

Research

Phytochemical Screening of Calpurnia Aurea Root ExtractDula DE^{1*}, Zelalem A²¹Department of Chemistry, Nekemte College of Teacher Education, Post Box No. 88, Nekemte, Ethiopia.²Department of Chemistry, College of Natural and Computational Sciences, College University, Nekemte, Ethiopia..**Received** June 06, 2018; **Accepted** August 02, 2018; **Published** August 06, 2018**Copyright:** © 2018, Dula DE et al

***Corresponding author:** Dula Daksa Ejeta, Department of Chemistry, Nekemte College of Teacher Education, Post Box No. 88, Nekemte, Ethiopia. E-mail: ladukan064@gmail.com

Abstract

In Ethiopia, any part of *Calpurnia aurea* is used for the treatment of different ailments. The researcher was interested to evaluate the phytochemical profile of the root extracts of *Calpurnia aurea*. *Calpurnia aurea* root extract contains cardiac glycosides, tannins, flavonoids, terpenoids, saponins, steroids, alkaloids and phenolic compounds but anthraquinones (generally anthocyanins) were failed to show positive results in all solvent extracts, yet there was difference observed based on extracting solvents used and strength of quality results in which cardiac glycosides, flavonoids, and tannins were strongly detected; phenolic compounds, steroids, alkaloids, terpenoids and triterpenoids were moderately detected, while saponins were weakly detected. Therefore, it is evident from this study that highest therapeutic efficacy possessing majority of secondary metabolite classes of compounds in root extract of *Calpurnia aurea*, which can be quantified for application in pharmaceutical industry. Especially, cardiac glycosides, flavonoids and tannins were strongly detected.

Keywords: *Calpurnia aurea*; phytochemicals; Phytochemical Screening; Qualitative Analysis; Standard Methods.**Introduction**

The medicinal plants are useful for healing as well as for curing of human diseases because of the presence of phytochemical constituents. Medicinal plant treatments are still used for many health problems [1]. They are safe, less toxic, economical and a reliable key natural resource of drugs all over the world. Medicinal herbs have been use in one form or another under indigenous systems of medicine [2]. Phytochemicals are naturally occurring in the medicinal plants, leaves, vegetables and roots that have defence mechanism and protect from various diseases. Phytochemicals are primary and secondary compounds. Chlorophyll, proteins and common sugars are included in primary constituents and secondary compounds have terpenoid, alkaloids and phenolic compounds [3]. The complete

phytochemical screenings of medicinal plants should be carried out, because these secondary metabolites are responsible for medicinal activity of the plant. Numbers of plants were screened for secondary metabolites for their medicinal values [4].

In order to promote herbal drugs, there is an urgent need to evaluate the therapeutic potentials of the drugs as per WHO guidelines and mentioned that 30% of the worldwide sales of drugs is based on natural products. Traditional indigenous medicine is limited to small tribal and geographical areas called “Little Traditions” are an excellent repository of knowledge about medicinal properties of botanical sources [5]. The bioactive extract should be standardized on the basis of phytochemical compounds. Phytochemical screening of medicinal plants is very important in identifying new sources of

therapeutically and industrially important compounds. It is imperative to initiate an urgent step for screening of plants for secondary metabolites. The present communication attempts to assess the status of phytochemical properties of medicinal plants to improve the health status of people and also to use in pharmaceutical products of commercial importance in the world [4].

Calpurnia aurea is a genus of Flowering Plants within the family of fabaceae. The genus comprises shrubs or small trees in or along the margin of forests in many parts of Ethiopia and widely distributed in Africa from Cape Province to Eritrea and which also occurs in Southern India [6]. Literature survey brings to light that, all parts of the plant species has been used for different human and animal disease [7]. In native countries like Ethiopia, traditionally, the leave and powdered roots of *Calpurnia aurea* is used for the treatment of syphilis, malaria, rabies, diabetes, lung TB, hypertension, diarrhoea, leishmaniasis, trachoma, elephantiasis, fungal diseases, different swellings, stomach-ache, abscesses, bowel, bladder disorders, to destroy maggots, to destroy lice, to relieve itches, used as a fish-poison or as a cure for dysentery, exhibit activity against amoebiasis and giardiasis, cough and snake bite [8,9]. Plant products have been part of phytomedicines since time immemorial. These can be derived from any part of the plant like stem bark, leaves, flowers, seeds and root i.e., any part of the plant may contain active components. Knowledge of the chemical constituents of plants is desirable because such information will be of value for the synthesis of complex chemical substances. Such phytochemical screening of various plants is reported by many research workers [4].

Thus, here in this present study, qualitative phytochemical analysis of the root extracts of *Calpurnia aurea* were screened by using standard methods. I hope that the findings from this work may add to the overall value of the medicinal potential of the plant species.

Materials and Methods

Collection and Identification

The plant sample of the study was collected from around Jimma Arjo highland (East Wollega Zone, about 50 km away from Nekemte town to the south-west direction, Western Ethiopia) at the end of February 2016 and the identity of the plant was confirmed by botanical scholars from Wollega University Biological Science Department

with the reference of National Museums of Ethiopia Herbarium. Appropriate voucher specimen designated was deposited at the School of Botanical science, Wollega University.

Extraction

The whole root part was washed in tap water and cut in to small bits to facilitate drying. After weighing the wet sample, it was hot-air oven-dried below 55°C for 14 h until it came to constant weight. Then the preliminary quantitative moisture difference was calculated and the complete dry sample was powdered to suitable size first the root bark and the inner part separately, and then homogenized. The powdered sample was stored in clean glassware container. The prepared powder weighed (680 g) and then macerated by using four organic solvents hexane (99%), chloroform (99.9%), ethanol (97%) and methanol (99.8%) according to their increasing polarity index for 72 hours with mechanical shaking within 4 hours interval in average and it was filtered through Whatman No.240 filter paper and the filtrate was dried using Rotary evaporator.

Phytochemical Screening

After preparation of Special and Standard Solutions, Preliminary qualitative phytochemical screening was carried out following methods described in different articles performed by many authors [10, 4, 11, 12, 13, 14].

Alkaloids

Wagner's test: About 10 mg of extract was taken and 3-5 drops of Wagner's reagent (1.27g of iodine and 2g of potassium iodide in 100mL of water) was added and the formation of a reddish brown precipitate indicates the presence of alkaloids. Reddish brown colored precipitate indicates the presence of alkaloids.

Hager's test

0.2 g of different solvent extracts of *Calpurnia aurea* were added in each test tube and 3 mL of hexane were mixed in each of it, shaken well and filtered. Then 5 mL of 2% HCl was taken and poured in each test tube having the mixture of plant extract and hexane. Each test tubes having the mixture had been heated, filtered and few drops of picric acid solution were poured into each mixture in the test tubes. Formation of yellow Colour precipitate is taken as indicator of the

presence of alkaloids. Yellow colour precipitate indicates the presence of alkaloids.

2 mL of 10% HCl was added to about 1 mg of the extracts in a test tube. The mixture was heated for 20 minutes. It was cooled and filtered. To 1 mL of the filtrate 5 drops each of Mayer's and Dragendorff's reagents were added. Formation of cream and orange colour precipitates respectively indicated the presence of alkaloids in the extracts. The appearance of whitish or cream colour precipitate indicates the presence of alkaloids; and for Dragendorff's test, mixing the same amount of the crude extract and the reagent as in Mayer's case. If orange-red precipitate colour is formed in later test, it indicates the presence of alkaloids in the crude extract.

Saponins

Froth test

0.5 mg of the extract was mixed with 5 mL of distilled water in a test tube and vigorously shaken for 2 minutes. Foam which persisted for 30 minutes and doesn't disappear upon warming was taken as indication of the presence of saponins in the extract. A stable froth (foam) up on standing indicates the presence of saponins.

Flavonoids

Shinoda Test

10 mg of extract was added to pinch of magnesium turnings and 3 drops of concentrated hydrochloric acid was added. Formation of orange-pink colour indicates the presence of Flavonoids.

Lead acetate test

10 mg of extract was taken and 20 drops of 10% lead acetate solution was added. Appearance of yellow or yellowish-orange colour precipitate indicates the presence of flavonoids.

Alkaline Reagent Test

NaOH Test

Crude extract was mixed with 2 mL of dilute NaOH (2% solution of NaOH). An intense yellow colour was formed which turned

colourless on addition of few drops of diluted hydrochloric acid (2% solution of HCl) indicating the presence of flavonoids.

Test for Terpenoids and Triterpenoids

Salkowski test

30 mg of extract mixed with 5 mL chloroform and warmed for 30 minutes. The chloroform solution is then treated with a small volume of concentrated sulphuric acid and mixed properly. The appearance of red colour indicates the presence of triterpenoids. Or 1 mL of methanolic extract of the sample was boiled with 2 mL chloroform, cooled; 1 to 2 drops of concentrated sulphuric acid were added slowly through the wall of the tube.

Shaken well and allowed standing for some time, red colour appearance at the lower layer indicates the presence of Steroids and formation of yellow coloured lower layer indicates the presence of triterpenoids.

Liebermann-Burchardt test

To 1 mL of methanolic extract, 1 mL of chloroform, 2-3 mL of acetic anhydride and 1 to 2 drops of concentrated sulphuric acid were added. Pink or red coloration is observed indicating the presence of terpenoid.

Additionally, 2 mg of the dry extract was dissolved in acetic anhydride; heated to boiling, cooled and then 1 mL of concentrated sulphuric acid was added along the sides of the test tube gently. Formation of pink colour indicates the presence of triterpenoids. Or 2 mL of methanol extract was added to 2 mL of acetic anhydride and concentrated H₂SO₄. Formation of blue or green rings indicates the presence of terpenoids.

Anthocyanins

Borntragers test

About 0.5 g of the extract was taken into a dry test tube and 5 mL of chloroform was added and shaken for 5 minutes. The extract was filtered and the filtrate was shaken with equal volume of 10% ammonia solution. A pink violet or red colour in the lower layer indicates the presence of anthraquinones.

Free Anthraquinones

A sample (5 g) of each plant extract was shaken with 10 mL of benzene and filtered. A 10% ammonium hydroxide solution (5 mL) was added to the filtrate, and the mixture was shaken. The presence of a pink, red or violet colour in the ammoniacal phase was taken as an indication of the presence of anthraquinones.

Test for Phenolic Compounds

Lead acetate test

10 mg of extract was taken and 0.5 mL of 1% lead acetate solution was added and the formation of precipitate indicates the presence of tannins and phenolic compounds.

Sodium hydroxide test

5 mg of extract was dissolved in 0.5 mL of 20% sulphuric acid solution. Followed by addition of few drops of aqueous sodium hydroxide solution, it turns blue which indicates the presence of phenols.

Tannins

Ferric chloride test

5 mg of extract was taken and 0.5 mL of 5% ferric chloride was added. The development of dark bluish black colour indicates the presence of tannins.

Lead acetate test

10 mg of extract was taken and 0.5 mL of 1% lead acetate solution was added and the formation of precipitate indicates the presence of tannins and phenolic compounds.

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

Test for steroids and sterols

Salkowski's test

5 mg of extract was dissolved in 2 mL of chloroform and equal volume of concentrated sulphuric acid was added along the sides of

the test tube. The upper layer turns red and lower layer turns yellow with green fluorescence, indicating the presence of the steroids and sterols compound, in the extract.

Liebermann-Burckhardt's Test

2 mg of dry extract was dissolved in acetic anhydride, heated to boiling, cooled and then 1 mL of concentrated sulphuric acid was added along the sides of the test tubes gently. Formation of green or dark green colour indicates the presence of steroids. Alternatively, 1 mL of methanol extract of each sample was boiled with 2–3 mL of acetic anhydride, and then cooled; 1 to 2 drops of concentrated sulphuric acid were added slowly through the wall of the tube. Dark green coloration of the solution indicates the presence of Steroids and dark pink or red coloration in the interface indicates the presence of Terpenoids.

Test for Glycosides and Cardiac glycosides

Glycoside test

0.5 mg of extract was dissolved in 1 mL of water and then aqueous NaOH solution was added. Formation of yellow colour indicates the presence of glycosides.

Liebermann's test

1 mg of crude extract was mixed with each of 2 mL of chloroform and 2 mL of acetic acid. The mixture was cooled in ice. Carefully concentrated H₂SO₄ was added. A colour change from violet to blue to green indicated the presence of steroidal nucleus, i.e., glycine portion of glycoside.

Salkowski's test

1 mg of crude extract was mixed with 2mL of chloroform. Then 2mL of concentrated H₂SO₄ was added carefully and shaken gently. A reddish brown colour indicated the presence of steroidal ring, i.e., glycone portion of the glycoside.

Keller-killani test

2 mg of crude extract was mixed with 2mL of glacial acetic acid containing 1-2 drops of 2% solution of FeCl₃. The mixture was then poured into another test tube containing 2mL of concentrated H₂SO₄.

A brown ring at the interphase indicated the presence of cardiac glycosides.

Results

Some Physical Properties and Fractional Extraction of the Plant Extracts

S/N	Solvent	Weight (g) of the crude extract	Percentage of the extract
1	n-hexane	10.10	1.48
2	Chloroform	15.78	2.32
3	Ethanol	38.92	5.72
4	Methanol	105.70	15.54

Table 1: Extracted sample weight and Percentage yields of the crude extracts of *Calpurnia aurea* root.

The ability of a solvent to extract the bioactive compounds from plant is determined by calculating the percentage yield of extraction. The percentage yields of plant extraction are mainly depending on the solvent used in the extraction. Different polarity index of solvent give different percentages yield and extract different phytochemical compounds.

Preliminary Phytochemical Test Results

Table 2 below shows the phytochemical profile of *Calpurnia aurea* root extract of four different solvents (n-hexane, chloroform, ethanol and methanol).

Here in this study, though the degree is different, almost all the phytochemicals investigated, except saponins, tannins and anthocyanins, were present in more than one solvent extracts of

Calpurnia aurea root. The extract contains cardiac glycosides, tannins, flavonoids, terpenoids, saponins, steroids, alkaloids and phenolic compounds but anthraquinones (generally anthocyanins) were failed to show positive results in all solvent extracts, yet there was difference observed based on extracting solvents used and strength of quality results in which cardiac glycosides, flavonoids, and tannins were strongly detected; phenolic compounds, steroids,

The dried n-hexane, chloroform, and ethanol extracts have waxy gel appearance whereas that of methanol has semi-solid liquid appearance. The four solvent extracts were reddish-brown color even though the deepness was slightly showing difference. The yield from different solvent extracts is presented in Table 1 below.

alkaloids, terpenoids and triterpenoids were moderately detected, while saponins were weakly detected.

Here Figure 1 below is showing some of the strong qualitative phytochemical screening test results observed during the experimentation.

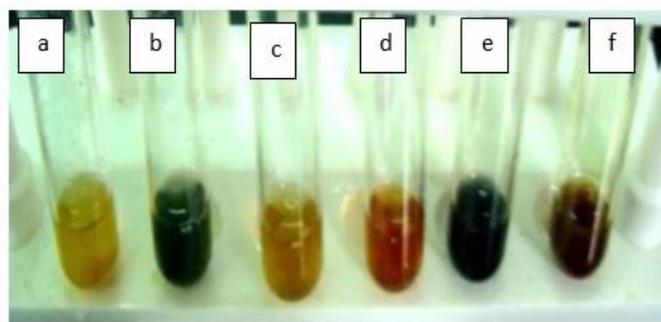


Figure 1: Preliminary phytochemical tests of Flavonoids (a), (c) and (d), Phenolic compounds (b), steroids and polyphenols (e), Cardiac glycosides (f) of the root extract of *Calpurnia aurea*.

S/N	Phytochemicals	Standard Tests	Solvent Extract			
			n-hexane	Chloroform	Ethanol	Methanol
1	Alkaloids	Dragendroff's Test	-	-	-	-
		Mayer's Test	-	+	+	+
		Wagner's Test	+	+	+	+
		Hager's Test	-	-	-	+
2	Flavonoids	Lead acetate Test	-	+	++	++
		Alkaline Reagent Test (NaOH Test)	+	-	++	++
		Shinoda's Test	-	-	++	++
3	Glycosides and Cardiac Glycosides	Salkowski's Test	++	+++	+++	+++
		Liebermann's Test	-	-	+	+
		Keller-Killiani's Test	++	+++	+++	+++
4	Terpenoids and Triterpenoids	Liebermann- Burckhardt's Test	-	-	+	+
		Salkowski's Test	+	+	+	-
5	Tannins	Braemer's Test	+	-	+	+++
		Lead acetate Test	+	-	+	+++
6	Anthocyanins	Borntrager's Test	-	+	-	-
7	Saponins	Foam Test	-	-	-	++
8	Phenolic Compounds	Ferric chloride Test	-	-	++	++
		Lead acetate Test	-	-	-	-
		NaOH Test	-	-	-	-
9	Steroids and Phytosterols	Liebermann- Burckhardt's Test	+	+	+	+
		Salkowski's Test	+	+	+	+

+++ Strongly detected; ++ moderately detected; + Detected but weak; - Negative or Not Detectable

Table 2: Results of Preliminary Phytochemical Screening of the root of *Calpurnia aurea* plant species Using Standard Chemical Test Methods.

Discussion

According to different findings made, preliminary phytochemical analysis of the plant extracts indicated presence of different phytochemicals. The hydro alcoholic extract of leaves of *Calpurnia aurea* was screened for the presence of bioactive plant chemical constituents as alkaloids, terpenoids, flavonoids, steroids and phytosteroids, tannins, saponins, phenols and cardiac glycosides using standard qualitative phytochemical screening test procedures [15, 16, 17, 4, 18]. The preliminary phytochemical analysis of 70% ethanolic extracts from the *C. aurea* seeds showed the presence of tannins, flavonoids, terpenoids, saponins, steroids, glycosides, alkaloids compounds [19]. The stem and bark hexane extract of *Calpurnia aurea* yielded the widely studied isoflavonoids and alkaloid type phytochemicals [5]. The phytochemical screening and qualitative estimation of *Calpurnia aurea* seeds and leaves showed that the leaves were rich in flavones and polyphenols than the seeds, yet the seeds are rich in alkaloids and tannins than the leaves *Calpurnia aurea* [4].

Here in this present study, though the degree is different in similar fashion, almost all the phytochemicals screened, except saponins, tannins and anthocyanins, were present in more than one solvent extracts of *Calpurnia aurea* root. As the study has revealed cardiac glycosides (very strong of all phytochemicals investigated in almost all solvents), phenols and polyphenols (strongly detected in both ethanol and methanol), flavonoids (strongly detected in both ethanol and methanol), saponins and tannins showed positive result in single solvent (methanol), alkaloids, terpenoids and triterpenoids, steroids and phytosterols showed weak positive result in all cases. In contrast, anthraquinones (generally anthocyanins) were failed to show positive results in all solvent extracts.

Conclusion

According to this study, the methanol extract of *Calpurnia aurea* root gave the highest percentage yield of extraction and followed by ethanol in contrast to n-hexane extract indicating that the root extract contained more polar compounds than non-polar compounds.

The plant root parts of different four solvent extracts were found to possess cardiac glycosides, tannins, flavonoids, phenolic compounds, alkaloids, steroids and saponins. However, as result data analysis revealed, there is difference in degree of contents of these phytochemicals based on the solvents used for sample extraction. The phytochemical screening test results have revealed that cardiac glycosides are the strongest detected components in the root part of the plant species next to which flavonoids and tannins were the strongly detected phytochemicals.

Finally, the medicinal plant *Calpurnia aurea* root extract appears to be rich in secondary metabolites as the same to that suggested by different authors on other parts of the plant species such as seeds and leaves widely used in traditional medicine to combat and cure various ailments, such as syphilis, malaria, rabies, diabetes, hypertension, diarrhoea, leishmaniasis, trachoma, elephantiasis, fungal diseases and different swellings. Exploitation of these pharmacological properties involves further screening of these active ingredients by

implementation techniques of extraction, purification, separation, crystallization and identification.

References

1. Nostro, M. P Germanò, V. D'angelo, A. Marino, M.A. Cannatelli (2000) "Extraction methods and bio autography for evaluation of medicinal plant antimicrobial activity". *Lett Appl Microbial* 30: 379-384.
2. N. K. Dubey (2004) "Global promotion of herbal medicine: India's opportunity". *Curr Sci* 86: 37-41.
3. D. Krishnaiah, R. Sarbatly, A. Bono (2007) "Phytochemical antioxidants for health and medicine: A move towards nature". *Biotechnol Mol Biol Rev* 1: 97-104.
4. G. Natesan, N. Haile, M. Umata, D. Seifu (2015) "Phytochemical Screening and Assessment of In Vitro Antioxidant Activities of *Calpurnia Aurea* Seeds and Leaves". *Ijppr.Huma* 2:1-12.
5. WHO Traditional Medicine Strategy (2002) World Health Organization, Geneva 2-5.
6. E. Korir, J. K.Joyce, R. C. Neil, M. Nivan, A. K. Neil (2014) "Isoflavones from *Calpurnia Aurea* Subsp. *Aurea* and Their Anticancer Activity." *Afr J Tradit Complement Altern Med* 11: 33-37.
7. Gemechu, G. Mirutse, W. Adane, A. Gobena (2013). "In vitro Anti-mycobacterial activity of selected medicinal plants against *Mycobacterium tuberculosis* and *Mycobacterium bovis* Strains". *BMC Complementary and Alternative Medicine* 13: 291.
8. H. Tadeg (2004) "Phytopharmaceutical Studies of Some Selected Medicinal Plants Locally Used in the Treatment of Skin Disorders". B. Pharm, Addis Abeba University, Ethiopia.
9. H. Tadeg, E. Mohammed, K. Asres, T. Gebre-Mariam (2005) "Antimicrobial activities of some selected traditional Ethiopian medicinal plants used in the treatment of skin disorders". *J. Ethnopharmacol* 100: 168-175.
10. H. G. Sunil, D. P. Shweta, S. U. Patil (2012) "Preliminary Phytochemicals Screening and TLC Analysis of *Ficus racemosa* Leaves". *J. Chem. Pharm. Res* 4:2380-2384.
11. S. Khalil, A. A. Rahim, K. K. Taha, K. B. Abdallah (2013) "Characterization of Methanolic Extracts of Agarwood Leaves". *Journal of Applied and Industrial Science* 1: 78-88.
12. G. A. Ayoola, H. A. Coker, S.A. Adesegun, A. A. Adepoju-Bello, K. Obaweya et al. (2008) "Phytochemical screening and antioxidant activities of some selected medicinal plants used for malaria therapy in South Western Nigeria". *Trop J Pharm* 7: 1019-1024.

13. J. Parekh, S. Chanda (2007) "Screening of aqueous and alcoholic extracts of some Indian medicinal plants for antibacterial activity". *Indian J. Pharm Sci* 68: 835-838.
14. S. Kumar, U.K. Sharma, A.K. Sharma, A.K. Pandey (2012) "Protective efficacy of Solanum xanthocarpum root extracts against free radical damage: phytochemical analysis and antioxidant effect". *Cellular and Molecular Biology* 58: 171-178.
15. K. Tiwari (2001) "Imbalance in antioxidant defense and human diseases: Multiple approach of natural antioxidants therapy". *Current science association* 81: 1179-1187.
16. Z. Birhanu, M. A. Wuhab, T. Abula (2015) "Antimalarial Activity of Calpurnia Aurea Hydro alcoholic Leaf Extract In Mice Infected With Plasmodium Berghei". *Archives* 2: 73-79.
17. E. Mebrahtu, S. Workineh, G. Mirutse (2013) "In vivo antimalarial activity of hydromethanolic leaf extract of Calpurnia aurea (Fabaceae) in Mice infected with chloroquine sensitive Plasmodium berghei". *Int. J. Pharm.Pharmacol* 2: 123-142.
18. Umer, K. N. Tekewe (2013) "Antidiarrhoeal and antimicrobial activity of calpurnia aurea leaf extract". *BMC complementary and alternative medicine* 13: 21.
19. N. Mulata, D. Seifu, M. Umata, E. Wendwesson, G. Natesan (2015) "Protective Effects of Calpurnia aurea Seed Extract on HAART Hepatotoxicity". *EJMP* 9: 1-12.