

## Phytochemical Screening of the Leaves *Calpurnia Aurea* (Ait.) Benth Extract

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### Abstract

**Background:** *Calpurnia aurea* is a genus of Flowering Plants within the family of fabaceae. *Calpurnia aurea* from Ethiopia is known locally as "digitta" (Amharic), "ሕግወቅ-Hitsawits" (Tigrigna). In native countries like Ethiopia, traditionally, the leave of *Calpurnia aurea* is used for the treatment of syphilis, malaria, rabies, diabetes, lung TB, hypertension, diarrhoea, stomach-ache, bowel, bladder disorders, to induce uterine contractions, tapeworm, trachoma, ringworm, as well as vomiting, Dandruff, headache, eye diseases, to destroy maggots, to destroy bedbugs, to relieve itches, used as a fish-poison or as a cure for dysentery, cough and snake bite.

**Objective:** The main purpose of this study was interested to investigate the phytochemical analysis of the leaves extracts *Calpurnia aurea*.

**Methods:** *Calpurnia aurea* leaves were collected from around debrekerbe kebele, medebay zana wereda and north western Zone of Tigray Region, Northern Ethiopia. The collected plant material was dried and powdered using mortar and pestle and then extracted using serial exhaustive solvent extraction methods within four organic solvents: petroleum ether, chloroform, ethyl acetate and ethanol 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.1 filter paper and the filtrate was dried using Rotary evaporator.

**Results:** *Calpurnia aurea* leaves extract contains cardiac glycosides, carbohydrates, phenolic compounds and tannins, flavonoids, fixed oil & fats, proteins, terpenoids, saponins, steroids and alkaloids, however there was difference observed based on extracting solvents used and strength of quality results in which, phenolic compounds and tannins, fixed oil and fats, steroids and cardiac glycosides were present in both petroleum ether and chloroform solvents; alkaloids, flavonoids, phenolic compounds and tannins, fixed oil and fats, amino acids and proteins, terpenoids, saponins and steroids were present in ethyl acetate solvent; carbohydrates, phenolic compounds & tannins, flavonoids, amino acids & proteins, terpenoids, saponins, steroids and alkaloids present in ethanol solvent.

**Conclusion:** Therefore, it is obvious from this study that this medicinal plant has possessed different vital phytochemicals that helps in the medicinal properties of the studied plant commonly used in Medebayzana woreda around Debrekerbe kebele.

**Keywords:** *Calpurnia aurea*; phytochemicals; Phytochemical Analysis; Qualitative Analysis, standard method

### 1. INTRODUCTION

Medicinal plants have been a major source of treatment for human diseases since time immemorial. One fourth of the world population i.e. 1.42 billion people are dependent on traditional medicines, particularly plant drug for curing ailments <sup>[1]</sup>. 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 <sup>[2]</sup>. Phytochemicals are bioactive chemicals of plant origin. They are regarded as secondary metabolites because the plants that manufacture them may have little need for them. They are naturally synthesized in all parts of the plant body; bark, leaves, stem, root, flower, fruits, seeds, etc. i.e. any part of the plant body may contain active components <sup>[3]</sup>.


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 [4]. 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 [5].

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 [6].

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 [5].

*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 [7]. Literature survey brings to light that, all parts of the plant species has been used for different human and animal disease [8]. *Calpurnia aurea* from Ethiopia is known locally as "digitta" (Amharic), "Hitsawits" (Tigrigna) see Figure 1. The plant is used in traditional medicine of Ethiopia to treat diverse medical conditions. 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, elephantiasis, fungal diseases, different swellings, stomach-ache, abscesses, bowel, bladder disorders, to induce uterine contractions [21], used as an insecticide to kill lice [20], and tapeworm, trachoma, ringworm, as well as vomiting, headache and eye diseases [22-25], to destroy maggots, to destroy bedbugs, 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].

**Table1.** Scientific classification of the plant

Scientific classification	
Kingdom:	Plantae
phylum	Angiosperms
class	Eudicots
division	Rosids
Order:	Fabales
Family:	Fabaceae
Genus:	Calpurnia
Species:	Aurea
	
Binomial name: <i>Calpurnia aurea</i> (Ait.) Benth.	
Table-1. Scientific classification of the plant	

**Fig1a.** leaves of *Calpurnia aurea* (By Hadera Brhane)

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 leaves 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.

## 2. MATERIALS AND METHODS

### 2.1. Plant Material Collection and Preparation

The leaves of *Calpurnia aurea* were collected from around debrekerbe kebele, medebay zana wereda and north western Zone of Tigray Region, Ethiopia in February 2019. The selected medicinal plant has been candidate for research because of their superficial medicinal properties, and its traditional use for medicine. The plant material was identified its scientific name by Leul Kidane W/michael (PhD) Assistant Professor in Plant Ecology & Biodiversity Conservation Department of Biology, Mekelle University, Ethiopia. The leaves were washed with tap water to remove dirt's and impurities, dried at 25°C for 10 days in the absence of sunlight and powdered well using mortar and pestle and stored in an air tight container for further use.

### 2.2. Preparations of the Extracts

The powdered medicinal plant material was taken and subject to serial exhaustive solvent extraction, during this solvents of increasing polarity from a low polar (Petroleum ether) to a high polar solvent (ethanol) to ensure that wide polarity range of compounds could be extract, during extraction solvents were diffuse in to the plant material and solubilize the phytocompounds with similar polarity. For qualitative determination, the extracts were place in pre-weighed flasks before drying. The remaining plant parts residues were extracted with other solvents sequentially [26].

### 2.3. Qualitative Phytochemical Analysis

The qualitative analysis of secondary metabolites was carry out by the following standard methods of [27, 28].

#### 2.3.1. Test for Alkaloids

Dragenderoff's Test: 2 ml aliquot of the extract was treated with the Dragenderoff's reagent. An orange red precipitate was produce immediately and it was indicate the presence of alkaloids.

Mayer's test: 1 ml aliquot of the extract was treated with few drops of Mayer's reagent. Formation of white precipitate or turbidity was produce and this shows the presence of alkaloids.

#### 2.3.2. Test for Flavonoids

Shinoda's Test: 1 ml of the extract was treating with magnesium turnings and 1-2 drops of concentrated HCl. Formation of pink or red colour was show the presence of flavonoids.

Alkaline reagent Test: 1 ml of the extract was treat with 5 ml of sodium hydroxide. The formation of intense yellow colour was confirming the presence of flavonoids.

#### 2.3.3. Test for Tannins and Phenolic Compounds

Ferric chloride test: 1 ml of the extract was treated with few ml of 5% neutral ferric chloride. A dark blue or bluish black colour product was show the presence of tannins.

Gelatin test: 1 ml of the extract was treated with few ml of gelatine solution; a white precipitate was form revealing the presence of tannins and phenolic compounds.

Lead acetate Test: 1 ml of the extract was treated with few ml of Lead acetate solution. A white precipitate production was show the presence of tannins & phenolic compounds.

#### 2.3.4. Test for Proteins

Xanthoproteic Test: 1ml of extract was treated with 2 drop of concentrated HNO<sub>3</sub>. Development of yellow colour was indicating the presence of proteins.

Biuret test: 1ml of extract was treated with 1 ml of 40% sodium hydroxide solution and 2 drops of 1% copper sulphate reagent. Appearance of violet colour was indicating the presence of proteins.

### 2.3.5. Test for Reducing Sugars

Benedict's test: To 1 ml of the extract, was added to 5 ml of Benedict's solution and keep in boiling water bath for 5 min. Red, yellow or green precipitate was indicate the presence of reducing sugars.

### 2.3.6. Test for Cardio Glycosides

Keller-Killani Test: To the solution of the extract in glacial acetic acid, few drops of ferric chloride and concentrated sulphuric acid was add, and observe for a reddish brown coloration at the junction of two layers and the bluish green colour in the upper layer was indicates the presence of glycosides.

### 2.3.7. Test for Saponins

Frothing Test: About 1 ml of the extract was dilute separately with 20 ml of distilled water and was shake in a graduated cylinder for 15 minutes. A 1 cm layer of foam was indicates the presence of saponins.

### 2.3.8. Test for Fixed Oils and Fats

Spot test: A small quantity of the extract was press between what man No 2-filter papers. Oil stains on the filter paper was indicate the presence of fixed oil.

### 2.3.9. Test for Terpenoids

Liebermann test: To 1 ml of extract 3ml of acetic acid and few drops of concentrated sulphuric acid were added. The colour change from red to blue was indicating the presence of terpenoids.

### 2.3.10. Test for Steroids

Liebermann-Burchards test: To 1.0 ml plant extract, 1.0 ml of concentrated sulphuric acid was added followed by the addition of 2.0 ml of acetic anhydride solution. A greenish colour was developed and it turns blue to indicate the presence of steroids.

Salkowski's test: To 1ml of extract, 1ml of concentrated sulphuric acid was added carefully along the sides of the tube. The red colour was produced in the chloroform layers then it indicated the presence of steroids.

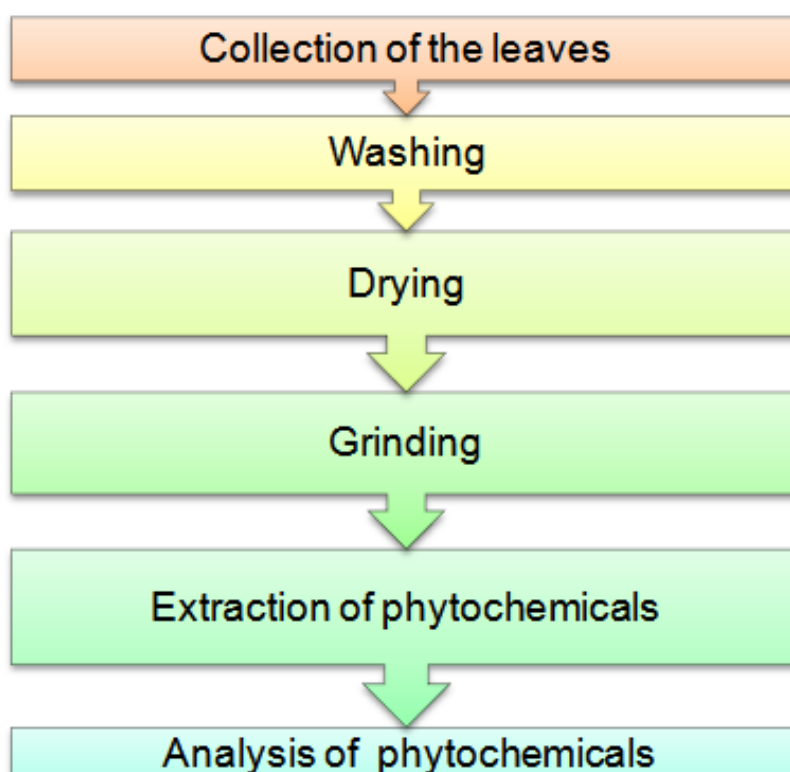


Fig1. Methodology summary

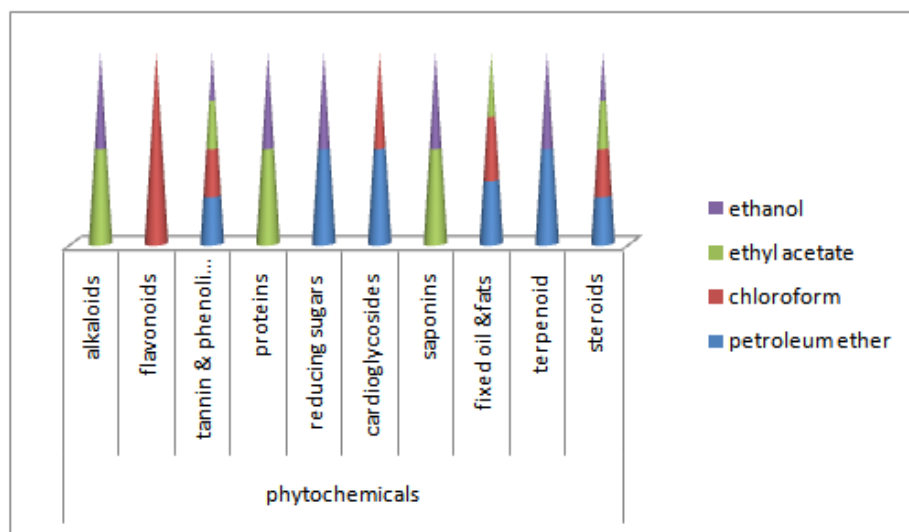
### 3. RESULTS AND DISCUSSION

#### 3.1. Results

**Table1.** Results of Preliminary Phytochemical Screening of the leaves of *Calpurnia aurea* plant species.

S/N	Phytochemicals	Standard Tests	Solvent Extract			
			Petroleum ether	Chloroform	Ethyl acetate	Ethanol
1	Alkaloids	Dragendroff's Test	-	-	+	+
		Mayer's Test	-	-	-	-
2	Flavonoids	Alkaline Reagent Test	+	-	+	+
		Shinoda's Test	+	-	-	-
3	Cardiac Glycosides	Keller-Killiani's Test	+	+	-	-
4	Terpenoids	Liebermann Test	+	-	-	+
5	Phenolic Compounds and Tannins	Ferric chloride Test	-	+	+	+
		Lead acetate Test	-	-	-	+
		Gelatin Test	+	-	+	-
6	Fixed oil and fats	Spot test	+	+	+	-
7	Saponins	Frothing test	-	-	+	+
8	Steroids	Liebermann-Burckhardt's Test	+	+	+	+
		Salkowski's Test	-	-	-	+
9	Reducing sugars	Benedict's test	+	-	-	+
10	proteins	Biuret test	-	-	+	-
		Xanthoproteic test	-	-	+	+

+ indicates = present, - indicates =absent



**Fig2.** Phytochemical constitutes v.s. solvent using for Extraction

#### 3.2. 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 manner,

As shown in the table -1- or figure-3 the preliminary phytochemical screening ascertained the presence of bioactive secondary metabolites such as flavonoids, Cardiac glycosides, steroids, tannins and saponin which confirmed that, presence pharmacologically active components, the polar compounds were detected in polar fractions and non-polar in non-polar solvents. Further it is clear from the data given in table -1- or figure-3 the number of phytochemicals extracted from leaves of *Calpurnia aurea* as the polarity i.e. ethanol shown the presence of maximum numbers of phytochemicals while the ethyl acetate and chloroform showed four classes of bioactive compounds, steroids, cardiac glycoside and terpenoids were found in all organic extracts, amusingly none of the extracts shown the presence of alkaloids.

#### **4. CONCLUSION**

According to this study, the ethanol extract of *Calpurnia aurea* leaves gave the highest phytochemicals of extraction and followed by ethyl acetate in contrast to petroleum ether and chloroform extract indicating that the leaves extract contained more polar compounds than non-polar compounds.

The plant leaves 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 phenolic compounds, tannins and steroids are the strongest detected components in the leaves part of the plant species next to which fixed oil and fats were the strongly detected phytochemicals.

Finally, the medicinal plant *Calpurnia aurea* leaves 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 roots 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. The study findings support the use of this one plant in the determinations of different functional groups in organic chemistry like alkenes, alcohols, carboxylic acids etc. This plant can also be used to find out bioactive natural products that may hand out as leads for the enlargement of latest pharmaceuticals.

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