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Phytochemical Screening and Assessment of *In Vitro* Antioxidant Activities of *Calpurnia Aurea* Seeds and Leaves

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ABSTRACT

Objective: In Ethiopia, *Calpurnia aurea* is used for the treatment of syphilis, malaria, rabies, diabetes, hypertension, diarrhoea, leishmaniasis, trachoma, elephantiasis, fungal diseases and different swellings. However, despite its traditional usage as an agent, there is limited or no information regarding the phytochemical and *in vitro* antioxidant profile of the leaves and seeds of *Calpurnia aurea* .Hence; we evaluated the phytochemical profile and *in vitro* antioxidant actives of this plant seeds and leaves extract.
Methods: *Calpurnia aurea* leaves and matured dried seeds were collected from south Gondar, northern Ethiopia. The collected plant materials were dried and powdered using electrical grinder and then macerated with 70% ethanol for 72 hours with mechanical shaking and it was filtered through Whatman No.1 filter paper and the filtrate was dried using Rota vapor. Preliminary phytochemical screening such as tannins, flavonoids, terpenoids, saponins, steroids, glycosides, alkaloids, anthraquinones and resins using standard methods and *in-vitro* antioxidant properties were screened through DPPH (1,1-diphenyl-2-picrylhydrazyl).
Results: *Calpurnia aurea* leaves and seed were contain tannins, flavonoids, terpenoids, saponins, steroids, glycosides, alkaloids but absent anthraquinone , yet seed containing more tannins and alkaloids than the leaves . Flavones and polyphenol levels were found more in leaves than the seeds. The extracts of both leaves and seeds of the plant indicated strong antioxidant activities.
Conclusion: It is evident from this study that highest therapeutic efficacy possessing majority of secondary metabolites classes of compounds in both leaves and seeds of *Calpurnia aurea*, which can be quantified for application in pharmaceutical industry. Conversely seeds contain more alkaloids and tannins than the leaves of *Calpurnia aurea*.

INTRODUCTION

The medicinal plants are useful for healing as well as for curing of human diseases because of the presence of phytochemical constituents [1].Medicinal plant treatment are still used for many health problems. 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 defense 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 investigations 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 [5]. 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 attempt 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

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, the leaf and stem of *C.aurea* has been used for different human and animal disease [8]. In Ethiopia, traditionally, the leave of *C.aurea* is used for the treatment of syphilis, malaria, rabies, diabetes, hypertension, diarrhoea, leishmaniasis,

trachoma, elephantiasis, fungal diseases and different swellings, stomach-ache, bowel, and bladder disorders [9, 10]. Plant products have been part of phytomedicines since time immemorial. These can be derived from any part of the plant like bark, leaves, flowers, seeds, etc. [11] 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 [12-14]. In the present work, qualitative phytochemical analysis was carried in *C.aurea* leaves and seeds.

MATERIALS AND METHODS

Plant materials

The *C.aurea* leaves and seeds were collected from northern Ethiopia, south Gondar in June 2013. The plant has identified by taxonomist and authenticated by Ethiopian National Herbarium of Addis Ababa University and its voucher number is 001/2006.

Extraction:

The leaves and seeds were washed thoroughly 2-3 times with running tap water, then air dried under shade after complete shade drying the plant materials were grinded in mixer, the powder was kept in small plastic bags with paper labeling. The grinded leaves and seeds were weighed 100 gm using an electronic balance and were macerated separately in 70% ethanol for 72 hours with mechanical shaking and it was filtered through Whatman No.1 filter paper. Then filtrates were evaporated using rotary evaporator and dried at 40^{O}C.

Phytochemical Screening:

Preliminary qualitative phytochemical screening was carried out with the following methods.

Steroids:

1 ml of the extract was dissolved in 10 ml of chloroform and equal volume of concentrated sulphuric acid was added by sides of the test tube. The upper layer turns red and sulphuric acid layer showed yellow with green fluorescence. This indicated the presence of steroids [15].

Terpenoids:

2 ml of extract was added to 2 ml of acetic anhydride and concentrated H_2SO_4. Formation of blue or green rings indicates the presence of terpenoids [16].

Tannins:

2 ml of extract was added to few drops of 1% lead acetate. A yellowish precipitate indicated the presence of tannins [17].

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 [18].

Anthocyanins:

2 ml of aqueous extract is added to 2 ml of 2N HCL and ammonia. The appearance of pink-red turns blue-violet, indicates the presence of anthocyanins [19].

Flavonoids

Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids [20].

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 [20].

Phenolic Compounds

To 2 g of the extract of the plant material, 3 drops of a freshly prepared mixture of 1 ml of 1 % $FeCl_3$ and 1 ml of potassium ferrocyanide was added to detect phenolic compounds. Formation of bluish-green color indicates the presence of phenolic compounds [20].

In-vitro Antioxidant Activity

The qualitative and quantitative analysis of *in-vitro* antioxidant activity was done to assess the antioxidant potential of the extract.

Thin Layer Chromatography Method

The *in-vitro* antioxidant of *C.aurea* seed was measured using DPPH according to Choi *et al*., 2002 [21]. In brief, 3 µl aliquots of freshly prepared 70% ethanol extract of the *C.aurea* seed at various concentrations were loaded individually in silica gel Thin-Layer Chromatography (TLC) plate and allowed to air dried for a few minutes. The TLC plate bearing the dry spots was placed upside down for 10 seconds in a solution of 0.1 millimole-ethanolic DPPH solution. The spot exhibiting radical scavenging activity of the antioxidant showed up as faint yellow spots against a purple background.

Spectrophotometric Method

The DPPH radical-scavenging activity of the test extracts was examined as previously described [22]. Different concentrations (31.25 - 1000 µg/ml) of each extract were added, at an equal volume, to methanolic solution of DPPH (100 mM). The mixture was allowed to react at room temperature in the dark for 30 minutes. Vitamin C was used as standard controls. Three replicates were made for each test sample. After 30 minutes, the absorbance (A) was measured at 518 nm and converted into the percentage antioxidant activity using the following equation: % scavenged. IC_{50} values denote the concentration of sample which is required to scavenge 50% of DPPH free radicals. The IC_{50} values were calculated by linear regression of plots, where the abscissa represented the concentration of the tested plant extracts and the ordinate the average percent of scavenging capacity from three replicates. IC_{50} value (concentration of sample where absorbance of DPPH decreases 50% with respect to absorbance of the control) of extracts were determined [23]. The higher the antioxidant activity, the lower IC_{50} value.

RESULTS

Table 1 shows the phytochemical profile of *C. aurea* seeds and leaves .These tests reveal the presence of various bioactive secondary metabolites which might be responsible for their medicinal attributes. The observations and inferences made in the phytochemical tests are

presented as follows:

Table 1: Preliminary Phytochemical tests and results of *C.aurea* seeds and leaves

S/N	Phytoconstituents	Test	Observation	Leaves	Seeds
1	Tests for alkaloids	Mayer's Test	Yellow color precipitate	+	+ +
2	Tests for flavonoids	Lead Acetate Test	Yellow color	++	+
3	Test for Saponin	Foam Test	Layer of foam	+	+
4	Test for Phenolic compounds	Ferric Chloride Test	Bluish-green color	++	+
5	Test for tannins	Braemer's Test	Bluish color	+	+ +
6	Test for terpenoids	Salkowski Test	Reddish brown color	+	+
7	Test for Anthocyanins	Borntrager's Test	No pink color	--	--
8	Tests for Cardiac glycosides	Keller-Killiani Test	Brown ring	+	+
9	Test for steroids/ Phytosterols	Libermann Burchard Test	Blue-green color	+	+ +

(+)= Presence; (–) =Absence; (++) = Higher proportion of the components.

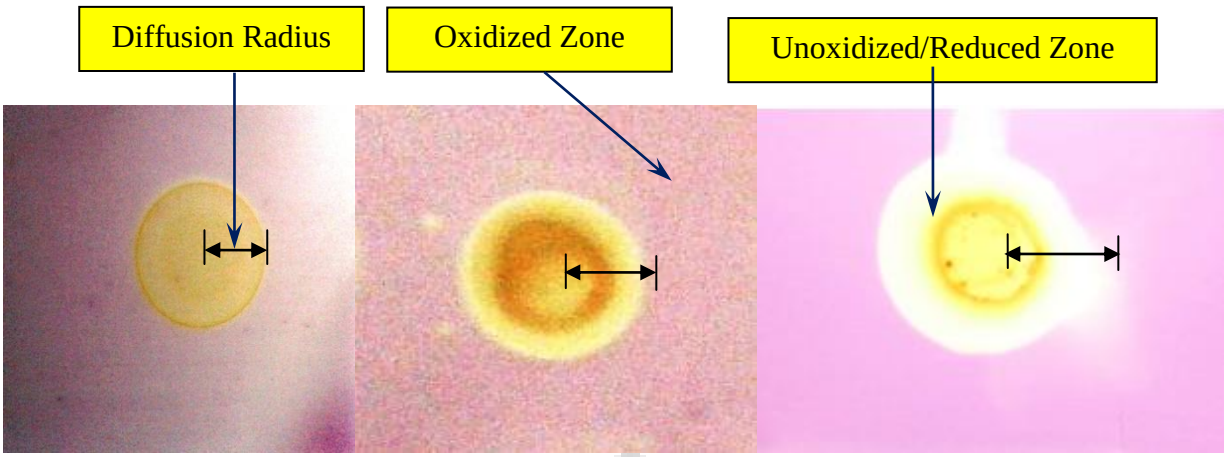
The phytochemical screening and qualitative estimation of *C.aurea* seeds and leaves showed that the leaves were rich in flavones and polyphenols then the seeds, yet the seeds were rich in alkaloids and tannins than the leaves *C.aurea*. Umer et al., reported that the 80% methanol extract of *C. aurea* leaf revealed the presence of alkaloids, tannins, flavonoids and saponins [24].

In-vitro Antioxidant Activity

Thin Layer Chromatography

The diameter of the diffused spots (Figure 1) indicating that the increase in concentration of *C.aurea* seed extract increased the anti-oxidant property, zone of oxidized inhibition directly proportional to the concentration of *C.aurea* seed in strong oxidizing agent (free radical) DPPH ethanol solution. The Thin-Layer Chromatography (TLC) plate immersed in DPPH solution

loaded with 300mg/100ml of the seed extract showed highest reducing ability. The similar results was obtained leaves extract also (the data is not yet shown).



100mg/100ml 200 mg/100ml 300mg/100ml

Figure 1 *In-vitro* anti-oxidant properties of various concentration of 70% ethanol extract of *C.aurea* seed extract (100, 200, 300mg/ 100 ml) from left to the right, respectively.

Spectrophotometric Method

The percentage inhibition in *C.aurea* seeds and leaves extract and standard Ascorbic acid Vs concentration shows that the antioxidant activities of 70% ethanol extract of *C.aurea* seed and the standard Ascorbic acid was found to be positively correlated with the % inhibition. The IC_{50} (Inhibition Concentration at 50%) value of *C.aurea* extract were calculated as 88.46 µg/ml (almost the same value for leaves extract) while that of Ascorbic acid was 51.41 µg/ml from their corresponding regression curves.

Table 2: Total antioxidant activity of the leaves and seeds extracts of *C. aurea*

Concentrations µg/ml	% inhibition of leaves extract	% inhibition of seeds extracts	% inhibition of ascorbic acid
31.25	22.5	23	45
62.5	31	30	55
125	39	41	63
250	55	57	65
500	75	73	85
1000	83	85	100

DISCUSSION

These secondary metabolites contribute significantly towards the biological activities of medicinal plants such as hypoglycemic, antidiabetic, antioxidant, antimicrobial, anti-inflammatory, anti-carcinogenic, anti-malarial, anti-cholinergic, anti-leprosy activities etc. [25]. The plant seeds and leaves were found to possess tannins, seed containing more tannins than the leaves. Tannins prevent plants from being edible for worms and confer resistance to microbes. They have been exploited as food and medicine for their effects against tumors, oxidants or microbes [26]. Tannins do not function solely as primary antioxidants (i.e., they donate hydrogen atom or electrons), they also function as secondary antioxidants. Tannins have the ability to chelate metal ions such as Fe^{2+} and interfere with one of the reaction steps in the Fenton reaction and thereby retard oxidation [27]. The inhibition of lipid peroxidation by tannin constituents can act via the inhibition of cyclooxygenase [28]. In some cases, alkaloids obtained from plants may cause serious illness, injury or even death. The manner of poisoning with plants can be divided into unintentional ingestion of plant material, intentional ingestion of plant material, and ingestion of abused plant material [29].

Flavonoids have been consumed by humans since the advent of human life on earth, that is, for about 4 million years. Flavonoids have been reported to exert wide range of biological activities. These includes: anti-inflammatory, antibacterial, antiviral, antiallergic [30], cytotoxic anti-tumour, treatment of neurodegenerative diseases, vasodilatory action [31, 32]. In addition flavonoids are known to inhibit lipid-peroxidation, platelet aggregation, capillary permeability and fragility, cyclooxygenase and lipoxygenase enzyme activities. They exert these effects as antioxidants, free radical scavengers, chelators of divalent cation [32, 33]. These are also reported to inhibit variety of enzymes like hydrolases, hyalouronidase, alkaline phosphatase, arylsulphatase, cAMP phosphodiesterase, lipase, α-glucosidase, kinase [34]. Several epidemiologic studies have suggested that drinking either green or black tea may lower blood cholesterol concentrations and blood pressure, thereby providing some protection against cardiovascular disease. Flavonoids are also known to influence the quality and stability of foods by acting as flavorants, colorants, and antioxidants [35, 36]. Flavonoids contained in berries may have a positive effect against Parkinson's disease and may help to improve memory in elderly

people. Intake of antioxidant flavonoids has been inversely related to the risk of incidence of dementia [37].

Saponins have been ascribed a number of pharmacological action [38-41]. The important ones being permeabilizing of the cell membrane [42] lowering serum cholesterol, stimulation of luteinizing hormone release leading to obortifacient properties [43], immunomodulatory potential via cytokine interplay [39], cytostatic and cytotoxic effect on malignant tumor cell [44], adjuvant properties for vaccines as immunostimulatory complexes [45] and synergistic enhancement of the toxicity of immunotoxins [46, 47].

Phenolics have been considered powerful antioxidants *in vitro* and proved to be more potent antioxidants than Vitamin C and E and carotenoids [48, 49]. The inverse relationship between fruit and vegetable intake and the risk of oxidative stress associated diseases such as cardiovascular diseases, cancer or osteoporosis has been partially ascribed to phenolics [50, 51]. It has been proposed that the antioxidant properties of phenolic compounds can be mediated by the following mechanisms (1) scavenging radical species such as ROS/RNS; (2) suppressing ROS/RNS formation by inhibiting some enzymes or chelating trace metals involved in free radical production; (3) up regulating or protecting antioxidant defense [52].

Terpenoids composed of “isoprenoid” units constitute one of the largest groups of natural products accounting for more than 40000 individual compounds, with several new compounds being discovered every year [53-55]. The diverse array of terpenoid structures and functions has provoked increased interest in their commercial use. Terpenoids have been found to be useful in the prevention and therapy of several diseases, including cancer, and also to have antimicrobial, antifungal, antiparasitic, antiviral, anti-allergenic, antispasmodic, antihyperglycemic, antiinflammatory, and immunomodulatory properties [56-59]. In addition, terpenoids can be used as protective substances in storing agriculture products as they are known to have insecticidal properties [60].

CONCLUSION

The medicinal plant *Calpurnia aurea* seeds and leaves appears to be rich in secondary metabolites and strong antioxidant capacity, 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 investigation of these active ingredients by implementation techniques of extraction, purification, separation, crystallization and identification.

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