Antibacterial activities of *Calpurnia aurea* and *Ocimum lamiifolium* extracts against selected gram positive and gram-negative bacteria

Addis Melese[1], Beyene Dobo[1,*], Abriham Mikru[1]

[1]Hawassa University, Department of Biology, PO Box 05, Hawassa, Ethiopia.

ABSTRACT

Indigenous knowledge, literature reports and ethnobotanical records suggest that plants are the basis for medicines. This study was designed to examine *in-vitro* antibacterial activity of *Calpurnia aurea* (leaf, bark) and *Ocimum lamiifolium* (leaf, flower) collected from Wonsho and Shebedino districts of Sidama Zone, southern Ethiopia, with different solvents against three Gram negative (*Escherichia coli, Salmonella typhimurium* and *Pseudomonas aeruginosa)* and one Gram positive *(Staphylococcus aureus*) bacteria in 2018. The leaf and bark of *Calpurnia aurea and* leaf and flower of *Ocimum lamiifolium* were dried, powdered and extracted with 80% acetone, ethanol, methanol and distilled water. Disc diffusion method was used for the antibacterial assay and measuring the zone of inhibition and minimum inhibition concentration (MIC) was determined by broth macrodilution method. The highest percentage yield of crude bioactive agents, i.e., 36.9% was obtained from *Ocimum lamiifolium* leaf with methanol as a solvent, while the lowest yield 12.6% was obtained from *Calpurnia aurea* bark with acetone extract. All crude extracts from the different plant parts showed antibacterial activity. Accordingly, *Calpurnia aurea* bark with methanol extract exhibited the highest antibacterial activity 22.64±0.95 (mm) against *S. aureus* which was comparable to standard antibiotic disc Ciprofloxacin with inhibition zone of 24.00 ±0.19 (mm), while the lowest inhibition of 6.12±0.41 (mm) was recorded from *Ocimum lamiifolium* flower with water crude extract against *P. aeruginosa*. The MIC of 3.13mg/ml was observed from methanol crude extract of bark of *Calpurina aurea* on *S. aurea*. Crude bark extract of Methanol showed the highest antibacterial activity. The studies revealed that antibacterial activity of the crude extracts from the different parts of the plant were variable when extracted by different solvents, however, possesses good antimicrobial activity which support the traditional use of the plant in the treatment of bacterial infections under study. Finally, to support the traditional users, scientific verification on phytochemical analysis and toxicity test should be carried out to confirm users' safety.

Key words: Antibacterial activity, Disc diffusion, MIC, Plant crude extract, Zone of inhibition

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[*]Corresponding author: beyeneashl@yahoo.co.uk

INTRODUCTION

Medicinal Plants are important antimicrobial agents used in different parts of the world. 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 (Owolabi *et al*., 2007). 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, such plants should be investigated for better understanding of their properties, safety and efficacy to develop antimicrobial drugs (Khulbe and Sati, 2009). Accordingly, the utilization of plants for the production of natural compounds of commercial interest draws increasing attention over the past decades (Canter *et al*., 2005). Furthermore, the emergence of microbial drug resistance and limited therapeutic efficacy of many of the available drugs necessitated search for potent antibacterial drugs with new modes of action. Local medicinal plants are potential source of novel antimicrobial agents and anti-Quorum sensing substances (Bacha *et al*., 2016). According to Abebe Dawit (2011), traditional remedies are the most important and sometimes the only source of therapeutics for nearly 80% of the population and 95% of traditional medicinal preparations in Ethiopia.

Ethiopia is a country known for its rich plant biodiversity and traditional use of plant-based drugs for curing or treating of many human and animal diseases. In Ethiopia alone, 1000 plant species are estimated to be in use for traditional medication (Tesema *et al*., 2002). WHO estimated that the majority of the population in developing countries, including 90% of African population, rely on traditional medicinal plants for their healthcare (WHO, 2011). Today, infectious diseases are responsible for mortality (particularly in third-world countries), and worse, synthetic (artificial) antibiotics are expensive. 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 urgent need to explore new effective drugs for the treatment of infectious diseases (Aiyegoro *et al*., 2011). Plant products and their active constituents play an important role in plant disease control by combating growth and development of pathogens. Plant based natural constituents can be derived from any part of the plant like bark, leaves, flowers, roots, fruits, seeds, etc. (Gordon and David, 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 disease causing pathogens. Antimicrobial research is geared towards the discovery and development of novel antibacterial and antifungal agents.

Ocimum lamifolium is an important medicinal herb belonging to family *Lamiaceae.* It is an indigenous plant in Ethiopia, locally called "dama-kassie'' (in Amharic) and is one of the most common traditional medicinal plants used in Ethiopia (Mirutse *et al*., 2010*). Ocimum lamiifolium* extracts are also known to have antibacterial, antifungal, insect repellent (Ermiyas Dagne, 2009), and anti-inflammatory activities in humans (Kashyap *et al*., 2011).

Calpurnia aurea is a plant within the family of *Fabaceae*. It is widely distributed in Ethiopia with a local name "digita" in Amharic and used in traditional medicine to treat diverse medical conditions in humans and animals (Zorloni, 2007). Abdella *et al*. (2013) reported that all parts of the plant are used for different human and animal diseases. Tadeg *et al*. (2005) have also reported that different parts of *Calpurina aurea* are used to treat different diseases such as diarrhea, stomach-ache, bowel, and bladder disorders. All these tell us that a range of medicinal plants with antimicrobial properties have been widely used by traditional healers. However, therapeutic potentials of some of these medicines have not been scientifically evaluated (Havagiray *et al*., 2004). Therefore, it is necessary to establish the scientific basis for the therapeutic actions of traditional plant medicines as these may serve as the source for the development of more effective drugs. The aim of the present study was, therefore, to evaluate the possible antimicrobial (in vitro) properties of the leaf and bark extract of *C. aurea* and leaf and flower of *O. lamiifolium* against four pathogenic bacterial strains (*Escherichia coli, Staphylococcus aureus, Salmonella typhimurium* and *Pseudomonas aeruginosa).*

MATERIALS AND METHODS

Description of study area

The study medicinal plants were collected from Boricha and Dale Woreda (District) of Sidama zone, southern Ethiopia. Sidama administrative Zone is found in Southern Nations Nationalities and Peoples Regional State (SNNPR), located 313 km away from Addis Ababa. It is located in the north eastern part of the region and bounded by Oromiya in the North, east and south east, with Gedieo Zone in the South, and Wolayta Zone in the West. Its geographic location lies between 6°14′ and 7°18′ North latitude and 37°92′ and 39°14′ East longitude. Total area of the

Sidama Administrative Zone is about 6981.8 km^2. The mean annual temperature is 19.5 °C. With 19 woredas and 4 administrative towns, it is one of the densely populated zones in the region (CSA, 2013; Sidama Zone FEDD Abstract, 2014).

Collection and authentication of plant material

Fresh leaf and barks of *Calpurnia aurea*, and also flowers and leaf of *Ocimum lamiifolium* were collected from randomly selected *kebeles* of Boricha district (Arossa and Galakohireye) and Dale district (Ferro I and Ferro II) of Sidama during the months of September and October 2018 and the identity of each plant specimen was confirmed at the National Herbarium, Addis Ababa University. Parts of plant separately collected were 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 final rinsing with distilled water. They were then placed on clean plastic plates and air dried at room temperature until their weight became constant. Then the dried samples were grounded to fine powder using electric grinder (FM100 model, China) and stored in sterile bottle at 4 °C for analysis of disc diffusion and MIC against test bacteria (Handa *et al*., 2008).

Preparation of plant extracts

The extracts of leaf and bark of *Calpurnia aurea*, and leaf and flower of *Ocimum lamiifolium* were prepared by dissolving 10 g of each fine plant powder separately in 100 mL of 80% of acetone, (80%) methanol, (80%) ethanol and water (CSLI, 2012). The extracts were prepared in 250 ml capacity conical flask by soaking 10 g of each plant powder separately in 100 ml of each solvent, methanol, ethanol, acetone and water for 3 days (hand-shaken several-to-many times a day) until the soluble material dissolved. Thereafter, each extract was filtered using Whatman no.1 filter paper and the filtrate was dried in oven with rotary fan at 40 °C until the solvent from extracts further evaporated. The resulting extracts were packed into a vial and stored at 20 °C until further investigation (Sukhdev *et al*., 2008).

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 the weight of the container alone and W_0 the weight of the dried plant material (Anokwuru *et al*., 2011).

Test organisms

The test bacteria one gram positive (*Staphylococcus aureus)* and three gram negative (*Escherichia coli, Pseudomonas aeruginosa* and *Salmonella*

typhimurium), all clinical isolates were obtained from Ethiopian Public Health Institute (EPHI), Addis Ababa, Ethiopia. The test bacteria were cultured on nutrient agar and stored at 4 °C until use (CLSI, 2012).

Preparation of media

The medium was prepared according to the manufacturer's instructions. 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-dishes and allowed to dry until the excess moisture from the surface of the agar was removed before use. The sterility of the prepared media was checked by incubation of randomly selected plates at 37 °C for 24 h (CLSI, 2012).

Preparation of inocula

This method assesses the antimicrobial activity of a bioactive compound by culturing bacteria in the presence of the compound/extract and measuring the zone of inhibition which corresponds to the area where no bacterial growth is observed under optimum conditions for bacterial growth. The higher the diameter (zone of inhibition) the more susceptible will be the bacteria to the bioactive compounds/extracts of the plant.

The method was executed 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 with the help of a sterile wire loop into a separately labeled test tube containing 5 ml of nutrient broth and incubated to grow at a temperature of 37 °C for two hours. The prepared culture was standardized to 0.5 McFarland turbidity standards using the spectrophotometer (optical density of 1.0 at 625 nm) by adding sterile nutrient broth to obtain the desired cell density of 1.5×10^8 (cells/ml). The 0.5 McFarland turbidity standard was prepared by adding 0.5 ml of 0.048 M $BaCl_2$ (1.17% w/v $BaCl_2.2H_2O$) to 99.5 ml of 0.18 M H_2SO_4 (1% v/v 0.05 ml of 1.175% of barium chloride dihydrate ($BaCl_2 \bullet 2H_2O$), with 9.95 mL of 1% sulphuric acid (H_2SO_4).

Preparation of Disc

Diffusion discs of 6 mm diameter were prepared from absorbent filter paper (Whatman no.1) by using a paper Puncher and sterilized at 120 °C for 1 h and dried in an oven. Then after, sterilized discs were soaked aseptically by applying 30 µl of each crude extract of plant at a concentration of 100 mg/ml using sterile digital

micropipette and then allowed to dry at a room temperature for 15 minutes and then placed in sterile container and stored at 4 ºC until further use (CLSI, 2012).

Disc diffusion test

The disc diffusion technique has been widely used to assay plant extract for antimicrobial activity (Taiwo *et al*., 2007). A sterile cotton swabs were dipped into the adjusted standardized broth inoculums suspension by rotating the swab. The swab was then evenly streaked over the entire surface of Muller Hinton agar plate. Streaking was repeated by rotating the plate approximately 60º each time to ensure an even distribution of inoculum. After inoculation, for each test bacterium, sterilized discs which were soaked under 30 µl of each crude extract of plant at a concentration of 100 mg/ml were applied while sterile, blank paper discs were soaked by each solvent (ethanol, methanol, acetone and water served as negative control), standard antibiotic ciprofloxacin disc 30 µg/disc was used as positive control.

Finally, the disc was applied on the inoculated 90 mm plates using flame sterilized forceps approximately equidistance to each other. Finally, all the plates were incubated at 37ºC for 24 hours. The antibacterial activities of the plant extracts were evaluated by measuring the diameter of the inhibition zone in each of the plates at the end of the incubation period. The diameter of the inhibition zone, including the diameter of the disc was measured by using sliding digital micro caliper. The bacterial activity of the crude extracts on the test bacteria were compared with those of the negative and positive controls according to CLSI (2012).

Minimum Inhibition Concentration (MIC)

The minimum inhibitory concentration is defined as the lowest concentration which can inhibit any visible bacterial growth on the culture plates (Radojevic *et al*., 2012). In this study Broth macro dilution assay were used. For each plant extract, a stock solution of 200 mg/ml was prepared. A 10 ml of Muller Hinton broth was added into each sterilized test tubes. From a stock solution, a double dilution method to bring 100 mg/ml, 50 mg/ml, 25 mg/ml, 12.5 mg/ml, 6.25mg/ml, 3.125mg/ml and 1.56mg/ml) were performed by using sterile digital micropipette (CLSI, 2012). Finally, 20 µl standard suspension of the test organism (which is adjusted to 0.5 McFarland standards) was added to each tube. Mixed by gently shaking the tubes and incubated the tubes at 37 ºC for 24 h. After 24 h incubation, the solution was further inoculated in agar plates. MIC was taken as the highest dilution of the extract that inhibited the growth of the bacteria. The lowest concentrations of the extracts, which inhibited the bacterial growth after a period of 24 h of incubation at 37 °C, were recorded as MIC. Broth inoculated with test organism without extract solution was used as a positive control and only broth was used as a negative control. All testes were done in triplicates (CLSI, 2012).

Data analysis

For each assay, all the measurements were triplicate and the results were presented as mean ± SD. The statistical analyses were performed using two-way ANOVA. Then Duncan`s test was used to compare means of antibacterial activity as compared to extraction solvents, plant parts and the difference in the sensitivity of the test microorganisms using the statistical package for social Sciences (SPSS) version 20 and P-values < 0.05 were considered as statistically significant.

RESULTS AND DISCISIONS

Percent extract yield

In this study the highest amount of extraction yield (31.9%) was obtained from 80% methanol extract of *Ocimum lamiifolium* leaf, followed by 30.4% from water extract of *Calpurnia aurea* leaf and 30.1% from *Ocimum lamiifolium* flower with 80% ethanol as a solvent, while the lowest (12.6%) was obtained from 80% acetone extract of *Calpurnia aurea* bark (Table 1).

Table 1. Percent extract yield of *Calpurnia aurea* and *Ocimum lamiifolium* extracted using different solvents

Plant type	Parts used	Extraction type	% yield (w/w)
Calpurnia aurea	Leaf	Water	30.4
		Acetone	28.5
		Ethanol	20.2
		Methanol	26.2
	Bark	Water	16.0
		Acetone	12.6
		Ethanol	19.0
		Methanol	21.0
Ocimum lamiifolium	Leaf	Water	22.3
		Acetone	15.7
		Ethanol	21.5
		Methanol	31.9
	flower	Water	23.5
		Acetone	17.3
		Ethanol	30.1
		Methanol	24.2

The highest amount of extraction yield (31.9%) was obtained from *Ocimum lamiifolium* leaf from 80% methanol followed by water (22.3%) and 80% ethanol (21.5%). This result showed better yield than that reported by Amare *et al*. (2013) on 80% ethanol (9.4%) and water (10.7%) crude extract. The lowest extraction

yield (15.6%) was observed from *Ocimum lamiifolium* leaf from 80% acetone solvent. The highest yield (30.1%) observed in the case of *Ocimum lamiifolium* flower with 80% ethanol was followed by 24.2% with 80% methanol and 23.5% with water extract. The lowest (17.32%) extract yield was observed from *Ocimum lamiifolium* flower with 80% acetone as a solvent.

Regarding *Caplurnia aurea* leaf the highest extraction yield (30.4%) observed from water extract was followed by (28.5%) from 80% acetone and (26.2%) from 80% methanol as extraction solvent. A comparable result (29.5%) was observed by Hailu *et al.* (2005) with 80% methanol extract of *Calpurnia aurea* leaf. In the case of *Calpurnia aurea* bark the maximum yield (21%) extracted from 80% methanol was followed by 19% extracted with 80% ethanol. The lowest extract yield (12.6%) was observed with 80% acetone extraction solvent.

This study showed solvent with varying polarity had a significant effect on the extraction capacity. Selecting appropriate extraction solvent is crucial in the extraction process. Different solvents have different extraction capacities and spectrum of solubility for phyto-constituents (Srinivasan *et al*., 2001). These bioactive compounds are maximally extracted in more polar solvents (Chenielle *et al*., 2009; Perumal *et al*., 2012). The addition of 20% water to 80% methanol, ethanol and acetone has enhanced the antimicrobial activity of the extract. Thus, the selective extraction of bioactive molecules from natural sources by appropriate solvents is important for obtaining compounds with high biological activities which can be used as preservative ingredients in the pharmaceutical industry (Perumal *et al*., 2012).

Antibacterial Assay

The antibacterial assay of the crude extract of all tested plant parts are shown in Table 2. The highest zone of inhibition were observed among all tested plant part on methanol crude extract of *Calpurnia aurea* bark (22.64±0.95mm) against *S. aureus* followed by methanol crude extract (19.18±0.66 mm) of the bark against *E. coli* and ethanol crude extract of *Calpurnia aurea* bark (17.49±2.56 mm) against *S. typhimurium*, while the least zone of inhibition was observed from *Ocimum lamiifolium* flower with water crude extract 6.12±0.41 mm) against *P. aeruginosa* (Table 2).

The results of the antibacterial assay of the crude extract of *Calpurnia aurea* leaf with four different solvents showed zone of inhibition against all tested bacteria. The highest zone of inhibition (14.59±0.72 mm) was observed with methanol extract against *E. coli* which was significantly different ($P<0.05$) from ethanol (11.24±1.18 mm) and water (10.63±0.85 mm) crude extracts against *E. coli*. The methanol crude extract of *Calpurnia aurea* leaf showed zone of inhibition of 13.86±0.33 mm against *S. aureus* and not significantly different ($P>0.05$) with

acetone crude extract (12.4±1.69 mm). The highest zone of inhibition (9.57±0.68 mm) was recorded against *S. typhimurium* with ethanol crude extract of *Calpurnia aurea* leaf. Also, methanol extract of *Calpurina aurea* leaf showed highest zone of inhibition (9.52±0.4 mm) against *P. aeruginosa.* This study demonstrated a comparable result with the findings obtained by Shemsu *et al*. (2013), which recorded inhibition zone of the methanol extract of *Calpurnia aurea* leaf to be 14 mm against *E. coli*, followed by 11 mm against *S. aurea,* 9 mm against *S. typhimurium* and 9 mm against *P. aerugenosa*.

In agreement to the current study, Binyam *et al*. (2014) reported high antibacterial activity (13.3 mm) against *E. coli* and (8.6 mm) against *S. typhimurium* with methanol extract of leaf of *Calpurnia aurea*. Acetone crude extract of *Calpurnia aurea* leaf showed the highest zone of inhibition (13.47±2.01 mm) against *E. coli* and 12.4±1.69 mm against *S. aureus* which was better antibacterial activity than ethanol crude extract (11.24 mm) against *E. coli* and 9.27 mm against *S. typhimurium*. The least zone of inhibition (6.82±0.92 mm) regarding *Calpurnia aurea* leaf was observed with water extract against *S. typhimurium.*

The methanol extract of *Calpurnia aurea* bark showed the high zone of inhibition (22.64±0.95 mm) against *S. aureus* which was comparable to that of the standard antibiotic *Ciprofloxacin* (24.00 ±0.19 mm), and was significantly different ($P<0.05$) with acetone (17.4±1.46 mm), ethanol (13.67±0.83 mm) and water (10.54±0.54 mm) crude extract of *Calpurnia aurea* bark against *S. aureus*. Also, methanol extract of *Calpurnia aurea* showed high zone of inhibition against *E. coli* (19.18±0.66 mm) which was significantly different ($P<0.05$) with ethanol (16.71±0.89 mm), acetone (15.53±0.27 mm) and water (7.29±1.61 mm) crude extract of *Calpurnia aurea* bark against *E. coli*. The ethanol extract of *Calpurnia aurea* bark showed the high zone of inhibition (17.49±2.56 mm) against *S. typhimurium*, and not significantly different ($P>0.05$) with methanol (16.13±1.67 mm) but they were significantly different ($P<0.05$) from acetone (11.82±1.19 mm) and water (8.61±1.1 mm) crude extract of *Calpurnia aurea* against *S. typhimurium*. The ethanol extract of *Calpurnia aurea* bark showed the high zone of inhibition (11.95±1.1 mm) against *P. aeruginosa,* which was not significantly different from methanol crude extract (11.78±1.01 mm) and acetone (10.04±1.57 mm) crude extract of *Calpurnia aurea* bark against *P. aeruginosa.*

The current results of the methanol crude extract of *Ocimum lamiifolium* leaf showed antibacterial activity against all tested bacteria; on *E. coli* (14.7±2.39 mm) followed by (13.2±1.49 mm) against *S. aureus* (11.84±0.74 mm) against *S. typhimurium* and (10.63±0.54 mm) against *P. aeruginosa*. In contrast, methanol extract of *Ocimum lamiifolium* didn't show any zone of inhibition against *P.*

aeruginosa (Teklit Gebregiorgis Amabye and Said Mussa, 2015) but in this study *P. aeruginosa* had zone of inhibition of 10.63±0.54 mm. This variation might be due to extraction solvent.

In this study ethanol and water crude extract of *Ocimum lamiifolium* leaf had antibacterial activity against *E. coli, S.aureus* and *P. aeruginosa.* Similarly, ethanol crude extract showed antibacterial activity against *E. coli* (13.5 mm) and against *S. aureus* (12 mm) and also water crude extract of *Ocimum lamiifolium* against *E. coli* (15.5 mm), *S. aureus* (13.5 mm) and *P. aeruginosa* (13 mm) (Amare *et al*., 2013).

The methanol extract of *Ocimim lamiifolium* flower showed the highest zone of inhibition (13.91±2.2 mm) on *E. coli*. The next highest (12.85±0.64 mm) was recorded for methanol crude extract against *S. aureus.* Zone of inhibition recorded for methanol extract against *S. typhimurium* and *P. aeruginosa* were 10.39±0.83 mm and 9.42±0.76 mm, respectively. The difference in zone of inhibition among the four tested bacteria depends on the extraction solvent, the bacteria inheritance behavior and the plant parts (Bhumika and Bijal, 2015).

The positive and negative controls were also carried out to monitor antimicrobial activity of the medicinal plants. Standard antibiotics Ciprofloxacin was taken as positive control. All tested bacteria were highly susceptible to standard antibiotic with different zone of inhibition for different microorganisms; in *E. coli* (26.15±0.38 mm)*, S. aureus* (24.00±0.19 mm), *P. aeruginosa* (27.70±0.45 mm) and *S. typhimurium* (26.83±0.27 mm). Also, a comparable result was recorded from the methanol crude extract of *Calpurnia aurea* bark to standard antibiotic against *S. aureus*. All negative controls didn't show any antibacterial activities (Table 2).

In the current study there was a significant difference ($p< 0.05$) in antibacterial activities among the crude extracts of the two plant parts of *Calpurnia aurea* (leaf and bark) depend on the extraction solvents against all tested bacteria. Comparing the mean inhibition zone of *Calpurnia aurea* bark, the crude extracts from bark with methanol as a solvent showed the highest inhibition zone (17.19 mm) against all tested bacteria and significantly different ($P<0.05$) from the three solvents.

The crude extract from the bark of *Calpurnia aurea* showed mean inhibition zone (14.12 mm) with ethanol extract and 13.70 mm with acetone extract had a better antibacterial activity and also had a significant difference ($P<0.05$) among them. The lowest antibacterial activity (8.90 mm) was recorded from the crude extract of *Calpurnia aurea* barks with water as a solvent. This indicated that the release of bioactive compounds on plant part depend on extraction solvent against all tested bacteria and comparing among *Calpurnia aurea* bark with different solvents, the methanol extract of *Calpurnia aurea* bark was effective to treat those tested bacteria (Table 3).

Table 2. (Mean±SD) of antibacterial activities of leaf and bark of *Calpurnia aurea* and leaf and flower of *Ocimum lamiifolium* crude extracts obtained using different solvents against four bacterial species.

Plant Part	Solvent	Zone of inhibition (mm)			
		E. coli	***S. aureus***	***P. aeruginosa***	***S. typhimurium***
Calpurnia aurea leaf	Water	10.63 ± 0.85^{ab}	9.03 ± 0.45^{ab}	8.78 ± 1.82^{a}	6.82 ± 0.92^{a}
	Acetone	13.47 ± 2.01^{cbd}	12.4 ± 1.69^{cd}	8.69 ± 1.27^{a}	9.15 ± 1.01^{bc}
	Ethanol	11.24 ± 1.18^{bca}	9.27 ± 1.47^{ba}	8.72 ± 0.2^{a}	11.07 ± 0.89^{dc}
	Methanol	14.59 ± 0.72^{dc}	13.86 ± 0.33^{dc}	9.52 ± 0.4^{a}	9.57 ± 0.68^{cbd}
Calpurnia aurea Bark	Water	7.29 ± 1.61^{a}	10.54 ± 0.54^{a}	9.16 ± 0.72^{a}	8.61 ± 1.1^{5ab}
	Acetone	15.53 ± 0.27^{bc}	17.4 ± 1.46^{c}	10.04 ± 1.57^{bcd}	11.82 ± 1.19^{ba}
	Ethanol	16.71 ± 0.89^{cb}	13.67 ± 0.83^{b}	11.95 ± 1.1^{dbc}	17.49 ± 2.56^{dc}
	Methanol	19.18 ± 0.66^{d}	22.64 ± 0.95^{d}	11.78 ± 1.01^{cbd}	16.13 ± 1.67^{cd}
Ocimum lamiifolium leaf	Water	11.16 ± 2.38^{ab}	10.3±0.52a	7.96 ± 3.53^{abc}	8.51 ± 1.52^{ab}
	Acetone	15.25 ± 1.17^{dbc}	13.8 ± 1.49^{dc}	8.05 ± 1.29^{bcd}	8.53 ± 0.74^{ba}
	Ethanol	13.57 ± 1.17^{bcd}	12.77 ± 1.52^{bc}	8.91 ± 1.01^{cbd}	10.4 ± 2.27^{cd}
	Methanol	14.7 ± 2.39^{cbd}	13.2 ± 3.2^{cdb}	10.63 ± 0.54^{d}	11.84 ± 0.74^{dc}
Ocimum lamiifolium Flower	Water	7.86 ± 0.4^{a}	7.13 ± 0.86^{a}	6.12 ± 0.41^{abc}	8.21 ± 0.98^{bac}
	Acetone	13.17 ± 0.78^{cdb}	12.42 ± 1.76^{cbd}	7.82 ± 0.61^{bacd}	8.16 ± 1.64^{abc}
	Ethanol	13.02 ± 0.4^{bcd}	10.41 ± 1.64^{bcd}	8.03 ± 1.54^{cbad}	9.86 ± 179^{cab}
	Methanol	13.91 ± 2.2^{dbc}	12.85 ± 0.64^{dbc}	9.42 ± 0.76^{dbc}	10.39 ± 0.83^{dc}
Ciprofloxacin(+ve control)		26.15± 0.38	24.00 ±0.19	27.70±0.45	26.83±0.27
Solvent(-ve control)		na	na	na	na

Mean values in the column that bear different superscript letters are significantly different at ($P<0.05$); *na* for not active

In the case of the leaf solvent extract of *Calpurnia aurea* the methanol extract had the highest (11.89 mm) antibacterial activity against all tested bacteria and significantly different ($p<0.05$) from the acetone (10.93mm), ethanol (10.08mm) and water (7.72 mm) crude extract of *Calpurnia aurea* leaf. There was no significance difference ($p>0.05$) among acetone (10.93 mm) and ethanol (10.08 mm) crude extract of *Calpurnia aurea* leaf but significantly different ($p<0.05$) from water (7.72 mm) and methanol (11.89 mm) crude extract in their zone of inhibition against all tested bacteria. The least zone of inhibition (7.72 mm) was observed from water crude extract of *Calpurnia aurea* leaf against all tested bacteria. Comparison between *Calpurnia aurea* leaf with different solvents shows that the methanol extract of *Calpurnia aurea* leaf was effective to treat those tested bacteria (Table 3).

Table 3. Plant parts by extraction solvent interaction effect on antibacterial activity of *Calpurnia aurea*

			95% Confidence Interval for Mean		
Plant Part	**Solvent**	**Mean**	**Std. Error**	**Lower Bound**	**Upper Bound**
Calpurnia aurea Bark	Water	8.90[a]	0.44	7.93	9.88
	Acetone	13.70[b]	0.93	11.65	15.75
	Ethanol	14.12[c]	0.71	12.56	5.68
	Methanol	17.19[d]	1.14	14.67	19.71
Calpurnia aurea Leaf	Water	7.72[a]	0.45	6.73	8.71
	Acetone	10.93[bc]	0.73	9.33	12.53
	Ethanol	10.08[cb]	0.42	9.16	11.00
	Methanol	11.89[d]	0.72	10.30	13.48

Mean values in the column that bear different superscript letters are significantly different at ($P<0.05$).

Regarding *Ocimum lamiifolium* flower, there was a significant difference ($p<0.05$) in antibacterial activities among the crude extracts of the two plant parts. Starting from *Ocimum lamiifolium* flower the crude extracts with methanol as a solvent showed the highest (12.64 mm) inhibition zone against all tested bacteria and significantly different ($p<0.05$) from three solvents acetone (10.40 mm), ethanol (9.92 mm) and water (7.46 mm), in their zone of inhibition against all tested bacteria. Also, there was no significant difference($P>0.05$) among acetone and ethanol crude extract of *Ocimum lamiifolium* flower and had better antibacterial activity than that of water which had a least (7.46 mm) zone of inhibition. Comparing among *Ocimum lamiifolium* flower with different solvents, the methanol extract of *Ocimum lamiifolium* flower was effective to treat those tested bacteria (Table 4).

In the case of the leaf solvent extract of *Ocimum lamiifolium*, the methanol extract showed the highest zone of inhibition (12.60 mm) against all tested bacteria and significantly different ($p<0.05$) from ethanol (10.42 mm) and water (7.46 mm) crude extracts. The lowest zone of inhibition (7.74 mm) was observed from water extract. Comparison among *Ocimum lamiifolium* leaf extract with different solvents, show that the methanol and acetone extract of *Ocimum lamiifolium* leaf was effective to treat those tested bacteria (Table 4).

Table 4. Plant parts by extraction with solvent interaction effect on antibacterial activity of *Ocimum lamiifolium*

Plant part	Solvent	Mean	Standard error	95% confidence interval	
				Lower	Upper
Ocimum lamiifolium leaf	Water	7.74[a]	0.67	6.26	9.21
	Acetone	11.41[cbd]	1.00	9.22	13.61
	Ethanol	10.42[bc]	0.51	9.28	11.55
	Methanol	12.60[dc]	0.68	11.09	14.10
Ocimum lamiifolium flower	Water	7.46[a]	0.27	6.86	8.06
	Acetone	10.40[cb]	0.80	8.64	12.15
	Ethanol	9.92[bc]	0.51	8.80	11.04
	Methanol	12.64[d]	0.63	10.26	13.03

Mean values in the column that bear different superscript letters are significantly different at ($P<0.05$).

Among all tested plant parts *Calpurnia aurea* (bark and leaf) and *Ocimum lamiifolium* (leaf and flower), *Calpurnia bark* had a better antibacterial activity (13.48 mm) and significantly different ($p<0.05$) from *Calpurnia aurea* leaf (10.15 mm), *Ocimum lamiifolium* leaf (10.54 mm) and *Ocimum lamiifolium* flower (9.86 mm) against all tested bacteria. Also, there is no significance difference ($p>0.05$) among *Ocimum lamiifolium* leaf (10.15 mm), *Ocimum lamiifolium* flower (9.86 mm) and *Calpurnia aurea* leaf (10.15 mm) in their zone of inhibition. This result indicated that *Calpurnia aurea* bark had high anti-bacterial activity than the others against tested bacteria (Table 5).

Minimum inhibitory Concentration (MIC)

The MIC assay was also employed to evaluate the effectiveness of the extracts to inhibit the growth of the tested bacteria. In the current study the MIC of water extracts of all tested plant parts were between 100-200mg/ml against all tested bacteria. The MIC of acetone extract of *Calpurnia aurea* leaf (25 mg/ml) inhibited *E. coli* and *S. aureus*. In this case ethanol extract (25 mg/ml) inhibited *E. coli* and 50mg/ml inhibited *S. aureus* and *S. typhimurium*.

Among methanol crude extracts of *Calpurnia aurea* leaf the MIC was 12.5 mg/ml which inhibited *E. coli* and 25 mg/ml inhibited *S. aureus*. The MIC value of methanol extract of *Calpurnia aurea* leaf against *S. aureus* was 25mg/ml which was agreed with the result of Hailu *et al*. (2005) in which the MIC value of 25mg/ml inhibited *S. aureus.* Also, a comparable result was observed from Shemsu *et al*. (2013) in which 62.5 mg/ml inhibited *S. typhimurium.*

Table 5. Plant parts of *Calpurnia aurea* and *Ocimum lamiifolium* interaction with bacterial species.

Plant part	Mean	Standard error	95% Confidence Interval	
			Lower	Upper
Calpurnia aurea Leaf	10.15^{bac}	0.37	9.42	10.89
Calpurnia aurea Bark	13.48^{d}	0.60	12.28	14.68
Ocimum lamiifolium Leaf	10.54^{cab}	0.44	9.65	11.43
Ocimum lamiifolium Flower	9.86^{abc}	0.36	9.13	10.58

Mean values in the column that bear different superscript letters are significantly different at ($P<0.05$).

In the case of acetone crude extract of *Calpurnia aurea* bark 12.5 mg/ml, 25 mg/ml and 50 mg/ml inhibited *S. aureas, E. coli* and *S. typhimurium* respectively. Among ethanol crude extracts of *Calpurnia aurea* bark 6.25 mg/ml inhibited *S. typhimurium,* 25mg/ml inhibited *E. coli* and 50mg/ml inhibited *S. aureus*. The MIC of methanol crude extract of *Calpurnia* bark was 3.12 mg/ml that inhibited *S. aureus* which had a potential to treat disease of *S. aureus*, also 6.25 mg/ml inhibited *E. coli and* 12.5 mg/ml inhibited *S. typhimurium* (Table 6).

The MIC of acetone crude extract of *Ocimum lamiifolium* leaf was 25 mg/ml which inhibited *E. coli* followed by 50 mg/ml inhibited *S. aureus*. MIC of ethanol crude extract of *Ocimum lamiifolium* leaf (50 mg/ml) inhibited *E. coli, S. aureus and S. typhimurium* respectively. In case of *Ocimum lamiifolium* leaf methanol crude extract 25 mg/ml inhibited *E. coli* and 50 mg/ml crude extract inhibited *S. aureus*, *P. aeruginosa* and *S. typhimurium.* The MIC of Water crude extract of *Ocimum lamiifolium* leaf which inhibited *S. aureus* at 200 mg/ml, also, inhibited *E. coli* and *P. aeruginosa* at 100mg/ml. A comparable report observed from Amare *et al*. (2013) 200mg/ml inhibited *S. aureus*, as well as 100 mg/ml inhibited *E. coli* and *P. aeruginosa*. The MIC of acetone crude extract of *Ocimum lamiifolium* flower (50 mg/ml) inhibited *E. coli* and *S. aureus*. Methanol extract of *Ocimum lamiifolium* flower inhibited *E. coli* at 25 mg/ml and also inhibits *S. aureus* and *S. typhimurium* at 50 mg/ml. The MIC of Ethanol crude extract of *Ocimum lamiifolium* flower inhibited *E. coli* at 50 mg/ml (Table 6).

Tested plant parts which were extracted with different solvents showed different MIC. The methanol crude extract of *Calpurnia aurea* leaf had a potential to inhibit *E. coli* at 6.25 mg/ml so, it had a potential to treat *E. coli*. Also, the methanol crude extract of *Calpurnia aurea* bark had a potential to inhibit *S. aureus* at 3.12 mg/ml, so it has a potential to treat of these bacteria. The ethanol crude extract of *Calpurnia aurea* bark had a potential to inhibit *S. typhimurium* at 6.25 mg/ml; so, it has a potential to treat *S. typhimurium* (Table 6).

Table 6. Minimum inhibitory concentrations (mg/ml) of *C. aurea* (leaf, bark) and *O. lamiifolium* (leaf, flower) in four different extraction solvents

Plant parts	**Extraction solvent**	**Bacteria species**			
		E. coli	***S. aureus***	***P. aeruginosa***	***S. typhimurium***
C. aurea Leaf	Water	200	100	200	200
	Acetone	25	25	100	50
	Ethanol	25	50	100	50
	Methanol	12.5	25	100	50
C. aurea Bark	Water	100	100	100	100
	Acetone	25	12.5	100	50
	Ethanol	25	50	100	6.25
	Methanol	6.25	3.12	50	12.5
O. lamiifolium Leaf	Water	100	200	100	100
	Acetone	25	50	100	100
	Ethanol	50	50	100	50
	Methanol	25	50	50	50
O. lamiifolium Flower	Water	200	200	200	200
	Acetone	50	50	100	100
	Ethanol	50	100	200	100
	Methanol	25	50	100	50

CONCLUSION

In conclusion, the demonstrated antimicrobial activities of the crude extracts of the four plant parts *Calpurina aurea* (leaf and bark) and *Ocimum lamiifolium* (leaf and flower) with 4 different solvents; methanol, acetone, ethanol and water have given a preface of their potentials as antimicrobial agents. Among the parts of the plants included in the current study, the barks of *Calpurnia aurea* were found to be the best source of antibacterial agents. Likewise, among the extraction solvents employed for the current study, 80% methanol was found to be the best extraction solvent. This shows the potential of the plants for developing drugs for treating various illnesses in human beings and Animals. The susceptibility of the four bacteria species appeared to be influenced by the plant parts and the extraction solvent used. This result confirmed that extracts of medicinal plants showed a comparable result to standard antibiotics and validates the use of the studied plant parts in traditional medicine.

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