to the socio cultural appeal, the cultural acceptability of healers and local pharmacopoeias, accessibility and effectiveness of medicinal plant against a number of health problems (Teferi and Hahn,2002).

Traditional medicinal practitioners used different type of medicine including plants, animals and minerals. The plant parts that are used in the preparation of medicine include roots, stems, barks, leaves flowers, and seeds (Mohsenzadeh, 2007). The traditional drugs are also prescribed in anon formulated form, additives are usually incorporated and more than one drug is used in a single dosage form (Rojas *et al.*, 2007). These drugs are administered using different roots. The major ones being: topical oral, respiratory and anal (Tena, 2008). Herbal medicines are parts of the entire system of traditional medicine (Taylor, 2003). An integration of traditional medicinal plants with modern medicine has been also practiced in countries such as Egypt, Ghana, India, China and Sudan (Penna et *al*., 2001).

According (Tena, 2008) at this moment, somewhere in the rural hinter land of the Ethiopia rural communities a local farmer may have just gathered leaves or root parts from local medicinal plants found near their homestead. In a nearby village a mother

might be in the midst of preparing a traditional plant treatment believed to restore strength, relieve stomach crumbs, and heal for a skin condition and ward of the evil eye or perhaps to help alleviate symptoms of a respiratory tract infection.

The indigenous traditional knowledge of medicinal plants of various ethnic communities has been transmitted orally from generation to generation (Velickovic *et al*., 2003). But now a day due to the advert of modern technology transformation of traditional culture migration from rural to urban areas, industrialization, rapid loss of natural habitat and changes in life style is on the ways of increasing the disappearance of this indigenous traditional knowledge from the surface of the earth (Semere, 2006).

Thousands of plant products with inhibitive effect on the micro organisms have shown in vitro activity and many of they have been used for centuries by various cultures in the treatment of different diseases (Ates and Erogrul, 2000). However these plants are used at a very high concentration with serious side effects on the patients. So, this requires the evaluation of the concentration against the standard antibiotics that have been ready on a market (Balagojevick *et al*., 2006).

Herbs, shrubs, higher plants and climbers remain as an important and reliable source of potentially useful chemical compounds for direct use as drugs and to synthesize prototypes for synthetic analogous in terms of drug efficacy (Dewanjee *et al*., 2007). Several studies have been undertaken in Ethiopia regarding medicinal plants that have been screened for their antimicrobial activities.

In Ethiopia, different plant parts have used as a source of traditional medicine from antiquity to solve different health problems and human sufferings. Due to its long period of practice and existence, traditional medicine has become an integral part of the culture of Ethiopian people (Haile, 2005).

As Dawit (2001) stated, there is large magnitude of use and interest in traditional medicinal plants in Ethiopia, due to cultural acceptability of healers, efficacy against certain types of diseases, physical accessibility and economic affordability as compared

to modern medicine. Herbal remedies are parts of the entire system of traditional medicine.

The use of plant extract (phytochemicals) believed to constitute the major parts of therapy apart from their use in the traditional systems of medical care at the local level (Desta, 1993). Medical plants currently used in the production of modern drug as source of direct therapeutics agents, as row material for the manufacturing of complex semi synthetic compounds and as taxonomic markers in the search of new compounds (Suppakul *et al*., 2003). The majority of Ethiopian rural areas were depends on the traditional medicinal plants as their only source of health care due to the lack of adequate clinics, hospitals, volunteers health care professionals, absence of vehicular roads and expensiveness of modern medicines(Ermias , 2005).

Veterinary traditional medicines are locally available and cheaper than the standard treatments as a result of this livestock holders can prepare and use a home made remedies with minimum expense (Fekadu, 2001). And also due to relatively few veterinarians and shortage of other facilities, traditional medicinal plants are the only choice to treat many ailments in developing countries like Ethiopia (Tena, 2008).

2.2. Current importance of medicinal plants

The uses of traditional medicinal plants for primary health care have steadily increased worldwide in recent years (Samie *et al.,* 2010). Scientists are in search of new phytochemicals that could develop as useful anti-microbial for treatment of infectious diseases (WHO, 2010). Currently, out of 80% of pharmaceuticals derived from plants, very few of them used as anti-microbial. But, Plants are rich in a wide variety of secondary metabolites that have found anti-microbial properties (Ramor and Ponnampulam, 2008).

Natural product drug discovery is a multi step, integrated process, requiring many key decisions (Farnsworth, 1985) including selection of target relevant for the therapeutic end points, choice of compounds for testing, the challenge of purification and structure

elucidation, pharmacological and toxicological characterization, formulation and other preclinical work required to move a lead compound to drug candidate status (Kumaraswamy *et al.*, 2008). Because of continual emergency of resistant organism, these considerations are especially critical to realization of major advances in the therapies available for the treatment of infectious disease (Haile, 2005).

The capacity of higher plants to produce inhibitors of microbial virulence factors have been reported (Blagojevich *et al*., 2006). But not widely exploited as source of new drug. Presumably these inhibitors function to protect the plant from microbial pathogens encountered in the environment, and thus would be expected to have potential utilities as antimicrobial drug or antibiotics (Stephen and Horace, 2000).

In the context of countries like Ethiopia the prohibitively expensive cost of efficacious antibiotics and the emergency of single and multiple antibiotic resistance of bacterial disease call for the search of alternative agents with possible antibacterial effect from natural products (Mesfin, 1986). One possible means to manage this problem is the search of pharmacologically active agents by screening traditionally claimed medicinal plants for their possible antibacterial effects (Ahmed *et al*., 2007)

Despite the wide utilization of medicinal plants in our country, a few researches have been conducted to evaluate the crude extracts of traditionally used medicinal plants (Iddock, 1990). Now a days because of steady increases of multi-drug resistant strain of bacteria in diseases causing there is a continuous and urgent need to discover new antimicrobial compounds with different chemical structures and novel mechanisms of action because there has been an alarming increase in the incidence of new and re-emerging infectious disease(Semere , *2006*).

The forests have been the source of valuable medicinal plants after human beings realized the preventive and curative properties of plants and stated to using medicinal plants for human health care (Balick and Cox, 1997). The medicinal plants of our planet faced with natural and man cause threats (Mesfin *et al*., 2006). Rapid increase in population, the need for fuel, urbanization, timber production, over harvesting, destructive harvesting invasive species, commercialization, degradation, agricultural expansion and habitat destruction are human caused threats to medicinal plants whereas recurrent drought, bush fire, disease and pest out breaks are the natural causes of treats of medicinal plants (Mesfi*n, 2006*). As a result this issue needs great attention from different sector including government, local community and traditional medicinal healer.

2.3. **Bacterial test organisms**

2.3.1. *Salmonella typhi*

The Salmonellae belong to a genus of the family Enterobacteriaceae. They are gram negative, facultatively anearobic, non-spore forming rods. Motile forms have peritrichous flagella. They are usually catalase positive, oxidase negative and reduce nitrates to nitrites (Adams and Moss, 2008). Salmonellae are recognized as a major cause of enteric fever and gastroenteritis. Many foods, particularly those of animal origin, have been recognized as vehicles for transmitting the organisms to human and to the food processing and preparation environment (Behailu, 2006).

Salmonella are known to cause disease in humans, animals, and birds (especially poultry) worldwide. The two major diseases caused by *Salmonella* spp. are gastroenteritis and typhoid fever (typhoid and paratyphoid fevers) in humans. Typhoid fever is a life-threatening illness caused by the bacterium S. *typhi* (Celikel and Kavas, 2008).

Typhoid fever is still common in the developing world, where it affects about 21.5 million persons each year. It lives only in humans; Persons with typhoid fever carry the bacteria in their bloodstream and intestinal tract. In addition, a small number of persons, called carriers, recover from typhoid fever but continue to carry the bacteria. Both ill persons and carriers shed *S. typhi* in their feces (stool) (Kumaraswamy *et al*., 2008).

Typhoid fever, also known as enteric fever, is potentially fatal, multi systemic illness caused primarily by *S. typhi.* The protean manifestations of typhoid fever make this disease a true diagnostic challenge (Ericsson and Sherris, 1971). The symptoms include prolonged fever, headache , malaise, diffuse abdominal pain, muscle pain and constipation. Untreated, typhoid fever is a grueling illness that may progress to delirium, obtundation, intestinal hemorrhage, bowel perforation, and death within one month of onset. Survivors may be left with long-term or permanent neuropsychiatric complications (Talaro and Talaro, 2002).

S. typhi has been a major human pathogen for thousands of years, thriving in conditions of poor sanitation, crowding, and social chaos. Although antibiotics have markedly reduced the frequency of typhoid fever in the developed world, it remains endemic in developing countries (Behailu, 2006).

The modes of transmission: of this disease takes place through oral transmission via food or beverages handled by an individual who chronically sheds the bacteria through stool or, less commonly, urine hand-to-mouth transmission after using a contaminated toilet and neglecting hand hygiene. The currently available treatments for *S. typhi* infection includes: rehydration and antibiotics in susceptible patients where as Vaccination prior to travel for Prevention.

2.3.2. *Staphylococcus aureus*

S.aureus is the species of the genus, Staphylococcus, which are Gram positive, facultative anaerobic, non motile and non-spore forming, catalase positive, coagulase positive cocci that divide in more than one plane to product irregular clusters of cells(Adams and Moss, 2008). *S. aureus* is a common case of skin and wound infection in humans and a significant proportion of the population also carries the organism as a commensal of the skin and nose. It is therefore frequently introduce into food by food handlers and indirectly by equipment.

Staphylococcus is quite resistant to desiccation and high-osmotic conditions. These properties facilitate their survival in the environment, growth in food, and communicability (Nita *et al*., 2002). Pathogenic staphylococci are usually opportunists and cause illness in compromised hosts (Penna *et al*., 2001).

S. aureus is the most pathogenic species. It causes skin and tissue infections and can invade many other organs and some strains produce toxins (Roberts and Greenwood, 2003). The major diseases are abscesses, osteomyelitis, endocarditis, toxic shock syndrome, bacterial pneumonia, common food poisoning, and other diseases. Food poisoning strains of *S. aureus* produce a heat-stable enterotoxin that has a direct effect upon the central nervous system (Celikel and Kavas, 2008). It is one of the four most common causes of Nosocomial infection, often causing post surgical wound infection. S. aureus remains an important pathogen, particularly among people who are hospitalized and some of these Staphylococci are resistant to penicillin (Mohsenzadeh, 2007).

2.3.3. *Shigella boydii*

Shigella is a genus of the Family Enterobactieracase. The organisms are Gram negative facultatively anaerobic, non- motile and non-spore forming rod. They are oxidase negative, urease negative, lactose and sucrose negative, and do not produce hydrogen sulfide (Adams and Moss, 2008). The most important reservoir of infection is the intestinal tract of humans and primates. The transmission is mainly person to person by the feacal- oral route and causes human shigellosis in primates and humans (Roberts and Greenwood, 2003).

S. boydii is one of the most dominant members of the genus that causes dysentery that result in the destruction of the epithelial cells of the intestinal mucosa in the cecum and rectum (Paton and Paton, 1998).Shigellosis is endemic throughout the world where it is held responsible for some 120 million cases of severe dysentery with the devastating majority of which occur in developing countries and involve children less than five years of age (WHO, 2009).

In tropical developing countries, shigellosis is endemic and a major killer of children under 5 years of age. Shigellosis occurs following ingestion of a very small number (100-1000) of bacteria, thus permitting easy spread of the disease by person-to- person contact as well as by the drinking of contaminated water (WHO, 2009).Transferable

antimicrobial resistance was described in Shigella and there has been a wide distribution of resistant strains and the development of resistance to an increasing number of antimicrobial agents (Talaro and Talaro, 2002).

A combined resistance to cotrimoxazole, chloramphenicol, ampicillin, and tetracycline has been widely described and resistance to nalidixic acid, the recent preferred treatment of choice, has also become increasingly common (Adwan *et al*., 2002). Resistance to nalidixic acid is associated with reduced sensitivity to ciprofloxacin and other fluoroquinolones, leaving few treatment options, particularly in low-income countries (Jenie *et al*., 2001).

2.3.4. *Escherichia coli*

Escherichia is the genus of the Enterobacteriaceae Family and *E.coli* is the species of the genus Escherichia. It is a catalase- positive, oxidase- negative, Fermentative, short, Gram- negative, non-spore forming rod (Adams and Moss, 2008). Genetically, *E.coli* is very closely related to the genus Shigella, although characteristically it ferments the sugar lactose and is otherwise far more active biochemically than shigella species (Parish and Davidosn, 1993). Late lactose fermenting, non- motile, biochemically inert stains of *E. coli* can be difficult to distinguish from shigella. *E.coli* can be differentiated from other members of the Enterobacteriaceae on the basis of a number of sugar fermentation and other biochemical test (Sakagami *et al*., 2007).

E. coli are the best-known coli form, its use as a focus for laboratory studies. It is called the colon bacillus and sometimes regarded as the predominant species in the intestine of humans. Its prevalence in clinical specimens and infections is due to its being the most common aerobic and non-fastidious bacterium in the gut (Paton and Paton, 1998).

As Mohsenzadeh (2007) reported because of its prominence as a normal intestinal bacterium in most humans, *E-coli* are currently one of the indicator bacteria to monitor

fecal contamination in water, food, and dairy products because they are present in larger numbers, can survive in the environment, and are easier and faster to detect than true pathogens.

Although many of the strains are not infectious, some have developed greater virulence through plasmid transfer, and others are opportunists. *E. coli* is the predominant cause of both community and nosocomial urinary tract infection (Behailu, 2006)

E. coli often invade sites other than the intestine. Urinary tract infections usually result when the urethra is invaded by these bacteria. The infection is more common in women because their relatively short urethras promote ascending infection to the bladder (cystitis) and occasionally the kidneys (Hart and Karriuri, 1998). Patients with bladder catheters are also at risk for *E. coli* urethritis. The rate of infection is higher in children and in crowded tropical regions where sanitary facilities are poor and water supplies are contaminated and adults carry pathogenic strains to which they have developed immunity.

2.3.5. *Pseudomonas aeruginosa*

The genus *Pseudomonas* contains a very diverse group of bacteria that are aerobic, oxidase-positive, catalase-positive, non- fermentative. Gram-negative rods, that is motile with polar flagella (Roberts and Greenwood, 2003). Some species attack sugars by oxidation and produce a diffusible fluorescent pigment; others produce alkali. The psychrotrophic strains are low temperature spoilage organisms of fresh egg, fish, meat and milk and are found widely in the soil, water and vegetation. *P. aeruginosa* commonly causes eye and ear infections as well as wound infections in burned body. It can sometimes be found in food, soil and water and should regard as a hygiene parameter; it is not though to cause gastrointestinal illness.

P. aeruginosa can able to grow and survive in almost any environment, soil, water and vegetation. This is due to their metabolic diversity and the production of siderophore and

fluorescein by some of the most dominant members of the group. *P. aeruginosa* is one of such members that are not only dominant in different habitats, but also associated with community- acquired and nosocomial infection in immune-compromised patients and hospitals. As a result, the pathogen is considered as an opportunist bacterium Most Pseudomonas species are naturally resistant to penicillin and the majority of related beta-lactam antibiotics, but a few of them are sensitive to piperacillin, imipenem, tobramycin, or ciprofloxacin.

One of the most striking characteristics of P. *aeruginosa* consists in its low antibiotic susceptibility. This low susceptibility is attributable to a concerted action of multi drug efflux pumps with chromosomally-encoded antibiotic resistance genes and the low permeability of the bacterial cellular envelopes. Besides intrinsic resistance, *P. aeruginosa* easily develop acquired resistance either by mutation in chromosomally-encoded genes, or by the horizontal gene transfer of antibiotic resistance determinants (Srikumar *et al*., 1998).

2.4 Fungal test organisms

2.4.1 Botrytis

Botryotinia is a genus of Ascomycete fungi causing several plant diseases (WHO, 1999). The anamorphs of Botryotinia are mostly included in the *imperfect fungi* genus Botrytis. The genus contains 22 species and one hybrid.

Plant diseases caused by Botryotinia species appear primarily as blossom blights and fruit rots .but also as leaf spots and bulb rots in the field and in stored products. The fungi

induce host cell death resulting in progressive decay of infected plant tissue, whence they take nutrients. Sexual reproduction takes place with ascospores produced in apothecia; conidia are the means of asexual reproduction (Mukherjee, *et al.*, 2001). Some species also cause damping off, killing seeds or seedlings during or before germination.

B. fuckelliana is an important species for wine industry as well as horticulture. Other economically important species include: *B. convoluta*, *B. polyblastis*, *B. allii* and *B. fabae* (WHO, 1999). *Botrytis* complicates wine making by making fermentation more complex. *Botrytis* produces an antifungal that kills yeast and often results in fermentation stopping before the wine has accumulated sufficient levels of alcohol.

Botrytis bunch rot is another condition of grapes caused by *B. cinerea* that causes great losses for the wine industry. It is always present on the fruit set; however, it requires a wound to start a bunch rot infection. Wounds can come from insects, wind, accidental damage, etc. To control botrytis bunch rot there are a number of fungicides available on the market. Generally, these should be applied at bloom, bunch closure (Lyon, 1976). In Horticulture *B. cinerea* affects many other plants. It is economically important on soft fruits such as strawberries and bulb crops. Unlike wine grapes, the affected strawberries are not edible and are discarded.

To minimize infection in strawberry fields, good ventilation around the berries is important to prevent moisture being trapped among leaves and berries. This is accomplished by slightly elevating the strawberry plants from the soil using straw, rather than planting them directly on the ground. In greenhouse horticulture, *B. cinerea* is well-known cause for considerable damage in tomato. In Human *B. cinerea* mold on grapes may cause "winegrower's lung", a rare form of hypersensitivity pneumonitis (Jorjandi, *et al*., 2009).

2.4.2 Fusarium

Fusarium is a large genus of filamentous fungi widely distributed in soil and in association with plants. Most species are harmless saprobes, and are relatively abundant members of the soil microbial community. Some species produce mycotoxins in cereal crops that can affect human and animal health if they enter the food chain. The main toxins produced by these Fusarium species are fumonisins and trichothecenes (Imamura *et al*., 2008).The genus includes a number of economically important plant pathogenic species. Such as *F*. graminearum commonly infects barley if there is rain late in the season. It is of economic impact to the malting and brewing industries, as well as feed barley. Fusarium contamination in barley can result in head blight, and in extreme contaminations, the barley can appear pink.

F. graminearum can also cause root rot and seedling blight. *F. oxysporum f.sp.cubense* is a fungal plant pathogen that causes Panama disease of banana (Musa spp.), also known as fusarium wilt of banana (Shahidi, *et al*., 2006). Panama disease affects a wide range of banana cultivars, which are propagated asexually from offshoots and therefore have very little genetic diversity. In humans some species may cause a range of opportunistic infections in humans.

In humans with normal immune systems, fusarial infections may occur in the nails and in the cornea (keratomycosis or mycotic keratitis).In humans whose immune systems are weakened in a particular way, (neutropenia, i.e., very low neutrophils count), aggressive fusarial infections penetrating the entire body and bloodstream may be caused by members of the *F.* solani complex, *F. oxysporum*, *F. verticillioides F. prolifurantum* and, rarely, other fusarial species (Aghighi, *et al*., 2004).

These fungal species also use as Biological warfare .when Fusarium-contaminated wheat flour was baked into bread, causing alimentary toxic; the Symptoms began with abdominal pain, diarrhea, vomiting, and prostration, and within days, fever, chills, and bone marrow depression and secondary sepsis occurred (Bibhuti *et al*., 2002). Further symptoms included pharyngeal or laryngeal ulceration and diffuse bleeding into the skin, bloody diarrhea, vaginal bleeding and gastrointestinal ulceration.

2.5. *Cucumis ficifolius*

It is a perennial herb climbing or trailing to 2m long from a perennial wood root stock up to 1mm long; stems are hairy with short fine hairs and also with small aculeate hairs of varying degrees or stoutness; older basal stems thickened and with whitish somewhat scaly fissured bark. Its vernacular name is Yemidier embuay (Amharic language);

Leaves are blade ovate to broadly rotundate in outline, subcordate, siluate- toothed, scabrid 30-76 mm long, 24-80mm broad, 0.8-1.3 times as loge as broad, usually palmately 3 -5 lobed. Sometimes unlobed or rarely lobed; lobes rounded above, narrowed or sometimes not narrowed bellow with the lateral sometimes almost obsolete and with the central larges broadly rounded or broadly ovate, 12-28 mm. long; 0.4-0.7 times as long as the whole blade, narrowed at its base to 0.8-1.0 of its breadth at its broadest; petiole hairy like the stem, 13-44 mm. long male flowers solitary on 4-22 mm long pedicels; receptacle tube green , 3-5 mm long; petals yellow 5-6 mm long 3-3.5 mm broad shortly united below (Mesfin, *et al*.,1995).

Fruit on a 30-70mm long stout stalk thickened up wards to abroad insertion on the fruit bluntly ellipsoid (52-60mm) long; 34-50mm across, pustulate with the pustules scattered broadly conical, 1-2 mm high each ending in hard blunt broad based hair, but sometimes very obscure, green sometimes faintly striped ripening; bright yellow and Seeds are elliptic (Hyde, *et al*., 2011). The habitat for this plant species is Grassland, wooded grassland, *Acacia* woodland, rocky slopes, secondary vegetation and cultivated places; in Ethiopia mainly distributed Tigray, Gojam, Shewa, Welega, Gamo Gofa and Harerge (Mesfin, *et al*.,1995). The altitude ranges from 1300-2400 m, mainly found in Ethiopia, Uganda, Kenya, Rwanda, and Tanzania (Burkill, 1985).

2.5.1. Medicinal value of *Cucumis ficifolius*

The plant provides grazing for all stocks in Senegal, Mauritania, Sudan, Somalia, Uganda and Kenya it is also said in Mauritania to be good for milk production (Burkill, 1985). However the plant part is unspecified but probably the fruit is used as an abortifacient for women and to hasten expulsion of the placenta for cows in Ethiopia. The fruits are recognized in Ethiopia as highly poisonous, and are reported to treat rabies.

In Nigeria and Ethiopia the fresh fruit with an end cut off is applied thimble like as addressing for inflamed fingers. The fruit has veterinary use as a vermifuge with the addition of natron for horses by the Hausa. It is also used as a medicine for fowls. In some places it is an ingredient of medicine for syphilis and as an emetic and in small doses with honey to relieve stomach ache for children, in Ethiopia it also used for the treatment of “Kuruba”, “Chiffea”, “Mageriat geter” (meningitis), “nessir” (epistaxis), “wefbeshita” (Teklehaymanot and Giday, 2007).and also the roots is remedy for malaria. The seeds are oil bearing. The Root extract of *C. ficifolius* is recorded to be used in local honey-wine or “Tej” to make beverage more intoxicating (Mesfin, *et al*., 1995).

2.6. *Zehneria scabra*

It is a perenniel climber plant having high medicinal values, which belongs to the Family Cucurbitaceae. It is distributed in hilly area; usually found covering fences, its vernacular name (Amharic language) *Etsesabek* or *Hareg eressa*. The leaves are heart shaped and lobed with distant spiny teeth on the margin (Mesfin, *et al*., 1995).

The flowers are small, white. Fruits look like miniature water melon and test like cucumber, and this species is a bit difficult to distinguish from *Z. maysorensis* which is native in South Africa. It is Prostate or climbing herb with annual stems, up to 6m long. Leaves more or less broadly ovate in outline; usually unlobed but occasionally 3-5 lobed, deep green and with rough hairs above, paler below, cordate at the base, margin prominently toothed. Flowers small unisexual on different plants male flowers in axillary clusters; female flowers solitary or in few flowered cluster, white.

Fruits are in clusters spherical, 8-13mm in diameter hairless bright red when ripe. The derivation of its specific name scabra is from its roughness referring to the leaves surface and its habitat is in forest or forest margin, riverine fringes and exotic plantations' and the altitude range where this species found is from 1200-3580m .In Ethiopia mainly distributed in Tigray, Gonder, Welo, Shewa, Arsi, Welega, Illubabor, Kefa, Gamo Gofa, Sidamo, Bale and Harerge (Mesfin, *et al*., 1995). Its distribution is worldwide especially wide spread in tropical Africa, South Africa Arabia, India, Java and the Philippines.

2.6.1. **Medicinal value of *Zehneria scabra***

The plant is useful in the treatment of jaundice and kidney infection other than stone. In kidney problems the boiled decoction of the shoots of the plant and touch me not in equal proportion mixed with molasses is prescribed as remedy. It is used to treat fever, "mich", "kintarot", common cold, skin problem and stomach pain in the form of root and leaf extracts by traditional healers (Abate, 1989). The ethanol root extract had significant

antibacterial activities against E-coli and *P. auruginosa*. The root extracts of the plant is used with milk to treat fever and diarrhea and the leaf extract is also used to treat skin rashes.

The leaf of *Z. scabra* was traditional used for the treatment of leprosy, measles, wound dressing and antihelminthes (Dawit *et al.*, 2005). In the present study an extensive antimicrobial profile was performed for two medicinal plants grown in Ethiopia around the town Sebeta 25 km far from Addis Ababa namely *C. ficifolius* and *Z. scabra* helminthes.

3. OBJECTIVES

3.1. General objectives

The overall objective of the present study is to determine the antimicrobial activity of *Cucumis ficifolius* and *Zehneria scabra* on selected test microorganisms.

3.2. Specific objectives

- To test the antimicrobial activity of *C. ficifolius* and *Z. scabra* on the basis of Minimum Inhibitory Concentration (MIC) of the crude extracts of *C. ficifolius* and *Z. scabra* against the test organisms.
- To compare the efficacy of the antimicrobial activity of the two plants extracts with the selected standard antibiotics against the test organisms.

4. MATERIALS AND METHODS

4.1. Plant Materials

4.1.1. Collection and identification

The traditional medicinal plants of *C. ficifolius* and *Z. scabra* were collected from Sebeta 25km far from Addis Ababa in August 2010. The voucher specimens of these plants were prepared and sent to the National Herbarium of Addis Ababa University for identification.

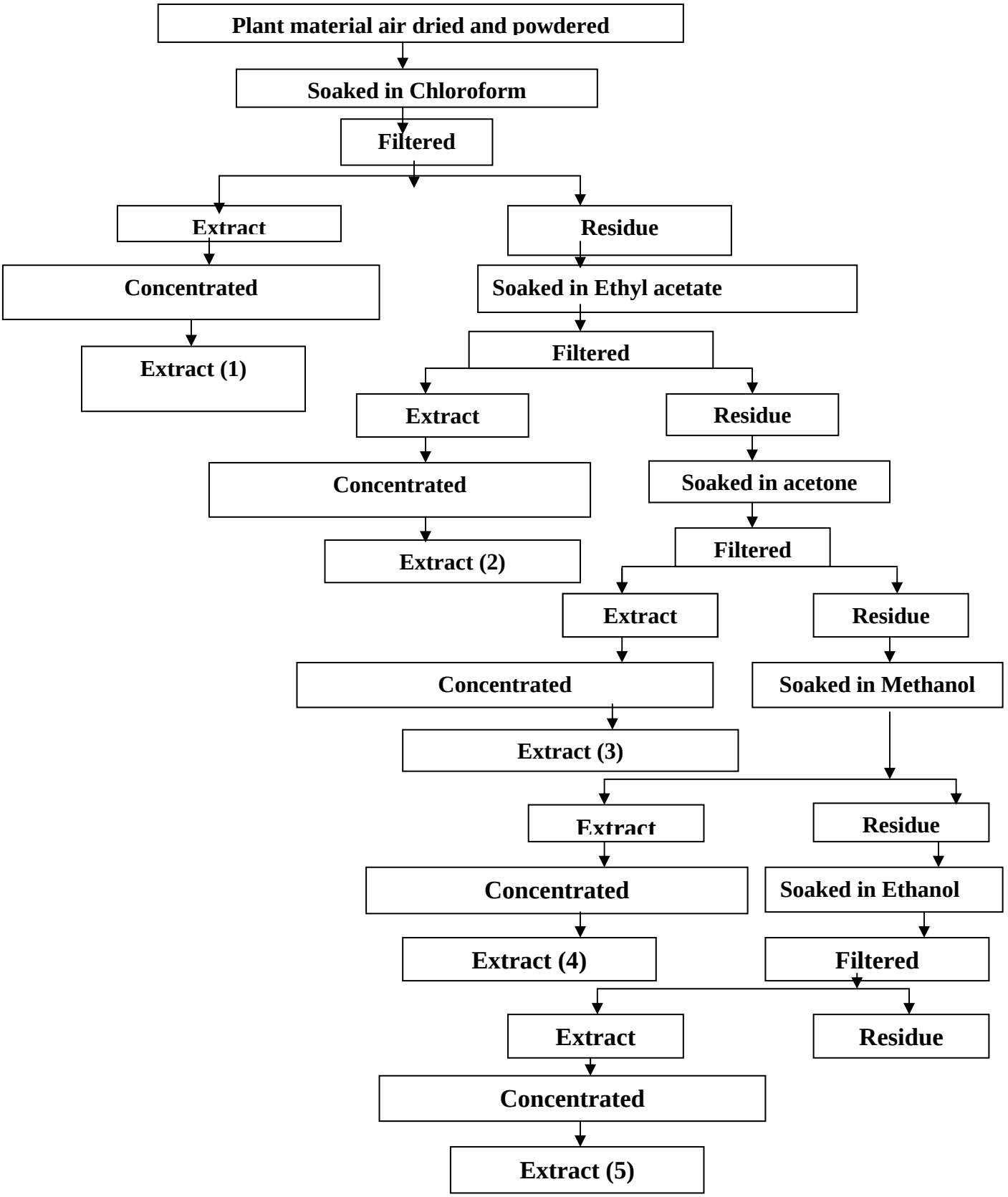
4.1.2. Extract preparation

The plant materials of both *C. ficifolius* and *Z. scabra* were air dried under shade in Biomedical Research Laboratory of the Faculty of Life Sciences of Addis Ababa University (AAU). They were then ground using mortal to powder form and sieved with a mesh to obtain a fine powder. The procedures of extraction are shown in the chart.

The sieved powder forms of both plant materials (125g) were extracted with 600ml of chloroform in 500ml Erlenmeyer flask on a rotary shaker ($120 r.pmin^{-1}$) at room temperature for 72 hours. The extracts were filtered using filter paper (whatman No.1) and the solvent was evaporated on the rotary evaporator (BUCHI-Germany) under reduced pressure at 45^0c. The extracts were then further dried at room temperature and extract fraction No. 1 was obtained.

The residues of both plants (120g) were extracted with 700ml of ethyl acetate at room temperature for 72 hours on a rotary shaker. The extracts were filtered using whatman No.1 and the solvent was evaporated on the rotary evaporator (with brand of BUCHI-Germany) under reduced pressure at 55^0c.The extracts were further dried at room temperature and extract fraction No. 2 was obtained.

Chart that shows Methods of Extraction



The ethyl acetate residues (fraction No. 2) of both plants (116g) were extracted with 80% acetone (640ml acetone to 160 ml of water) at room temperature for 72 hours on a rotary shaker. The extracts were filtered using filter paper (whatman No. 1) and the solvent was evaporated on the rotary evaporator under reduced pressure at 40^0c. The extracts were further dried at room temperature and extract fraction No. 3 was obtained.

The acetone residues (fraction No. 3) of both plants (110gm) were extracted with 80% methanol (640ml methanol to 160 ml of water) at room temperature for 72 hours on a rotary shaker. The extracts were filtered with (whatman No. 1) and the solvent was evaporated on the rotary evaporator under reduced pressure at 40^0c. The extracts were further dried at room temperature and extract fraction No.4 was obtained.

The methanol residue (fraction No.4) of *C. ficifolius* (106g) was extracted with 800ml of ethanol at room temperature for 72 hours on a rotary shaker. The extracts were filtered using filter paper (whatman No.1) and the solvent was evaporated on the rotary evaporator under reduced pressure at 55^0c. The extracts were further dried at room temperature and extract fraction No.5 was obtained. Five extracts of *C. ficifoius* and four extracts of *Z. scabra* were stored at-20^0c until the antimicrobial tests were done.

4.2. **Test Organisms**

4.2.1. **Bacterial strains**

The bacterial test micro-organisms used in this investigation were *Escherichia coli (*ATCC25922*), Pseudomonas aeruginosa (*ATCC25853*), Staphylococcus aureus (*ATCC25923*),* which were donated from Ethiopian Health and Nutrition Research Institute (EHNRI): *Shigella boydii* and *Salmonella typhi* that were obtained from the Microbiology Laboratory of the Faculty of Life Sciences of AAU.

4.2.2. **Fungal strains**

The fungal pure culture of Botrytis and Fusarium were donated from Mycology Laboratory of the Faculty of Life Sciences of AAU.

4.2.3 Standard antibiotics

Tetracycline and erythromycin were used as positive control for the antibacterial susceptibility test; whereas Tween 20 served as a negative control. Ketoconazole and Tween 80 were used as positive and solvent controls for the antifungal test respectively.

4.3. Inoculum Preparation

4.3.1. Bacterial test organisms

To obtain pure culture and to avoid contamination the standard test organisms (*P. aeruginosa, S. typhi, E. coli, S. aureus*) and the clinical test organism (*S. boydii)* were streaked on differential or selective media that is S-S agar (OXOID, code CM99) for *S.*

boydii, Brilliant green agar for *S. typhi*, Mannitol salt agar (BBL,product No.211407) for *S. aureus*, *Pseudomonas* isolation agar for *P. aeruginosa and* MacCONKEY agar (batch No.8195) for *E. coli.* To determine the exponential (active state of the bacterial test organisms), each bacteria was grown in 100ml nutrient broth in 250ml Erlenmeyer flask on a rotary water shaker (120r.pmin^{-1}) at 37^{0}c. Samples were taken every 2hrs for a total of 24hours and the optical density reading was taken to determine the exponential phase using spectrophotometer at 625nm (NCCLS, 1993). The exponential phase of each test organism was distinguished by constructing a growth curve of optical density against time.

After determining the active phase approximately at18hours, inoculums preparation was standardized by inoculating the bacteria in peptone water 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_2SO4 (1%v/v) (McFarland, 1979).

4.3.2. Fungal test organisms

Loopful of the fungal test organisms Botrytis and Fusarium was sub cultured on acidified Potato Dextrose Agar (PDA) slants at 30^{0}C for five days. The slants were then washed with 10ml 0.05% Tween 80 to obtain a spore suspension (Bullerman *et al*., 1977). The OD_{625} of the fungus suspension was measured and adjusted to 0.1 by using 0.05% Tween 80 to obtain the size of 10^{5}cfu/ml.

4.4. Antimicrobial activity assay

4.4.1. Anti bacterial Susceptibility Test

4.4.1.1. Disk diffusion

The inhibitory activity of the extracts against test organisms was determined by using the agar disc diffusion method as described by (Arthur *et al*., 1979; Rios *et al.*, 1989; Piddock, 1990). Inoculums were prepared by mixing a few microbial colonies (1ml) from exponential phase with 9ml of sterile peptone water and comparing the turbidity with that of the standard 0.5 McFarland solution which is equivalent to 10^6-10^8cfu/ml (Wanger, 2007). The sterile swab was dipped into the properly adjusted inoculums and the excess was removed by gentle rotation of the cotton swab against the surface of the tube.

The test microorganisms were uniformly swabbed on the Mueller Hinton Agar (MHA) 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 (NCCLS, 1996). Each extract was dissolved in 10% of Tween 20 to a final concentration of 400mg/ml; approximately ten micro litres (1 mg) of each plant extract was transferred onto a sterile filter paper disc (Whatman No.1; 6 mm in diameter) and allowed to dry for 15 min (Chantana *et al*., 2005). Using aseptic conditions all the selected antibiotic and the plant extracts were applied on the MHA and left for 15 minutes to allow the extract to diffuse (NCCLS, 2000). 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 from the edge of each disc after the incubation period by using Sliding Callipers and ruler (Shahwar and Raza, 2009).

4.4.2. Antifungal test

Agar disc diffusion method (Arthur *et al*., 1979; Rios *et al.,* 1989; Piddock, 1990) was used to determine the antifungal activity of the plant extracts; 60ml of MHA were poured in 15-cm petri plates and allowed to solidify. The standard size (approximately 10^5) of *Botrytis* and *Fusarium* was spread on the surface of MHA and allowed to dry for 15 minute. The sterile 6-mm disc impregnated with different leaves extracts, the standard drug ketokonazole and Tween 80 were then distributed on the surface of the agar. The plates were then left at room temperature for 30 minutes to let the extracts and controls diffuse in the agar medium and incubated at 30^0C for 48 to 96 hours. The inhibition zone was measured in millimeter by ruler.

4.4.3. Determination of Minimum Inhibitory Concentration (MIC)

The MHA was prepared as recommended by the manufacturing industry, and the sterilized medium was allowed to cool to 50^oC in a water bath. Different dilutions of the plant extracts were prepared (from 400mg/ml-0.195mg/ml). Nineteen (19ml) of molten agar and 0.1ml of the test organism were added in to each 250ml Erlenmeyer flask, mixed thoroughly and poured into prelabeled sterile petri dishes on a level surface. The plates were allowed to stand at room temperature to solidify the agar. Then the plate was incubated at 37^0c for 18h and after the incubation period the MIC of each antimicrobial agent was determined by observing the inhibition or the growth of the micro-organism with the naked eye (NCCLS, 2000).

5. **Data analysis**

The data are presented as the mean ± SEM for the group of the experiments. Zone of inhibition was analyzed using windows SPSS version 17.0 to compare means.

6. RESULTS

6.1 Antibacterial activity of *C. ficifolius* and *Z. scabra*

In this investigation, the antimicrobial activity of the chloroform, ethyl acetate, acetone, ethanol and methanol extracts of the leaves of *C. ficifolius* and all extracts of *Z. scabra* but not ethanol were evaluated using disk diffusion method. Table 1 and 2 show the antibacterial activity of the different extracts of the leaves of the two medicinal plants against five pathogenic bacteria species. The extracts of these two plant species showed varying degree of inhibitory activity against *S. aureus, E. coli, P. aeruginosa* and *S. boydii* at concentration of 400mg/ml.

Table1.The effect of the different extracts of the leaves of *C. ficifolius* against the bacterial test organism using disk diffusion method (zones of inhibition in mm) at concentration of 400mg/ml

	Inhibition zone of the leaves extract of *C.ficifolius* in mm					
Test organisms	$CHCl_3$	MeOH	EtOAc	Ac	EtOH	Tc
S. typhi	-	-	-	-	-	23.3±0.33
E. coli	-	-	-	-	-	20.3±0.33
P. aeruginosa	-	11±0.577	14±0.577	13±0.577	12±0.577	25.3±0.33
S. boydii	-	10±0.577	-	12±0.577	11±0.577	7±0.577
S. aureus	-	-	-	9±0.577	-	12±0.0

Note: - $CHCl_{3:}$ chloroform, EtOAc: ethyl acetate, MeOH: methanol, EtOH: Ethanol, Ac: acetone, Tc: tetracycline (positive control), (-): no inhibitory activity, negative control did not show any activity, the number indicate zones of inhibition in millimeter.

Among the extract fraction of the leaves of *C. ficifolius*, ethyl acetate exhibited the highest inhibition zone against *P. aeruginosa* (14±0.577mm) whereas; acetone was the second with inhibition zone of 13±0.577mm against *P. aeruginosa.*

The least inhibition zone was observed for acetone extract fraction against *S. aureus* with inhibition diameter of 9±0.577. The chloroform extract fraction was ineffective against all test organisms. *P. aeruginosa* was the most susceptible test organism for all extract fractions except chloroform. *S. boydii* was the second susceptible test organism for the extract fraction of methanol, acetone and ethanol.

S. aureus was the least susceptible test organisms that inhibited by the acetone extract fraction only. S. typhi and E. coli were resistant to all extract fractions of the leaves of *C. ficifolius*. The inhibition zones showed by the methanol, acetone and ethanol extract fraction were greater than the drug tetracycline against *S. boydii*, but the inhibitory activity of tetracycline against all test organisms except *S. boydii* were better than the plant extract fraction.

Table 2 .The effect of the different extracts of the leaves of *Z. scabra* against the bacterial test organism using disk diffusion method (zones of inhibition in mm) at concentration of 400mg/ml

	Inhibition zone of the leaves extract of *Z. scabra* in mm					
Test organisms	$CHCl_3$	MeOH	EtOAc	Acetone	Ery.	T20
S .typhi	-	-	-	-	16±0.577	-
E. coli	-	9±0.577	11.3±0.33	14±0.577	9±0.577	-
P. aeruginosa	-	-	10.3±0.33	-	25.6±0.33	-
S. boydii	-	-	6.3±0.33	6.6±0.33	6.3±0.33	-
S. aureus	-	-	22.6±0.33	19.3±0.33	23±0.577	-

Note: - $CHCl_3$: chloroform, EtOAc: ethyl acetate, MeOH: methanol, EtOH: Ethanol, Ery: erythromycin (positive control), T20:Tween20, (-): no inhibitory activity, negative control did not show any activity, the number indicate zone of inhibition in millimeter

Among the extract fraction of *Z. scabra*, ethyl acetate showed the highest inhibition diameter against *S. aureus* (22.6±0.33mm) and acetone was the second with inhibition diameter of 19.3±0.33 against *S. aureus.*

The least inhibitory activity was exhibited by ethyl acetate against *S. boydii* with inhibition diameter of 6.3±0.33 whereas moderate inhibition zones were observed for extract fraction of acetone, methanol and ethanol against *E. coli.* The chloroform extract fraction was in effective against all test organisms.

S. aureus was the most susceptible test organism for ethyl acetate and acetone extract fraction. *E. coli* was the second susceptible test organism for all extract fractions except the chloroform.

S. boydii was the least susceptible test organism for ethyl acetate extract fraction and *S .typhi* was resistant to all extract fraction of *Z. scabra*. The inhibitory activity of the extract fractions of ethyl acetate and acetone were much more than that of erythromycin against *S. aureus* and *E.coli* respectively.

The ethyl acetate and acetone extract fraction showed relatively the same inhibitory activity with erythromycin against *S. boydii*. The drug erythromycin showed the highest inhibitory effect against *S. boydii* and *S .typh.*

6.2. Minimum Inhibitory Concentration (MIC)

The Minimum Inhibitory Concentration assay was also employed to evaluate the effectiveness of the extracts to inhibit the growth of the bacterial test organisms. The extracts of the two medicinal plants were subjected to the concentrations ranging from 0.195mg/ml up to 400mg/ml and the results were recorded on Table 3.

Table 3.Minimum Inhibitory Concentration (MIC) of the extracts of the leaves of *C. ficifolius* and *Z. scabra* against bacterial test organism in mg/ml

Bacteria spp.	Extracts	0.781	1.56	3.125l	6.25	12.5	25
E. coli	EtOAc [b]	+	*				
	MeOH[b]	+	+	+	+	+	*
	MeOH[b]	+	+	*			
P.aeruginosa	EtOAc [b]	+	+	+	+	*	
	EtOAc [a]	+	*				
	Ace [a]	*					
	MeOH[a]	+	+	*			
	EtOH[a]	+	+	+	*		
S. boydii	Ace [a]	+	+	+	*		
	Ace [b]	+	+	+	+	+	*
	EtOAc [b]	+	+	+	+	+	*
	MeOH[a]	+	+	+	+	+	*
	EtOH[a]	+	+	+	+	*	
S .aurues	EtOAc [b]	*					
	Ace [b]	+	*				
	Ace [a]	+	+	+	*		

Note: - [a] for *C. ficifolius*, [b] for *Z. scabra*, + = growth of bacteria observed, Ace: acetone, *=for Minimum Inhibitory Concentration

Among the extract fraction of the leaves of *C. ficifolius*, acetone exhibited the lowest MIC (0.781mg/ml) against *P. aeruginosa.* The extract fraction of ethyl acetate showed an MIC of (1.56mg/ml) against *P. aeruginosa.*

The highest MIC was exhibited by methanol extract fraction (MIC of 25mg/ml) against *S. boydii*. *P. aeruginosa* was the most inhibited test organism with an MIC of 0.78 mg/ml of acetone extract fraction.

Of all extract fraction of *Z. scabra*, ethyl acetate extract showed the least MIC of (0.78mg/ml) against *S .aurues.* The extract fraction of acetone and ethyl acetate were the second that exhibited an MIC of (1.56mg/ml) against *S .aurues* and *E. coli* respectively.

S .aurues was the highly inhibited test organisms with an MIC of 0. 781 mg/ml of the ethyl acetate extract fraction. When *E. coli* was the second test organisms inhibited by an MIC of 1.56mg/ml of the ethyl acetate and acetone extract fraction.

The highest MIC was exhibited by acetone and ethyl acetate extract fraction with an MIC of 25mg/ml against *S. boydii*. Among all test organisms S. *boydii* was relatively resistance to both plant extract fraction that was inhibited by the highest MIC25mg/ml.

6.3 Anti fungal activity of *C. ficifolius* and *Z. scabra*

According to the data obtained from this investigation the chloroform, ethyl acetate, acetone, methanol, ethanol extracts of the leaves of *C. ficifolius* and the extracts of the leaves of *Z. scabra* excluding the ethanol extract did not show inhibitory effect against the two fungal test organisms (Botrytis and Fusarium*)*.

7. DISCUSION

The ethyl acetate and acetone extracts of the leaves of *C. ficifolius* showed sensitivity towards *P. aeruginosa* with inhibition zone of 14±0.577and 13±0.577mm at concentration of 400mg/ml respectively (Table1). The ethanol extracts of the leaves of *C. ficifolius* also showed significant antibacterial activity against *S. boydii* at concentration of 400mg/ml.

The extracts of the leaves of *C. ficifolius* showed inhibition diameter ranging from 9±0.577-14±0.577 at 400mg/ml for all test organisms except *E. coli* and *S. typhi* .The inhibitory activity of the extracts of the leaves of *C. ficifolius* was not as potent as the positive control (tetracycline) but, the ethanol and acetone extracts of the leaves of this plant species have showed inhibition zones of 11±0.577, 12±0.577 against *S. boydii* respectively (Table1).

The antibacterial activity of *C. anguri* was analyzed against six bacterial strains. The maximum antibacterial activities were observed in ethanol extract. Among the six bacterial organisms maximum suppression was observed in *E-coli* (4.6±1.4) when moderate inhibitory activity was shown against *P.aeruginosa* (3.8±0.06), *S.aureus* (2.8±0.13) and *S.typhi* (2.6±0.16) (Kumar and Kamaraj, 2010). When the present study contrasted with this study the antibacterial activity of the extracts of the leaves of *C. ficifolius* has shown relatively good inhibitory activity.

The antimicrobial activity of *C. ficifolius* was done for the first time but, many other plants have shown inhibitory activity. For example Pavithra and co-workers (2011) reported that the methanol extracts of *Mollugo cerviana* inhibited the growth of S. *aureus* and *E. coli* with zones of 7.33±0.57 to 11±1, while chloroform extracts were ineffective against these bacterial strains. The methanol extract of *Merremia tridentata* was found to have strong antibacterial activity against gram-positive strains *S. aureus* with inhibition zones of 11.33±0.57 and *P. aeruginosa* was the least susceptible test organism to these plant extracts. But in the present investigation the methanol extracts of *C. ficifolius* did

not show positive inhibition against S. *aureus* and *E. coli* in contrast to this the growth of *P. aeruginosa* and *S. boydii* was inhibited by the methanol extracts of *C. ficifolius.*

The methanol extract of the *M. cerviana* and *M. tridentata* was most effective in inhibiting the bacterial growth suggesting that polar solvent methanol was most successful in extracting secondary metabolites responsible for the antibacterial property than the non polar solvent chloroform contrast to this in the present study the extracts of the polar solvent ethyl acetate and acetone was active. Similar to the study conducted by (Pavithra *et al*., 2011) the extracts of the chloroform did not show inhibitory activity against all test organism

On the other hand, the extracts of the leaves of Z. *scabra*; the ethyl acetate extract exhibited the highest antibacterial effect followed by acetone extracts. Growth inhibition was recorded from all the test organisms excluding *S. typhi* at concentration of 400mg/ml of the extracts of the leaves of *Z. scabra* ranging from inhibition zones of 6.3±0.33 to 22.6±0.33mm (Table 2)

The most pronounced activity of inhibition zones was shown on the ethyl acetate extracts of the leaves of *Z. scabra* against *S. aureus* and acetone extracts of the leaves (inhibition zone of 19.3±0.33mm) against *S. aureus* at concentration of 400mg/ml. *E. coli* were the second sensitive bacteria while *P. aeruginosa* was the third sensitive test bacteria against the ethyl acetate extracts of the leaves of *Z. scabra* (Table 2).

The fourth sensitive bacteria were *S. boydii* for the acetone and ethyl acetate extract. Whereas; of all the test bacteria in this study *S.typhi*, was relatively found to be resistant to all extracts of the leaves of *Z. scabra* at the highest concentration tested in the present study.

The study conducted by (Dawit *et al.,* 2005) confirmed the antimicrobial activity of the methanol extract of the leaves of *Z. scabra* against *N. gonorrhoea* at concentration of 2000µg/ml and the classes of the compounds also identified as tannins, unsaturated sterol/triterpens and saponins. Similar to the studies reported by Dawit et *al.,* (2005) the

present study of the methanol extracts of the leaves of *Z. scabra* also showed inhibitory effect against *E. coli.*

In the present investigation the different test bacteria showed variations in their sensitivity and resistance to the different extracts of the leaves of *Z. scabra*. Among the solvent needed to extract the biologically active substances from this medicinal plants, ethyl acetate was the best; whereas acetone was the second. But, the amount of the extract that was obtained from acetone was much more than that of the ethyl acetate. This indicates that the extraction of medicinal plants with different solvents may show different result which based on the potential of the solvents used to extract the biologically active constituents (George *et al*., 2010).

The ethyl acetate extracts of the leaves of *Z. scabra* showed relatively the same antibacterial activity as the positive control against *S. aureus, E- coli,* and *S-boydii* and the methanol extracts of the leaves of this plant also showed the same result with the erythromycin against *E- coli* (Table 2). In the same ways the acetone extract of this plant showed inhibitory activity against *E-coli* and *S. boydii* but, the drug is more potent than the extract against *S.typhi* and *P. aeruginosa*

In the study of (Anand *et al*., 2004) the extracts of *Zehneria scabra* tubers were studied for their antibacterial activities against *E-coli*, *P-aeruginosa* and *S-typhi* using disc diffusion method. Aqueous, chloroform and ethanolic extracts were screened for the inhibitory activity and the results suggest that the enthanolic root extract had significant antibacterial activities against *E. coli*, and *P. aeruginosa*. Similarly in the current study the chloroform extract did not show inhibitory activity against all bacterial test organisms (Table 1 and 2)

In the present study *S. boydii* and *P. aeruginosa* were more susceptible for the methanol, ethyl acetate, acetone, and ethanol extracts of the leaves of *C.ficifolius* than *Z. scabra.* And conversely, *E. coli* and *S. aureus* gave higher inhibition zones for the test

concentrations of 400mg/ml of the ethyl acetate and acetone extracts of the leaves of *Z. scabra* than *C. ficifolus*. This shows that the extract of different plant with different or similar solvents show significantly different results. The ethyl acetate extracts of the leaves of Z. *scabra* were found to be more active on *S. aureus* than *S. typhi, S. boydii* and *P. aeruginosa*. In most test organisms, the extract of the leaves of *Z. scabra* was relatively effective as compared to the extracts of the leaves of other plant species based on the size of inhibition diameter on the tested bacteria species. Philip *et al*., (2009) reported that the ethyl acetate extract of the leaves of *C.aeruginosa* showed inhibition diameter of 7.8mm and 6.7mm against *P. aeruginosa* and *S. aureus* at concentration of 500 mg/ml respectively.

S. typhi was the most resistant test bacterium that was not inhibited at concentration of 400mg/ml of both plant extracts of the present investigation. The acetone extracts of the leaves of *C. ficifolius* showed positive inhibition against *P. aeruginosa, S.boydii* and *S. aureus*. Whereas, the ethyl acetate extracts of the leaves of Z. *scabra* showed inhibitory effect against *E. coli, P. aeruginosa, S. boydii and S. aureus*. When the present study compared with the studies conducted by (Philip *et al*., 2009) the antibacterial activity of the presently investigated medicinal plants showed relatively better results.

The differences between the antibacterial activity of the two medicinal plant species show variations not only among different chemical extraction, but also among different species of plants; and in inherent resistance of the tested bacteria species (Nayan *et al*., 2011).

The present phyotochemical study of *C. ficifolius* and *Z. scabra* are similar to those plant extracts that have been reported to possess strong antimicrobial activity against gram positive, and gram negative bacteria. It is likely that the presence of the same types of compounds may have contributed to the antimicrobial activity of this plant species (Baldemir *et al*., 2007).

The plant extracts which demonstrated relatively good results were selected for MIC. *S. aureus, E. coli, P. auroginosa* and *S.boydii* were found to be susceptible to most of the extracts tested in this study, only the chloroform extracts of the two selected medicinal

plants *C. ficifolius* and *Z. scabra* were not active, were not considered for MIC. *S. boydii (*ethanol extract*)* and *P. aeruginosa (*acetone extract*)* were inhibited at MIC of 12.5mg/ml and 0.781mg/ml by the extracts of the leaves of *C. ficifolius* respectively (Table3).

The Acetone and ethyl acetate extracts of the leaves of *Z. scabra* showed best activity against *S. aureus* exhibited an MIC of 1.56mg/ml and 0.781mg/ml respectively (Table3). All taken together the extracts of the leaves of *Z. scabra* indicates relatively lower MIC values to inhibit *S. aureus* and *E. coli* than *C. ficifolius* leaves extracts. On the other hand the acetone extracts of the leaves of *C. ficifolius* showed an MIC of 0.781mg/ml against *P. aeruginosa.*

The chloroform and methanol extracts of *Delonix elata* inhibited the growth of gram-positive strains *S. aureus* and gram-negative strains *E. coli* with MIC ranging from 0.78 to 3.125 mg/ml and 12.5 to 25 mg/ml, respectively. However, these extracts did not exert any inhibitory activity on *P. aeruginosa* (Pavithra *et al*., 2011).

The acetone extract and the ethanol extract of *Alpinia nigra* showed interesting antibacterial activity against *S. aureus* with a MIC = 1.32 mg/ml. Furthermore, the water extract of *Muntingia calaiura* was also active against *S. aureus* with a MIC = 1.32 mg/ml (Chantana *et al*., 2005). *Alpinia nigra* is one of the most herbal remedies and it used in Thai traditional medicine for stomachicum, gastric diseases, antibacterial, antifungal and antioxidant activities (Habsah *et al*., 2000). The inhibitory activity of the plant extracts against both gram-positive and gram-negative bacteria may be an indication of the presence of broad spectrum antibiotic compounds.

Naser and Kamel, (2004) reported that the growth of *P. aeruginosa* was remarkably inhibited by the ethanol extract of *S. aromaticum* (MIC 0.781mg/ml) it seems very likely, therefore that the antibacterial compound extracted from *S. aromaticum* may serve as a source to develop antibacterial agent against this multi-drug resistant bacteria. In the same way the present study also reported that the acetone extracts of the leaves of C. *ficifolius* inhibited the growth of *P. aeruginosa* at MIC 0.781mg/ml.

Naser and Kamel, (2004) also showed that the ethanol extracts of the leaves of *R. officinalis* and its combination with *T. vulgaris* leaves had strong inhibitory effects against *P. aeruginosa, S. aureus* and *E. coli* with an MIC of 0.781mg/ml indicating that these plants had significant antibacterial activity as compared to the extracts of the leaves of *C. ficifolius* and *Z. scabra*.

In this study *S. aureus* and *P. aeruginosa* were the only bacterial species that was inhibited by the acetone extracts of the leaves of *C. ficifolius* and the ethyl acetate extracts of the leaves of *Z. scabra*. But in the previous studies the mixture of both *R. officinalis* and *T. vulgaris* showed inhibitory effect against *E. coli, S. aureus* and *P. aeruginosa* at an MIC of 0.781mg/ml.

Besides their antibacterial activity the antifungal activity of *C. ficifolius* and *Z. scabra* also employed but, negative result was observed. The positive control Ketoconazole showed the most pronounced activity on the tested fungus *Botrytis* and *Fusarium* species.

8. **CONCLUSION**

With the exception of the chloroform extracts the other solvent extracts' showed different levels of antibacterial activity on the tested bacterial test organisms. Some of these extracts were more effective than the others.

The results of this study provide an insight into the anti microbial properties of the extracts of C. *ficifolius* and *Z. scabra*. As well as it created an opportunity for selection of bioactive extracts for initial fractionation and further studies of these two medicinal plants in the antibacterial assays.

This *in vitro* study demonstrated that these two folklore medicinal plants have good potential. From the present study the chance to find antimicrobial activity was more apparent in the ethyl acetate and acetone extracts of the leaves of *C. ficifolius* and *Z. scabra.*

The acetone extract of the leaves of *C. ficifolius* and the ethyl acetate extract of the leaves of Z. *scabra* presented the lowest MIC value and these plants could be a source of new antibiotic compounds after further detailed study.

9. RECOMMENDATION

These medicinal plants may have synergistic effect to inhibit micro-organisms. Therefore, further investigation is needed to evaluate the antimicrobial activity of the integrated medicinal plants.

It can be recommended that further work is needed to isolate the secondary metabolites from the extracts of *C. ficifolius* and *Z. scabra* in order to test specific antimicrobial activity.

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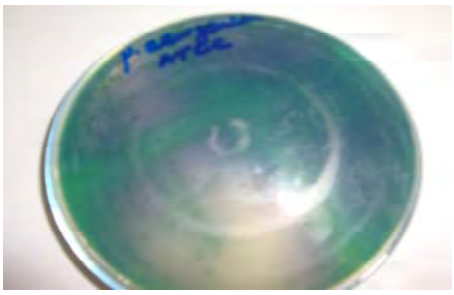
ANNEX

Annex1.*Zehneriascabra* (leaves)

Annex 2. *Cucumis ficifolius*(leaves)

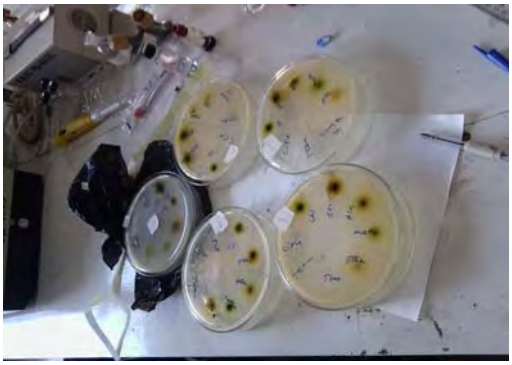
Annex 3. Sample collection

Annex 4. Extraction

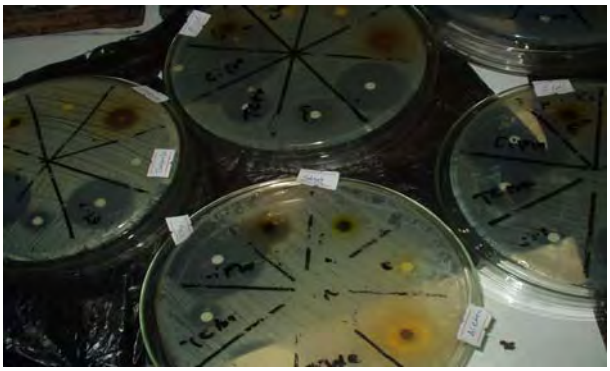


Annex 5.Pure culture

Annex 6. Observation of results



Annex 7. Antibacterial activity of *C. ficifolius*



Annex 8. Antibacterial activity of *Z. scabra*

Annex 9. Determination of Minimum Inhibitory Concentration

DECLARATION

I, the undersigned, declare that this thesis is my original work. It has not been presented in others University, colleges or institutions, seeking for similar degree or other purposes. All sources of materials used for the thesis have been totally acknowledged

Name: Tegenu Gelana

Signature: ----------

Date----------

This work has been done under my advisor

Professor Yalemtsehay Mekonnen (PhD)

Signature: ----------

Date----------