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Ethnobotanical and associated antibacterial activities of selected medicinal plant leaf extracts against human pathogenic bacteria

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ETHNOBOTANICAL AND ASSOCIATED ANTIBACTERIAL ACTIVITIES OF SELECTED MEDICINAL PLANT LEAF EXTRACTS AGAINST HUMAN PATHOGENIC BACTERIA

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A Thesis Submitted to the Department of Biology in Partial Fulfillment of the Requirements for the Degree of Master of Science in Biology (Botanical Sciences)

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As thesis research advisor, I hereby certify that I have read and evaluated this thesis prepared, under my guidance, by Samuel Fentie entitled **"Ethnobotanical and associated antibacterial activities of selected medicinal plant leaf extracts against human pathogenic bacteria"**. I recommend that it be submitted as fulfilling the Thesis requirement.

Getahun Yemata (Ph.D., Assoc. Professor) _____ _____

Advisor Signature Date

As member of the Boards of Examiners of the MSc Thesis Open Defense Examination, we certify that we have read, evaluated the Thesis prepared by Samuel Fentie and examined the candidate. We recommended that the Thesis be accepted as fulfilling the Thesis requirement for the Degree of Master of Science in Biology (Botany).

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Internal Examiner	Signature	Date
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External Examiner	Signature	Date

Declaration

This work is the result of my own investigation. All sources of materials used for the thesis have been duly acknowledged.

Samuel Fentie

Signature_____

Date_____

List of acronyms and abbreviations

ANOVA	Analysis of variance
APHI	Amhara Public Health Institute
CLSI	Clinical and Laboratory Standards Institute
EUCAST	European Committee for Antimicrobial Susceptibility Testing
FAO	Food and Agriculture Organization
MBC	Minimum Bactericidal Concentration
MIC	Minimum Inhibitory Concentration
WHO	World Health Organization

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Abstract

Plant based traditional medicine plays an essential role in human and animal medication and a significant number of world population rely on traditional medicines for their primary health care. However, scientific evaluation and confirmation of traditionally used medicinal plants is scarce. Thus, the present study was aimed to evaluate the antibacterial activity of four medicinal plants namely *Buddleja polystachya*, *Osyris quadripartita*, C*lausena anisata* and *Premna schimperi* against pathogenic bacteria *Staphylococcus aurous, Staphylococcus epidermises, Escherichia coli* and *Klebsiella pneumonia*. The selected medicinal plants were collected from the field and the bioactive ingredients of plant leaf samples were extracted using maceration method. The antibacterial activity of leaf extracts was evaluated by disc diffusion method. The minimum inhibitory concentrations (MIC) and minimum bactericidal concentration (MBC) were determined by agar dilution method. The phytochemical analysis revealed the presence of alkaloid, flavonoid, phenolic compounds, saponins, tanins and terpenoids. The extracts of each species showed antibacterial activity and there is significance difference among medicinal plant extracts. The extracts of *Buddleja polystachya* exhibited the highest antibacterial activity at 100mg/ml followed by *Osyris quadripartita* against all the tested bacteria. The MIC and MBC values ranged from 25-100mg/ml. *Buddleja polystachya* and *Osyris quadripartita* showed the lowest MIC and MBC values against *Klebsiella pneumonia.* The medicinal plant *Buddleja polystachya* was more potent against each human pathogen. It is recommended that further research in a similar manner be done on and unexplored parts of the studied plants.

Keywords: Antibacterial Activity, Medicinal Plant, Minimum Bactericidal Concentration Minimum Inhibitory Concentrations, Phytochemical analysis

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1. INTRODUCTION

1.1. Background of the study

Medicinal plants are important antimicrobial agents used in different parts of the world. Plants produce a diverse range of bioactive molecules, making them rich source of different types of medicines. Plant based traditional medicine plays an essential role in human and animal medication and a significant number of the world population rely on traditional medicine for their primary health care (Owolabi *et al.,* 2007). Therefore, plants are valuable source of secondary metabolites, which are used as pharmaceuticals, flavours, fragrances, colors, biopesticides and food additives. Indeed, plants are a natural gift to human beings to protect their health (Al-snafi, 2013). Many communities in Asia, Africa and South America used medicinal plants for the treatment of diseases for centuries. In spite of the great advances achieved in modern medicine, thousands of rural communities in developing countries still depend on folklore medicine to cure diseases mainly because of economic and cultural factors (Kamatenesi and Oryem-Origa, 2007).

However, plants with high potency safety and efficacy to develop antimicrobial drugs should be investigated (Khulbe and Sati, 2009). In order to do this, over the past few decades, the use of plants to produce naturally occurring chemicals of commercial interest has gained more attention (Hussain *et al.,* 2012). Moreover, the emergence of anti-microbial resistance and limited therapeutic efficacy of many of the available drugs necessitated search for potent antibacterial drugs with new modes of action. Medicinal plants are potential source of novel antimicrobial agents (Bacha Ketema *et al.,* 2016). According to Abebe Dawit (2011), for over 80% of the population, traditional remedies constitute the most significant and occasionally the only source of therapies.

Ethiopia is a country known for its rich plant biodiversity and traditional use of plant-based drugs for curing or treating many human and animal diseases. Most of the evidence of plant use as medicine is most visibly seen in the rural people and indigenous community. In Ethiopia alone, 1000 plant species are estimated to be in use for traditional medication (Tesema Tesfaye *et al.,* 2002). WHO estimated that the majority of the population in developing countries, about

90% of African population, relies on traditional medicinal plants for their healthcare (WHO, 2011).

Today, infectious diseases are responsible for most of the mortality particularly in third-world countries. The increasing global trend of resistance to drugs among Gram-positive and Gram-negative bacteria poses major challenges (Bassetti *et al.,* 2011). Multidrug resistant bacteria are resistant to several different antibiotics. The management of multi-drug resistant bacterial strains are difficult because treatment options are limited and beyond the reach of healthcare systems (Miyakis *et al.,* 2011). Therefore, there is an urgent need to explore new effective drugs for the treatment of infectious diseases (Aiyegoro *et al.,* 2011). Pathogen growth and development can be prevented in part by using plant products and their active ingredients. Bark, leaves, flowers, roots, fruits, and seeds are only a few examples of plant parts from which natural substances generated from plants can be obtained (Cragg and Newman, 2001). These plant products possess various secondary metabolites with significant inhibitory effect against the growth of pathogens; hence, the plant and their products should be utilized to combat pathogens. The goal of antimicrobial research is to find and create new antibacterial agents. The presence of phytochemicals in the plant extracts makes them crucial antibacterial agents (Cristina Abreu *et al.,* 2011). Therefore, the aim of this study was to evaluate the antibacterial activities of selected medicinal plant leaf extracts *in vitro* against some human pathogens. The research aids in facilitating the development of modern pharmaceutical drag against human infections.

1.2. Statement of the problem

The emerging of antibiotic resistance has brought most of the serious public health problems. It is therefore, important to look for more effective, safer and less toxic alternate options of treatment. Nearly eighty percent of Ethiopians rely on traditional medicinal plants to treat various types of infectious diseases causing agents such as bacteria, fungi and protozoa (Hiwot Ayalew *et al*., 2022). So, the use of plants for medicinal purpose requires preliminary study of extracts for their inhibitory effects on test organisms. The present study, thus, aims to evaluate the antibacterial activity of widely used medicinal plants *Buddleja polystachya*, *Osyris quadripartita*, C*lausena anisata* and *Premna schimperi* against four pathogenic bacteria namely *S. aurous, S. epidermises, E. coli* and *K. pneumonia*.

1.3. Objectives of the study

1.3.1. General objective

- The aim of this study was to assess the trends of ethnobotanical research and associated antibacterial activities of selected medicinal plants against human pathogenic bacteria.

1.3.2. Specific objectives

- To assess the trends of ethnobotanical studies and the potential efficacy of the selected medicinal plants against human pathogens.
- To evaluate the antibacterial activities of selected medicinal plant leaf extracts against some human pathogenic bacteria.
- To qualitatively analyze the potential phytochemicals present in the leaf extracts of the selected medicinal plants.

1.4. Research questions

- ✓ What are the trends in ethnobotanical research on chosen plants used as remedies for human pathogens?
- ✓ How effective is the antibacterial activity of the selected medicinal plants' methanol extracts against human infections?
- ✓ What are the major phytochemicals in the leaf extracts of the selected medicinal plants?

1.5. Significance of the study

The main aim of this study is to evaluate the antibacterial activities of some selected medicinal plants against human pathogens. So, a result of the study is helpful for identification of medicinal plants to use themselves as raw materials in development of drugs in pharmaceutical industries of Ethiopia. The result of this study is encouraging other researcher to investigate the antimicrobial potential of plant species. In addition to this once completed the thesis paper is used as reference for upcoming graduate students while they are compiling their thesis works.

2. LITERATURE REVIEW

2.1. Medicinal plant

A medicinal plant is any plant which, in one or more of its organs, contains substances that can be used for therapeutic purposes or which are precursors for the synthesis of useful drugs. Medicinal plants are important, fundamental and most useful to almost all life on the earth; of which, one of the most significant use is the phytomedicinal role. Plants have contributed to modern medicine, through providing ingredients for drugs or having played central role in drug discovery. Some drugs have botanical origin, extracted from plants. Traditional medicinal plants have remained as the most affordable and easily accessible sources of treatment in the primary healthcare system of resource poor communities and the local therapy is the only means of medical treatment for such communities. Like in other developing and developed countries, the majority of the population lacks access to modern health care services, which makes them unaffordable (Haile Yineger *et al*., 2008). Due to this issue, as well as the country's fast growing population and cultural opposition to the use of modern medications, the majority of Ethiopians rely on traditional remedies, primarily those made from plants, to treat a wide range of illnesses (Dawit Abebe, 2001).

The use of medicinal plants is commonplace in the lives of many people, providing them with support for their health, financial security, and means of subsistence (Belay Beyene *et al*.,2016). Plants have been indispensable and the most important sources of both preventive and curative traditional preparation for human beings and livestock since time immemorial. By their capacity of photosynthesis, plants form the basis of the biological food web and producing oxygen which is the key for our lives and they are balancing the gases of our environment. Plants are also recycling essential nutrients, establishing soils and soil fertility, protecting areas of water catchments. Through the process of transpiration, they help to regulate rainfall and maintain biological and climatic balances. All of these advantages of plants have a direct or indirect relationship to health care (Kelbessa Urga *et al*., 2004). As a result, botany and health care have become inseparable spheres of human activity. This is because traditional health care systems place a high value on a variety of plant products.

In addition to playing a crucial part in the supply of healthcare for a large portion of the world's population, medicinal plants are also significant economically. These plants are frequently traded in a variety of ways across nations (Payyappallimana, U. 2010). Currently large number of medicinal plants has been found their way as raw materials of modern bio-pharmaceutical industries (Rai *et al.,* 2000). Ethiopia is endowed with a number of economically useful medicinal plants. But Ethiopia is not known in developing the law for importing and exporting medicinal plants legally. The country exports only some agricultural products such as coffee, cotton, niger seed, linseed, castor seed and *catha edulis* as a means of getting foreign currency.

Traditional systems also address physiological demands in a way that contemporary medicine does not, and they are more culturally acceptable (Fassil Kibebew, 2001). In fact, there is growing agreement that the most effective way to guarantee accessible and long-lasting health care for underprivileged people throughout Africa is to revitalise and promote traditional health practises alongside contemporary health services (Cunnigham, 1993). Traditional medicine can save foreign exchange. Moreover, the development of medicinal plants in primary health care not only will save the foreign exchange but also will aid in conserving our national heritage (Abiot Birhanu *et al.,* 2006).

Due to the traditional medicinal herbs' socio-cultural acceptability, accessibility, cost, and biological advantages, there is a significant amount of use and interest in them in Ethiopia. In other words, because traditional medicine is deeply ingrained in every region of the nation, people frequently rely on it as an effective and affordable form of alternative health care (Mwambazi, 1996;). The majority of Ethiopians rely on medicinal plants to treat themselves and their animals since they are frequently more accessible than contemporary medications that are administered in far-off medical facilities (Alemayehu Kefalew *et al,*. 2015)

2.2. Use of Medicinal plant

As the use of particular medicinal plants for treating particular ailments came to light, the use of these plants gradually moved away from the empiric framework and towards the foundation of explicatory facts. However, the use of natural pharmaceuticals is once again a hot topic due to the decreasing efficacy of synthetic drugs and the rising contraindications to their use(Petrovska 2012).

Direct food for humans medicinal plants, like other plants, enter into the daily human food directly, such as pimples rich in carbohydrates and vitamins such as beans, lentils, chickpeas, beans, and other grains rich in proteins, as well as vegetables that are eaten directly such as basil and mint Coast, parsley, spinach, as well as figs, olives, pomegranate and apples, all of these medicinal plants are entered as a direct food for humans (Samiha 2022).By acting as a springboard for the creation of innovative pharmaceuticals, medicinal plants play a crucial role in the growth and advancement of contemporary research. (Wright, 2005). Various modern drugs were extracted from medicinal plants through the use of plant material as indigenous cure in folklore or traditional system of medicine (Verma and Singh, 2008).

Estimate that, outside of Western nations, between 75 and 90 percent of the world's rural population uses herbal treatment exclusively. According to the WHO, traditional medicine is the primary source of healthcare for 70–80% of people in underdeveloped nations, including Africa (Cunningham, 1994). The inability of people to acquire expensive contemporary pharmaceuticals is not the only factor contributing to this; traditional methods are also more culturally acceptable and address psychological requirements in a way that modern medicine does not (Brown, 1994). A better public health service will be offered with the merging of traditional medicine with the modern health system.

The number of higher plant species (angiosperms and gymnosperms on this planet is estimated between 250,000-500,000 (Mahesh and Satish, 2008)**.** Only 6% of them have reportedly undergone screening for biologic activity, while 15% reportedly underwent phytochemical evaluation. Estimates show that about 25,000 to 75,000 species of higher plants have been used in traditional medicine (Farnsworth, 1980). Evidently, traditional knowledge of medicinal plants is important in development of new modern drugs. Currentlythere are more drugs (e.g.

aspirin from *Ulmaria* (Rosaceae), Quinine from *Cinchona pubescens* (Rubiaceae), Morphine from *Papaver somniferum* (Papaveraceae) and ephedrine from *Ephedra sinica*) prescribed in North America and Europe. In the industrialised world as a whole, about 80 medications are given; the majority of them were found using data from ethnobotanical research (Farnsworth ,2007).

Ethiopia is a rich source of medicinal plants however, the knowledge and use of plant is an integral part of many ethnic rural cultures, the extent of which has not yet been studied in depth (Abbink, 1995). Perhaps the best-known species is *Phytolacac dodecandra*. In United States of America, plant derived anti-cancer drugs save at least 30,000 lives per year (Roberson, 2008) and even drug for deadly diseases like HIV/AIDS could be discovered by ethnobotanical approaches, by taking indigenous knowledge as a base.

2.3. Role of plant extracts as control agent of pathogenic bacteria

There is increased interest in the quest for new medications made from natural resources because medicinal plants are essential in the treatment, prevention, and promotion of many diseases (Ovais *et al*., 2018). Because they contain a variety of bioactive chemical components (phytochemicals) that can function as antimicrobial agents, medicinal plants present a significant possibility.

Moringa seeds broaden the range of application of Moringa seeds as a water purifier and water treatment agent by protecting against diarrheal agents, Escherichia coli, and Shigella flexneri. Additionally, this suggests that moringa seeds may help treat various gastro-intestinal and wound infections brought on by gram-negative bacteria. **(**Lar *et al*., 2011).

Essential oils from the leaves of two species of Eucalyptus (globulus and camaldulensis) were tested for their antibacterial effects on Escherichia coli and Staphylococcus aurous. The leaf essential oils of the two species showed an excellent inhibitory effect on *Staphylococcus aurous* than that of *Escherichia coli* (Bachir *et al.,* 2008).

Datura stramonium extracts exhibited significant zone of inhibition and good antimicrobial activity against the majority of the selected strains of microorganisms, such as *Staphylococcus aurous, Proteus vulgaris, Pseudomonas aeruginosa, Escherichia coli, Aspergillusniger* and *Fusarium species* (Reddy, 2009). *Croton macrostachyus* is a multipurpose plant that has been

used for various remedies as constituents of traditional medicine. Up to now the treatment of infectious diseases caused by bacteria is mainly depend on the use of commercial antibiotics. Currently, due to the emergence of multi-drug resistant strains and widely distributed infectious diseases caused by bacteria unable to treat by the existing drugs (Sendeku *et al*., 2015).

Numerous studies have found that medicinal plants are particularly successful in treating infectious disorders. The plants have a lot of potential as a source of cutting-edge antibacterial compounds. They are inexpensive, easily accessible, and nearly without any negative side effects. Phytochemicals, which are plant derivative substances, have even been used to treat numerous infectious diseases and have displayed intriguing antibacterial efficacy against a number of human infections (Bibi *et al* .,2011).

2.4. Application of Traditional Medicinal Plants

In communities with little resources, traditional medicine has continued to be the most accessible and reasonably priced form of therapy. The local population has a long history of using medicinal plants in traditional ways. Plants have long been used for therapeutic purposes. According to texts, the usage of plants for therapeutic purposes dates back to between 4000 and 5000 B.C. The Chinese were the first to use natural herbal concoctions as medicines. In India earliest references of use of plants as medicine appear in Rig-Veda, which is said to be written between 1600 - 3500 B.C. Later, the ancient physicians (an indigenous school of medicine) examined the characteristics and therapeutic applications of medicinal plants in-depth and empirically recorded their findings, which forms the fundamental basis of ancient medical knowledge in India (Hosseinzadeh *et al* ,.2015).

Medicinal plant is an important element of indigenous medical systems in all over the world. The ethno botany provides a rich resource for natural drug research and development (Farnsworth, 1990). 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). 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).

Historically, medicinal plants were employed to treat a variety of illnesses. Different ailments have been treated with plant parts including leaves, stems, roots, bark, twigs, tubers, bulbs, exudates, flowers, and fruits. According to van wyk and Gericke (2000), these plant elements are utilised to create enemas, extracts, infusions, teas, snuffs, and many more forms that are delivered in various ways. Many modern medicines are created indirectly from medicinal plants, which are sources of novel pharmaceuticals. There are more than 250,000 species of flowering plants, according to estimates. Understanding plant toxicity and defending people and animals against natural poisons are two benefits of studying medicinal plants.

According to (Regassa Tena 2008), a local farmer may have only gathered leaves or root parts from regional medicinal plants found close to their homestead. This may have happened somewhere in the rural hinterland of the Ethiopian rural communities. A mother might be creating a traditional plant remedy for a skin issue, relieving stomach cramps, healing from an infection of the respiratory tract, protecting against the evil eye, and other ailments in a local hamlet.

Different plant components have been used in Ethiopia as a source of traditional medicine since antiquity to treat a variety of illnesses and human miseries. Traditional medicine has been practised and used for a very long time, and as a result, it is now deeply ingrained in Ethiopian culture. Ethiopia's traditional medical system is diverse and influenced by the nation's varied environment, the sociocultural circumstances of the various ethnic groups, as well as historical events connected to migration, the introduction of foreign culture, and religion (Mersha Ashagre Eshete and Ermias Lulekal Molla 2021).

Traditional medical practitioners treat people and most of the health services rendered by these practitioners are focused on communicable diseases among people. Proper management of traditional medicinal plant resources is essential, not only because of their value as a potential source of new drugs but due to reliance on traditional medicinal plants for health. Ethno botanical studies can indicate management problems of medicinal plants through interviews and market surveys and it gives solutions by promoting local traditions and customs that had conservation merits (Turner N, 2000).

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 Cephaelisipecacuanha and related species, has been used for many years as amoebicidal drug as 18 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 Hydrastiscanadensis are also examples of medicinal plants that have been used as sources of antibiotics.

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, Aframomummelegueta, Xylopiaaethiopica, Cryptolepissanguinolent and Chasmantheradependens 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, Hydrastiscanadensis 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).

2.5. Human pathogenic bacteria used in this study

2.5.1. Staphylococcus epidermises

When compared to the more pathogenic, coagulase-positive Staphylococcus aurous, Staphylococcus epidermidis is a ubiquitous and mostly un-harmful commensal of human skin (Becker *et al.,* 2014). Human skin is nutrient-poor, dehydrated, acidic, and has a high osmolarity. The relative abundance of bacteria at various skin areas has been determined thanks to shotgun metagenomic whole genome sequencing (Byrd *et al.,* 2018).

In places with sebaceous glands as well as dry and moist skin, Staphylococcus epidermidis is a dominant part of the microbiome (Liu *et al*., 2020). Growing evidence suggests that Staphylococcus epidermidis is not just a benign resident but also actively participates in the host immune system's regulation to support commensal survival and shape the skin's and the nose's microbiome development (PrabhuDas et al., 2011; Naik *et al*., 2012, 2015*).*

Staphylococcus epidermidis strains usually resist against several types of antibiotic classes such as tetracyclines, aminoglycosides, cephalosporins, fluoroquinolones, penicillins, and macrolides. Nowadays, resistant S. epidermidis has become a serious problem in hospitals (Chabi and Momtaz, 2019).

2.5.2. Staphylococcus aurous

Staphylococcus aurous is a Gram-positive, non-spore forming spherical bacterium that belongs to the Staphylococcus genus. There are 32 species and subspecies of the Staphylococcus genus. Almost of staphylococcal food poisoning is caused by S. aurous, which also produces staphylococcal enterotoxin (SE) (FDA, 2012). Human skin and wound infections caused by Staphylococcus aurous are prevalent, and a sizable fraction of the population also carries the germ as a commensal of the skin and nose. As a result, equipment and food handlers frequently incorporate it indirectly into meals.

Catalase and coagulase positivity are used to identify Staphylococcus aurous from other strains. It grows best between 7 to 480C and is mesophilic (Ebrahimi and Akhavan, 2009). According to Addis *et al*. (2011), growth is best at pH levels of 6-7, with lowest and maximum limits of 4.0 and 9.8–10.0, respectively

Numerous toxin-mediated diseases, including food poisoning, impetigo, toxic shock syndrome, and necrotizing pneumonia, are caused by Staphylococcus aurous. Eating contaminated food that includes Staphylococcal enterotoxins causes self-limiting Staphylococcal food poisoning. Staphylococcal food poisoning symptoms include nausea, vomiting, headaches, and, less frequently, diarrhoea (Tenover and Gorwitz, 2006).

2.5.3. 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).

Escherichia coli is a typical mesophile growing from 7-100C up to 500C with an optimum around 37°C, although there have been reports of some ETEC strains growing at temperatures as low as 40C. 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).

On the basis of various sugar fermentation and other biochemical tests, Escherichia coli can be distinguished from other Enterobacteriaceae members (Sakagami *et al*., 2007). The most well-known strain of coli is Escherichia coli, which is often the subject of laboratory research. It is known as the colon bacillus and is occasionally regarded as the dominant species in the human intestine. Being the most prevalent aerobic and non-fastidious bacterium in the gut accounts for its predominance in clinical specimens and illnesses (Paton and Paton, 1998).

Infections of the urinary tract, wounds, lungs, meninges, and septicemia have all been linked to Escherichia coli. Traveler's diarrhea and the uraemic syndrome have both been linked to specific Escherichia coli strains (Spicer, 2000; Mittal *et al.*, 2009).

2.5.4. ***Klebsiella pneumonia***

The well-known genera Salmonella and Escherichia are members of the Enterobacteriaceae family, which also contains Klebsiella pneumonia. Since Carl Friedländer first identified it as a cause of pneumonia in 1882, klebsiella pneumonia has been known to be a disease agent and is still one of the most prevalent nosocomial pathogens worldwide (Pendleton et al., 2013).

A mobile pool of virulence and antimicrobial resistance genes is accessible to virulent and multidrug-resistant Klebsiella pneumonia clones (Holt et al. 2015; Lam et al. 2018; MC Lam et al. 2018), allowing for the emergence of a multidrug, hypervirulent Klebsiella pneumonia clone that can infect healthy people with untreatable infections. Unfortunately, studies detailing the isolation of such strains have already been published (Zhang *et al*. 2016, Gu *et al*. 2018, and Yao *et al*. 2018).

The most prevalent gram-negative pathogen linked to a variety of infections, including meningitis, intra-abdominal infection, pneumonia, and pyogenic liver abscess (PLA), is klebsiella pneumonia, which is second only to Escherichia coli in prevalence (Anderson et al., 2014). Extended-spectrum -lactamases (ESBLs), which are produced by extended-spectrum

cephalosporin-resistant K. pneumonia, have dramatically increased in prevalence over the past few decades worldwide. In most of the world, K. pneumoniae is the pathogen most closely linked to the spread of ESBLs and other horizontally transmissible resistance genes.

3. MATERIALS AND METHODS

3.1. Review Methodology

The methodological approach in this review was based on the broader literature search and synthesis of peer-reviewed articles and empirical findings, extracted from international databases such as Web of Science, Scopus, Goggle Scholar, University repositories using the following search terms for English articles: "ethnobotanical studies", "medicinal plants", "*B.polystachya*", "*P.schimperi*", "*C. anisata*", and "*O. quadripartita*", "human pathogens", AND "Ethiopia". Studies that dealt with ethnobotanical studies of the selected medicinal plants were included, whereas studies that were not relevant to the desire outcome were not included. Out of the eligible studies that were screened, 23 publications were finally used to synthesize knowledge.

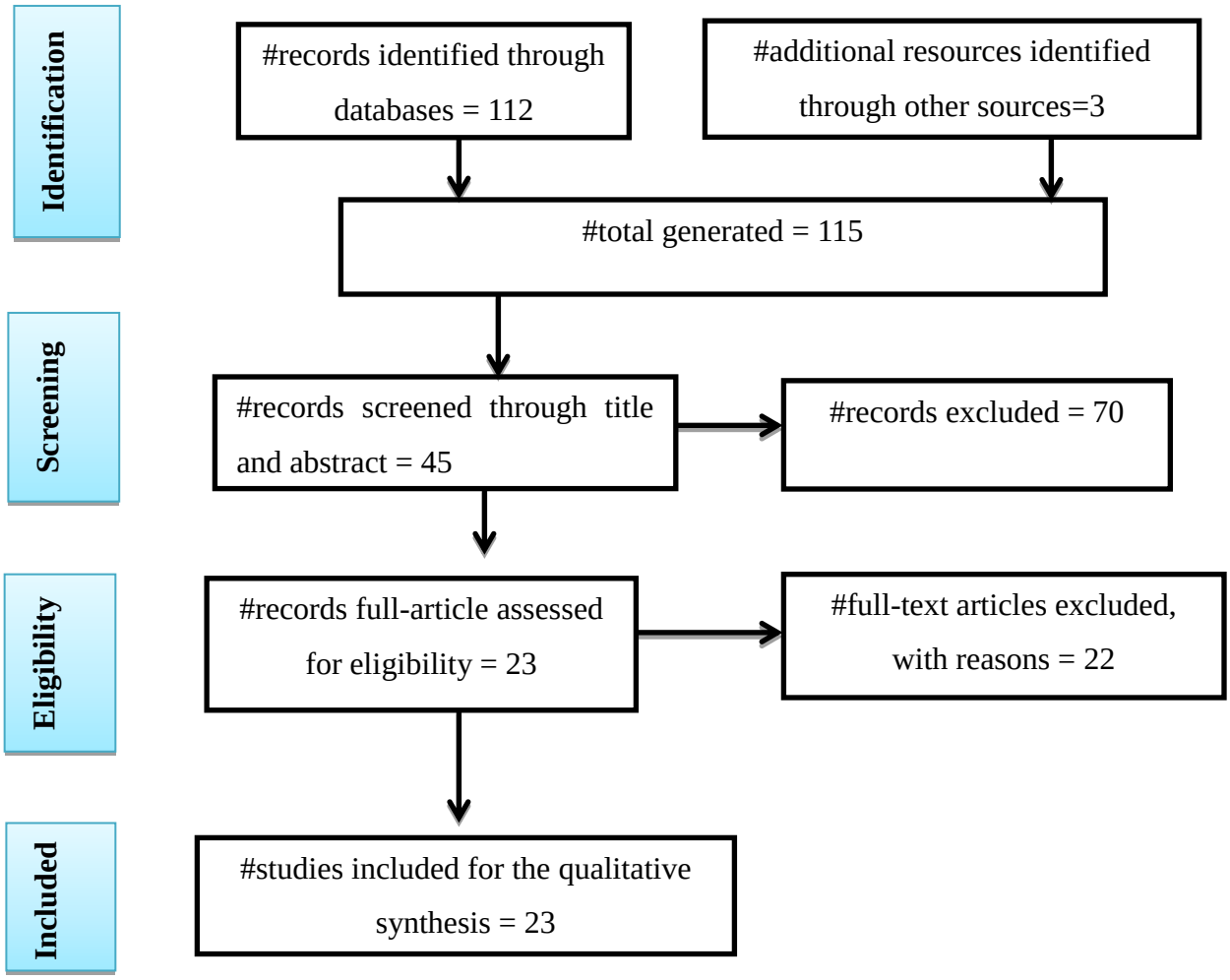

Figure 1 Flowchart for selecting eligible studies on the trends of ethnobotanical studies of the selected medicinal plant resources against human pathogens

3.2. Medicinal plant Sample collection

Fresh leaves of *B. polystachya*, *O. quadripartita*, C. *anisata* and *P. schimperi* were collected from November to December, 2022. Parts of plants were collected separately and transported in plastic bags to the laboratory for further processing. In the laboratory, the samples were washed under running tap water to remove dust particles and followed by rinsing with distilled water. They were placed on clean plastic and air dried at room temperature until their weight became constant. Then the dry samples were ground to fine powder using electric grinder and stored in sterile bottle at 4 °C for bioassay test using disc diffusion and MIC against test bacteria (Handa *et al.*, 2008).

3.3. Identification of medicinal plants

Sample specimens of the selected medicinal plants were taken to the biology department at Bahir Dar University, where they were identified with the assistance of experts in the area and based on flora of Ethiopia and Eritrea books.

3.4. Preparation of medicinal plants extraction

The bioactive ingredients of *B. polystachya, O. quadripartita,* C. *anisata* and *P. schimperi* plant leaf samples were extracted using maceration method (Tmušićet al., 2021). The absolute methanol solvent was used in the process. The leaf powder of each species and solvents were added into separate conical flasks in the ratio of 1:10 (for every g of leaf powder 10 ml of solvent was added). The flasks were tightly closed and placed on an orbital shaker at a speed of 150 rpm for three days at room temperature. The extracts of *B. polystachya , O. quadripartita*, C. *anisata* and *P. schimperi* were decanted with flasks in the presence of cheese cloth and cotton. The residue was squeezed to the flask and removed. The extracts were further filtered through Whatman no. 1 filter paper. Then, the solvent was removed using rotary evaporator. Dry extract of each species was stored at 4ºC for further investigation (Sukhdev *et al.*, 2008).

3.5. Determination of extraction yield

Percentage yield of *B. polystachya*, *O. quadripartita*, C. *anisata* and *P. schimperi* of leaf crude extracts were calculated as described by Joshi and Kaur (2013).

$$\text{Extract yield (\%)} = \frac{Dry\ weight\ of\ extract\ (gm)}{Powder\ weight\ of\ plant\ material\ used\ for\ extraction\ (gm)} * 100$$

3.6. Test organisms

All clinical isolates of two gram-positive bacteria (*Staphylococcus aurous and Staphylococcus epidermidis*) and two gram negative bacteria (*Escherichia coli and Kelbsela pneumoniae*) were obtained from Amhara Public Health Institute (APHI) Bahir Dar, Ethiopia. The test bacteria were cultured on Mueller-Hinton agar and store at 4°C until use (CLSI, 2012).

3.7. Preparation of media

The medium was prepared according to the manufacturer's instructions. About 38 g Mueller Hinton Agar was added to a flask containing 1000 ml of distilled water and gently heated until the medium is completely dissolved. The medium was sterilized by autoclaving at 121 °C for 15 minutes. After cooling to about 50°C, approximately 25 ml of the sterilized medium was aseptically poured into 90 mm diameter sterilized Petri-dish and allowed to solidify (CLSI, 2012).

3.8. Preparation of inoculum

The method was complete according to the procedures described before (CLSI, 2012). Accordingly, three to five colonies from pure cultures of each of the four selected bacterial species were transferred to 5ml of distilled water with the help of a sterile wire loop. The prepared culture was standardized to 0.5 McFarland turbidity standards using the spectrophotometer to obtain thedesired cell density of 1.5×10^8 (cells/ml).

3.9. Evaluation of antibacterial activities

3.9.1. Preparation of sterile discs

Whatman No.1 filter paper was punched into 6 mm disc form and sterilized. Each sterile disc was rinsed in each test concentration of the selected medicinal plant extracts for 24 hrs. After this period, the discs were taken out and dried under room temperature. Precautions were taken to prevent contamination of the discs during the process. Then they were stored at 4°C.

3.9.2. Evaluation of the antibacterial activity of extracts

Approximately 20 ml of sterilized Muller Hinton Agar was poured into sterile Petri-dish and allowed to solidify. Suspension of the study bacterial pathogens was prepared from a fresh culture and its optical density was adjusted to 0.5 McFarland turbidity standards. This was followed by inoculation with sterilized cotton swab. The discs prepared as mentioned above were kept over the inoculated agar plates using sterile forceps the plates were incubated for 24 hours at 37°C (Zaidan *et al.,* 2005). The inhibition zone was observed and measured in millimeter using a transparent meter or ruler. The potency of each test concentration was evaluated against the standard antibiotic (gentamycin) and negative control.

3.9.3. Determination of the minimum inhibitory concentration (MIC) of plant extracts

The minimum inhibitory concentration (MIC) of leaf extracts was determined using agar dilution method as described in EUCAST (2000). One ml of each test concentration of each crude extract was thoroughly mixed with 19ml of Muller Hinton agar medium and poured to Petri dishes. The medium was allowed to solidify at room temperature. The inoculum adjusted to turbidity of a 0.5 McFarland standard was inoculated at each Petri dish. The inoculated Petri dishes were incubated at 37^{o}C for 24hr. Parallel to this, Petri dishes without extract were used as controls and the results were compared against these controls.

3.9.4. Determination of the minimum bactericidal concentration (MBC) of plant extracts

Minimum bactericidal concentration (MBC) of leaf extracts was determined as described in Ninga *et al*. (2014). Muller Hilton agar medium was prepared and autoclaved at 121^{o}C for 15 minutes. The medium was poured in to sterile Petri-dishes and allowed to cool and solidify. The contents of the MIC Petri-dishes that did not show growth or showed growth less than 80% of the controls were sub-cultured on to the prepared Petri-dishes. After that, the Petri-dishes were incubated for 24 hours at 37°C. The minimal bactericidal concentration (MBC) is represented by the Petri dishes without any growth after that.

3.10. Qualitative Phytochemical Testing

Phytochemicals are chemical compounds that occur naturally in plants. The extraction, screening, and identification of the medicinally active compounds present in plants referred to as phytochemical screening. According to Ahmad *et al*. (2006), phytochemical screens of flavonoids, alkaloids, carbohydrates, proteins, tannins, terpenes, and phenolic compounds some of the bioactive components that can be obtained from plants were made.

3.10.1. Test for Phenols

Half g of each of the crude extracts was placed in a separate test tube and treated with a few drops of 2% FeCl3; the presence of phenols was detected by bluish green or black coloration (Harborne, 1998).

3.10.2. Test for Terpenoids

Two millilitres of the plant extract were placed in a test tube, treated with one millilitre of chloroform, and then 1.5 millilitres of concentrated H_2SO_4 were applied along the tube's edges. The interface's reddish brown hue is seen as a sign that terpenoids are present.

3.10.3. Test for Tannins

In a test tube, 10 mL of water were used to boil 0.5 g of each crude extract before it was filtered. To give the color of brownish green or blue-black, a few drops of 0.1% ferric chloride were applied (Ayoola et al., 2008).

3.10.4. Test for flavonoids

Half g of crude extract was added, along with 10 millilitres of ethyl acetate, and boiled in a steam bath for three minutes. A yellow colouring indicates a positive test after the mixture was filtered and 4 mL of the filtrate was agitated with 1 mL of diluted ammonia solution (Sofowora, 1993).

3.10.5. Test for saponins

The "froth test" was used to determine whether saponins were present. Each extract was taken in a graduated cylinder, diluted with distilled water to a volume of 20ml, and agitated for 15 minutes. The presence of saponins was assumed to exist when a 1 cm layer of foam formed (Pandey and Tripathi.2014).

3.10.6. Test for alkaloids

Mayer's technique was able to identify the presence of alkaloids in the sample. 100ml of distilled water was used to dissolve 1.3g of mercuric chloride and 5g of potassium iodide to create Mayer's reagent. Separate plant extracts (1 ml) were added to a test solution in a test tube along with 1 ml of Mayer's reagent, and the test tube was gently shaken to ensure proper mixing. It was assumed that the presence of yellow precipitate indicated the presence of alkaloids (Pandey and Tripathi 2014).

3.11. Data analysis

General Linear Model of Univarate analysis was used to manage the laboratory data using plant extracts, pathogens, and test concentrations as predictor variables and the zone of inhibition as a response variable at $p<0.05$ significance level. Post Hoc test (LSD) was also used to compare the mean difference among the treatments (bacterial pathogen and extract concentrations). Also descriptive statistics was employed to compare the factors with their corresponding mean. All the generated data was managed by using statistical Packages for Social Sciences (SPSS v.24)

4. RESULTS

4.1. Ethno-medicinal studies of the selected medicinal plants in Ethiopia

4.1.1. Plant parts used in the illegible ethnobotanical studies

In this study, a total of 23 original ethno-medicinal studies were included, comprising the published data that solely to explore the traditional medicinal practices pertaining to the selected medicinal plants. The findings of the researches indicate that the leaves of *B. polystachya* 20(74.1%), *P. schimperi* 10 (62.5%), *C. anisata* 13 (56.5%), and *O. quadripartita* 3(37.5%) are the most frequently utilized plants as an important ingredient in formulating traditional remedies for treating various human ailments (Figure 2; supplementary Table 1). Roots of *B. polystachya* 3 (11.1%), *P. schimperi* 5(31.2%), *C. anisata* 7(30.4%), and *O. quadripartita* 3(37.5%) were identified as the next important plant parts for treating human pathogens in the included studies (Figure 2; supplementary Table 1). On the other hand, seed, barks and fruits of these plants were mentioned less frequently in the studies and were considered to be of lower importance compared to the leaves and roots.

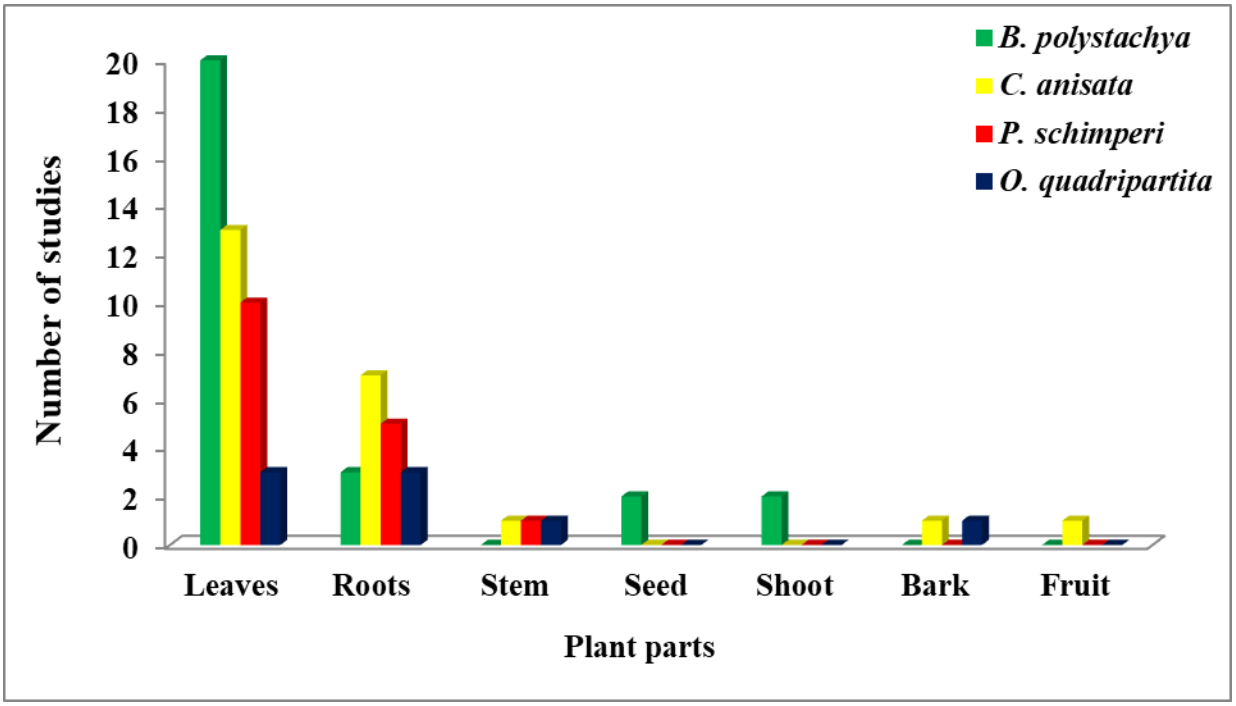

Figure 2 Ethnobotanical studies related to plant parts of the selected medicinal plants used for treating different human ailments

4.1.2. Mode/method of preparation

The studies employed various methods in preparing traditional medicinal remedies from selected medicinal plants against human pathogens. These herbal formulas were made through a single method (12), and some in a combination of two methods (10). Only three studies documented multiple methods to prepare these remedies for human health issues for each of the selected traditional medicinal plants. The study findings indicated that the most commonly used techniques for *B. polystachya* 14(60.9%), *C. anisata* 7(46.7%), *P. schimperi* 7(46.7%), and *O. quadripartite* 3(50%) were pounding and crashing, followed by direct application and squeezing/juice (Fig 3; supplementary Table 1).

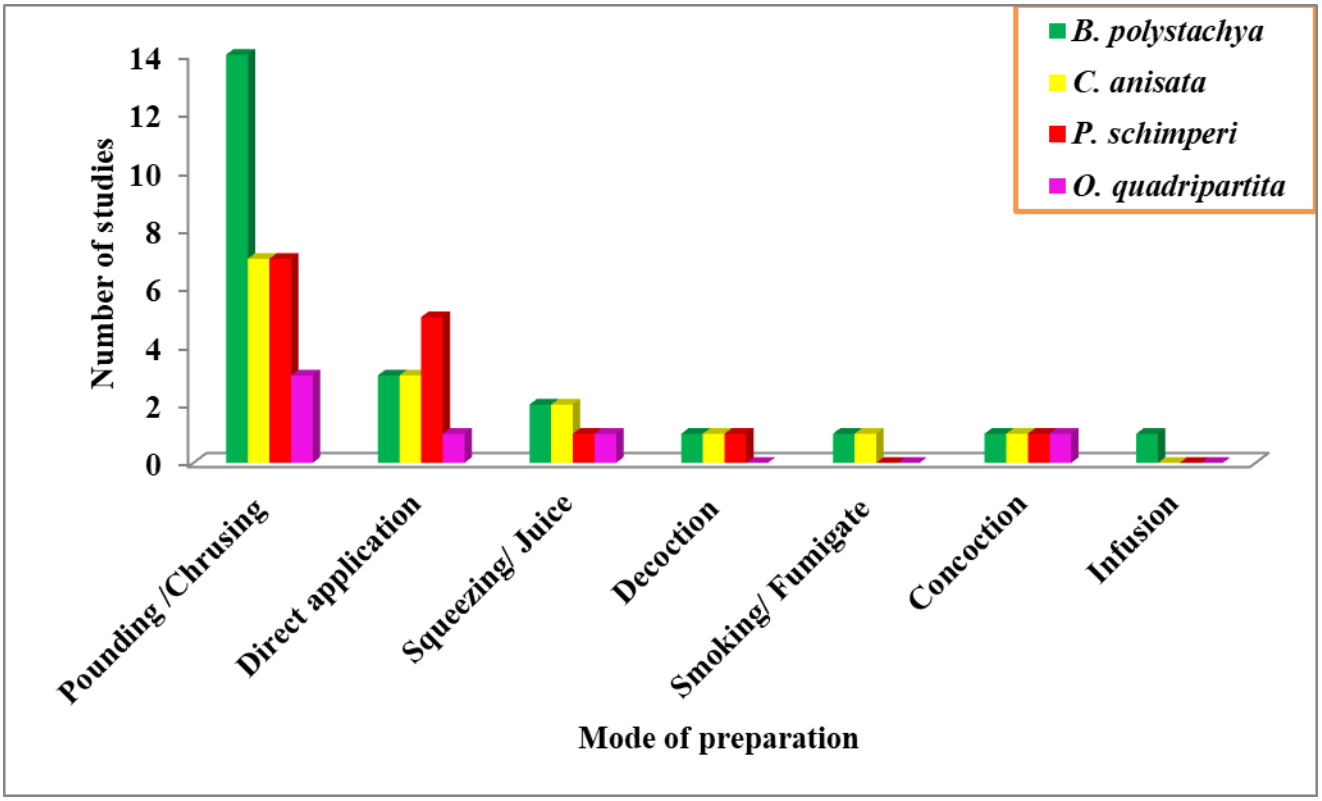

Figure 3 Ethnobotanical studies on the method of preparation on the selected medicinal plant species in Ethiopia

4.1.3. Route of administration

According to studies, medicinal plants such as *B. polystachya* (40.9%), *C. anisata* (72.9%), and *O. uadripartite* (80%) were often taken orally, followed by dermal application 22.7%, 13.6%, and 20%, respectively. However, for *P. schimperi*, dermal application (50%) followed by oral intake (28.6%) were more commonly used for treating human ailments (Figure 4; supplementary Table 1).

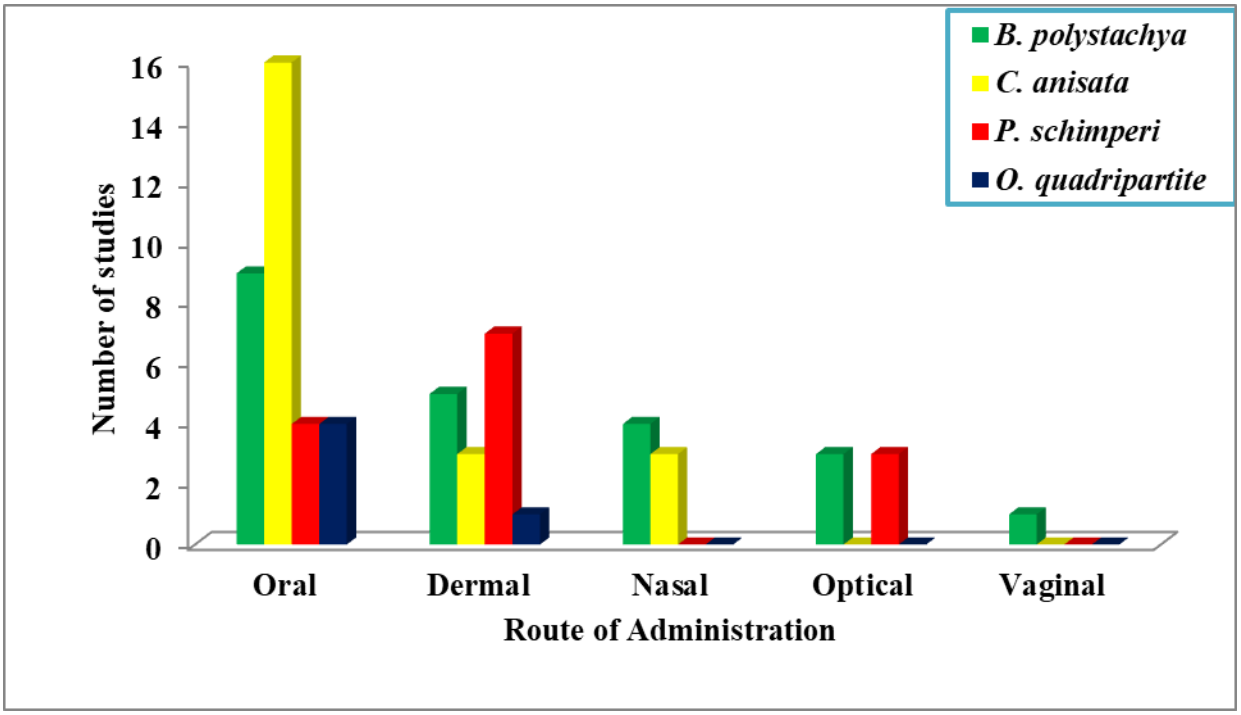

Figure4.Ethnobotanical studies associated to the route of tradition medicinal remedies of the selected medicinal plants in Ethiopia

4.1.4. Diseases treated by the selected plants

These plants are used to treat various ailments, for instance *B. polystachya* used for eye, tonsil, animal bite, and wound treatments, *C. anisata* mainly for evil eye, animal bites, and skin problems, and interestingly *O. uadripartite* for treating Rabbis and TB (supplementary Table 1).

4.2. Extract yield (%)

The extract yield is a measure of the solvent efficiency to extract specific components from the original material (Dhanani, *et al.*, 2017).The percentage yield of crude extract in solvent. Thus, the present study showed a variation in extract yield between the studied medicinal plants. Irrespective to significant difference, the highest extract yield was obtained from the leaves of *P. schimperi* **(**27.40%), followed by *B. polystachya* (23.80%), and *O. quadripartita* (22.80%), whereas the lowest extract was obtained from *C. anisata* (14.60%).

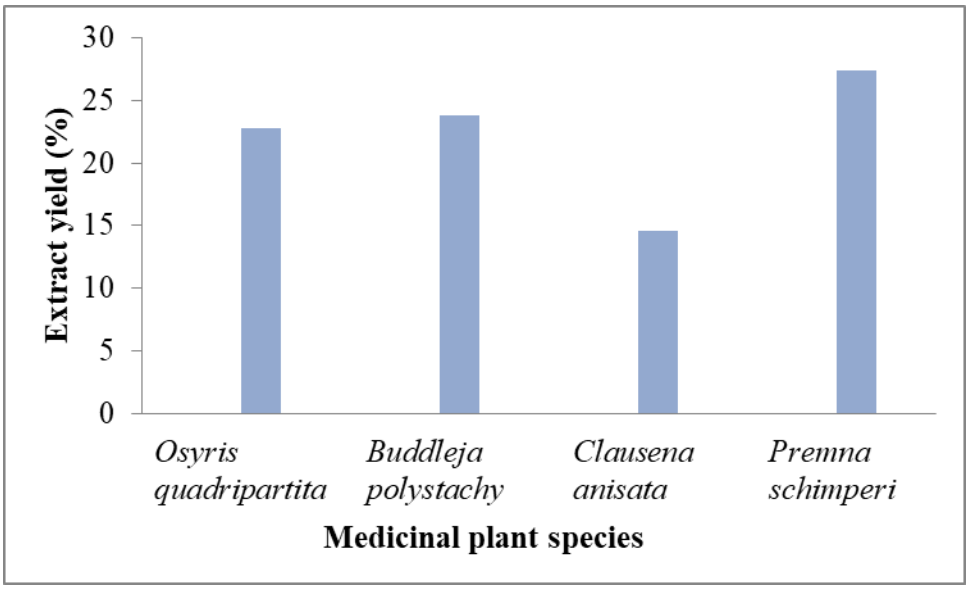

Figure 5: Extract yield (%) comparison of crude leaf extract of medicinal plant species

4.3. Phytochemical screening

Table 1 showed the preliminary phytochemical screening of extracts . Thus, *O. quadripartite* leaf extract contained saponin, tannins, alkaloids, flavonoids, and phenolic substances, however, the amount of terpenes was negligible. In the *P. schimperi* extract, phenols, tannins, flavonoids, and alkaloids were present, but there was no saponin. The study further noted that the alkaloids and tannins compounds detected in the leaf extract of selected medicinal plants.

Table 1 Phytochemical screening of leaf extract of medicinal plants.

Medicinalplant Species	Phytochemicals					
	Alkaloid	Saponin	Terpenoid	Flavonoid	Tannins	Phenol
B. polystachya	+	-	-	+	+	+
C. anisata	+	-	-	-	+	-
P. schimperi	+	-	+	+	+	+
O. quadripartite	+	+	-	+	+	+

Key, + = the presence of phytochemical constituents
- = the absence of phytochemical constituents.

4.4. Antibacterial susceptibility testing of the crude leaf extracts

Table 2 indicates the antibacterial susceptibility of methanol crude leaf extracts of the selected medicinal plants along with extract concentration against human pathogenic bacteria. . Thus, the crude extracts showed that a variation in mean inhibition zone of diameter on the test pathogens such as *E.coil, K.pneumonia, S. aureus, and S.epidermidis*. In the same ways, there was a considerable difference ($p<0.05$) in the pathogens zone of inhibition among medicinal plant extracts. In all aspects, there was an incremal trends in zone of inhibition with increasing extract concentration of the studied medicinal plants.

Among medicinal plant extracts, *B. polystachya* had the highest inhibition zone (33.0±2.1), followed by *O. quadripartita* (32.3±0.57), and *C. anisata* (29.0±1) at 100mg/ml in *S.epidermidis*. Comparatively, *S.epidermidis* was shown to have the highest (32.33±0.57) inhibition zone among human pathogenic bacteria. However, *P. schimperi* species showed less antibacterial

activity compared to extract of *O. quadripartita, B. polystachy and C. anisata* at 100mg/ml test concentration (Table 2). The extract of *O. quadripartita* had significantly higher antibacterial activity than extract of *B. polystachy*, *C. anisata*, and *P. schimperi* at 25mg/ml test concentrations in *S.epidermidis.*. At the concentration of 50mg/ml and 12.5mg/ml *O. quadripartita* showed maximum antibacterial activity against *K. pneumonia (*29.00±1.00) and (23.66±4.72) zone of inhibition respectively of the *(*Table 2*).*

The extract of *P. schimperi* showed maximum antibacterial activity against *E.coil (*25.33±0.57), (24.00±3.46) and (22.00±1.73) at 100mg/ml, 50mg/ml and 25mg/ml concentrations, respectively *(*Table2*).* On the other hand, the extract of *P. schimperi* showed minimum antibacterial activity against *S. aureus (*22.33±2.08), (20.00±2.64), (18.33±1.52) and (15.66±0.57) at concentration of 100mg/ml, 50mg/ml, 25mg/ml and 12.5mg/ml, respectively*(*Table 2*).*The extract of *C. anisata* showed maximum antibacterial activity against *S.epidermidis (*29.00±1.00), (27.66±0.57) and (23.00±3.00) at 100mg/ml, 50mg/ml and 12.5mg/ml concentrations, respectively of the *(*Table2*).* The extract of *B. polystachya* showed minimum antibacterial activity against *K.pneumonia (*29.66±0.57), 26.33±0.57 and (21.66±1.52) at 100mg/ml, 50mg/ml and 12.5mg/ml concentrations, respectively of the *(*Table2*).*

Extracts of B. polystachya, O. quadripartita, C. anisata and P. schimperi had significantly ($P < 0.05$) lower antibacterial activity as compared with standard drug (Table 2). The inhibition of crude extract of the B. polystachy, O. quadripartita, C. anisata and P. schimperi was less potent against S. aureus, E. coli, K.pneumonia and S.epidermidis compared to positive control (Gentamicin) which mean that positive control was highly inhibited growth but the negative control did not show any zone of inhibition against S. aureus, E. coli, K. pneumonia and S.epidermidis (Table 2).

Table 2Growth inhibition zone of methanol Crude leaf extract of medicinal plants at different concentrations.

Medicinal plant species	Test concentration (mg/ml)	Study pathogens *E.coil*	*K.pneumonia*	*S. aureus*	*S.epidermidis*
O. quadripartite	100	29.33 ± 1.52^{Ac}	31.00 ± 1.73^{Aab}	30.00 ± 1.00^{Ab}	32.33 ± 0.57^{Aa}
	50	27.70 ± 0.6^{ABb}	29.00 ± 1.00^{ABa}	28.00 ± 1.00^{ABab}	28.00 ± 1.00^{Bab}
	25	24.00 ± 2.64^{BCb}	24.00 ± 1.00^{Bb}	25.33 ± 0.57^{BCa}	25.00 ± 1.00^{Ba}
	12.5	20.00 ± 2.64^{Cb}	23.66 ± 4.72^{Ba}	22.33 ± 3.21^{Ca}	20.00 ± 1.00^{Cb}
P. schimperi	100	25.33 ± 0.57^{Aa}	24.66 ± 1.52^{Aa}	22.33 ± 2.08^{Ab}	24.33 ± 1.15^{Aa}
	50	24.00 ± 3.46^{Aa}	21.33 ± 2.08^{Bb}	20.00 ± 2.64^{Bb}	23.00 ± 2.64^{Aa}
	25	22.00 ± 1.73^{Ba}	20.33 ± 2.51^{Cb}	18.33 ± 1.52^{Cc}	20.33 ± 0.57^{Bb}
	12.5	19.00 ± 1.00^{Ca}	19.33 ± 2.08^{Da}	15.66 ± 0.57^{Dc}	17.33 ± 1.15^{Cb}
C. anisata	100	28.00 ± 1.00^{Aa}	28.00 ± 2.64^{Aa}	22.66 ± 0.57^{Ab}	29.00 ± 1.00^{Aa}
	50	26.33 ± 1.52^{Ba}	24.66 ± 3.05^{Bb}	22.66 ± 1.52^{Ac}	27.66 ± 0.57^{Aa}
	25	24.66 ± 1.15^{Ca}	19.33 ± 2.08^{Cb}	19.00 ± 1.00^{Bb}	23.66 ± 3.21^{Ba}
	12.5	20.00 ± 1.00^{Db}	17.66 ± 1.52^{Cc}	18.00 ± 2.00^{Bc}	23.00 ± 3.00^{Ba}
B. polystachya	100	33.00 ± 2.64^{Aa}	29.66 ± 0.57^{Ab}	33.00 ± 2.00^{Aa}	33.33 ± 2.08^{Aa}
	50	29.33 ± 0.57^{Bb}	26.33 ± 0.57^{Bc}	28.33 ± 0.57^{Bb}	31.00 ± 1.00^{Ba}
	25	27.00 ± 3.60^{Ba}	21.66 ± 1.52^{Cc}	24.00 ± 3.60^{Cb}	23.33 ± 2.08^{Cb}
	12.5	19.33 ± 1.52^{Cb}	21.00 ± 1.73^{Cab}	22.00 ± 2.64^{Da}	20.66 ± 0.57^{Db}
Gentamicin		36.00 ± 1.00^{a}	35.66 ± 0.57^{ab}	34.00 ± 1.00^{bc}	33.33 ± 1.52^{c}
Methanol		-	-	-	-

Note: (-) = no inhibitory effects

Mean values in a column followed by the same uppercase letters are not significant ($P<0.05$) among the concentration gradients, whereas mean values in a row followed by the same lowercase letters are not significant difference ($P<0.05$) among the bacterial pathogens

4.5. Minimum inhibitory and bactericidal concentrations of extracts

The MIC value of crude extracts of plant parts against the tested bacteria ranged from 6.25 to 100 mg/ml. The potency of extracts against tested bacteria was demonstrated by their minimum

inhibitory and bactericidal concentration values. The study also shown that variation in the tested bacteria growth among the studied medicinal plant species. Their effect on the growth of tested bacteria was variable among species. The most frequent MIC and MBC value of the extracts were 25 mg/ml and 50 mg/ml, respectively. The highest MIC and MBC values were recorded by extracts of *O. quadripartita*, C. *anisata* and *P. schimperi*. Extract of *B. polystachya* inhibited the growth of the test bacteria at relatively low concentration as compared to others. The higher MIC and MBC indicates that the plant extracts had weaker activities of killing or inhibiting the test pathogens and vice versa. The leaf extracts *B. polystachya, P. schimperi and O. quadripartita* had lowest MIC value of 25mg/ml against *Klebsiella pneumonia*. All plant extracts had MIC value of 50mg/ml against *Staphylococcus aurous.* The MBC values of *O. quadripartita*, C. *anisata, P. schimperi* and *B. polystachya* extracts were determined at 100mg/ml against *Staphylococcus aurous. B.polystachya* and *O. quadripartita* of the MBC values showed 50mg/ml against *Klebsiella pneumonia.* In *Clausena anisata* the MIC and MBC values were found to be 50mg/ml and 100mg/ml, respectively all tested bacteria.

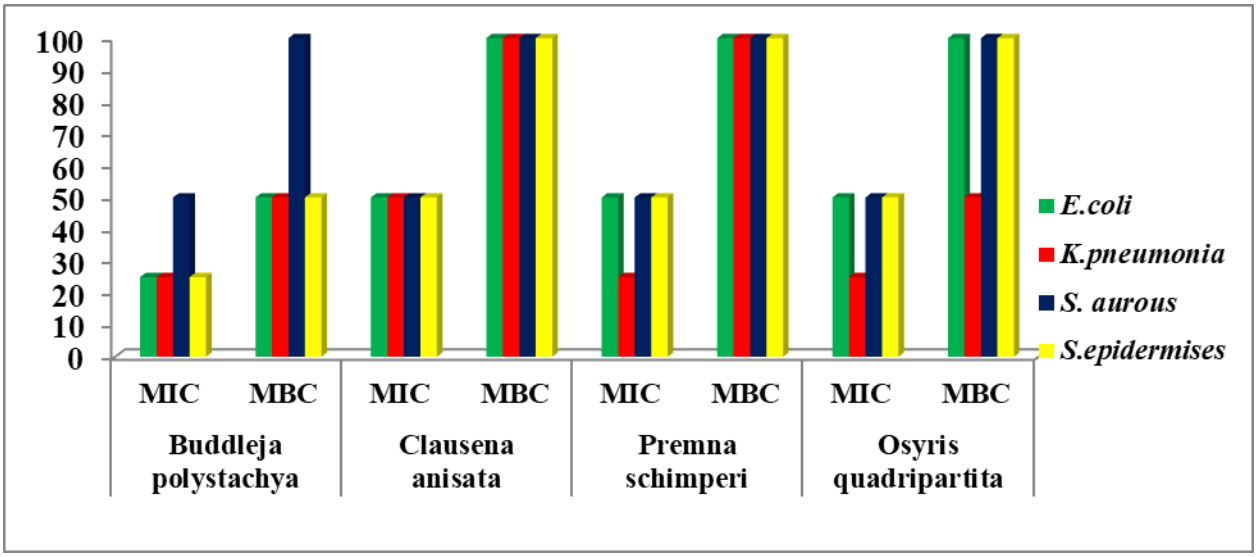

Figure 6:MIC and MBC of crude leaf extracts of medicinal plants

4.6. Minimum inhibitory and bactericidal concentrations of ratio

The MBC/MIC ratio was determined to identify whether the active principle was a bactericidal or a bacteriostatic compound. The MBC/MIC ratio is less than 1where the active principles can be considered to be a bactericidal agent (Santhanam *et at.,* 2008,). The results of MIC and MBC indicated that the active principles from *B. polystachya, C. anisata, P. schimperi and O. quadripartita* are presente.

Table 3. MIC, MBC, and MIC:MBC values of the active principles against the pathogens

Medicinal plants	Pathogens	MIC	MBC	MIC:MBC (ratio)
B. polystachya	*E.coli*	25	50	0.5
	K.pneumonia	25	50	0.5
	S. aurous	50	100	0.5
	S.epidermises	25	50	0.5
C. anisata	*E.coli*	50	100	0.5
	K.pneumonia	50	100	0.5
	S. aurous	50	100	0.5
	S.epidermises	50	100	0.5
P. schimperi	*E.coli*	50	100	0.5
	K.pneumonia	25	100	0.25
	S. aurous	50	100	0.5
	S.epidermises	50	100	0.5
O. quadripartia	*E.coli*	50	100	0.5
	K.pneumonia	25	50	0.5
	S. aurous	50	100	0.5
	S.epidermises	50	100	0.5

5. Discussion

According to the studies that were reviewed, it was found that leaves were the most commonly used part for making remedies for human ailments. This agrees with Tilahun Teklehaymanot and Mirutse Giday, (2007), Bogale Haile, (2018); Derebe Alemneh, (2021); Seyoum Getaneh and Zerihun Girma, (2014) and Getenet Chekole, (2015) who have reported that leaves are the most significant component utilized in formulating traditional remedies used to treat human diseases. In terms of preparation and administration, the findings revealed that the traditional remedies were usually made by crushing and pounding of the leaves. And hence the remedies were given through different routes principally via oral and dermal. This is in lines with the findings of Megersa Feyisa (2021) and Derebe Alemneh (2021) who have reported that pounding is the major ways of traditional medicinal plants preparation that administered principally through the oral and dermal to provide remedies for animal ailments.

In the present study, the highest extract yield was obtained from the leaves of *P. schimperi (*27.40%). When *C. anisata* had the lowest yield extract*, B. polystachya and O. quadripartita* had moderate percent yield extracts. Similar results have been reported in *B. polystachya* with ethanol extraction (Berhanemeskel Atsbeha *et al*., 2014) and low yield has been reported from methanol extracts by Gebregergs Tesfamaryam *et al. (*2015) as 19.5%. Higher extract yield has been reported by Alemtshay Teka *et al.,* (2015) as 31%. In the present study, the extract yield was obtained from the leaves of *O. quadripartita* (22.80*%).* A higher and lower extract yield have been reported by Samuel Taddese *et al.,(*2003*)* (40.11%) and Senait Girma *et al.*,(2015) as (6.60%),(32.75%)and(1.447%) the solvents Aqueous, Methanol and Chloroform, respectively. According to Ermiyas Solomon (2020), extract yield (8.5%) has been obtained from the leaves of *O. quadripartita* but, in the present study higher extract yield was obtained from the leaves of *O. quadripartita*. The differences in the extract yields might be ascribed to the different availability of extractable components, resulting from the varied chemical composition of plants (sultana *et al.,* 2009). In the present study, a lower extract yield of *B. polystachya (*23.80%) as compared to Firnus Haile *et al*.,(2022) who have reported the extract yield 45.37% of the same species

The phytochemical compound screening test of the methanol crude extract of the selected medicinal plants revealed the presence of alkaloid, tannins, saponin, flavonoid, phenol and terpinoid. Methanol is an all-purpose solvent that dissolves most secondary metabolites in plants

and enhances the release of these chemicals from cellular matrix (Visht and Chaturvedi, 2012; Moteriya *et al.,* 2014). In the present study, the methanol extract of *P. schimperi* to the presence of alkaloid, flavonoid, phenol, tannin and terpinoid. In traditional medicine, the leaf extracts of *P. schimperi* are used to treat external injuries and secondary infection in wounds (Solomon Habtemariam *et al*., 1990). In the present study, phytochemical screening test results showed the presences of alkaloid and tannin in *C. anisata*. On the contrary Dandena Tamene and Milkyas Endale (2019) have reported the presences of alkaloids, tannins, flavonoid, phenol and terpenoid in the extract of the same species.

According to Agyepong *et al.,* (2014) the preliminary phytochemical screening of ethanol leaf extract of *C. anisata has* shown the presence of tannins, saponins, flavonoids, steroids, phenolics, anthraquinonnes, glycosides, cardiac glycosides and alkaloids. Phytochemical constituents such as flavonoids have been found *in vitro* to be effective against a wide range of microorganisms (Harborne, 1993; Owoyele *et a*l., 2008). Tannins and saponins have been reported to prevent the growth of microorganisms by precipitating microbial protein and making nutritional proteins unavailable for them (Sodipo *et al.,* 1991 ;Panda and Tripathy, 2009). Studies have also shown that the growth of many fungi, bacteria and viruses are inhibited by tannins (Chung *et al.,* 1998; Panda and Tripathy, 2009) and the presence of flavonoids, tannins and saponins in the ethanol leaf extract. Therefore be responsible for the antimicrobial properties of the *C. anisata.*

In the present study the Antibacterial activities of the extracts of the *C. anisata* at 100mg/ml, 50mg/ml and 25mg/ml of the 22.66±0.57, 22.66±1.52, 19.00±1.00and18.00±2.00 as *S. aureus*, respectively. On the contrary, Agyepong *et al.,* (2014) had reported an inhibition zone diameter of use ethanol solvent 20.8±0.33, 18.3± 0.33, 16.7±0.67and 14.0±0.58 against *S. aureus at* 20mg/ml, 15mg/ml, 10mg/ml and 5mg/ml concentrations, *respectively.* If we consider the use of solvents that may affect the growth of the microorganism tested.

According to Firnus Haile *et al*. (2022) terpenoids, alkaloid, flavonoids, phenols, tannin, saponin and glycosides have been reported in the methanol extracts of *B. polystachya*. The phenolic compounds are among secondary metabolites found in the plant extract and reported to have antimicrobial activity (Thippeswamy *et al*., 2013). On the present study the crude extract of *P. schimperi* had phenolic compounds. The phenolic compounds contribute to the antimicrobial

properties of plant extracts whereby the extent of inhibition depends on the concentration of these compounds (Moteriya *et al.,* 2014). This study highlights the potential of *P. schimperi* rich in phenolic compounds to be used in pharmaceutical and cosmetic fields.

In the present study the Antibacterial activities of the extracts of the *O. quadripartita* at 100mg/ml, 50mg/ml and 25mg/ml of the 30.00±1.00, 28.00±1.00and 25.33±0.57 as *S. aureus*, respectively. On the contrary, Samuel Taddese (2003) has reported an inhibition zone diameter of 15.5± 0.17, 17.0± 0.17 and 19.0± 0.33 against *S. aureus* at 100mg/ml, 50mg/ml and 25mg/ml concentrations, *respectively*. The result of this study indicates methanol leaf crude extracts of *B. polystachya* showed excellent inhibitory activity against *S. aureus*. Similarly, Firnus Haile *et al*., 2022 have shown an excellent inhibitory activity of *B. polystachya* against *S. aureus.*

In the present study, the MIC values of the crude extracts of *B. polystachya* against *S. aureus* was 50mg/ml and MBC values of the plant against S. aureus was at 100mg/ml. On the contrary, According to Firnus Haile (*et al*., 2022) the MIC value of ethanol and methanol crude extracts of the plant against *S. aureus* has been 6.25% and MBC values of the plant against *S.aureus* 12.5% ethanol crude extract of *B. polystachya and* MBC value of 25% was observed on methanol crude extract of *B. polystachya* against *S. aureus*. Therefore, this may be due to the difference in solvent used for extraction. In the present study the MBC values of the crude extracts *O. quadripartita* against of *S. aureus* was at 100mg/ml. Similarly, the MBC values of the crude extracts of *O. quadripartita* against of *S. aureus* has been found 100mg/ml (Eyob tilahun, 2020).

The MBC/MIC ratio is less than1 where the active principles can be considered to be a bactericidal agent (Santhanam *et at.,* 2008,). The results of MIC and MBC indicated that the active principles from *B. polystachya, C. anisata, P. schimperi and O. quadripartita* are presente.

Generally, the methanol crude leaf extracts of the studied medicinal plant species showed different antibacterial activity attributed to the concentration and diversity of secondary metabolites. Extracts with abundant amount and large number of secondary metabolites had higher antibacterial activity. Accordingly, extracts of B. polystachya had higher antibacterial activity showing their potential in each human pathogen bacterial.

6. Conclusions and Recommendations

6.1. Conclusions

The leaves are the most frequently utilized plan parts used to treat human pathogens. In terms of preparation and administration, the findings revealed that the traditional remedies were usually prepared by crushing and pounding the leaves. The highest extract yield was obtained from the leaves of *P. schimperi* **(**27.40%), followed by *B. polystachya* (23.80%), and *O. quadripartita* (22.80%), whereas the lowest extract was obtained from *C. anisata* (14.60%). *B. polystachya*, *O. quadripartita*, *C. anisata*, and *P. schimperi* were contained potential antibacterial components that might be used for the development of pharmaceutical products against human pathogenic bacteria. The maximum percent extract yield and the highest antibacterial activity against pathogens could be designated as distinctive features of these medicinal plants for further development, formulation and commercialization as biocides with broad spectrum activity. The minimum inhibitory concentration value ranged from 25 to 50 mg/ml. Traditional knowledge might provide some clues to elucidate potential candidates for future development of new antibiotic agents. This study supported the view that certain medicinal plants are promising sources of potential antibacterial and effective as preventive agents in the pathogenesis of some diseases. Therefore, this study unveiled that the medicinal plant *B. polystachya* was more potent against each human pathogen, and thus more research on the effectiveness of its powerful chemical using an in vivo approach and additional phytochemical screening is required.

6.2. Recommendations

Based on results of the present study, the following recommendations are forwarded.

- Similar studies must be conducted to investigate how the selected medicinal plants and their phytochemical active influence other human pathogenic bacteria.
- It is recommended that further similar studies should be conducted on other parts of the plants such as root, fruit, shoot and bark.
- Further studies need to be conducted on the phytochemical screening or phytochemical active ingredients of the plant extracts.

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8. Appendices

Appendices A- Photo of medicinal plants collected and drying of medicinal plants in laboratory

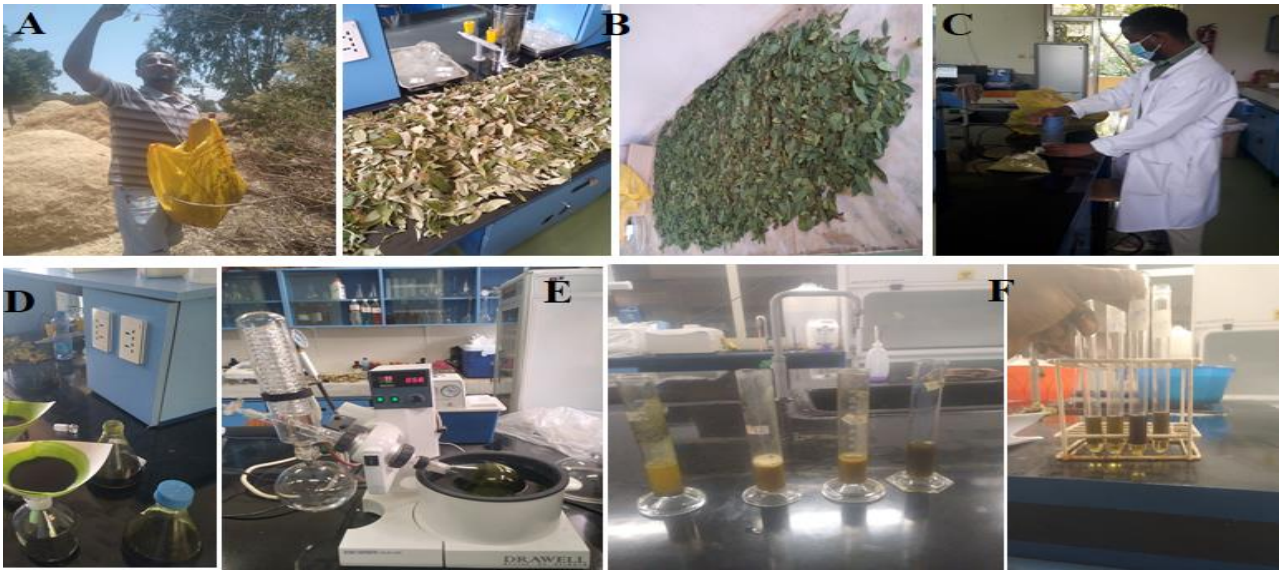

Figure. Illustrative showing the methodological protocols followed in the field and laboratory condition. A, Leaves sample collection; B, Drying out the collected medicinal plants under laboratory condition; C, Pulverizing the leaves of medicinal plants; D, Soaking followed by filtration; E, Vaporizing the solvent using rotary vapor; F, Phytochemical tests

(Photo by Samuel Fentie 2023)

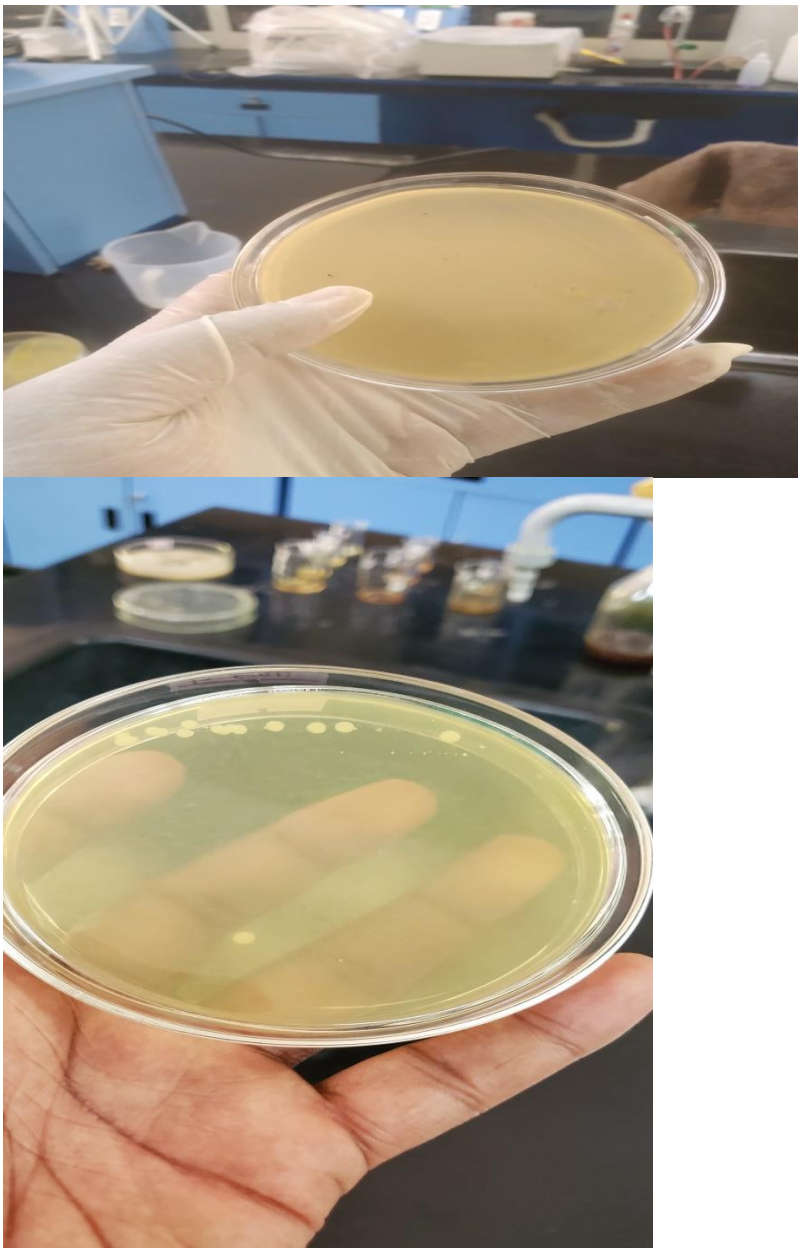

figure.MICand MBC

Table 1.Ethno-medicinal studies of the selected medicinal plants in Ethiopia

Plants	Part used	Mode of preparation	Administration	Disease treated	Reference
B. polystachya	Leaf Root	Pounding	Nasal	Headache	Derebe Alemneh 2021
B. polystachya	Root	chopped and soaked in warm water over night and filtrate applied topically to treat dermatophytes and tick mite infestations	Dermal	Dermatophilous and mite infestation	Ketema Tolossa *et al* 2013
B. polystachya	Seed	The dried Seeds are pounded, mixed with honey and eating	Oral	Diarrhea	Bekele Kindie *et al.*, 2021
B. polystachya		Add to fire and expose to the smoke		Saitan	Meaza Gidey,*et al.*, 2015
B. polystachya	Leaf Root	Pounding	Nasal	Headache	Derebe Alemneh 2021
B. polystachya				Tonsillitis	Getnet Chekole *et a*l 2015
B. polystachy	Leaf	**Leaf** of *B. polystachya* is chewed and spitted on cattle eye.	Optical	Eye disease	Abera Balcha2014

a					
B. polystachya	Leaf	Inflorescence with the leafofPhytolaccadodecandra is given in nose and ear to expel the parasite	Oral	Leech (Alekit	Alemayehu Kefalew *et al* (2015)
B. polystachya	Leave	Fresh leaves smashed and the extracts dropped in the eyes of affected cattle	Optical	Eye disease	Moa Megersa, *et al* 2013
B. polystachya	Leaf	Squeeze the leaf and drop on the infected eye	Optical	Eye disease	Seyoum Getaneh and ZerihunGirma 2014
B. polystachya	Leaf	Infusion of crushed dry leaves is taken orally	Oral	'Dingetegia'	Genene Bekele, and Reddy 2015
B. polystachya	Leaf		Oral	Uterine pain	Melesse Maryo *et al* 2015
B. polystachya	Leaf	Crushing and diluting	Nasal	leech infestation of cattle	Megersa Feyisa *et al* 2021
B. polystachya	Leaf	The leaf of BuddlejaPolystachya is pounded, powdered and applied on wound	Dermal	Wound	Eskedar abebe 2011
B. polystachya	Leaf	Leaf will be crushed with that of Rhamnusprinoides and put on the affected area	Dermal	Tonsillitis	Bogale haile bayuh 2018
	Leaf	Leaf will be crushed/ powdered, mixed with water and drunk	Oral	Intestinal parasite	
B. polystachya	Leaf	Fresh leaf is crushed, mixed with little water, and		Eyedisease s	AmareBitew Mekonnen *et al.*,

		dropped on the eye			2022)
B. polystachya	Leaf	Dry leaf is pounded, powdered, mixed with butter, and painted on the wound		Wound	„
B. polystachya	Seed	Seeds are pounded, mixed with honey or sugar in water solution and drunk.	Oral	Diarrhea	Mengist gebrehiwot 2010
B. polystachya	Leave	Leaves are pounded together with Calpurnia aurea, mixed with water and the infusion is drunk to cure or prevent the disease.	Oral	Malaria	
B. polystachya	Leaf	Crush and add liquid through the nose	Nasal	Leeches	Abraha Teklay *et al.*, 2013
B. polystachya	leaves		Oral	Uterine pain	Melesse Maryo *et al*., 2015
B. polystachya	Shoot	Tie and cream concoction	Dermal	Tonsillitis	Getnet Chekole and Zemede Asfaw. (2015).
B. polystachya	Leave	Crush and powder, immerse in TEJ then drink the juice	Oral	Intestinal parasite	
B. polystachya	Leave	Make soft by rubbing, and insert with new cloth until bleeding stops	Vaginal	Excessive menstruation	
B. polystachya	Shoot	Crush and tie	Dermal	Wound	
B. polystachya	Leave	Squeeze the leave and drop on the infected eye		Eye disease	Seyoum Getaneh and Zerihun Girma,

					(2014)
C. anisata	Leaf	Decoction	Oral	Snake bite toothache	Tegegne Bayih and Abduselam Usman. (2018
C. anisata	Leaf fruit	Chewing	Oral	Madness/mental illness	DerebeAlemneh (2021)
C. anisata	Bark	Bark of Clausenaanisata, leaves of Sidarhombifolia, root of Cucumisficifolius, bark root of Bruceaantidysentrica powdered together and mixed in milk then drunk a cup of tea for three days in order to get cured from Rabies disease	Oral	Rabies disease	Moa Megersa, *et al* 2013
C. anisata	Leaves	Dry leaves are ground, mixed with honey, and eaten	Oral	Breast cancer	Solomon Tesfaye (2020)
C. anisata	Root	Sniff, drink and fumigate with concoction	Na, O & De	Evil eye	Getenet Chekole *et al(*2015)
C. anisata	**Leaf**	**Leaf** of *C. anisata*, *Solaneciogigas* and *Justiciaschimperiana* are pounded together	Dermal	Skin irritation	Abera Balcha2014
C. anisata	root or stem	Cutting root or stem, chew, place and hold on the aching tooth	Oral	Toothache	Alemayehu Kefalew *et al*(2015)
C. anisata	Root	Inhaling its root powder		Devil	

		with roots of croton macrostachyus			
C. anisata	Leaves	Juice of leaves is used as ear drop		ear sickness	Tilahun Teklehaymanot and Mirutse Giday((2007)
C. anisata	Leaf	Leaves powdered and mixed with water and given immediately for the victimed		Snake bite	Mersha Ashagre Eshete, *et al* 2016
C. anisata	Leaf		Oral	Sunbur	Melesse Maryo et al 2015
	Leaf		Oral	Ascariasis	
	Leaf		Oral	Rheumatism	
	Leaf		Oral	Evil eye	
C. anisata	Leaf	Crushing and squeezing	Oral	Diarrhea	Megersa Feyisa 2021
C. anisata	Leaf and root			Stomach ache,ear infection	TamruTemam and AsalfewDillo (2016)
C. anisata	Root	The fresh root of Clausenaanisata is crushed and mixed with water and drunk	Oral	Evil eye	Eskedar abebe (2011)
C. anisata	Leaf	----------	Nasal	Evil eye	Mirutse Giday,*et al*., (2010)
C. anisata	Root	Crush and fumigate	DNO	Evil spirit	
C. anisata	Root	Sniff, drink and fumigate with concoction	Na, O & De	Evil eye	Getnet Chekole and Zemede

					Asfaw 2015
P. schimperi	Leaf	Chew and spit	Optical	Eye problem	Getenet Chekole *et al(*2015
P. schimperi	Leaf	Squeeze fresh leaves and drop a drop of the extract on the affected eye.	Optical	Eye disease	Seyoum Getaneh and Zerihun Girma,(2014)
P. schimperi	Leaf	Leaf pounded with leaf of Buddlejiapolystachya, and the juice is dripped on the eye	Optical	Eye disease	Alemayehu Kefalew et al(2015)
P. schimperi	Leaf	Pounding the leaves and making s/n to take it orally and applying through the opening. The residue should be closed on the opening.	Oral and dermal	Cancer (Swelling and forming deep opening)	Mersha Ashagre Eshete, et al 2016
P. schimperi	Root	Root chopped and soaked in warm water over night and filtrate applied topically to treat dermatophytes and tick mite infestations	Dermal	Dermatophilous and mite infestation	Ketema Tolossa et al 2013).
P. schimperi	Leaves		Dermal	Eye ache	Melesse Maryo et al 2015
P. schimperi	Leaf, root, stem	Grinding, chewing, rubbing, boiling	Oral	Toothache, coughing somachache	Banchiamlak Nigussie and Kim, (2019).
P. schimperi	Roots	The powdered roots are mixed with butter then painted on the breast of	Dermal	Mastitis	Tilahun Tolossa and Moa Megersa, 2018

		cows			
P. schimperi	Leaves	crushed & pounded leaves are tied on the infected body	Dermal	Boils	
P. schimperi	Root	Remove the bark from the root, chew the remaining part and kept on the teeth.	Oral	Toothache	Mengistu gebrehiwot(2010)
P. schimperi	Leave	Fresh leaf is rubbed again and again on the affected part.	External	Eczema	
P. schimperi	Leaf	Chew and spit	Optical	Eye problem	Getnet Chekole and Zemede Asfaw.2015
P. schimperi	Leaf	Crush, powder then cream with butter or honey	Dermal	Wound	
P. schimperi	Root	Chew and take with teeth	Oral	Toothache	
O. quadripartita	*Stem*	Chewing the stem and swallowing the fluid only. Crush and boil with water and drink.	*Oral*	Abdominal pain Urine problem	Seyoum Getaneh and Zerihun Girma, (2014)
O. quadripartita	*Leaf and root*	Pounding these parts, making s/n & drinking one water glass daily for a month.	*Oral*	*TB*	MershaAshagre Eshete, *et al* 2016
O. quadripartita	Barks		*Oral*	Jaundice	Melesse Maryo *et al* 2015
O. quadripartita	Leaf, root,	Squeezing, powdering, grinding, liquid form		Stomachache, cough, swelling	Banchiamlak Nigussie and Kim, (2019).

O. quadripartita	Leaf	The leaf *Osyris quadripartite* is crushed, powdered and then applied on the wound part	Dermal	Wound	Eskedar abebe (2011)
O. quadripartita	Root	Crush the concoction then drink with milk	Oral	Rabies	Abraha Teklay *et al* 2013