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Evaluation of Antibacterial Activity of Ocimum *Gratissimum* Against Selected Bacterial Pathogens of Public Health Concern

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Abstract

Ocimum Grattissimum is used to treat several infectious and non-infectious diseases. Antimicrobial resistance (AMR) among bacteria to conventional antimicrobials becomes a serious challenge and threat worldwide. However, the medicinal plants of Ocimum Grattissimum, which are used for two Gram-positive (Staphylococcus aureus and Enterococcus faecalis) and two Gram-negative (Escherichia coli, and Pseudomonas aeruginosa) bacteria, was collected from Jimma town of Ethiopia. Therefore, the antibacterial activity of leaf extracts of selected plants was determined in vitro, using disc diffusion and minimum inhibitory concentration (MIC) determined against selected zoonotic pathogenic bacteria. Aqueous extraction, ethanol, and methanol were the extraction solvents used in this study. The means inhibition zone among three, methanolic and ethanolic crude extract, were higher and aqueous was lowest extract while compared using analysis of significant difference test. The antimicrobial assay showed that the zones of inhibition produced using the disc diffusion method ranged from 2±0.26mm lower to 30±0.34 mm highest for the three extraction methods, with the highest value of 30±0.34 mm obtained with methanol extraction. The zones of inhibition for dried Ocimum grattissimum methanol extract were 15±0.26mm lowest to 30±0.34mm highest and for were 15±0.26 to 28±0.11, Ethanol extract from dried leaf Ocimum grattissimum was 10±0.22mm lowest to 26±0.34mm highest and 7±0.11mm to 24±0.26mm respectively. Regarding ethanol, methanol extract, and hot and cold aqueous extract, there was a significant difference (P<0.05) seen for all tested bacteria. This study's results suggest that Ocimum grattissimum extracts may be useful in the search for an antibacterial agent to help create novel medications that combat animal pathogenic bacteria that cause life-threatening illnesses.

Keywords: ***Antibacterial activity, Life-threatening infections, Ocimum grattissimum, Pathogenic bacteria.***

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1. Introduction

For pharmaceutical intermediates, food supplements, nutraceuticals, and chemical entities for synthesized medications, plants are the most abundant source of drugs in both conventional and modern medical systems [1]. The earliest recorded instances of human civilization involve the use of plants and plant products as medicines. For both individual and community health, medicinal plants are extremely important. An ethnobotanical and ubiquitous plant serves as a rich resource of natural drugs for research and development [2]. Medicinal plants-based drugs owe the advantage of being simple, effective, and exhibiting broad-spectrum activity. 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 infections [3]. Herbal medicine has been widely used all over the world and formed an integral part of primary health care in many countries. Since ancient times, many plant species reported having pharmacological properties as they are known to possess various secondary metabolites utilized to combat disease-causing pathogens [4].

Antimicrobial resistance (AMR) is the capacity of a microbe to live and grow when exposed to dosages of antibiotics that were once thought to be effective against it. This is a growing issue on a global scale. AMR is currently considered a serious developing danger to global public health and food security since it has reached alarming levels in the majority of the world, both in human and veterinary medicine [5]. Although antimicrobial usage (AMU) has become an increasingly important aspect of animal production worldwide, misuse or overuse of AMU can lead to the emergence of bacteria resistant to antibiotics [6]. In the food production chain, a number of antimicrobial-resistant food-borne bacteria have recently surfaced that can infect humans and spread to other people. Antimicrobial resistance in *Salmonella*, *Staphylococcus aureus*, and *Escherichia coli* is one of the problems that the global healthcare system is facing [7].

The problem of antimicrobial resistance is spreading globally. Investigating new and creative antimicrobials is one way to improve the situation at hand. Antibiotics and chemotherapeutic medications have proven helpful in the treatment of many infections, but their use must be carefully monitored to prevent the emergence of resistant forms. Both in developed and developing countries, there is an increasing demand for herbal medicines, which are utilized by about 85% of people globally for the prevention and treatment of sickness [8]. Approximately, 25% of drugs contain compounds that are derived from higher plants [9]. Traditional medicine provides primary medical care to up to 80% of the population in Africa [10], [11].

In Ethiopia, around 80% of the human population and over 90% of the livestock population still rely on plant treatments as their primary source of medicine, sometimes even their only one [12], [13]. A significant portion of the knowledge of ethnomedicinal plants is in danger of being lost forever and deteriorating because herbal heritage is passed down orally, rather than in writing, from generation to generation, despite the fact that these plants are essential to maintaining the health of both humans and livestock populations [14].

About 160 species make up the genus *Ocimum* (Family *Lamiaceae*), often known as basil. It is distributed across the tropical, subtropical, and parts of the temperate regions of both hemispheres, from sea level to 180 attitudes [15]. Naturally, *Ocimum gratissimum* (Lamiaceae) is used to treat skin conditions, ophthalmic, fever, diarrhea, headaches,

and pneumonia. Additionally, *Ocimum* oil exhibits antibacterial activity against a variety of bacterial species, including *Shigella, Salmonella, Proteus, Listeria monocytogenes*, and *Staphylococcus aureus* [16]. Numerous research have looked into the antibacterial qualities of plants all around the world, and because of these qualities, several of them have been used as therapeutic substitutes [17]. Alkaloids and phenolic chemicals, among other secondary metabolites, are abundant in antibacterial capabilities found in plants.

Escherichia coli is a Gram-negative bacillus found in the large intestine and excreted naturally through the feces and urinary tracts. It is one of the most frequent causes of many common bacterial diseases including cholecystitis, bacteremia, cholangitis, urinary tract infections and travelers' diarrhea, and other clinical infections, such as neonatal meningitis and pneumonia [18].

Additionally, *Pseudomonas aeruginosa* is a rod-shaped, mono flagellated, gram-negative bacteria. It manifests as a grape or tortilla and has a pearly appearance. This bacterium is capable of growing above 42°C, which sets it apart from other *Pseudomonas* species [19]. It grows well around 25–37°C. Growing evidence points to *Pseudomonas aeruginosa* (P. aeruginosa) as a significant nosocomial organism that can cause serious infections, particularly in hospitalized burn victims [20]. Numerous nosocomial infections, such as bacteremia, endocarditis, urinary or wound infections, and in certain cases, fatalities, are caused by these opportunistic and extremely resistant bacteria. *Pseudomonas aeruginosa* infections are linked to higher rates of death and morbidity in patients with cystic fibrosis, immunocompromised or disabled individuals, and hospitalized burn victims [21].

Staphylococcus aureus is a Gram-positive coccus that colonizes the nasal mucosa and skin of healthy individuals. The organism can cause a wide range of diseases ranging from skin or soft tissue infections to systemic and fatal diseases [22]. *Staphylococcus aureus* possesses a specific virulence factor called coagulase, which plays a significant role in biofilm formation during *S. aureus* infection. Coagulase binds to host prothrombin and forms active staphylothrombin complexes that convert soluble monomeric fibrinogen into self-polymerizing insoluble fibrin and activate a coagulation cascade [22].

Gram-positive, cocci-shaped, non-spore-forming, fermentative, facultatively anaerobic bacteria is called *Enterococcus faecalis*. In smaller quantities, they can also be found in the oral cavity and female genital tracts of humans. Numerous energy sources, such as carbs, glycerol, lactate, malate, citrate, arginine, and other keto acids, are catabolized by them [23]. Extremely alkaline pH levels and high concentrations of salt are among the harsh conditions in which E. faecalis can thrive [23], [24]. They are resistant to desiccation, ethanol, zeolite, detergents, heavy metals, and bile salts. They can withstand a temperature of 60 ^{o}C for 30 minutes and grow in the range of 10 to 40^{oC} [23].

Nevertheless, resistant or multi-resistant strains continue to emerge despite the availability of strong antibiotic and antifungal medicines, necessitating a constant search for and development of new medications. Thus, it is imperative that the hunt for fewer sources of antibiotics be an ongoing endeavor. [25]. According to reports, the least expensive and most secure alternative source of antimicrobials is plants. Of the native species of Ethiopia, *O. grattissimum* is the

most frequently grown. Despite being native to our nation, the plant's antibacterial properties have not yet been investigated.

1.1. Statement of the Problem

Emergency and spread of bacterial resistance against formulated conventional antibiotics are increasingly becoming a concern to public health. Besides the antibacterial efficacy of Ocimum grattissimum against *Staphylococcus aureus*, *Enterococcus faecalis, Escherichia coli,* and *Pseudomonas aeruginosa* bacteria at Jimma town were not yet known. Therefore, the significance of this study was undertaken with the aims of:-

- ✓ Evaluate the antibacterial efficacy of Ocimum grattissimum leaf extracts against selected bacterial pathogens at different concentrations.
- ✓ Assess the better solvent for extraction of targeted plant materials amongst selected extraction solvents.
- ✓

1.2. Objectives

1.2.1. General Objectives:

- ➢ The main objectives of this study were to determine the antimicrobial activity of extracts of *Ocimum grattissimum* plant for selected zoonotic antibiotic resistance bacteria.

1.2.2 Specific Objectives:

- ➢ To evaluate the in-vitro antibacterial activities of *Ocimum grattissimum* plant extract against Gram-positive (*S. aureus and E. faecalis*) and two Gram-negative (*E. coli* and *P. aeruginosa*) bacteria.
- ➢ To assess better solvents from the extracts of *Ocimum Grattissimum* plants materials amongst selected extraction solvents (methanol, ethanol, and aqueous).

2. Materials and Methods

2.1. Study Period and Study Area

The study was carried out between February 2023 and July 2023 in Jimma town of Oromia Regional State. The investigation was initially carried out in the southwest of Ethiopia; Jimma Town is situated 357 kilometers from Addis Ababa. Jimma is one of Ethiopia's largest and most prominent towns. At a height of 1704 meters above sea level, Jimma was situated between latitudes of 7° 13'20" and 8° 53' 16" North and longitudes 35° 51' 07" and 37°36' 16" East. The average yearly temperature is roughly 18.5°C, with the minimum and maximum values ranging between 6°C and 31°C, respectively. Unpredictable rainfall and temperature swings play a significant influence on the spread of disease in both animals and people [26].

Two main soil types make up the Jimma region: alluvial soils that are brownish-gray and grayish-white clay soils and reddish-brown residual soils. The bulk of people focus on working in shops, metalwork, woodwork, and other business-related fields that deal with various commodities. The town's cattle are managed under conventional extensive and intensive husbandry systems with communal herding, and they are a mix of native and exotic breeds. With a mixed farming system as its foundation, agriculture provides the majority of the society's means of subsistence, and livestock is essential to this system [27].

2.2. Plant Collection and Preparation

Ocimum gratissimum plant's fresh, healthy leaves were subsequently recognized and verified by a botanist from plant science at Addis Ababa. The plant parts were collected, cleaned with sterilized distilled water, cut into smaller sizes of about 1-2 cm long, and dried for 15 (fifteen) days in the shade at room temperature in the Jimma University College of Natural Science and Department of Chemistry laboratories. It was then crushed into a fine powder using an electric grinder and then stored in a refrigerator (4°C) until needed. The grinding process began with a traditional wooden pestle and mortar [28], [29] (Fig. 1).

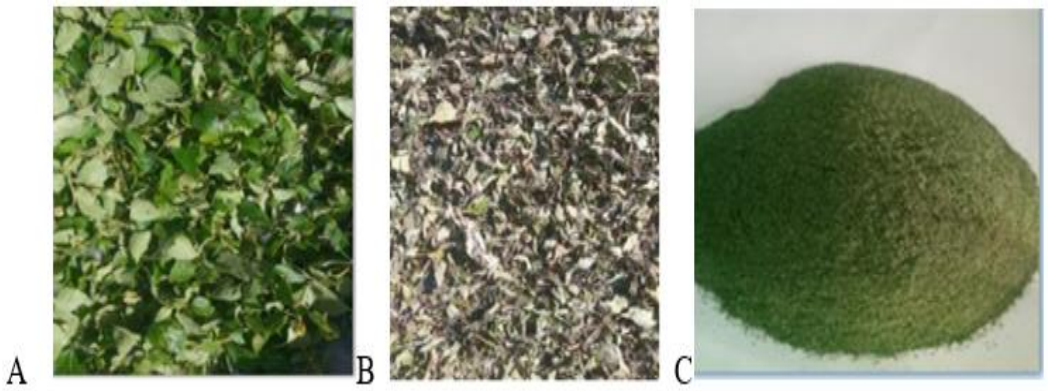

Fig. 1. Key: A) Collected sample of O. gratissimum B) Drying time for 15 days C) Powdering of sample.

Preparation of extract leaves of *Ocimum grattissimum* collected from Jimma town. Fresh leaves of mature and healthy Ocimum grattissimum washed in tap water for 5 minutes and cleaned with sterile distilled water. The drained gel was dried in the oven at 80° C for 48 hours. Dried was ground to obtain powder by using mortar and pastel and 30g of this powder was soaked into 300ml of solvents (ethanol, methanol, and aqueous) for 4 days for proper extraction of the active ingredients at room temperature [30]. This was later filtered by Whatman filter paper No.1 and the filtrates evaporated to dry the extract using a rotatory evaporator. The supernatant was stored in a refrigerator at 4°C after collection. Before being used for antimicrobial susceptibility testing the extracts was dissolved in Dimethyl Sulfoxide (DMSO).

2.3. Plant Extraction

Although a standard extraction procedure for herbal extracts has not been established, the business for herbal medicine routinely prepares plant crude extracts using 20–95 percent of the solvents (polar or/and non-polar). Based on data from traditional claims and earlier investigations, all medicinal herbs, *Ocimum gratissimum* was extracted using methanol (99.8%), Aqueous, and ethanol (99.5%). A flask with a flat bottom that contained 200 mL of extracting solvents was used to macerate 50 grams of *Ocimum gratissimum* air-drying powder plant materials for 24 hours while it was shaken at 121 rpm. With Whatman No. 1 paper, the suspension was filtered. The filtrate that was left over was subsequently concentrated in a rotary evaporator at reduced pressure. After further drying, a water bath and an oven set at 45°C and 42°C, respectively, were used to extract the solvent from the sticky residue. The remaining crude extracts

were kept in the refrigerator in an airtight bottle until the experiment was carried out after the solvent had evaporated [28].

2.3.1. Preparation of aqueous extracts

i. Cold aqueous Extracts of Fresh and dried Leaves

A conical flask is filled with 100 ml of cold distilled water, and 25 grams of fresh plant leaves (Ocimum Gratissimum), and a rubber stopper is placed on top. The mixture was allowed to steep for seven days while being sometimes shaken. Then, it was transferred to a different clean conical flask using sterile filter paper (Whitman no. 1). For a test of their antibacterial activity, the standard extracts were then kept in the refrigerator at 4 °C [31]. In a different instance, fresh leaves that had been air-dried thoroughly were being ground. 25g of the ground leaves were weighed out, dipped into 100 ml of cold distilled water, and sealed with rubber corks in a conical flask. The mixture was allowed to sit for 7 days while being occasionally shaken. The other steps were the same as follows in the case of cold-water extracts of fresh leaves.

ii. Hot aqueous Extracts of Fresh and dried leaves

Here, the same step was used as in cold water treatment with 30 minutes of boiling while plant material was dipped in distilled water.

2.3.2. Methanol extraction

The plant's (*Ocimum Gratissimum*) newly harvested leaves were first cleaned with distilled water before being air dried at 20 to 30 degrees Celsius. Following regulated circumstances and powder drying in a table dryer. 200 ml of methanol was combined with 50g of plant leaf of *Ocimum Gratissimum* material for powdering (50 percent). The combinations were mixed many times using a sterile glass rod while being maintained in tight-seal jars for 24 hours at room temperature to protect them from sunlight. This mixture was filtered through Whitman No. 1 filter paper, the residue was adjusted to the necessary concentration (50 ml of methanol for every 50g of plant material powder residue), and the extraction of fluid for further extraction was repeated three times until clear colorless supernatant extraction liquid was finally obtained.

The methanol was removed from the extracted liquid using rotary evaporation. After being frozen overnight at -80°C, the semisolid extract was dried in a 200ml vacuum freezer for 24 hours at -60°C. To be used later, the extract was then kept in an airtight container and chilled to 4^{o}C in a refrigerator. The concentration extracts were marked with the names OGLM (*Ocimum Gratissimum* leave methanol extract). A similar procedure was followed for ethanol (Fig. 2).

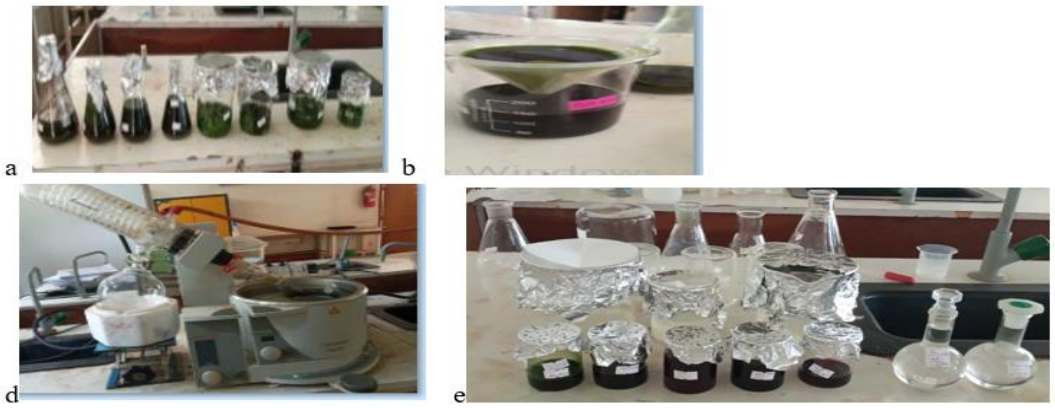

Fig. 2. Key: a) mixing powder with solvents b) mixing gel with solvents c) Filtering of each by filter-paper d) Evaporation by rota vapor e) After vapor keeps until solvents completely evaporate at Jimma chemistry laboratory.

2.4. Determination of Extraction Yield

The percentage yield of each extract was obtained using the formula

$$\text{Percent extracts} = \frac{W_2 - W_1}{W_0} \times 100$$

Where W_2 is the weight of the dried extract and the container, W_1 is the weight of the container alone and W_0 is the weight of the dried plant material [32].

2.5. Test Bacteria

To assess the antibacterial efficacy of crude plant extracts, reference bacterial strains of *S. aureus* (ATCC 25923), *E. faecalis* (ATCC 29212), *E. coli* (ATCC 25922), and *P. aeruginosa* (ATCC 27853) were used. The Jimma Public Health Institute's main campus was the source of these four American-type cell cultures (ATCC), which were aseptically collected and transported through a cold chain. In the study, two Gram-negative and two Gram-positive bacterial strains each made up one of the four employed. The bacteria were grown on nutrient agar slants at 4°C and maintained on all of the test's strains, which are reference strains.

2.6. Media for Test Bacteria

The media was set at 1000 ml of distilled water and 38 g of Mueller Hinton Agar were placed in a flask, and the mixture was heated gradually until the media was completely dissolved, as per the manufacturer's instructions. To sterilize the media, it was autoclaved for 15 minutes at 121 °C. Once the media had cooled to about 50 oC, 25 ml of the sterilized medium were aseptically placed into 90 mm-diameter sterilized Petri plates. The plates were then allowed to dry until all moisture that remained on the agar surface was removed before being used. The produced media's sterility was assessed by incubating a group of randomly selected plates at 37 °C for 24 hours [33].

2.7. Standardization of Inoculum

The zone of inhibition, which corresponds to the area where no bacterial growth was seen under ideal conditions for bacterial growth, is measured by culturing bacteria in the presence of the chemical/extract, and this method was used to evaluate the antimicrobial activity of a bioactive component. Bacterial susceptibility to the bioactive substances/extracts of the plant increased with increasing diameter (zone of inhibition). The technique was put into practice using the pre-outlined steps [34].

As a result, three to five colonies from pure cultures of each of the four chosen bacterial species were transferred with the use of a sterile wire loop into a different label test tube containing 5 ml of a nutrient broth and allowed to grow for two hours at 37 ^{o}c. The inoculums were made from the stock cultures, which were subcultured onto nutrient broth using a sterilized wire loop while being kept on a nutrient agar slant at 4^{o}C. Comparison with the 0.5 McFarland standard of sodium chloride solution was used to calculate the density of suspension inoculates onto the media for the susceptibility test [35].

2.7.1. Preparation of disc

Diffusion discs of 6 mm diameter were prepared from absorbent filter paper (Whatman no.1) by using a paper Puncher and sterilizing at 120 °C for 1 hr and drying in an oven. Then after, sterilize discs were soaked aseptically by applying 30 µl of each crude extract of the plant at a concentration of 100 mg/ml using a sterile digital micropipette and then allow to dry at room temperature for 15 minutes and then place in a sterile container and store at 4 ^{o}C until further use [33].

2.7.2. Preparation of the test bacteria

The test bacteria were grown on new plates using the isolate cultures that were acquired on agar slants. After choosing several bacterial isolate colonies, the colonies were suspended in 5 milliliters of nutritional broth in sterile vials with clearly labeled labels. For twenty-four hours, they were incubated at 37 °C. A 0.5 ml syringe was used to choose 0.02 ml of dilutions ranging from 10-4 to 10-9 for each of the bacteria in order to calculate the population density. After being incubated, different isolates of bacteria produced variable numbers of colonies at different dilutions [36].

2.7.3. Antimicrobial Activity (Disc Diffusion Test)

A standard inoculum dilution of the test bacterial isolates in 1 ml was used to seed semi-solid nutrient agar plates. The inoculum on the agar surface was distributed by swirling the plates, and any extra was thrown away in a sterile Jar. After setting for around 20 minutes on the bench, the plates were dried in the incubator for 30 minutes at 37 degrees Celsius. Six wells were drilled around the plates using the sterile standard Cork borer. To stop the extracts from diffusing beneath the agar, the bottoms of the wells were sealed with one drop of sterile nutritional agar. Positive and negative controls were used in the fifth and sixth wells, respectively. The negative control well was filled with sterile distilled water. Tetracyclines/ ciprofloxacin for *Ocimum grattissimum* was used as the positive control [37].

The disc diffusion technique had been widely used to assay plant extract for antimicrobial activity [38]. A sterile cotton swab was dipped into the adjusted standardized broth inoculums suspension by rotating the swab. The swab was then

evenly streaked over the entire surface of the Muller Hinton agar plate. Streaking was repeated by rotating the plate approximately 60° each time to ensure an even distribution of inoculum. After inoculation, for each test bacterium, sterilize discs that were soaked under 30 µl of each crude extract of the plant at a concentration of 100 mg/ml were applied while sterile, white paper discs were soaked by each solvent (Ethanol, Methanol, and Water) and distilled water served as a negative control, standard antibiotic tetracycline/ciprofloxacin disc 30 µg/disc was used as a positive control.

Lastly, the disc was placed on the 90 mm inoculate plates using flame-sterilized forceps that were roughly equally spaced from one another. After that, each plate was incubated for a full day at 37 °C. The antibacterial properties of the plant extracts were evaluated by measuring the diameter of the inhibitory zone in each plate following the incubation period. The diameter of the disc as well as the inhibitory zone was measured with a sliding digital microcaliper. The test bacteria's bacterial activity with the crude extracts was compared to that of the positive and negative controls, per CLSI [33].

2.8. Antibacterial Assay

The antibacterial activity of the different samples of fresh leaves and drying leaves and powder from fresh leaf juice was individually tested against the studied bacteria. In vitro, an antibacterial test was then carried out by disc diffusion method [39, 40] using 25µl of standardized suspension of testes bacteria (108cfu/ml) spread on Mueller-Hilton agar plates. The dry and sterile disc (6mm in diameter) impregnated with 0.1 ml of standard plant extracts was placed on the seed agar plates with the aid of sterile forceps. The plates were incubated at 37^{0}C for 24 hours. Antibacterial activity was evaluated by measuring in millimeters (mm) the zones of inhibition against the tested bacteria [39].

All extracts were put under the control of DMSO. Aqueous extracts were compared to sterile, distilled water. Following our control studies, 1 percent (V/V) DMSO assisted in the dissolving of organic extracts (methanol) and of aqueous extracts with water, which did not affect the proliferation of microorganisms [39]. The positive control was ciprofloxacin and tetracycline, while the negative control well contained sterile distilled water. The extracts were aseptically injected into wells, 1-4 with 0.2ml of each concentration that had been made. Pre-diffusion was allowed to occur on the plates for 40 minutes before the plates underwent an overnight incubation at 37°C. A ruler calibrated in millimeters was used to measure the resulting inhibition zones. The zone of inhibition of the bacterial isolates under consideration at that specific concentration was determined to be the average of the three values [37].

2.9. Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration is the lowest dose at which no detectable bacterial growth can occur on the culture plates [41]. For every plant extract, a stock solution containing 200 mg/ml was prepared. Ten milliliters of Muller Hinton broth were added to each sterile test tube. A double dilution method was used with a sterile digital micropipette and stock solution to create 100 mg/ml, 50 mg/ml, 25 mg/ml, 12.5 mg/ml, and 6.25 mg/ml) [34]. Lastly, each tube received 20 µl of the test organism's standard suspension, which had been adjusted to 0.5 McFarland standards. After giving the tubes a little shake to mix them, they were incubated for 24 hours at 37°C. The solution was further injected into agar plates after a 24-hour incubation period. MIC was determined to be the maximum dilution of the extract that prevents bacterial growth. The MIC values are the lowest doses of the extracts that, after 24 hours of

incubation at 37 °C, inhibit bacterial growth. All tests were done in triplicates [33]. Breakpoint/interpretive criteria-minimal inhibitory concentration (MIC) or zone diameter value used to indicate susceptible, intermediate, and resistant.

2.10. Data Management and Analysis

Before being transferred to statistical tools for analysis, data were entered and coded in Microsoft Excel spreadsheets and then transferred to (SPSS 20). To determine whether there was any variation in the antibacterial effects of the four extracts—aqueous, ethanol, methanol, and fresh leaf one- way Anova analysis of variance was conducted. The mean comparative value of the zone of inhibition obtained against each particular bacterium was computed for the chemical extracts of *Ocimum gratissimum*. A P-value of less than 0.05 was considered significant for all analyses.

3. Results

3.1. Antibacterial Assay

The antibacterial activity of the dried leaves of *Ocimum gratissimum* extracted by cold and hot water, ethanol, and methanol. The extract had more antibacterial action against gram-positive species (*S. aureus and E. fecalis*) than gram-negative pathogens (*E. coli and P. aeruginosa*). Every time, the activity of the extracts was compared to that of the tetracycline antibiotic for *Ocimum grattissimum*. The yield of the extracts in different solvents of various forms of extract of leaf of *Ocimum grattissimum* against aqueous (cold), methanol, ethanol, and aqueous (hot) are shown in Table 1. The extraction solvents by methanol and ethanol highly yield percent and aqueous Extraction is variable under different forms of the extract.

Table 1: Yield of the Extracts in Different Solvents.

Scientific name	Solvents	Weight of dry Powder	Weight of extract powder	% Yield
Ocimum grattissimum	Methanol	50gm	25.6gm	51.2%
	Ethanol	50gm	15.7gm	11.4%
	Aqueous (cold)	25gm	5gm	20%
	Aqueous (hot)	25gm	4gm	16%

Key: gm=gram Source: [31], [30], and [28].

The different effects on the four test bacteria by Ethanol extracts of *Ocimum grattissimum* is shown in Table 2 below. Both show the four micro-organisms were significantly different in response to ethanol extracts of *Ocimum grattissimum* compared to standard antibiotics (Tetracycline and Ciproflaxin for bacteria). In the current investigation, three of the examined pathogens *E. fecalis, Staphylococcus aureus, and P. aeruginosa* were susceptible to fresh leaf ethanol extract of Ocimum gratissimum, with variable degrees of inhibition. Table 2 displays the diameter of their inhibition zones. Aside from *E. coli* which showed low inhibition on the extract of the dried leaves, all pathogens were

sensitive to ethanol extracts of fresh and dried leaves, with their corresponding diameter zones of inhibition shown in Table 2.

Table 2: Antibacterial Activity of Medicinal Plant Extracts by Ethanol Against Selected Bacteria.

Name of Bacteria		Zones of inhibition (mm) Mean ±SE				
		Ethanol extract of			Tetracycline/ Ciproflaxin	
		O.grattissimum				
		Fresh leaf	Driedleaf		(Standard)	Sig.
Gram Positives	*S. auerus*	15 ±0.25	26 ±0.34		18 ±0.34	.003
	E. fecalis	10 ±0.32	22 ±0.37		20 ±0.37	.001
Gram Negatives	*E. coli*	5 ±0.12	12 ±0.26		12 ±0.26	.000
	P. aeruginosa	8 ±0.13	10 ±0.22		19 ±0.22	.000

Sig.= significance, Ns =No significance (P>0.05, S= Significant (P<0.05).

Ocimum gratissimum methanol extract was tested against two gram-positive and gram-negative bacteria. The extract was inhibitory to some of the pathogens tested, with the corresponding diameter zone of inhibition shown in Table 3. For *P. aeruginosa and E. coli,* the fresh leaf of methanol extract had low inhibitory effects. Among the four studied pathogens, *S. aureus* was likewise found to be highly sensitive (p< 0.05) to both fresh and dried *Ocimum gratissimum* extract, with inhibitory zones measuring 20±0.28 mm and 30±0.34 mm, respectively, in diameter. Distilled water and tetracycline were typically used as negative and positive controls, respectively in this extract form.

Table 3: Antibacterial Activity Plants Extracted by Methanol Against Selected Bacteria.

Name of Bacteria		Zones of inhibition (mm) means ±SE		Methanol extract		
	Fresh O.g	Dried O.g		D. water	Tetracycline (Standard)	Sig.

Gram Positives	*S. auerus*	20 ±0.28	30 ±0.34		N	18 ±0.34	.003
	E. fecalis	13 ±0.17	18 ±0.19		N	20 ±0.37	.001
Gram Negatives	*E. coli*	12 ±0.12	15 ±0.29		N	12 ±0.26	.000
	P. aeruginosa	10 ±0.16	18 ±0.11		N	19 ±0.22	.000

Data are presented as means ±SE, the Value of the triplicate experiment, O. g= *Ocimum grattissimum*, DMSO – Dimethyl sulfoxide, sig: significance, Ns =No significance (P>0.05) S= Significant (P<0.05).

Table 4 below shows significant differences in activity among the hot and cold aqueous extracts of *Ocimum grattissimum* parts used compared to standard antibiotics (Tetracycline). For *S. aureus and E. fecalis,* the diameter of the inhibitory zones was 8±0.34 and 12±0.37mm, respectively, when dried leaf extracts were cold aqueous extracted and also *E. coli* 6±0.26mm *and P. aeruginosa* 12±0.22mm could grow by these extracts. Additionally, warm aqueous extracts of dried leaves had a comparable antibacterial effect on *S. aureus and E. fecalis* but have a moderate inhibitory impact on *E. coli and P. aeruginosa*.

Table 4: Antibacterial Activity of Two Plants Extracted by Aqueous Against Selected Bacteria.

Name of Bacteria				Zone of inhibition (mm) Aqueous extraction means ±SE						
		Fresh leaves		*Dried leaves O.g*				D. water Water	Tetracycline	Sig.
		Hot	Cold	Hot		Cold				
Gram Positives	*S. auerus*	3 ±0.24	4 ±0.29	6 ±0.34		8 ±0.34		N	18 ±0.34	.003
	E. fecalis	7 ±0.37	9 ±0.37	4 ±0.37		12 ±0.37		N	20 ±0.37	.001

Gram Negatives	*E. coli*	2 ±0.26	4 ±0.26	10 ±0.26		6 ±0.26		N	12 ±0.26	.000
	P. aeruginosa	3 ±0.22	6 ±0.22	8 ±0.22		12 ±0.22		N	19 ±0.22	.000

Diameter of the zone of inhibition including a diameter of disc 6mm. Data are presented as means ±SE, the Value of a triplicate experiment. *O.g=Ocimum grattissimum*, Ns=No significance (P>0.05), N = No inhibition.

Table 5: ANOVA Table for the Significance tests at different concentrations (mg/ml)

a) ANOVA table of *Ocimum grattissimum* extract against bacteria of *S. aureus*

Source of variation	Df	Sum of Squares	Mean Square	F value	Sig.
Treatment	21	7186.364	342.2078	2932.71	.003
Residual	44	5.1342	0.1167		
Total	65	7191.498			

Key: Df- Degrees of freedom. Based on this analysis, extraction solvent, plant (Treatment) used, and test-organism factors are significant at p-value e <0.05.

b) ANOVA table type of *Ocimum grattissimum* extract against bacteria of *E. fecalis*

Source of variation	Df	Sum of Squares	Mean Square	F value	Sig.
Treatment	21	3891.955	185.3312	1376.3	.001
Residual	44	5.925	0.1347		
Total	65	3897.88			

Key: Df- Degrees of freedom. Based on this analysis, extraction solvent, plant (Treatment) used, and test-organism factors are significant at p-value e <0.05.

c) ANOVA table type of *Ocimum grattissimum* extract against bacteria of *E. coli*

Source of variation	Df	Sum of Squares	Mean Square	F value	Sig.
Treatment	21	1904.864	90.70779	1359.84	.000
Residual	44	2.935	0.0667		
Total	65	1907.799			

Key: Df- Degrees of freedom. Based on this analysis, extraction solvent, plant (Treatment) used, and test-organism factors are significant at p-value e <0.05.

d) ANOVA table type of *Ocimum grattissimum* extract against bacteria of *P. aeruginosa*

Source of variation	Df	Sum of Squares	Mean Square	F value	Sig.
Treatment	21	2306.318	109.8247	2279.38	.000
Residual	44	2.12	0.04818		
Total	65	2308.438			

Key: Df- Degrees of freedom. Based on this analysis, extraction solvent, plant (Treatment) used, and test-organism factors are significant at p-value e <0.05.

3.2. Minimum Inhibitory Concentration (MIC)

The following table displays the minimal inhibitory concentrations of the various extracts and the significance tests against the tested bacterial pathogens. The *S. aureus, E. fecalis, and P. aeruginosa* pathogens were evaluated, and the ethanol extracts of the fresh and dried leaves of *Ocimum grattissimum* showed inhibitory activity against all four of the pathogens. Their MIC values ranged from 5±0.12 to 26±0.34 mg/ml. With MIC values ranging from 3±0.24 to 30±0.34mg/ml, the methanol extract of the fresh and dried leaves *Ocimum grattissimum* inhibits all of the examined organisms. The highest minimum inhibitory concentration was 8±0.34 mg/ml for *S. aureus*, while it is 15±0.26 mg/ml for *E. fecalis*. The maximum inhibitory concentration was 30±0.34 mg/ml for *S. auerus*. A minimum inhibitory concentration (MICs) of each extract that was able to kill or inhibit the growth of one or four microorganisms in this study was exhibited by extracts that were active at a concentration of 100 mg/ml to 6.25 mg/ml. The lowest MICs (15-30 mg/ml) indicate the highest activity, while 10-15 mg/ml (moderate activity), and 3-10 mg/ml indicated low activity. In the current investigation, two of the examined pathogens *E. fecalis* and *Staphylococcus aureus,* were susceptible to fresh leaf ethanol extract of *Ocimum gratissimum*, with variable degrees of inhibition. Aside from *E. coli* and *P. aeruginosa* which showed low inhibition on the extract of the dried leaves, all pathogens were sensitive to ethanol extracts of dried leaves, with their corresponding diameter zones of inhibition shown in Table 6.

Table 6: *Ocimum Grattissimum* in Different Concentrations of Extracts by Ethanol

Conc. in g/ml	Zone of inhibition			
	Ethanol extract			
	S. auerus	*E. faecalis*	*E. coli*	*P. aeruginosa*
1	26 ± 0.34^{c}	22 ± 0.37^{a}	12 ± 0.26^{d}	10 ± 0.22^{e}
0.5	20 ± 0.34^{f}	20 ± 0.37^{c}	8 ± 0.26^{g}	9 ± 0.22^{f}
0.25	18 ± 0.34^{g}	12 ± 0.37^{g}	6 ± 0.26^{h}	7 ± 0.22^{h}
0.125	9 ± 0.34^{h}	6 ± 0.37^{j}	4 ± 0.26^{i}	5 ± 0.22^{i}
Distilled water (2ml)	0 ± 0.00^{m}	0 ± 0.00^{l}	0 ± 0.00^{k}	0 ± 0.00^{l}
Positive control	18 ± 0.34^{d}	20 ± 0.37^{b}	12 ± 0.26^{a}	19 ± 0.22^{a}

Mean values with different superscripts in the same column are significantly different at P<0.05.

Analysis of variance showed that the antimicrobial activity (production of inhibition zones) of Methanol extracts was influenced by plant use, test organisms, and extraction solvent (Table 7). The *Ocimum grattissimum dried* extracts (mean inhibition zone 30± 0.34mm) appear more effective than the fresh extracts (MIZ of 20± 0.28mm) against *S. aureus*, with a significant difference in activity from each other.

Table 7: *Ocimum Grattissimum* in Different Concentrations of Extracts by Methanol.

Conc. in g/ml	Zone of inhibition			
	Methanol extract			
	S. auerus	*E. faecalis*	*E. coli*	*P. aeruginosa*
1	30 ± 0.34^{a}	18±0.37^{d}	15 ±0.26^{b}	18±0.26^{b}
0.5	28±0.34^{b}	15±0.37^{e}	14±0.26^{c}	14±0.22^{c}
0.25	22±0.34^{e}	13±0.37^{f}	11±0.26^{e}	12±0.22^{d}
0.125	20±0.34^{f}	10± 0.37^{h}	6±0.26^{h}	3±0.22^{i}
Distilled water (2ml)	8±00.34^{i}	0±0.00^{m}	0±0.00^{l}	0±0.00^{k}
Positive control	18±0.34^{d}	20±0.37^{b}	12±0.26^{a}	19±0.22^{a}

Mean values with different superscripts in the same column are significantly different at P<0.05. *Means inhibition zones (mm), within a column, followed by the same letter are not significantly different from each other at $p < 0.05$.

Table 8 below shows significant differences in activity among the cold aqueous extracts of *Ocimum grattissimum* parts used compared to standard antibiotics (Tetracycline). For *S. aureus and E. fecalis,* the diameter of the inhibitory zones was 8±0.34 and 12±0.37mm, respectively, when dried leaf extracts were cold aqueous extracted and also *E. coli 6*±0.26mm *and P. aeruginosa* 12±0.22mm could grow by these extracts. Additionally, warm aqueous extracts of dried leaves had a comparable antibacterial effect on *S. aureus and E. fecalis* but have a moderate inhibitory impact on *E. coli and P. aeruginosa*.

Table 8: *Ocimum Grattissimum* in Different Concentrations of Extracts by Aqueous (cold).

Conc. in g/ml	Zone of inhibition			
	Aqueous(cold) extract			
	S. auerus	*E. faecalis*	*E. coli*	*P. aeruginosa*
1	8 ± 0.34^{i}	12±0.37^{g}	6 ±0.26^{h}	12±0.22^{d}
0.5	6±0.34^{j}	8±0.37^{i}	2±0.26^{k}	10±0.22^{e}
0.25	2±0.34^{l}	0±0.00^{m}	0±0.00^{l}	4±0.22^{j}
0.125	0±0.00^{m}	0± 0.00^{m}	0±0.00^{l}	0±0.00^{l}
Positive control	18±0.34^{d}	20±0.37^{b}	12±0.26^{a}	19±0.22^{a}
Distilled water (2ml)	0±0.00^{m}	0±0.00^{m}	0±0.00^{l}	0±0.00^{l}

Mean values with different superscripts in the same column are significantly different at P<0.05.

Table 9: *Ocimum Grattissimum* in Different Concentrations of Extracts by Aqueous (hot).

Conc. in g/ml	Zone of inhibition			
	Aqueous(hot) extract			
	S. auerus	*E. faecalis*	*E. coli*	*P. aeruginosa*
1	6± 0.34^{j}	4±0.37^{k}	10±0.26^{f}	8±0.22^{g}
0.5	3±0.34^{k}	3±0.37^{l}	8±0.26^{g}	3±0.22^{k}
0.25	2±0.34^{l}	0±0.00^{m}	6±0.26^{h}	0±0.00^{l}
0.125	0±0.00^{m}	0± 0.00m^{l}	3±0.26^{j}	0±0.00^{l}
Distilled water (2ml)	0±0.00m^{l}	0±0.00^{m}	0±0.00^{l}	0±0.00^{l}
Positive control	18 ±0.34 ^{d}	20 ±0.37 ^{b}	12 ±0.26 ^{a}	19 ±0.22 ^{a}

Mean values with different superscripts in the same column are significantly different at P<0.05.

4. Discussion

4.1. Antibacterial Assay

The goal of the current investigation was to gather preliminary data on the antibacterial activity of *Ocimum grattissimum leaf* fresh leaf juice, water, ethanol, and methanol extracts. The Ethanol extracts of fresh and dried leaves of *Ocimum grattissimum* have strong antibacterial activity in the growth of the organisms tested except on *E. coli and P. aeruginosa* where it had a low inhibitory effect as compared with the standardized control tetracycline and Ciproflaxin (p>0.05). Ethanol extract from dried leaf *Ocimum grattissimum* showed an extensive antibacterial effect against the tested Gram-negative bacteria (*E. coli and P. aeruginosa*) and Gram-positive bacteria (*S. aureus* and *E. fecalis*) and their respective diameter zones of inhibition were 12±0.26mm, 10±0.22mm, 26±0.34mm, and 22±0.27mm respectively. The inhibitory effect of ethanol extracts from fresh leaves and *dried Ocimum grattissimum* was also noticed. This result is in agreement with the studies of [42]**.** However, *Staphylococcus aureus, and E. fecalis*, have been reported to be less susceptible to plant extracts in an earlier study conducted by other researchers [43], [44].

There are differences in the antimicrobial effects of various extracts of *O. grattissimum*. *Ocimum grattissimum* extract of methanol had the maximum antibacterial activity as compared to other solvents ethanol, and aqueous. This was similar to a study by [45], to investigate the antimicrobial evaluation of plant pathogens by disc diffusion method, which was done with three different forms (ethanol, methanol, and aqueous extracts) [45]. And for *O. grattissimum* also, this was similar to a study by Junaid, to investigate the antimicrobial evaluation of *O. gratissimum* extracts on some selected bacterial gastrointestinal isolates, which was done by [46].

In this study, methanol and ethanol extract from dried leaves exhibited higher antibacterial activity in the growth of Gram-positive bacteria tested as compared to tetracycline. This is because methanol and ethanol can extract the active ingredient of the plant more than aqueous extracts. Earlier investigators have shown that plants inhibited the growth of Gram-positive bacteria more than gram-negative bacteria [47], [48]. This may be attributed to the high permeability barrier of Gram-negative bacteria to numerous antibiotic molecules similar to the aqueous and ethanol. This

corroborates the work of Nair [49] who had similar findings. Conversely, when contrasting the two types of extract, the dried leaf *Ocimum grattissimum* methanol extract shown extensive antibacterial activity against every tested bacterium, including *S. auerus, E. fecalis, E. coli*, and *P. aeruginosa*. The plants' ability to combat both Gram-positive and Gram-negative bacteria may be a sign that their leaves contain broad-spectrum antibiotic chemicals [50], [51].

The hot and cold aqueous extract of the fresh and dried leaves of *Ocimum grattissimum* showed appreciable antibacterial activity on *S. aureus and E. fecalis*, while it does low affect *E. coli and P. aeruginosa*. The cold water extract of dried leaves displayed a relatively better antibacterial effect against *S. aureus* and, *E. fecalis* with their diameter zones of inhibition recorded at 8±0.34, and 12±0.37 mm respectively. According to the results of the present study, aqueous extract of dried leaf plants against *S. aureus* and *E. coli* showed the lowest activity when compared with others. Our results go parallel with the observations of Odebisi-Omokanye and co-investigators [42]. In earlier studies conducted by other researchers, hot water extracts could not inhibit any organisms [43], [44].

However, in this study, hot water extract of fresh and dried leaves of *O. grattissimum* were able to inhibit two organisms, namely *S. aureus* and *E. fecalis*. This result is interesting because, in the traditional method of treating a bacterial infection, a decoction of the plant part or boiling the plant in water is employed. Another reason for the ineffectiveness of aqueous extracts could be that the active compounds were not soluble in water, or the aqueous extracts in this study were not prepared according to traditional methods, which in many cases involve boiling and soaking with water for several hours [52]. Success in traditional medicines may be due to the administration of the extracts in large quantities and over a long period [53]. Evidences indicate that the global rise of antimicrobial resistance is mainly due to in discriminatory use of a drug for the treatment of both human and animal diseases [54], [55]. Although different antibiotic classes of drugs are used in animal health management and human medicine, the selection of resistance to one drug class may lead to cross-resistance to another [56].

In general, the antibacterial activity of the crude plant extracts on the test organisms supports the active ingredient found in herbal remedies, and the bioactive substance's antibacterial potential was quite equivalent to the antibiotic utilized in the sensitivity test. Additionally, the involvement of plants in the development of the chosen bacteria may be an indication of the presence of broad-spectrum antibiotic chemicals in the plant's leaf. To inhibit pathogenic bacteria, *Ocimum grattissimum* may thus develop into promising natural antibacterial agents with prospective applications in the pharmaceutical sector. However, if plant extracts are to be used for medicinal purposes, issues of safety and toxicity should be taken into consideration.

The potency of the extract depends on the method used to obtain it, the failure of some of the extracts to have an antibacterial effect on the test organism is not sufficient to conclude that the extract does not contain substances that can have an antibacterial effect on the test organism [57]. The age of the plant utilized, the freshness of the plant material, physical factors (temperature, light, or water), contamination by field bacteria, improper processing of the plant, etc. may all have a role in the antimicrobial activities of different plant extracts [50].

5. Conclusion and Recommendations

The need to find plant-based antimicrobials is growing as a result of the high cost, decreased efficacy, and rising resistance to conventional medications. A new method for revealing an effective antibacterial agent from *Ocimum grattissimum* is suggested by several discoveries. The current result generally showed that the active principle of the plants, which had effective antibacterial results towards the tested pathogens (*S. aureus, E. fecalis, E. coli, and P. aeruginosa*) which are responsible for the most common bacterial infection, could become a promising natural antimicrobial agent with potential applications in pharmaceutical industries for controlling pathogenic bacteria. However, the value of the extract for bacterial cultivation depends on the anti-bio gram output of the experiment. The findings of this research demonstrated that all of the extracts have antibacterial activity against the examined pathogens to varying degrees. Thus, based on the above concluding remarks, the following recommendable points are forwarded:

- People ought to be advised on possible ways of cultivating plants.
- Attempts should be made to evaluate the antimicrobial impact of the plant's other components, including roots, stems, etc.
- These plants naturally grow in a variety of settings; it would be naive to anticipate a significant diversity in chemical constituent quantity and composition in the tree's various portions.
- To determine the potential variables influencing the antibacterial effect of the plant extract, further research should be conducted.
- It is important to raise awareness of the plant's historical use in the region as a remedy for various illnesses.

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7. Contribution of Authors

All the authors contributed for the preparation of the manuscript.

8. Conflict of Interest

There was no conflict of interest.

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REFERENCES

1. Hammer KA, Carson CF, Riley TV. Antimicrobial activity of essential oils and other plant extracts. J Applied Microbiol. 1999;86(6):985-990.
2. Kong, J.M., Goh, N.K., Chia, L.S., and Chia, T.F. Recent advances in traditional plants drugs and orchads. Acta Pharmacol Sci. 2008; 24: 7-21.
3. Ijeh II, Njoku OU, Ekenze EC. Medicinal evaluation of extracts of Xylopia aethiopica and Ocimum gratissimum. J Medicinal Aromatic Plat Sci. 2004; 26(1):4-44.

4. Redda YT, Kebede E, Cruz C, et al. Potential antibacterial activity of crude extracts from medicinal plants, Zingiber officinale and Vinca major medicinal plants. Int J Microbiol Res. 2014; 5(3):202-207.
5. Chuanchuen R, Pariyotorn N, Siriwattanachai K, et al. Review of the literature on antimicrobial resistance in zoonotic bacteria from livestock in East, South, and Southeast Asia. Bangkok. FAO Regional Office for Asia Pacific. 2014;60(7):437-443.
6. Phu VD, Wertheim HF, Larsson M, et al. The burden of hospital-acquired infections and antimicrobial use in Vietnamese adult intensive care units. PloS One. 2016;11(1):44.
7. Kashef N, Djavid GE, and Shahbazi S. Antimicrobial susceptibility patterns of community-acquired uropathogens in Tehran, Iran. Journal Infect Develop Countries. 2010;4(04):202-206.
8. Abramov, V. Traditional medicine. World Health Organization, 1996; 134,pp. 1-3.
9. Krogsgaard-Larsen, P., Christensen, B., and Kofod, H. The role of medicinal plants in drug development. Natural Products and Drug Development, 1994; pp. 34-45.
10. Sinha P, Govil NJ and Singh VK. Diseases and their management, recent progress in medicinal plants. Sci Tech Pub LLC, USA, 2002;100-105.
11. Yineger HA. Study on the ethnobotany of medicinal plants and floristic composition of the dry Afromontane forest at Bale Mountains National Park, Ethiopia. Addis Ababa University; 2005.
12. Mengistu A.K. The Effect of Herbal Preparations on Staphylococcus aureus and Streptococcus agalactiae isolated from clinical bovine mastitis (Unpublished MSC thesis Faculty of veterinary medicine, AAU); 2004.
13. Tadesse B and Dereje A. Survey of ethnoveterinary medicinal plants at selected Horro Gudurru Districts, Western Ethiopia. African Journal of Plant Science. 2015;9(3):185-192.
14. Tadesse B, Mulugeta G, Fikadu G, et al. Survey on ethnoveterinary medicinal plants in selected Woredas of east Wollega zone, western Ethiopia. J Biol Agricult Healthcare. 2014;4(17):97-105.
15. Junaid SA, Olabode AO, Onwuliri FC, et al. The antimicrobial properties of Ocimum gratissimum extracts on some selected bacterial gastrointestinal isolates. African J Biotechnol. 2006;5(22):2315-2321.
16. Janine AL, Xisto SP, Orionalda FF, et al. Antifungal activity from Ocimum gratissimum L. towards Cryptococcus neoformans. Mem Inst Oswaldo Cruz. 2005;100:55-58.
17. Bugno A, Nicoletti MA, Almodóvar AA, et al. Antimicrobial efficacy of Curcuma zedoaria extract as assessed by linear regression compared with commercial mouthrinses. Brazilian J Microbiol. 2007;38:440-445.
18. Singh RK, Chang HW, and Yan D. Influence of diet on the gut microbiome and implications for human health. J Transl Med. 2017;15(1):73.
19. Wu M, and Li X. Klebsiella pneumoniae and Pseudomonas aeruginosa. In Molecular Medical Microbiology. 2015; 1547-1564pp.
20. Zhapouni, Z, Fashad S, and Alborzi A. Pseudomonas aeruginosa: burn infection, treatment and antibacterial resistance. Iranian Red Crescent Med J. 2009;11(3):244–253.
21. El Solh AA, and Alhajhusain A. Update on the treatment of Pseudomonas aeruginosa pneumonia. Journal of Antimicrobial Chemotherapy, 2009;64(2):229–238.
22. Taylor TA and Unakal CG. Staphylococcus Aureus; StatPearls. Publishing: Treasure Island, FL, USA; 2017.
23. Stuart CH, Schwartz SA, Beeson TJ et al. Enterococcus faecalis its role in root canal treatment failure and current concepts in retreatment. J Endodontics. 2006;32(2):93-98.

24. Hedge V. Enterococcus faecalis; clinical significance and treatment considerations. Endodontology, 2009; pp. 48-54.
25. Doughari JH, Pukuma MS, and De N. Antibacterial effects of Balanites aegyptiaca L. Drel and Moringa oleifera Lam. on Salmonella typhi. African J Biotechnol. 2008;6(19):2212- 2215.
26. CSA. Central Statistical Authority, Federal Democratic Republic of Ethiopia, Statistical Abstract; 2007.
27. Jimma Zone Health Office. Disease Prevention and Control Department Annual Report. Jimma, Ethiopia; 2021, 96-97pp.
28. Mulatu G. Antibacterial activities of Calpurnia aurea against selected animal pathogenic bacterial strains. Adv Pharmacol Pharm Sci. 2020;2020(1):8840468.
29. Sulaiman SF, Sajak AAB, Ooi KL, et al. Effect of solvents in extracting polyphenols and antioxidants of selected raw vegetables. Journal of Food Composition and Analysis. 2011;24(4-5):506-515.
30. Agarry OO, Olaleye MT, and Bello-Michael CO. Comparative antimicrobial activities leaf. Afr. J. Biotechnol. 2005; 4(12):1413-1414.
31. Akueshi CO. Antimicrobial potentials of hyptis sauvedens Poit (Lamiaccae), Nigeria. J Bot. 2002;15:37-41.
32. Anokwuru CP, Anyasor GN, Ajibaye O, et al. Effect of extraction solvents on phenolic, flavonoid, and antioxidant activities of three Nigerian medicinal plants. Nat Sci. 2011:9(7):53-61.
33. CLSI, (Clinical and Laboratory Standards Institute). Performance standards for antimicrobial susceptibility tests for bacteria that grow aerobically approved standards. 30th ed. *Wayne. www.clsi.org*, USA, 2012.
34. CLSI, (Clinical and Laboratory Standards Institute). Performance standards for antimicrobial susceptibility tests for bacteria that grow aerobically approved standards. 5th vol. *Wayne. www.clsi.org*, USA, 2020.
35. Cheesbrough M. Biochemical tests to identify bacteria. District laboratory practice in tropical countries, 2002; Part, 2, 63-70p.
36. Miles AA, Misra SS, and Irwin JO. The estimation of the bactericidal power of the blood. Epidemiol Infect. 1938; 38(6):732-749.
37. Abayomi S. The state of medicinal plants research in Nigeria. University of Ife Press. 1982; 200p.
38. Taiwo SS, Oyekanmi BA, Adesiji YO, et al. In vitro antimicrobial activity of crude extracts of Citrus aurantifolia Linn and Tithonia diversifolia Poaceae on clinical bacterial isolates. Int J Trop Med. 2007;2(4):113-117.
39. Bauer AW, Kirby WM, Sherris JC, et al. Antibiotic susceptibility testing by a standardized single disk method. Am J Clin Pathol.1986;45(4):493-496.
40. Barry AL. Procedure for testing antimicrobial agent in agar media. In Lorian Antibiotica in Laboratory Medicines. Willims and Wikins Co. Baltimore. 1980; 1-23p.
41. Radojevic ID, Stankovic MS, Stefanovic OD, et al. Great horsetail (Equisetum telmateia Ehrh.): Active substances content and biological effects. Excelsior J. 2012;11:59.
42. Odebisi-Omokanye MB, Agbabiaka TO, Zakariyah RF, et al. Antibacterial effect of Ocimum gratissimum against bacteria from pediatric diarrhoeal stool samples in the tropics. J Sci Technol (Ghana). 2016;36(3):64-74.
43. Kuhnt M, Pröbstle A, Rimpler H, et al. Biological and pharmacological activities and further constituents of Hyptis verticillata. Planta Medi. 1995;61(3):227-232.
44. Afolabi CA, Emmanuel AE, and EO F. Phytochemical constituent and antioxidant activity of extract from the leaves of Ocimum gratissimum. Sci Res Essays. 2007;2(5):163-166.

45. Irshad S, Butt M, and Younus H. In-vitro antibacterial activity of Aloe barbadensis Miller. Int Res J Pharm. 2011;1:60–65.
46. Junaid SA, Olabode AO, Onwuliri FC, Okwori AE, et al. The antimicrobial properties of Ocimum gratissimum extracts on some selected bacterial gastrointestinal isolates. Afr J Biotechnol. 2006;5(22):2315-2321.
47. Doughari JH and Manzara S. In vitro antibacterial activity of crude leaf extracts of Mangifera indica Linn. Afr. J. Microbiol. Res. 2008;2(1):67-72.
48. Nalubega R, Kabasa JD, Olila D, et al. Evaluation of the antibacterial activity of selected ethnomedicinal plants for poultry in Masaka district, Uganda. Res J Pharmacol. 2011;5(2):18-21.
49. Nair R, Kalariya T, and Chanda, S. Antibacterial activity of some selected Indian medicinal flora. Turk J Biol. 2005;29(1):41-47.
50. Vaghasiya Y and Chanda S. Screening of methanol and acetone extracts of fourteen Indian medicinal plants for antimicrobial activity. Turk J Biol. 2007;31(4):243-248.
51. Kedarnath M Vishwanath B, Patil C. Antimicrobial activity of Aloe Vera leaf extract. India Department of Biotechnology, B.V. Bhoomreddy College of UG and PG Studies, Bidar, Karnataka, India. 2013; 287-288p.
52. Clarkson C, Maharaj VJ, Crouch NR, et al. In vitro antiplasmodial activity of medicinal plants native to or naturalized in South Africa. J Ethnopharmacol. 2004;92(2-3):177-191.
53. Haile Y, Ensermu K, Tamirat B, et al. Plants used in the traditional management of human ailments at Bale Mountains National Park, Southeastern Ethiopia. J Med Plants Res. 2008;2(6):132-153.
54. Akoachere JT, Tanih NF, Ndip LM, et al. Phenotypic characterization of Salmonella Typhimurium isolates from food animals and abattoir drains in Buea, Cameroon. J Health Popul Nutr. 2009;27(5):612.
55. Mahami T, Odonkor S, Yaro M, et al. Prevalence of antibiotic-resistant bacteria in milk sold in Accra. Int Res J Microbiol. 2011;2(4):126-132.
56. Haran KP, Godden SM, Boxrud D, et al. Prevalence and characterization of staphylococcus aureus, including methicillin-resistant Staphylococcus aureus, isolated from bulk tank milk from Minnesota dairy farms. J Clin Microbiol. 2012;50(3):688-695.
57. Unease NC and Abarikwa PO. Antimicrobial activity of certain medicinal plants used in traditional medicine in Nigeria. A preliminary study. J Microbiol 1996;69(1):32-40.

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