OPEN ACCESS Freely available online

Natural Products Chemistry & Research

Research Article

Phytochemical Investigation and Assessment of Antibacterial Activities of Calpurnia Aurea Root Bark.

Gebretsadik Tebabal Kebede[1*] and Zelalem Abdisa Negero[2]

[1]*Department of chemistry, college of natural and computational science, Raya University, post, box, 92, maichew, Ethiopia*

[2]*Department of chemistry, college of natural and computational science, Wollega University, post, No. 395, nekemte, Ethiopia,*

ABSTRACT

The plant Calpurnia aurea has been used in Ethiopia as a folk remedy to treat diseases like malaria, rabies, stomachache, treatment of extoparasites and different diseases caused by bacteria and fungus. The present study aimed to screen phytochemicals and evaluate the in vitro antioxidant and antibacterial activities of the root bark extract of Calpurnia aurea. The root bark of Calpurnia aurea was collected from Wollega University compound, dried and extracted sequentially with hexane, ethyl acetate, acetone, and methanol. The preliminary phytochemical analysis was conducted using standard test methods. Phytochemical screening was found positive for alkaloids, Terpenoids, flavonoids, saponins, tannins, anthraquinones, and anthocyanins in the root bark even though different from extract to extract. Antibacterial activity was performed against Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, and Salmonella typhirium bacterial strains. Following the good activities of the extract the minimum inhibition concentration (MIC) have be performed and the methanol extract found to be effective at lower concentration (6.25mg/ml) for E. Coli whereas the ethyl acetate extract showed no or little activities below 100mg/ml except for E. Coli which inhibit at 25mg/ml. The ethyl acetate, acetone and methanol extract showed good antibacterial activities but the hexane extract didn't show inhibition against tested bacterial strains. The MIC value also determined by serial dilution and the methanol extract was found effective at a lower concentration which is 6.25mg/ml in Escherichia coli, the ethyl acetates extract could not kill bacteria in dilution below 100mg/ml except Escherichia coli which inhibited at 25 mg/ml. Generally, the result obtained from this study is similar to the result reported in other parts of the plant species and support the medicinal values of the plant Calpurnia aurea. Thus the root bark of the plant was found to exhibit moderate antibacterial properties.

Keywords: Calpurnia aurea, Phytochemical, Infectious disease, DPPH, Biological activity.

INTRODUCTION

Background of the study

Plant medicines have a historically significant role in different parts of the world such as Africa and Asia in different cultural systems for human and animal health management. This is because, the people considered as the simplest and widely available health care options (Welch, 2010).

The role of plant medicines in human life is predated with his history for therapeutic purposes and the traditional practitioners have great contribution to popularizing these purposes worldwide.(Maridass et al 2008). Plant based drugs provide outstanding contribution to modern therapeutics (Abiyu et al., 2014)

Medicinal plant and herbal products have now become a key issue in developing and industrialized country. The extraction of bioactive compounds from the medicinal plant can be used as a reference for synthesis of new drugs. Widespread use of herbal

[*]**Corresponding to**: Gebretsadik Tebabal Kebede, Roya University, Ethiopia; Email: gebretsadiktebabal@gmai.com

Received date: October 13, 2020, 2020; **Accepted date:** August 30, 2021, 2021**; Published date:** September 11, 2021

Citation: Tebabal Kebede G (2021) Phytochemical Investigation, And Assessment of Antibacterial Activities of Calpurnia Aurea Root Bark. Nat Prod Chem Res. 9:p383

remedies and healthcare preparations is described in the Vedas and the Bible (Singh, 2015).

Figure 1: Some chemical drugs obtained from medicinal plant

Plants have a broad spectrum of structural and biological diversity. They contain biologically active compounds called phytochemicals which can be present in different part of the plant such as root, stem, leaves, barks, flower, and fruits. This is the reason why plants are referred to as the pharmaceutical industry (Ingle et al., 2017). .

The individuals or groups in Ethiopia us medicinal plants or traditional medicines based on their ethnic and cultural diversity and employed as the primary tools in the fight against numerous health problems. It is believed to be 80 % of people and 90 % of the animal in Ethiopia is still depend on traditional plant medicines. Ethiopia is estimated to contain between 6500 and 7000 species of higher plants of which about 12 % are endemic (Negero et al., 2015).

Calpurnia aurea is one of the plants in the genus Calpurnia of flowering plants (angiosperm) in the fabaceae (legueminocae) family. The genus Calpurnia comprises shrubs to small trees 2-15 m height. The Calpurnia aurea species is mostly distributed in African continents especially South Africa around capeprovinces and eastern margin of the continent like Ethiopia and Eritrea and used for treatment of many human diseases like syphilis, malaria, rabies, diabetes, hypertension, diarrhea, leishmaniasis, trachoma, elephantiasis, fungal diseases and different swellings, stomach-ache (Natesan et al., 2015).

MATERIAL AND METHODS

PLANT MATERIAL COLLECTION AND SAMPLE PREPARATION

The root bark of Calpurnia aurea was collected around uncultivated area of Wollega University school compound, located around Nekemte city, East Wollega zone of Oromia region, , in April, 2018 and identified by Dr. Tena Regasa (botanist) from department of biological science, Wollega University. The collected root bark of Calpurnia aurea was washed with running tap water followed by distilled water to remove unwanted dusts like soil and sand and it was air dried at room temperature in the research laboratory of chemistry department until having constant weight. The dried root bark was ground in to an appropriate size by using analytical miller TW135 to increase the surface of contact between the solvent and sample. The powder was kept packed with glass jar until needed for extraction.

PREPARATION OF PLANT EXTRACT

The powder was weighed with an electronic balance and 560 g of the powder was soaked and extracted with 99 % hexane, two times for 48 h each with random mechanical shaking. The filtrates in each step were collected and combined. The marc was dry again and further soaked and extracted with 99.5 % ethyl acetate in the same way two times for 48 hrs with mechanical shaking. Then the extract was collected and put together. The same steps were followed with 99.5 % acetone and 99.8 % methanol. The filtrates were collected separately and filtered with a cotton plug and then with Whatman No.1 fitter paper in suction filtration. The filtrates were collected from each soak and concentrated by using rotary evaporator under reduced pressure. The obtained semi-solid materials were kept for further analysis. The mass yield of the extract were evaluated and presented in Table 3.

PRELIMINARY QUALITATIVE PHYTOCHEMICAL SCREENING OF EXTRACT

The preliminary qualitative phytochemical screening of Calpurnia aurea root bark was conducted to investigate the presence or absence of different secondary metabolites such as alkaloids, terpenoids, tannins, flavonoids, saponins, anthraquinones, and anthocyanins. The screening was conducted based on the methods used in the literature (Natesan et al., 2015, Zohra et al., 2012 & Shuma et al., 2018) and the results are confirmed after three repetitive trails . The investigated phytoconstituents and respective standard tests reagents are listed below (Table 1).

Table 1: Phytoconstituents and respective test reagents used for screening

S.No	Phytoconstituentes	Standard tests
1	Alkaloids	Mayer's test
		Wagner's test
2	Terpenoids	Salkowski Test
3	Tannins	Braemer's Test
		Ferric chrloride test
4	Saponins	Foam test
5	Anthocyanins	Borntrager's Test
6	Flavonoids	Alkaline reagent(NaOH) test
		Shinoda test(Mg tunning test)
7	Anthraquinones	CHCl3 & HCl test

A.TEST FOR ALKALOIDE

Mayer test: 1ml of extract was added in test tube and treated with 1ml of Mayer's reagent (potassium mercuric iodide solution). The sample was then observed for the formation of whitish or cream precipitate.

Wagner test: 1ml of extract was added into the test tube and treated with 2ml of Wagner's reagent (iodine in potassium iodide). The sample was then observed for the formation of reddish brown precipitate.

B. TEST FOR TERPENOIDES

Salkowski Test: 2 ml of extract was added into the test tube and treated with 2 ml of acetic anhydride and a drop of concentrated H2SO4 was added. Then observed for formation of Blue or green rings to confirm the presence of terpenoids.

C. TEST FOR TANNINS

Braemer's Test: 2 ml of extract was added into the test tube and few drops of 10% lead acetate were added to it. Then it was observed for the formation of yellowish precipitate to indicate the presence of tannins.

Ferric chrloride test: 2 ml of extract was added to 2 ml of water into the test tube and 2 drops of 10 % ferric chloride solution was added and observed for formation of dark green or blue green precipitate for the presence of tannins

D. TEST FOR SAPONINS

Foam test: 5 ml of extract was mixed with 20 ml of distilled water into the test tube and then it was shacked for 15 minutes and stand. Then it was observed for the formation of Up to 1cm foam to show the presence of saponnins.

E. ANTHOCYANINS

Borntrager's Test: 2 ml of extract was added into the test tube and 2 ml of 2 % HCl and 2 ml of 2 % ammonia was added and it was observed for the formation of pink-red turns to blue-violet color.

F. FLAVONOIDS

Alkaline reagent: 2 ml of extract was treated with few drop of 10 % sodium hydroxide solution. Then it was observed for the formation of intense yellow color.

Shinoda test: 5 ml of extract was treated with a concentrated sulfuric acid (1 ml) and 0.01 g of Mg. Then it was observed for the pink or red coloration.

G. TEST FOR ANTHRAQUINONES

2ml of extract was mixed with 2ml of 2 % HCl and 3ml of CHCl3 in the test tube and from the mixture small drop of 10 % ammonia was added. Then it was observed for the formation of rose-pink color

ANTIBACTERIAL ACTIVITY ASSAY

TEST STRAIN COLLECTION

Total of four types of bacterial species were subjected for antibiotic assay these are Staphylococcus aureus (ATCC12022) (gram positive), Pseudomonas aeruginosa (ATCC27853) (gram negative), Salmonella typhirium (ATCC20911) (gram negative) and Escherichia coli (ATCC25922) (gram negative). All of these were obtained from microbiology laboratory of biological science department in Wollega University.

INOCULUMS PREPARATION

Stock cultures were maintained at 4oC on nutrient agar slants and the active culture bacteria for the test were prepared by taking loop full of bacterial cell from stock cultures and streaked on Mueller Hinton plate and it was incubated at 37oc for 24hrs. The cell suspension of the above four bacteria was freshly prepared by transferring the isolated colony which is 24hrs old and it was mixed by gently swirling the test tube with sterile physiological saline(0.85%) solution. The turbidity of the suspension was adjusted to standard inoculums to McFarland standards (108CFU/ml) (which is prepared by adding 0.5 ml of 0.048 M BaCl2 to 99.5 ml of 0.36 M H2SO4) in sterile saline solution (Tendencia, 2004).

THE DISK DIFFUSION METHODS

About six mm diameter paper disk were prepared from Whatman No 1 filter paper and it was sterilized at 1210C for 15 minute in an autoclave. The sterilized paper discs were impregnated with 50 µl of the prepared solution of crude extract. It was allowed to diffuse for 15 minute before added to the inoculated Petri dish. Bacterial suspensions obtained from above were spread on Petri dish about 100 mm diameter containing nutrient agar using a sterile cotton swab. The dry paper disk impregnated with 50 µl sample and standards were placed on the inoculated Petri dish using sterile forceps. They were then pressed down with a slight pressure to ensure complete contact of the disc with the inoculated agar surface and incubated at 37 0C aerobically in an inverted position for 24hrs. Commercially available broad spectrum (Gentamicin and tetracycline) were used as positive control and the disk socked with DMSO as negative control to compare the result of effects of plant extract. After 24 hrs the antibacterial activity was evaluated by measuring the diameter of growth inhabited zone and compared with standard antibiotics tablets (Gentamicin and Tetracycline) to determine whether the tested bacterial species are sensitive or resistant to the plant extract (Javid et al., 2015). The half fold dilution methods were used to determine the MIC values (Shemsu et al., 2013).

RESULT AND DISCUSSION

Result

PHYTOCHEMICAL EXTRACTION OF CALPURNIA AUREA ROOT BARK

The phytochemical extraction of Calpurnia aurea root bark was conducted with four different organic solvents such as n-hexane, ethyl acetate, acetone, and methanol sequentially with increasing their polarities index. The total mass of 560 g powder was used for extraction and the mass of extract obtained was different from each other in each solvent and resulted in 3.5 g in hexane extract, 10 g in ethyl acetate extract, 4 g in acetone extract and 119 g in methanol extracts as presented below(Table 2).

Table 2: The percentage yield of extract in gram

S. No	Extraction solvents	Yield(gms)	Percentage (%) yield
1	Hexane	3.5	0.625
2	Ethyl acetate	10	1.78
3	Acetone	4	0.71

4	Methanol	119	21.25

The yield of Methanol extract was the highest with 21. 25 % and it was red oily in nature followed by Ethyl acetate (1.78 %) and it was reddish brown solid. Hexane extract had the lowest percentage with 3.5 g and it was brown oily in nature and acetone extract has 4 g with red solid. The yield of Ethyl acetate extract is higher next to methanol. This is because of ethyl acetate is moderately polar solvent it can absorb both moderately polar and polar compounds and methanol is highly polar solvent so it can extract the remaining polar compounds.

PHYTOCHEMICAL ANALYSIS OF CRUDE EXTRACT BY STANDARD TEST METHODS

In this study phytochemical investigation was conducted on different phytoconstituents using different standard test reagents. This preliminary qualitative phytochemical analysis gives positive results for most phytoconstituents and the result is expressed using positive for the presence and negative for absence. As observed from the result the solvents extraction have an influence on the type of phytoconstituents extracted because the results are different among four different solvents extract of secondary metabolites. Phytochemical screening was performed on sucsessive hexane, ethyl acetate, acetone and methanol extract.

The preliminary qualitative phytochemical screening test of hexane revealed the presece of alkaloids, terpenoids and flavonoids, and negative for all other tests. The ethyl acetate extract was positive for terpenoids, flavoids, anthocyanins and anthraquinones. The acetone extract showed positive result for tannins and flavonoids. Methanolic extract revealed positve results for most tests except for terpenoids and anthocyanins as shown below (Table 3).

Table 3: Phytochemical screening result of Calpulnia aurea root bark extract

Phytoconsitituents	Test or Reagent	Result			
		Acetone	E. Acetate	Hexane	Methanol
Alkaloids	Mayer's reagent	-	-	-	+
	Wagner's reagent	-	-	+	+
Flavonoides	AlkalineTest(NaOH).	+	+	+	+
	Mg Tunning Test	+	+	+	+
Tannins	Braemer's Test	+	-	-	+
	Ferric Chrloride	+	-	-	+
Terpenoides	Salkowski Test	-	+	+	-
Saponinin	Foam Test	-	-	-	+
Anthocyanins	Borntrager's Test	-	+	-	-
Antraquinones	CHCl3 & HCl	-	+	-	+

Antibacterial activities assay

In this study, different solvent fractions of Calpurnia aurea root bark were subjected for antibacterial effect tests against both gram positive and gram negative bacterial pathogens like Staphylococcus aureus (positive), Escherichia coli (negative), Salmonella typhirium (negative), and Pseudomonas aeruginosa (negative) using disc diffusion methods. Acetone, ethyl acetate and methanol extracts showed promising antibacterial effects against the tested strain while hexane extract didn't show at all at test concentration of 100mg/ml. The ethyl acetate extract showed the highest antibacterial activities (diameter of inhibition 25 mm) against S. aureus. All the remaining test strains namely Escherichia coli, Pseudomonas aeruginosa and Salmonella typhirium showed 15 mm diameter of inhibition zone. Acetone extract of this sample also showed promising growth inhibition effects (15-22 mm) in which 22 mm inhibition was observed for Salmonella typhirium. The most promising zone of inhibition was exhibited by methanol extracts with 20-25 mm inhibition zone in which 25 mm inhibition was recorded for Escherichia coli (Table 6). The methanol extract showed roughly similar antibacterial activity with standards reference. Ethyl acetate and methanol extracts demonstrated equal diameter of inhibition zone with standard reference gentamicine (25 mm) against Staphlococcus aureus. Methanol extract also showed equal inhibition diameter with gentamicine against E. coli. Based on the result displayed in Table 4, one can probably arrange the four solvent extracts of the plant root bark in increasing order as follows: Hexane < Ethyl acetate < Acetone < Methanol (Table 4). All extracts except hexane showed broad spectrum antibacterial activities which can inhibit both gram negative and gram positive bacteria.

Table 4: Antibacterial properties of different organic extracts of Calpurnia aurea root bark against selected human pathogens at concentration of (100 mg/ml) in diameter of inhibition zone.

Solvent used for extraction	Diameter of Inhibition zone			
	S. aureus	E. coli	P. aeruginosa	S.typhirium
Hexane	n. a	n. a	n. a	n. a
Ethyl acetate	25	15	15	15

Acetone	20	15	20	22
Methanol	20	25	22	20
Gentamicin e	25	25	21	24
Tetracycline	25	23	22	24
DMSO	n. a	n. a	n. a	n. a

Where n. a =not active, DMSO=Dimethyl sulfoxide

DETERMINATION OF MINIMUM INHIBITORY CONCENTRATION (MIC)

Since the methanol, ethyl acetate and acetone extracts of Calpurnia aurea root bark showed promising inhibition zone as compared with the standard reference antibiotics it was considered to continue further test in bacterial activities. Serial dilution method was used to obtain the minimum of concentration that can inhibit the visible growth of each bacteria in the disk diffusion methods. The 100mg/ml concentration of the extract was diluted to 50 mg/ml, 25 mg/ml, 12.5 mg/ml, 6.25 mg/ml and 3.125 mg/ml serially. The minimum inhibitory concentration (MIC) is the lowest concentration of an antimicrobial chemical that can inhibit the visible growth of microorganism after an overnight incubation. The minimum inhibitory concentration of each extract varies from species to species. The MIC of methanolic extract was 50mg/ml in Pseudomonas aeruginosa, 25 mg/ml in Staphylococcus aureus and Salmonella typhirium, and 6.25 mg/ml in Escherichia coli. Ethyl acetate extract couldn't inhibit the growth of bacteria below 100 mg/ml for three species except Escherichia coli which is at 25 mg/ml and acetone extract showed the minimum inhibition at 50 mg/ml in Staphylococcus aureus, 100 mg/ml in Salmonella typhirium, 50 mg/ml in Escherichia coli and 25 mg/ml in Pseudomonas aeruginosa. This indicates that methanol and ethyl acetate extracts are active against Escherichia coli relative to the other species with MIC value of 6.25 and 25 mg/ml, respectively. The acetone extract is also active against Pseudomonas aeruginosa since the best inhibition at low concentration is observed in this spp. which is 25 mg/ml as shown below (Table 5).

In the over all of the observation Escherichia coli were sensitive to the plant extract and the inhibitory activities at small concentration were obtained in this species which is 25, 50 and 6.25 mg/ml in ethyl acetate, acetone and methanol, respectively. As observed in the data the minimum bactericidal concentration is at high dosage in all extract especially in ethyl acetate (100 mg/ml) in most bacteria. This result supports that the traditional practitioners should use this plant at high dosage.

Table 5: The minimum inhibitory concentration (MIC) of ethyl acetate, acetone and methanol extract against human pathogen

Extracts	Minimum inhibitory concentration in (mg/ml)			
	Staphylococcus aureus	**Escherichia coli**	**Psedomonas aeruginosa**	**Salmonella typhirium**
Ethyl acetate	100	25	100	100
Acetone	50	50	25	100
Methanol	50	6.25	50	25

Figure 7: The antibacterial properties of methanol extract in serial dilution and diameter of inhibition

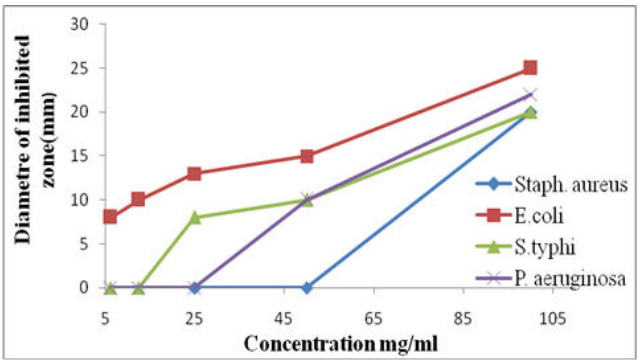


Figure 8: The antibacterial properties of acetone extract in serial dilution and diameter of inhibition

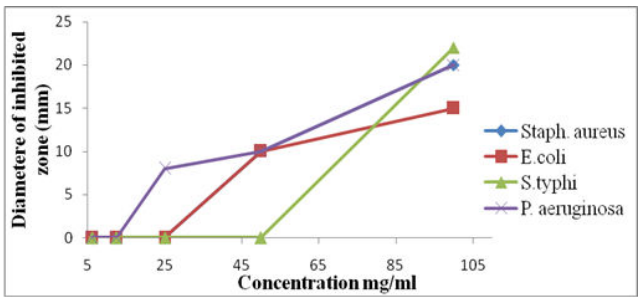
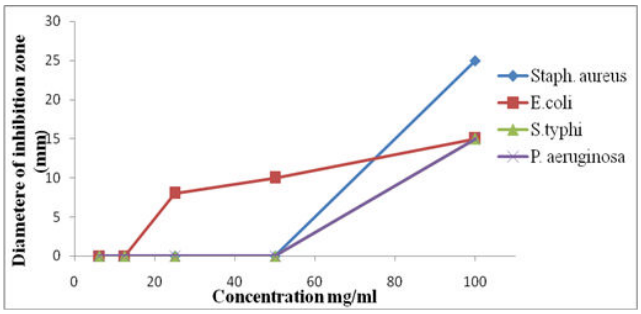


Figure 9: The antibacterial properties of ethyl acetate extract in serial dilution and diameter of inhibition zone



DISCUSSION

The major classes of phytochemicals with disease preventing functions are antioxidants, anticancer, detoxifying agents, immunity potentiating agents and neuropharmacological agents. Each class of these functional agents consists of a wide range of chemicals with differing potency (Saxena et al., 2013). Phytochemicals like alkaloids are used as a direct medicine for treatment of several diseases. including antihypertensive effects (many indole alkaloids), antimalarial activity (quinine), and

anticancer actions (dimeric indoles, vincristine, vinblastine) (Nyamai et al., 2016).

Phenolics apparently are responsible pharmacological properties including antioxidant, anti-inflammatory, wound-healing, antimicrobial and antiviral activities(Wink, 2015). Since most of the listed diseases are caused by microorganisms like bacteria, virus and protozoa it can be treated effectively with root bark of Calpurnia aurea plant because it is found to contain many secondary metabolites. In the present study the root bark extract of Calpurnia aurea can inhibit the growth of bacteria strains like E. coli, S. aureus, S. typhi and P. aeruginosa. The inhibition of these bacteria with this plant extract indicates that the plant used to prevent the disease caused by these strains effectively. The Escherichia coli is commonly known to cause of the disease diarrhea primarily in children in a poor hygiene, and severe water diarrhea in the human being and dysentery in children and adults are caused by mostly E. Coli (Allocate et al., 2013). Pseudomonas aeruginosa is the pathogen in hospitalized or immune-compromised patients, causing infections, such as pneumonia, wound, urinary tract, and gastrointesteinal infections (Sousa & Pereira, 2014). Therefore the use of Calpurnia aurea root bark for treatment of diarrhea, dysentery, wound healing, abdominal pain and other infectious disease in traditional societies can be justified by the capacity of this plant secondary metabolite for inhibition of Escherichia coli and other pathogenic species like Pseudomonas aeruginosa strain and the treatment of skin infection by Calpurnia aurea is also probably related to inhibition of Staphylococus aureas. Plant secondary metabolites are used to prevent disease caused by microorganisms by acting in different ways include the disruption of membrane function and structure interruption of DNA or RNA synthesis and function. The antibacterial activities are the sequence of actions that phytochemicals interact with cell membrane compound is thought interaction with both outer and inner Cytoplasmic cell membranes by integrating at the polar head group region of the lipid bilayer. This alternates the cell membrane and leads to its increased permeability or disintegration. The primary target site of plant secondary metabolite is the Cytoplasmic membrane and phytochemicals can affect its structure and integrity, permeability or functionality to prevent growth and reproduction and this can be done by the reactive hydroxyl groups or conjugated electron systems (Radulovic et al., 2013).

CONCLUSION

The result showed that the percentage yield of the crude extracts is less in n-hexane but highest in methanol. This suggests that the chemical composition of Calpurnia aurea root bark is more of polar metabolites. The ethyl acetate, acetone and methanol extract showed antibacterial activities against both gram negative and gram positive bacteria tested. This indicates that these extracts contain biologically active constituents while hexane extract couldn't show any antibacterial activity. This is probably due to the non polar nature of the constituents. In addition, the concentration of the extract at which it becomes active inhibitor showed that the root bark of Calpurnia aurea should be effective at relatively comparable dosage.

REFERENCES

1. Abiyu, E., Zemede, A., Ensermu, K. & Raja, N. Ethnobotanical study of traditional medicinal plants in & around Fiche district central Ethiopia. Current Research Journal of Biological Sciences, 2017; 6: 154-167.
2. Adedapo, A. A., Jimoh, O. F., Koduru, S., Afolayan, A. & Masica, J. P. Antibacterial & antioxidant properties of the methanol extracts of the leaves & stems of Calpurnia aurea. Alternative & Complementary Medicine, 2008; 8: 53-61.
3. Ahmed, D., Khan, M. & Saeed, R. Comparative analysis of phenolics, flavonoids & antioxidant & antibacterial potential of methanolic, hexanic & aqueous extracts from Adiantum caudatum leaves. Antioxidants, 2015; 4: 394-409.
4. Allocati, N., Masulli, M., Alexeyev, F. & Ilio, D. Escherichia coli in Europe: An overview. International Journal of Environmental Research Public Health, 2013; 10: 6235-6254.
5. Daksa. D &, Abdisa. Z. Antioxidant activity assessment of Calpurnia aurea root extract. Natural Product Chemistry & Research, 2018; 6: 307.
6. Ergene, B., Acikara, B. O., Bakar, F., Saltan, G. & Nebdoglu, S. Antioxidant activity & phytochemical analysis of Alchemilla persica rothm. Jornals of Faculty of Pharmacology of Ankara University, 2018; 39: 145-154.
7. Hailemichael, T. Isolation & structure elucidation of natural products from plants (Unpuplished Ph.D dissertation). University of Hamburg, Hamburg, Germany.2005.
8. Ingle, P. K., Deshmukh, G. A., Padole, A. D., Dudhare, S. M., Moharil, P. M. & Khel urkar, C.V. Phytochemical extraction, method of identification & detection of bioactive compounds from plant extracts. Journal of Pharmacognosy & Phytochemistry, 2017; 6: 32-36.
9. Korir, E., Kiplimo, J., Crouch, R., Moodley, N. & Koorbanally, A. Isoflavones from calpurnia aurea subsp. aurea & their anticancer activity. African Journal of Traditional, Complement & Alternative Mededicine, 2014; 11: 33-37.
10. Maridass, M., John, A. & Britto, D. Origins of Plant Derived Medicines. Ethnobotanical Leaflets, 2008; 12: 373-387.
11. Mebrahtu, E., Workineh, S. & Mirutse, G. Invivo Antimalarial activity of hydromethanolic leaf extract of Calpurnia aurea (Fabaceae) in mice infected with chloroquine sensitive Plasmodim berghei. International Journal of Pharmacy & Pharmacology, 2012; 2: 131-142.
12. Mirutse, G., Tilahun, T., Abebe, A. & Yalemtsehay, M. Medicinal plants of the Shinasha, Agew-Awi & Amhara peoples in northwest Ethiopia. Journal of Ethnopharmacology, 2007;110: 516–525.