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In Vitro Antimicrobial Evaluation of Aqueus Methanol Extract from *Calpurina Aurea* (Fabaceae) Leaves

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ABSTRACT: In Ethiopia, *Calpurnia aurea* is used for the treatment of syphilis, malaria, rabies, diabetes, hypertension, diarrhoea, leishmaniasis, trachoma, lymphatic filariasis, fungal diseases and different swellings. However, despite its traditional usage as an agent, there is limited information regarding the phytochemical and *in vitro* antimicrobial profile of the leaves of *Calpurnia aurea*. *Calpurnia aurea* leaves were collected from Gondar area, northern Ethiopia and dried under shed. The collected plant material were powdered using electrical grinder and then macerated with 99.5% methanol for 72 hours with mechanical shaking repeated three times and it was filtered through Whatman No.1 filter paper and the filtrate was dried using rotary evaporator at 50^0C. Preliminary phytochemical screening such as test for tannins, flavonoids, terpenoids, saponins, glycosides, alkaloids and anthraquinones were done using standard methods; antimicrobial activity by agar well diffusion and micro well dilution methods were performed. *Calpurnia aurea* leave contained tannins (+), terpenoids (+), saponins (+), flavonoids (+), alkaloids (+) but lacked glycosides and anthraquinone (-). The extracts of leaves of the plant indicated good antimicrobial activity in both crude and fractionate especially methanol extract against *E. coli* (ATCC 25922), *S. auras* (ATCC 29213) and *S. typhi* (ATCC 6539). It is evident from this study that the highest therapeutic efficacy possessing majority of secondary metabolites with strong antimicrobial property were present in leaves of *Calpurnia aurea*, which can be quantified for application in pharmaceutical industry for treatment of different disease.

Keywords: Antimicrobial Effect, Calpurnia Aurea, Phytochemicals

INTRODUCTION

Traditional medicine has been practiced in Ethiopia since long time ago. The knowledge, largely oral, has been transferred from one generation to the next through professional healers, knowledgeable elders and/or ordinary people. It is estimated that about 80% of the Ethiopian population is still dependent on traditional medicine, which essentially involves the use of plants (Giday et al., 2007). *Calpurnia aurea*, a member of the subfamily Papilionoideae of the family, is a plant commonly used in traditional medicine to treat diverse medical conditions and parasitic infestation, both in humans and animals. It is a small, multi-stemmed tree, 3–4 m tall, occurring widespread in bushland and grassland in sub-Saharan Africa and India. In southern Ethiopia, it is called *cheka* by the Borana people. It is often found in overgrazed areas and is easily cultivated (Zorloni et al., 2010). The leaves of *Calpurnia aurea* (Ait.) Benth are used for wound healing activities through topical application in different communities in Ethiopia. The leaves had many confirmed in vitro activities that can promote wound healing effects. In spite of many claims and in vitro studies with supportive results in wound healing, no scientific study has been conducted on the woundhealing and anti-inflammatory activity of leaves of *Calpurnia aurea* (Ait.) Benth on animals (Ayal et al., 2019). *Calpurnia aurea* is an African medicinal plant used in many countries in Africa to treat a range of medical conditions or disorders. Extracts of the plant were shown to be active in antibacterial and antioxidant assays as well as against lice, ticks and maggots. The aim of the study was to isolate the phytochemical constituents from the plant and to test them in appropriate bioassays dependent on the compounds isolated in order to provide a rationale for the use of the plant in ethno-medicine or to provide some information on its constituents (Korir et al., 2014). The study of Ethiopian medicinal plants has not been realized as fully as that of India or other traditional communities elsewhere. In Ethiopia, though there have been some organized ethno-medicinal studies, there is limited development of therapeutic products and the indigenous knowledge on

usage of medicinal plants as folk remedies are getting lost owing to migration from rural to urban areas, industrialization, rapid loss of natural habitats and changes in life style. In addition, there is a lack of ethnobotanical survey carried out in most parts of the country. In view of these, documentation of the traditional uses of medicinal plants is an urgent matter and important to preserve the knowledge. Furthermore, most of the ethnomedicinal studies in northern part of Ethiopia are focused on 'Medihanit Awakie' (professional traditional practitioners) and the ancient medico-magical and/or medico-spiritual manuscripts and old Gee'z manuscripts, and ignore the knowledge of ordinary people in the locality. Thus, the purpose of this study is to investigate the traditional uses of medicinal plants by the ordinary people in ZegiePeninsula and to provide baseline data for future pharmacological and phytochemical studies (Teklehaymanot and Giday, 2007). Herbal medicines are plant-derived remedies that are used for their therapeutic properties and they have been an important tradition of many cultures and beliefs of African people. Sanitation and hygiene levels for the majority of people in Africa are not comparable to those of the first world countries. This exposes African people to a wider array of microbial pathogens, which increases their susceptibility to bacterial, fungal and viral infections. Indigenous plants are often the only available means of treating such infections. Since there is an increasing resistance to antibiotics by many pathogenic and opportunistic bacteria, plant extracts and plant-derived compounds have emerged as potential and promising antimicrobial agents (Rates, 2001).

After the revolution in the “golden era”, when almost all groups of important antibiotics (tetracyclines, cephalosporins, aminoglycosides and macrolides) were discovered and the main problems of chemotherapy were solved in the 1960s, the history repeats itself nowadays and these exciting compounds are in danger of losing their efficacy because of the increase in microbial resistance. Currently, its impact is considerable with treatment failures associated with multidrug-resistant bacteria and it has become a global concern to public health. About 80% of the population in developing countries use traditional medicines because they cannot afford the high cost of western pharmaceuticals and for health care, though the drugs have adverse effects like hyperuricemia, diarrhea, nausea, myositis, gastric irritation, flushing, dry skin and abnormal liver function (Harikumar et al., 2014). The capability of natural defense systems of living organisms against excess production of these species decreases when influenced with negative environmental factors, life style or aging. As a result, different cellular and extracellular components, and especially nucleic acids, are damaged, causing or enhancing a number of degenerative diseases. That is why natural products with antioxidant properties become more and more popular all over the world (Olga et al., 2004). As a result, the increase in antibiotic resistance by microorganisms and the often-lethal diseases caused by free radicals is posing serious ramifications to the lives and health of humans. Thus, there is a need to identify and process naturally occurring compounds

MATERIALS AND METHODS

Plant Materials Collection and Preparation

The *C.aurea* leaves were collected from their natural habitat around Tara Gedam, north-wetern Gondarin The plant was identified by experienced botanist in Gondar University College of Natural and Computational Science, Department of Botany. The collected leaves of *C.aurea* were air dried under shade. After complete shade drying the plant materials was grinded in grinder, the powder was weighed using an electronic balance and kept in flask with paper labelling.

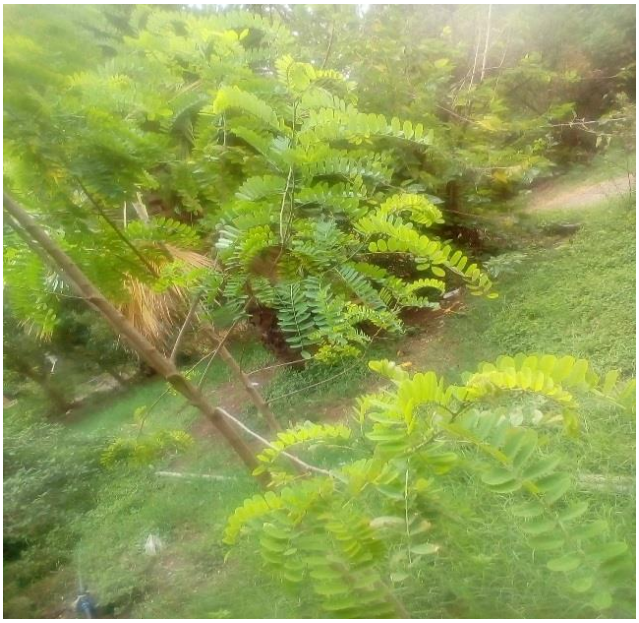

Figure 1. Tree of *Calpurina aurea*

Extraction

The methods described by Nostro et al. (2000) were used for extraction of *Calpurina aurea* by maceration. The grinded leave were weighed 836 gm using an electronic balance and were macerated in 99.5 % methanol for 72 hours with mechanical shaking and it was filtered through

Whatman No.1 filter paper and the method repeated three times. Then filtrates were evaporated using rotary evaporator at 60^0C. After remaining methanol evaporated, the final extract which is almost free of methanol were lyophilized. Finally, the extract obtained was weighed using an electronic balance to determine percentage yield. Then the final extract was packed in flask and kept at $+4^0$Crefrigerator for further phytochemical test.

Fractoination

The methods described by Nostro et al. (2000) were used for fractionation of *Calpurina aurea* by using solvents in order of increasing polarity. Obtained methanolic extract were soaked in in distilled water to make a suspension in a separatory funnel and then equal volume of hexane were added, shaked carefully to mix well, and allowed to stand until two clear layers formed. After separated hexane layer (upper one) were collected, dicholoromethane were added to the bottom layer and extracted in the same way to get dicholoromethane fraction. Then, ethyl acetate was added to the bottom layer and get ethyl acetate fraction. Finally, all collected fractions were evaporated on water bath and dried in deep freeze and percent yield were calculated.

Phytochemical Screening

Preliminary qualitative phytochemical screening was carried out with the following methods described by Harborne (1978) were used to test for the presence of the active ingredients in the test sample.

Terpenoids

2 ml of extract was added to 2 ml of acetic anhydride and concentrated H 2SO4. Formation of blue or green rings indicates the presence of terpenoids.

Tannins

2 ml of extract was added to few drops of 1% lead acetate. A yellowish precipitate indicated the presence of tannins.

Saponins

5 ml of extract was mixed with 20 ml of distilled water and then agitated in a graduated cylinder for 15 minutes. Formation of foam indicates the presence of saponins.

Flavonoids

Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colorless on addition of dilute acid, indicates the presence of flavonoids.

Alkaloids

0.2 g of extracts of *C.aurea* added in each test tube and 3 ml of hexane were mixed in it, shaken well and filtered. Then took 5 ml of 2% HCL and poured in a test tube having the mixture of plant extract and hexane. Heated the test tube having the mixture, filtered it and poured few drops of picric acid in a mixture. Formation of yellow color precipitate indicates the presence of alkaloids.

Detection of glycosides

Extracts were treated with Ferric Chloride solution and immersed in boiling water for about 5 minutes. The mixture was cooled and extracted with equal volumes of benzene. The benzene layer was separated and treated with ammonia solution. Formation of rose-pink colour in the ammonical layer indicates the presence of anthranol glycosides.

Anthraquinone

50mg of extract were heated with 1ml 10% ferric chloride solution and 1ml of concentrated hydrochloric acid. The extract was cooled and filtered. The filtrate was shacked with equal amount of diethyl ether. The ether extract was further extracted with strong ammonia. Formation of Pink or deep red coloration of aqueous layer indicates the presence of Anthraquinone.

Antimicrobial activity

Antimicrobial activity of the plant crude extracts and fractionates (ethyl acetate fraction, n hexane fractoin and DCM fraction) were tested individually by the same procedure and on the same pathogens. Antimicrobial activity was evaluated on the following pathogens: *Salmonella typhi*, *Staphylococcus aureus*, *citrobacreria*, *klebsella pseudomonas* and *Escherichia coli*, all are standard.

Agar well diffusion

The methods described by (Das et al., 2010) were used to test for the Antibacterial assay of the extracts by measuring the zone of inhibition against the test organisms. Test organisms were diluted and diluted inoculums (0.1 mL) of test organism were spread on Muller-Hinton agar plates by swab. Six wells of 8 mm diameter were punched into the agar medium with sterile cork borer under aseptic conditions. The extract was diluted by DMSO to make 500mg/ml, 250 mg/ml, 125 mg/ml and 62.5 mg/ml. The first four well was filled with 50 µl of different extract concentration and then with 50 µl of solvent blank and standard antibiotic oxytetracycline. The plate was kept at

room temperature for 2 h for diffusion and was then incubated for 24 h at 37°C. Antimicrobial activity was evaluated by measuring the zone of inhibition against the test organisms. Oxytetracyclinewas used as a reference standard and dimethylsulphoxide (DMSO) was used as a control. The growth was compared with the reference as well as the control.

Broth micro dilution method

The methods described by CLSI, 2012 were used to test for the Antibacterial assay of the extracts using 96-well micro-titration plate for determination of MBC and MBC. Serial dilutions were prepared from 500 mg/ml of the plant extract using DMSO. The wells were inoculated with 50 µl aliquot of test organisms having serial dilutions of the extract (100 µl) and50 µl broth. The micro plate was incubated at 37°C ± 1°C for 24 h. Dilution of the extract corresponding to respective test organism showing no visible growth was considered as MIC. The MBC were determined after broth microdilution by sub-culturing a sample from wells, yielding a negative microbial growth after incubation on the surface of non-selective agar plates to determine the number of surviving cells after 24 h of incubation.

Statistical analysis

The experimental results were expressed as mean ± standard error of mean (SEM) of the replicates. Where applicable, the data were subjected to one way analysis of variance (ANOVA) and differences between samples were determined by using the Statistical Analysis System (SPSS 16.0 SAS, 1999) program. P values < 0.05 were regarded as significant.

RESULT

Extraction and Fractionation yield

According to described method of fractionation mentioned above (in section 3.2 and 3.3), obtained results are presented as follows:

Therefore, crude extract % yield of c.a = (251.14 gm/836 gm) 100% = 30.04%

Percent yield = % yield = (Weight of the dried fraction/Weight of dried crude) 100%

Percent yield of n hexane = (10.83g / 760g)*100 = 1.425

Phytochemical screening

The preliminary phytochemical screening of calpurina aurea leaf methanol extracts has revealed the presence of secondary metabolites of therapeutical importance. The major phytochemicals found were: Saponin, tannins, terpenoids, alkaloids, and flavonoids. However, the extract tested showed the absence of anthraquinones and cardiac glycosides. The observations and inferences made in the phytochemical tests are presented table 1.

Antimicrobial activity

Agar well diffusion of methanol extract represented the following results (Table 2, Figure 2).

Table 1. The results of the phytochemical compound assay of the methanol extract of C. *aurea*

No	Phytoconstituents	Test	Observation	Interpretation
1	Test for Saponin	Foam Test	Layer of foam	Positive
2	Test for tannins	Braemer's Test	Bluish color	Positive
3	Test for terpenoids	Salkowski Test	Reddish brown color	Positive
4	Tests for alkaloids	Mayer's Test	Yellow color precipitate	Positive
5	Tests for Cardiac Glycosides	Keller-Killiani Test	No Brown ring	Negative
6	Tests for flavonoids	Lead Acetate Test	No Yellow color	Positive
7	Test for Anthraquinone	Borntrager's test	No Pink or deep red coloration of aqueous layer	Negative

Table 2. Descriptive analysis; inhibition of various concentration of *C.aurea* against different organisms expressed by mean ± standard error and Post Hoc Tests Multiple Comparisons of mean by one way ANOVA (Tukey).

Concentration	Mean ± St. Error Of Methanol Extract Against Bacterial Strains				
	S. Auras	***S. Typhi***	***K. Pneumonia***	***Citrobacter***	***E. Coli***
500mg/Ml	14.96±0.372 ^{a3d3e3}	15.4300±0.055^{a3c3d3e3}	10.3767± 0.038^{a3c3d3e3}	12.6267±0.263^{a3c2d3e3}	15.6333±0.117^{a3c1d3e3}
250mg/Ml	14.0933±0.047 ^{a3d3e3}	13.1767±0.216^{a3b3d3e3}	8.3833± 0.02^{a3b3d3e3}	10.0400± 0.095 ^{a3b2e2}	13.8900±0.605^{a3b1d3e3}
125mg/Ml	10.4767±0.238 ^{a3b3c3e2}	11.1567±0.023^{a3b3c3e3}	Not effective	8.8900± 0.061 ^{a3b3}	8.7300± 0.265^{a3b3c3e2}
62.5mg/Ml	8.5333± 0.277 ^{a3b3c3d2}	8.9900± 0.020^{a3b3c3d3}	Not effective	7.3767± 0.188 ^{a3b3c2}	Not effective

Values are expressed as Mean ± S.E.M (n=3), analysis was performed with One-Way ANOVA followed by Tukey test; **a** compared to positive control, **b** to 500mg/ml, **c** to 250mg/ml, **d** to 125mg/ml, **e** to 62.5mg/ml; **1**P<0.05, **2**P<0.01, **3**P<0.001. The negative control has shown no antibacterial activity. Oxy= oxtetracycline. The mean-difference is significant at the 0.05 level. Confidence Interval of the mean is 95%.

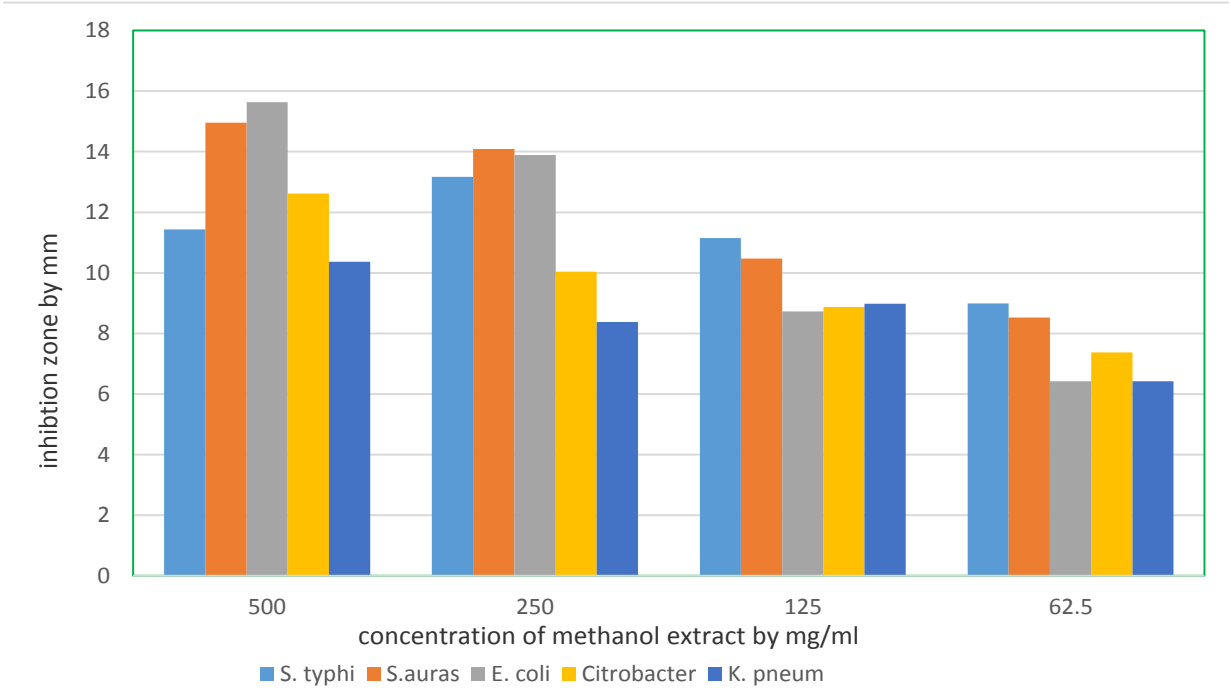

Figure 2. Agar well diffusion concentration difference between Inhibitions zones of methanol extract of Calpurina aurea against different bacterial strains

As shown on table 3 and on chart 1 above, the growth of all test bacterial strains were inhibited by the tested concentrations of the crude (99.5% methanol) extract of the plant in concentration dependent manner. But, the observed zone of inhibition of the crude extract at the tested concentrations were statistically different compared to that of their respective positive control (P<0.05) against all test bacteria (Table 3). Among the test bacteria, gram negative bacterial species of *E. coli and S. typhie* were slightly more susceptible than that of the gram positive bacterial species at the corresponding tested concentrations of the crude extract, especially at 500 mg/ml. As depicted in Table 3, at

500 mg/ml, the most susceptible bacterium was *E. coli* followed by *S. typhie* from gram negative and *S. auras* from gram positive with a mean zone of inhibition of 15.63 mm, 15.43 mm and 14.96 mm, respectively. 50 µl Methanol extract of *C. Aurea* possess high antibacterial activity with inhibition zone 8.99± 0.02 mm at lower concentration (62.5mg/ml) was observed against *Salmonella typhi*, followed by *S. Aureus* with inhibition zone of 8.53±0.27 mm at lower concentration (62.5mg/ml), which was significantly lower ($P < 0.05$) than the positive control. Similarly, *Citrobacter* responded to the extract with moderate inhibition zone (7.37± 0.18) at 62.5mg/ml. 50 µl Methanol extract of *C. Aurea* don't possess antibacterial activity at lower concentration against *Citrobacter* (62.5mg/ml) and K. pneumonia (62.5mg/ml and 125 mg/ml)was observed, but it possess antibacterial activity at higher concentration.

MIC AND MBC of methanol crude extract and solvent fractions

As presented in Table 3 and figure 3 above, the MIC figures of the methanol crude extract were in agreement with its preliminary antibacterial activities i.e the more susceptible is the bacterium, the lower is the concentration of the extract required for growth inhibition in most of the test bacteria. The crude extract of the plant was almost equally potent against both gram positive bacteria and gram negative bacteria. The maximum MIC (less diluted) obtained by methanol extract was 8.73 mg/ml (against *K. pneumonia*) and the minimum MIC (highly diluted) was 0.65 mg/ml (against *S. typhi*). The MIC figures of the ethyl acetate fraction ranged from 0.96 mg/ml (against *Citrobacter*) to 11.83 mg/ml (*E. coli*). Ethyl acetate fraction MIC value indicate that, citrobacter were completely inhibited at 0.96 mg/ml concentration. The MIC figures of the DCM fraction ranged from 26.08mg/ml (against *s. auras*) to 115.74 mg/ml (*k. pneumonia*). DCM fraction indicate that, s. auras completely inhibited at 26.08 mg/ml. The MIC figures of the n-hexane fraction ranged from 5.80mg/ml (*citrobacter*) to 97.22 mg/ml (s. auras). DCM fraction indicate that, *citrobacter* were completely inhibited at 26.08 mg/ml. When the MIC value of the crude extract and that of the active solvent fractions are compared, methanol extract inhibits all test bacteria except *K. pneumonia* at lowest concentration followed by ethyl acetate fraction. Based on the MBC determination method, the crude extract and active solvent fractions were bactericidal even though the methanol extract showed bactericidal activity at lower concentrations. The maximum mean MBC (least dilution) of methanol extract was 11.83 mg/ml (against *Citrobacter*) and the minimum mean MBC (highest dilution) of the crude extract of the study plant was 0.81 mg/ml (against *S. typhie*). The corresponding values of the ethyl acetate fraction were 18.0 mg/ml (against *E. coli*) and 1.08 mg/ml (against *K. pneumonia*). Similarly, the ranges for the mean MBC values of the DCM fraction was range from 125 mg/ml to 39.76 mg/ml and mean MBC values of the n-hexane ranges from 106mg/ml to 14.91 mg/ml against the growth of the bacterial species which were susceptible in its antibacterial activity testing experiment. Taken together the MIC and MBC, the methanol crude extract and ethyl acetate was more potent to inhibit and kill the bacteria at lower concentration compared to that of the DCM and n-hexane fractions.

Table 3. The mean of MIC and MBC (by mg/ml) of the crude extract and the solvent fractions of the leaves of calpurina aurea against gram positive and gram negative bacteria

Bacteria	Methanol extract		Ethyl acetate fraction		DCM fraction		N-hexane fraction	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
E. coli	1.65	2.23	11.83	18	29.02	35.49	78.7	97.22
S. typhi	0.66	0.81	9.77	10.8	41.51	60.18	29.29	47.69
S. auras	0.85	1.08	10.8	15.94	26.08	39.76	97.22	106.48
K. pneumonia	8.74	11.83	0.96	1.08	115.74	125	9.77	10.8
Citrobacter	2.23	2.57	2.91	3.53	97.22	106.48	5.8	14.91

MIC= Minimum Inhibitory Concentration, MBC= minimum bactericidal concentration.

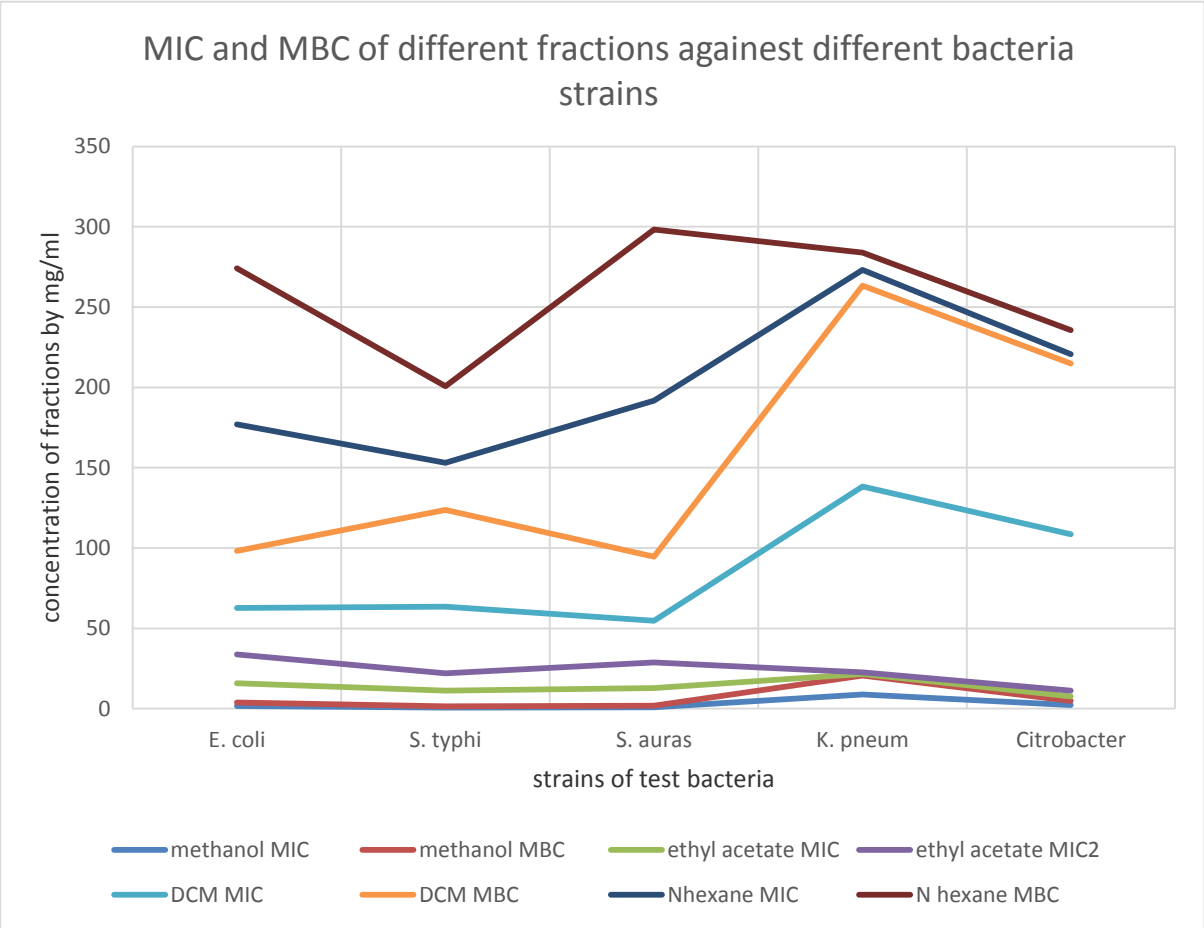

Figure 3 MIC and MBC of different fractions of Calpurina aurea against different bacteria strain

DISCUSSION

Since the discovery and introduction of antibiotics into clinical use, there has been an increase in reported cases of bacterial antibiotic resistance. Frequent multiple antibiotic resistance in bacteria makes it desirable to identify novel antimicrobial agents from plants. This study was aimed at screening *calpurina aurea* for the presence of phytochemical compound, antibacterial and antioxidant compound(s). Screening medicinal plants is one of the first steps towards the isolation and characterisation of potentially bioactive compound(s).

The preliminary phytochemical screening of *calpurina aurea* leaf methanol extracts has revealed the presence of secondary metabolites of therapeutical importance (Patel et al., 2009; Fullas, 2001; Yanping et al., 2005). The major phytochemicals found were: Saponin, tannins, terpenoids, alkaloids, and flavonoids. However, the extract tested showed the absence of anthraquinones and cardiac glycosides. Literature review on the phytochemical constituents of these plants revealed that quinolizidine alkaloids, lectins, non-protein amino acids and tannins are the major components of *Calpurnia aurea* (Radema et al., 1979).

The antidiarrheal and amoebic dysentery in animal's effect of *C. aurea* as reported by (Fullas, 2001) may be due to the presence of phytochemicals such as tannins and flavonoids Manach et al. (1996). The wound healing property of this plant can be attributed to the presence of tannins (Kozioc and Marcia, 1998). Elmarrie and Johan (2001) have reported tannin to have antibacterial activity. Tannins and flavonoids are thought to be responsible for antidiarrheal activity (Enzo, 2007). Flavonoids were found in the extract and are potent water soluble antioxidants which prevent oxidative cell damage suggesting antiseptic, anticancer, anti-inflammatory effects and mild hypersensitive properties (Okwu, 2004). Chemical investigations of *C. Aurea* by Asres, (1986), resulted in the isolation of a series of quinolizidine alkaloids. Saponin

fraction from leaf extract have anti-obesity activity as reported by (Marrelli et at., 2016), which have the potential to lower lipid such as Triglycerides, total cholesterol, low-density lipoproteins and very low-density lipoproteins. It was reported that the presence of plants flavonoids and saponins compound of plant has potential to change lipids levels in hypercholesteremic (Yanping et al., 2005; Patel et al., 2009). Previous reports have demonstrated that the alkaloid virgiline isolated from *Calpurnia aurea* possesses a potent molluscicidal shown to possess a significant radical scavenging property (Asres and Bucar, 2002). As can be seen from the results, *C.aurea* methanol extract and its fractions (ethyl acetate) were active against all bacteria. All extract and fractions of plant included in the present study were found to be active on at all of the selected microbial with different concentration. The antibacterial activities of the calpurina aurea medicinal plant were determined against five pathogenic microorganisms' *S. aureus, S. typhi, Citrobacter, E. coli* and *K. pneumonia*. The clear zones on the plates, with no bacteria colony, indicate antibacterial property of the plant when tested against the five pathogenic microorganisms. This observation was similar with the previous observations made by Adepo et al., (2008), where the plant extracts used showed antibacterial activity against Gram-positive *S. aureus* and gram negative *E. coli*. *S. typhi, E. coli* and *S. auras* was the most sensitive strain of all the bacteria used in this study despite the fact, Gram-negative bacteria are frequently reported to have developed multi drug resistance to many of the antibiotics currently available in the market of which *Escherichia coli* is the most prominent (Alonso et al., 2000). It is surprising to learn that *Escherichia coli* is the most responding bacterial strain to *Calpurina aurea* extract and fractions. However, some extract and fractions of *Calpurina aurea* are still of special interest for further investigations in this regard as in the case of methanol extract and ethyl acetate of *Calpurnia aurea*, which showed exceptionally stronger activity against *S. typhi* and *Escherichia coli* also having good activity on Gram-positive bacteria. The antibacterial activity was more pronounced on the Gram-negative bacteria (*Escherichia coli* and *Salmonella typhi)* than the Gram-positive bacteria except *Staphylococcus aureus*. The Gram-positive bacteria on the other hand are more susceptible having only an outer peptidoglycan layer which is not an effective permeability barrier (Nostro et al., 2000). In spite of this permeability differences, however, the extract and some of fractions have still exerted high degree of inhibition against Gram-negative organisms. Several reports (Dagnew and Gunther, 1990) indicate that infectious skin disorders are very common in Ethiopia. Among the pathogens most commonly known to cause infectious disorders of the skin is *Staphylococcus aureus* (Jones et al., 2003). Thus, the fact that extract and some fraction of the tested plant showed activity against *Staphylococcus aureus* might justify the extensive use of these agents for the treatment of skin disorders.

CONCLUSION

The present study revealed that the methanol extract and fractions of the leaves of *Calpurina aurea* have antibacterial activities against the growth of the test bacteria with varying antibacterial spectrum. Therefore, the study provides scientific basis on the traditionally claimed use of the medicinal plant for the treatment of bacterial infections like infectious skin disorders, wound healing problem, antidiarrheal or others which are probably caused by the susceptible bacteria. The antibacterial activities of the plant might be linked with the presence of non-polar and/or intermediately polar bioactive secondary metabolites in the chloroform and methanol fractions including alkaloids, terpenoids, tannins, saponins and flavonoids that can act either individually or synergistically. But, in addition to those bioactive secondary metabolites covered by this study, other bioactive principles which were not addressed might contribute their own share for the antibacterial activities of the crude extract and the active solvent fractions of the study plant. The antibacterial properties of *Calpurnia aurea* are not as effective as the standard drug (Ciproflox and Gentamycin). The data obtained in this study suggest a possible use of *Calpurnia aurea* as a source of natural antimicrobial agents. Therefore, based on the above results the following recommendation are forwarded:

- Antibacterial activities of the plant should also be done on other bacterial species which were not addressed by this study
- Mechanistic studies for the responsible antibacterial agent of the study plant have to be conducted for the antibacrerial activity
- Using different range of solvents, further studies should be conducted to isolate, purify and identify bioactive principle(s) responsible for the antibacterial activities of the plant.
- In vivo antibacterial studies of the crude and active solvent fractions should be conducted to confirm the antibacterial effectiveness of the plant.
- The acute, sub-chronic and chronic toxicological studies should be done for the of the extracts of the plant.

DECLARATIONS

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

MB, MY and AK conceived the review, coordinated the overall activity, and reviewed the manuscript.

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