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Antibacterial activity of the ethanolic extracts of the leave and bark of *Calpurnia aurea* against *Escherichia coli* O157:H7 and *Staphylococcus aureus*

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Key words: Antibacterial activities, *Calpurnia aurea,* Disc diffusion, *Escherichia coli* O157:H7, *Staphylococcus aureus.*

Abstract

The main purpose of this study was to analyze the major groups of secondary compounds of *Calpurnia aurea* and evaluate the antibacterial activity of their crude extracts against specific bacterial strains as *(Escherichia coli O157:H7,* and *Staphylococcus aureus).* Fresh leaves and bark of *Calpurnia aurea* were randomly collected from the Hosanna district in southern Ethiopia. Extraction of the sample was done by using ethanol and phytochemical analysis was carried out using a standard protocol and the contents of extracts were determined using spectrophotometric. The antibacterial effectiveness of extracts was examined against *Escherichia coli O157:H7* and *Staphylococcus aureus* using the disc diffusion method. The antibacterial activity of ethanol extract from leaves and bark revealed of inhibition against *Escherichia coli O157: H7* and *Staphylococcus aureus*. The average suppression zone in the diameter range was between 11.8 and 17.4 millimeters ethanolic leaf extract of *Calpurnia aurea* showed significant inhibition zones in *Escherichia coli O157: H7* and *Staphylococcus aureus*. Dimethyl sulfoxide used as a negative control showed no activity. This study depicts the presence of phenolic compounds, saponins, flavonoids, alkaloids, and other phytochemicals associated with *Calpurnia aurea* leaves and bark. Ethanol extract showed antibacterial activity against *Escherichia coli O157: H7* and *Staphylococcus aureus*. Therefore, the results of the current study suggest that the ethanol extract of *Calpurnia aurea* has an antibacterial activity on the test pathogens for the pathogen used in the current study. Further research on total microorganisms is needed.

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Introduction

The use of traditional medicinal plants in the treatment of various human diseased and non-pathological traditional medicinal plants is on the rise, thanks to the worldwide expansion of multidrug resistance identified in bacteria. In developed countries, traditional medicinal plants account for around 80% of the flora. As a result, such plants have been studied from time to time in order to gain a better understanding of their medical characteristics (Dawit, 2011). Ethiopians have a wide range of medicinal plant knowledge, which has been used in healing methods for both humans and cattle (Bekele, 2007). The usage of herbs or medicinal plants has a long cultural and social history in Ethiopia, owing to its medical significance. In Ethiopia, medicinal herbs are widely used due to their accessibility, real chemical, biological, and physiological effects, as well as radiation and behavioral toxicity consistency.

Herbs have become widely used due to their bio-chemicals and medicinal properties (Akroum and Satta, 2009). Infections and communicable diseases have been linked to the widespread use of extracts from therapeutic herbs. This has to do with their antibacterial properties (Ashraf *et al*., 2018). Important substantial compounds with pharmacological effects derived from natural composite sources serve as new anti-infectious agents and aid in the development of synthetic drugs, chemistry, and antibiotic manufacture. Plants continue to play an essential role in both traditional and modern systems around the world. The research plant contains essential substances that have historically been employed to conform pharmacological properties and natural components (Lin *et al*., 2002).

Calpurnia aurea, widely grown in Ethiopia and locally known as 'Digita' in Amharic. Since ancient times, the extract of this plant has been used in human pathological and non-pathological treatments to cure disorders of the animal and human body. Extracts from this plant are used to treat infectious diseases such as diarrhea, stomach diseases, bowel disorders, and bladder disorders. *Calpurnia aurea* is a plant with yellow flowers widely distributed in tropical regions of Africa. Its use is in the treatment of disorders such as amoebic dysentery, diarrhea, syphilis, leishmaniasis, scabies, elephantiasis and various swellings in humans and animals (Asres *et al.,* 1986; Tadeg *et al.,* 2005). *Calpurnia aurea* excipients are natural particles found in completely different parts. The profile of this plant's by-metabolites can shift from species to species within this plant, among other things, due to genotype or imprinting changes and topographical regions (Penuelas and Louisa, 2001). Therefore, it is necessary to find new and successful medicines for treating infectious infections. Plant yields and their essential components play an essential role in plant disease control by controlling the development and outbreak of pathogens.

The current shortage of new antimicrobials and the prospect of replacing those plants, which are becoming unproductive, underscores the need to protect the long-term efficacy of medicines and the growth of many powerful medicines (Umer *et al.,* 2013). Therefore, the main purpose of this study was to analyze the major groups of secondary compounds of *Calpurnia aurea* and evaluate the antibacterial activity of their crude extracts against specific gut bacterial strains.

Material and methods

Experimental Study Design

Experimental studies should be built on the basis of standard laboratory research in commercial databases. Two parts each were harvested from a single plant and two test pathogens were harvested using antibacterial strain extracted from ethanol.

Collection and verification of plant materials

The completely fresh leaves and bark of *Calpurnia aurea* used in this study were randomly collected from wosheba kebele in the sorro woreda Hadiya zone in the Hosanna district in southern Ethiopia. It is at latitude 7 ° 29 '59 .99 "N, longitude 37 ° 34 '59.99" E. The plant parts (leaves and bark) have been certified by the experienced Botanists Erchafo Mohamed and Tewolde-Behan G/Egziabher at the National Herbarium of Addis Ababa University, and the

specimen 016AKWGA has been deposited at the National Herbarium. The method we followed for this investigation complies with all institutional, national and international guidelines and laws.

Ethical considerations

The Wachemo University research ethics review committee gave their clearance for the project (November 18, 2021, Decision no: 2021/259). The wosheba kebele soro woreda government was informed of the grounds for the probe. *Calpurnia aurea's* leaves and bark were collected with the government's written consent on September 8, 2020, observation was formally authorized for usage with the reference number (Hadiya zone wosheba kebele /318/2020) by the observatory enforcement site's government. The resulting plant species don't have to worry about going extinct. The ethical procedure we used comply with the declaration of Helsinki.

Preparation of crude extract

The collected *Calpurnia aurea* leaves and bark were washed separately with water and cut with a knife. The leaves and bark of the plant were dried in a standard laboratory. The remaining dried plant portion was ground with a grinder and stored at 4 °C until used for extraction for qualitative and quantitative measurements and antibacterial bioassays. Extraction was performed. 4 grams of each dried sample of *Calpurnia urea* plants were dissolved in 400mL of ethanol and held in a rotary shaker at 190-220 rpm for 24 hours (Verastegui *et al.,* 1996). The solvent in the filtrate was evaporated using rotating steam and extracted. Plant samples were weighed, stored and placed in sample vials at 4 °C until further use (Yadav, 2015).

Qualitative screening of important secondary metabolites

Using qualitative screening methods, the presence or absence of metabolites like tannins, flovatannins, steroids, terpenoids, phenolic compounds, saponins, flavonoids, glycosides, and alkaloids in *Calpurnia aurea*, were tested using methods demonstrated earlier elsewhere. It was confirmed that it was performed using a solidified ethanolic plant extract sample (Umer *et al.,* 2013; Brain and Turner, 1975).

Tannin test: 1 gram of *Calpurnia aurea* leaf and bark powder, were measured and added one by one to 20mL of distilled water. After boiling the sample in a water bath, 1mL filtrate sample was diluted with distilled water to 5mL solution and 10 percent ferric-chloride was added to a 2-3 drop test tube. Correspondingly, color formation was observed with tannins (Ajayi, 2011).

Steroid test (Lieberman-Bur Chard test): 2mL of chloroform and 10 drops of acetic acid were added. Color changes have been observed from steroids. 0.5mL of *Calpurnia aurea* leaves and concentrated ethanol bark extract were added to a sample tube and mixed. Later, a corresponding change in steroid color was observed (Gayathri and Kiruba, 2014).

Test of terpenoids based on Salkowish test: 5mL of concentrated ethanol extract from *Calpurnia aurea* leaves and bark is mixed with 2mL of chloroform in individual tubes, then carefully added 2mL of concentrated sulfuric acid and gently shaken to form layer. Therefore, interphase reddish-brown coloring could be confirmed by terpenoids (Biswas *et al.,* 2013).

Test of saponin based on the (Ajayi *et al.,* 2011): place1 gram of *Calpurnia aurea* leaves and powdered bark in separate test tubes and mix with 10mL of distilled water. The mixture was then boiled in a water bath and filtered. After cooling, tests show that saponins were performed.

Alkaloid test: 1.5mL of 1% HCl was added to 4.5mL of concentrated ethanol extract from *Calpurnia aurea* leaves and bark in different in vitro. The filtered mixture was tested for the presence of alkaloids using Wagner by adding a 1mL filtrate sample to the 0.4mL Wagner test. Reddish brown was an indicator of alkaloids performed according to the protocol (Adachukwu, 2013). Phenolic compound testing: Two drops of 5 percent of ferric-chloride were added individually to 5mL of concentrated ethanol leaf and

bark extract in vitro. The greenish precipitate indicated the presence of phenolic compounds (Adachukwu, 2013).

Quantitative analysis of major secondary metabolites

Measurement of alkaloid content by (Harborne, 1973)

Remove 3 g of *Calpurnia aurea* leaves and bark and place in separate flasks. Next, 200mL of ethanol was added to 10 percent acetic acid in a cotton-covered flask, and the solution was allowed to stand for 4 hours. Next, ammonium hydroxide was added drop wise to the filtrate and filtered until precipitation stopped. The alkaloid content was calculated asmg/g of the powder used.

Method for determining total phenolic content by (Cavalcanti de Amorim et al., 2012)

10mg of tannic acid was dissolved in 100mL of pure ethanol to make a stock solution of 0.1mg/mL, w/v. 500 µl of a 10 percent Folin-ciocalteu solution was added to each of the pint flasks. Mixture was stirred for 10 seconds and mixed with 1mL of 7.5 percent sodium carbonate. The absorptions were then measured by spectrophotometer (LT-2204, Labtronics, India) at 560nm.

Method for determining tannin content by (Van-Burden, 1981)

Powder 0.5 gram was put to a small flask with 10mL of 2M HCL and agitated for 5 minutes. The contents were completed, then moved to Erlenmeyer flasks, where they were made up to 100mL sample contents, and filtered. In a test tube, put 5mL of filtrated solution, 3mL of 0.1 M $FeCl_3$ in 0.1 M, and 3mL of 0.008M potassium. Within 10 minutes, Ferro-cyanide was added, and the absorbance at 720 nm was measured using a spectrophotometer.

The content of saponins was determined by (Obadoni and Ochuko, 2001)

The amount of saponins in each sample was calculated. At 55°C, stirring, 15mL of 20% ethanol was added to a conical flask containing 3 gram of ethanolic extracts from leaves and bark, and heated for 4 hours. The residue was re-extracted, and 30mL of 20% ethanol was produced, then reduced to 10mL using conventional laboratory conditions. The reduced solution had been transferred to a separator flask, and 5mL of diethyl ether had been added and vigorously agitated. The mixed aqueous layer solution was then added 15mL n-butanol, and the resulting solution was violently agitated. Then, in a pre-weighed Petri-plate, add 2.5mL of 5 percent aqueous sodium chloride. Due to the weight of the samples, they were dried and weighed in a conventional laboratory setting. The saponin content was measured in mg/g.

Antibacterial Assay

4 gram *Calpurnia aurea* leaves and bark dried powder were reconstituted in 400mL ethanol to make a stock of 10mg/mL. *Calpurnia aurea* leaves and bark extracts were produced at varied concentrations of 0.3125mg/mL, 0.625mg/mL, 1.25mg/mL, and 2.5mg/mL from their respective stocks (Alabi *et al.,* 2012).

Collection of test strains and their culture method

In an antibacterial bioassay, *Escherichia coli* O157: H7 and *Staphylococcus aureus* have a clinically isolated human bacterial strain collected from Addis Ababa, Ethiopia's Institute for Public Health (Kiehlbauch *et al.,* 2000). The culture was cultured on a nutrient agar plate for 24 hours. To obtain colonies, they were picked up in a loop at 37°C, transferred to a test tube, and vortexes. The turbidity of each bacterial suspension corresponded to 0.5 McFarland standard turbidity as described in (Clinical and Laboratory Standards Institute, Wayne, 2012). The resulting suspension was prepared and used as an inoculum for *Escherichia coli*0157: H7 and *Staphylococcus aureus* for antibacterial susceptibility testing. The recorded suppression zones were measured with mill meter accuracy along the two axes via the recorded laboratory equipment (Biswas *et al.,* 2013).

Determination of Minimum Inhibitor Concentration

The minimum inhibitory concentration of leaves and bark extract was tested with reduced concentrations of ethanol extract, where 10mg/mL stock solution was made and concentrations at 0.3125mg/mL,

0.625mg/mL, 1.25mg/mL, and 2.5mg/mL were used for the experiment. Therefore, 2mL of nutrient solution was added to 0.1mL of concentrated ethanol leaf and bark extract prepared in vitro and mixed. Then, 0.1mL of the prepared clinical test pathogen was added to the test tube together with the nutrient solution and the suspension of the ethanol extract. And the lowest concentration with no visible growth of the pathogen was recorded at the minimum inhibitory concentration. Ciprofloxacin disc of 5µg was used as positive control in this study (Alabi *et al.,* 2012).

Data management and analysis

The recorded test data were expressed as mean ± standard deviation. Data were statistically measured and mean difference samples were compared using the Pairing Statistics Package for Social Science Software v. 20 (SPSS; Chicago, IL, USA). A P-value of < 0.05 was considered statistical significant at 95% CI.

Result and discussion

Crude yield extract

Various studies have investigated the recent development of extracts and synthetics such as *Calpurnia aurea,* which have pharmacological effects and can be a source of natural compounds that act as new anti-infective agents, and plants with antibacterial activity.

As result shown in Table 1, the yield percentage of the bark of 4 gram and 400mL ethanol of powder obtained from the crude ethanol extract of *Calpurnia aurea* leaves and the extract.

Table 1. Yield Percentage of Crude Ethanol Extract from *Calpurnia aurea* Leaves and Bark.

Plant type	Parts used	Extraction type	Weight (in gram)	Percentages (in%)
Calpurnia aurea	Leave	Ethanol	20.168	51.08
	Bark	Ethanol	9.072	22.68

The results showed that the yields of crude ethanol extracts of *Calpurnia aurea* leaves and bark differed from other polar solvents. Ethanol has excellent solubility in many organic compounds, but is inherently co-soluble with other solvents such as water, so it can be used to extract dissolved polar and non-polar substances. Ethanol leaf extract had the highest yield, followed by the lowest yield of ethanol bark.

Disk diffusion method

The antibacterial activity of ethanol extract from *Calpurnia aurea* leaves and bark revealed a clear zone of inhibition against *Escherichia coli* O157: H7 and *Staphylococcus aureus*. The average suppression zone in the diameter range was between 11.8 and 17.4 millimeter. Ethanol leaf extract of *Calpurnia aurea* showed significant inhibition zones in *Escherichia coli* O157: H7 and *Staphylococcus aureus*. The ethanol extracts in the leaf suppression zone were found to be 12.7 ± 0.058 and 11.8 ± 0.15, respectively. Ethanolic bark extract of *Calpurnia aurea* showed significant inhibition zones in *Escherichia coli* O157: H7 and *Staphylococcus aureus*. Ethanol extracts in the cortical suppression zone were found to be 13.7 ± 0.15 and 17.4 ± 0.06, respectively. Ethanol bark extract was the most active and lowest inhibitory zone of *Staphylococcus aureus*, while water in ciprofloxacin showed activity with an average zone diameter of 21 and 37 millimeters'. From current studies, the most active crude ethanol extract against microorganisms was the P-value. It is 0.05 compared to other polar extracts, but does not contain positive controls. The average zone diameter ranges from 21 and 37 millimeters'. In this experiment I wanted to use DMSO as negative control, which offered no interference with the results obtained in the assay. And there is no effects in the antibacterial test, and it is both polar and non-polar compound dissolving purpose, act as uniform solvents showed no activity in Table 2.

Table 2. Antibacterial activity of *Calpurnia aurea* leaf and bark ethanol extract against *Escherichia coli* O157: H7 and *Staphylococcus aureus*.

Test sample	*Escherichia coli O157:H7*	*Staphylococcus aureus*
Leave extract	12.7±0.058	11.8±0.15
Bark extract	13.7±0.15	17.4±0.06
Positive control	37±0.19	21±0.52
Negative control	NA	NA

The values are shown as Mean ± Standard deviations (n=4).

Ethanol leaf extract had the highest yield, followed by the lowest yield of ethanol bark. As previously reported cited accordingly, the most antibacterial agents that have been identified from plants are soluble in organic solvents and this reveals the better effectiveness of ethanol as extracting solvent than other solvents *Calpurnia aurea*, *Escherichia coli* O157: H7 leaf ethanol, and the highest activity extract obtained from the water extract was active against *Staphylococcus aureus* of the same value with different standard deviations and different p-values (HA, 2013; Cowan, 1999).

Ciprofloxacin inhibited the concentration of each test organism, resulting in the lowest activity against all other solvent extracts against some pathogens (Tadeg *et al.,* 2005; Rojas *et al.,* 2001). Most of the crude ethanol extracts from *Calpurnia aurea* leaves showed significant growth inhibition for the two test organisms, described in Table 2. Earlier studies demonstrated the antibacterial activity of *Calpurnia aurea* leaves and bark containing ethanol extract using the disc diffusion method (Bibitha *et al.,* 2002; Akharaiyi and Boboye, 2010). The lowest activity was obtained in some extract portions against these test organisms (0.42mg /mL). (Leaves and bark) of *Calpurnia aurea*. Several plant phytochemicals are known and their antibacterial properties and studies have been widely reported (Roy *et al.,* 2006).

Minimum inhibitory concentration (MIC)

The bioassay was aggressive to assess the effectiveness of the ethanol extract in inhibiting the growth of the bacterial pathogens tested. Minimum inhibitory concentration values correlate with *Escherichia coli* O157: H7 and *Staphylococcus aureus* leaving an ethanol extract. The minimum inhibitory concentrations between the ethanol extracts of the leaves of *Calpurnia aurea* were 0.325mg /mL, which inhibits *Escherichia coli* O157: H7, and 2.5mg /mL, which inhibits *Staphylococcus aureus* [Fig 1]. Whereas ethanolic extracts of the bark of *Calpurnia aurea Escherichia coli* O157: H7 was inhibited with a minimum growth inhibitory concentration of 0.625mg /mL [(Fig 2] and *Escherichia coli* O157:H7 inhibited at 1.25mg /mL are shown. The ethanolic bark extracts of *Calpurnia aurea* had a potential to inhibit *Staphylococcus aureus* at 1.25mg/mL so, it had a potential to treat *Staphylococcus aureus* [Fig 4]. Also, the ethanolic leaf extracts of *Calpurnia aurea* had a potential to inhibit *Escherichia coli* O157:H7 *at* 0.325mg/mL, so it has a potential to treat of these bacteria [Fig 3] are shown in Table 3.

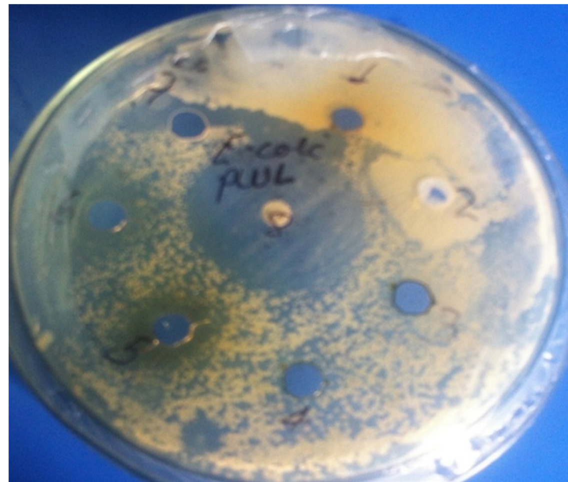

Fig. 1. Minimum inhibitory concentration on leave *Escherichia coli O157:H7.*

Noted: [(Fig 1: 1) negative control, 5) 0.3125mg /mL, 2) 0.625mg /mL, 3) 1.25mg /mL, 4) 2.5mg /mL, 6) 5mg /mL, 7) 7.5mg /mL, 8) positive control, and pw L plant wosheba leaves)].

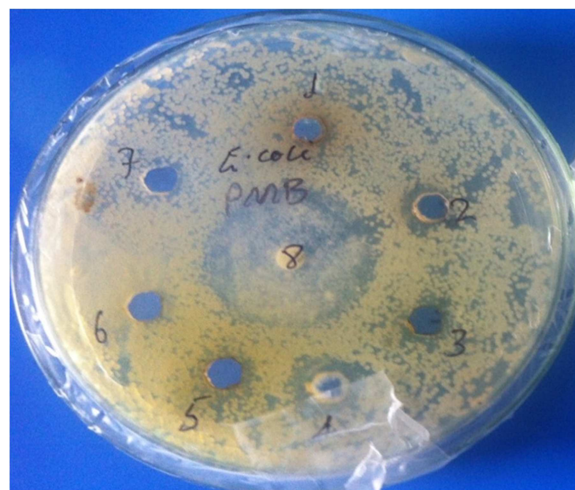

Fig 2. Minimum inhibitory concentration on bark *Escherichia coli O157:H7.*

1) negative control, 5) 1.25mg /mL, 2) 7.5mg /mL, 3) 0.3125mg /mL, 4) 0.625mg /mL, 6) 5mg /mL, 7) 5mg /mL, 8) Positive controls and pw B plant wosheba bark).

Fig 3. Minimum inhibitory concentration on leave *Staphylococcus aureus.*

[(Fig 3: 1.) Negative control, 5) 1.25mg /mL, 2) 7.5mg /mL, 3) 0.3125mg /mL, 4) 0.625mg /mL, 6) 5mg /mL, 7) 5mg /mL, 8) Positive controls and pw B plant wosheba bark)]

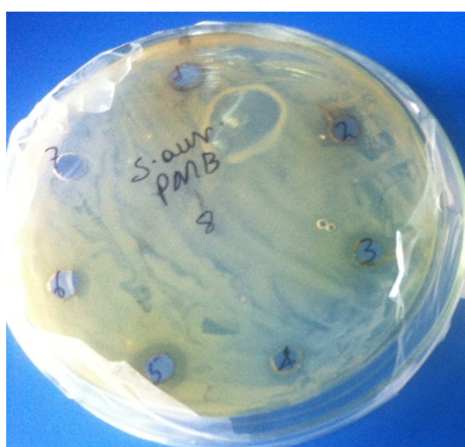

Fig 4. Minimum inhibitory concentration on bark *Staphylococcus aureus.*

[(Fig 4: 1.) Negative control, 5) 1.25mg /mL, 2) 7.5mg /mL, 3) 0.3125mg /mL, 4) 0.625mg /mL, 6) 5mg /mL, 7) 5mg /mL, 8) Positive controls and pw L plant wosheba leaves

Table 3. Minimum inhibitory concentration of leaf and bark of *Calpurnia aurea* on ethanolic extract against *Escherichia coli* O157:H7 and *Staphylococcus aureus.*

Minimum Inhibitory Concentration [(MIC) (mg/mL)] values for clinical isolations		
Bacterial pathogen	Leave extract	Bark extract
Escherichia coli O157:H7	0.3125mg/mL	0.625mg/mL
Staphylococcus aureus	2.5mg/mL	1.25mg/mL

In these study the minimum inhibitory concentration of the ethanolic extract of *Staphylococcus aureus* leaves against 1.25mg /mL, consistent with the result that the minimum inhibitory concentration inhibited the 25mg /mL value of *Staphylococcus aureus.* Similar reports were observed from *Staphylococcus aureus* inhibited at 25mg /mL and *Escherichia coli* O157: H7 inhibited at 1.25mg /mL (Shadrach, 2013; Adedapo *et al.,* 2008).

Phytochemical screening

The phytochemical screened effects found out the presence and shortage of the subsequent principal secondary compound exhibited that the ethanolic extracts of the plant the tannin, flavonoids, steroids, alkaloid, terpenoids, phenol, decreasing sugar, glycosides and saponins had been gift with inside the extracts of leaf and bark with exception of terpenoid and glycosides depart and decreasing sugar and saponnin bark while phlabotannin, and flavonoids had been absent in all maximum in all of the extracts primarily based totally at the studies in the Table 4.

Table 4. Major secondary compounds report of leaf and bark ethanolic extracts of Calpurnia aurea.

Screening compounds	Plant parts	
	Leave	Bark
Terpenoids	-	+
Alkaloids	+	+
Phlabotannin	-	-
Saponnin	+	-
Steroids	+	+
Phenolic comp	+	+
Glycosides	-	+
Reducing sugars	+	-
Tannin	+	+
Flavonoids	-	-

Key: (+) refers presence & (-) absence

In the view of the antibacterial activity of ethanolic plant extracts is associated with the presence of bioactive compounds in plants against bacterial infections. Phytochemical screening revealed is that a water extract of *Calpurnia aurea* containing tannins, flavonoids, steroids, alkaloids, terpenoids, phenols, reducing sugars, glycosides and saponins excludes steroids and resins, leaves and reducing sugar bark. It has been shown to be present in all leaf and bark extracts. On the other hand, phlabotannin, reducing

sugars and flavonoids were not contained in almost all ethanolic extracts as shown in Table 4. This may be due to differences in plant parts in physiology and plant survival in different regions and seasons that affect the accumulation of secondary metabolites. The results of the current study were compared with previous studies performed using similar standard methods to quantify phytochemicals in soaked leaves and bark (Dula and Zelalem, 2018; Adedapo *et al.,* 2008).

Quantitative determination

For the effect of quantitative determination of phytochemicals was concise of the crude phenolic compound (4.04mg/g and 3.97mg/g), alkaloid (39mg/g, and 34.4mg/g), saponnin (313mg/g, and 176mg/g) and tannin contents (2.24mg/g, and 2.87mg/g) were determined on leave and bark of plant part ethanolic extract contents respectively. The leaf crude extract had higher than in crude bark ethanolic extracts; whereas alkaloids and tannin crude extract bark is higher than crude leaves respectively it shown in Table 5.

Table 5. The contents of major phytochemicals (mg/g of crude extract) in ethanolic extracts of leaves and bark of *Calpurnia aurea.*

Ethanolic extracts	Leave	Bark
Crude phenolic compound content	4.04±0.4	3.97±0.21
Crude tannin content	2.24±0.15	2.87±0.15
Crude alkaloid content	39±1.02	34.4±0.58
Crude saponnin content	313±0.58	176±2.52

The values are represented as Mean values with ± Standard error (n=3)

In these study crude tannins, alkaloids, saponins, and phenols in ethanolic leaf extracts are significantly higher than in ethanolic bark extract. On the other hand, the bark of the crude extract of alkaloids and tannins is higher than the leaves as shown in Table 5. Similar studies found that ethanolic leaf extracts had higher levels of crude alkaloids and tannins than ethanolic bark extracts (Shadrach, 2013). The content of crude alkaloids in the ethanolic leaf extract is higher than that in the ethanolic bark extract. Leaf ethanol extracts with crude phenol and tannin content had significantly higher bark ethanol extract content, whereas bark ethanol extract had significantly higher saponin levels than leaf ethanol extract. Among the quantified phytochemicals, the content of the ethanolic leaf extract of *Calpurnia aurea* was higher than that of the bark extract. This may be due to the different roles of plants in physiology and the survival of plants in different regions and seasons that affect the accumulation of secondary metabolites (Akharaiyi and Boboye, 2010).

Limitation of the study

There are several limits to this study, some of which have been nice to check the antibacterial activity with multiple solvents. Apart from the bark and leaves, other plant samples such as roots and seeds are used as test samples, and using only two pathogens is one of the limitations of this study.

Conclusion

This study depicts the presence of phenolic compounds, saponins, flavonoids, alkaloids, and other phytochemicals associated with *Calpurnia aurea* leaves and bark. Ethanol extract showed antibacterial activity against *Escherichia coli* O157: H7 and *Staphylococcus aureus*. Therefore, the results of the current study suggest the use of an ethanol extract from *Calpurnia aurea* for the pathogen used in the current study. Further research on total microorganisms is needed.

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Source of fund: None

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