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IN-VITRO ANTIOXIDANT ACTIVITY AND QUANTITATIVE ANALYSIS OF TOTAL PHENOLIC AND FLAVONOID COMPOUNDS OF *Sebastiania chamaelea* Muell. Arg. LEAF EXTRACTS

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ABSTRACT

The importance of antioxidant constituents in maintenance of health attracted research in plant based antioxidants. Presence of amino acid argenine and secondary metabolites like flavonoids and phenolic compounds and antibacterial activity leads for the evaluation of antioxidant activity of *Sebastiania chamaelea* leaf aqueous extracts. *In-vitro* free radical scavenging LC $_{50}$ at 100-90 µg/ml, and r educing ability EC_{50} at 175µg/ml are closely resembled to that of the standard drug ascorbic acid. The antioxidant activity also supported by the evaluation of the total phenolic compounds 235 µg/ml Gallic acid equivalent and flavonoids 210µg/ml as Quercetin Equivalent. *S. chamaelea* antioxidant activity may also support the herbal usage against vertigo, astringent, remedy for syphilis and diarrhoea.

KEYWORDS:Freeradical,IC50,EC50,Reducingpower,Phenoliccompounds,Sebastianiachamaelea, Flavonoids.

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INTRODUCTION

The antioxidants present in food can limit the damage of somatic cells and pathogenesis of diseases by acting directly on reactive species or by stimulating endogenous defence systems. Polyphenols can accept an electron to form relatively suitable phenoxyl radicals, thereby disrupting chain oxidation reaction in cellular components [1]. Flavonoids and phenolic acids are widely distributed secondary metabolites with antioxidant and antiradical properties [2]. The importance of antioxidant constituents of plant materials in maintenance of health and protection from ageing related diseases has attracted interest in natural antioxidants [3]. Decoction of *S.chamaelea* leaf in ghee is given as tonic and applied to the head in vertigo. Leaf juice is astringent and is used as a remedy for syphilis and diarrhea [4, 5]. It also possesses 77.5% free amino acids, as argenine with 60% of total free amino acids [6]. Preliminary phytochemical screening of methanolic leaf extract of *S. chamaelea* revealed the presence of Phenols, Flavonoids, Tannins, Saponins, Steroids and Glycosides were proved to possess antibacterial activity [7].

MATERIALS AND METHODS

Collection and identification of plant material

S.chamaelea leaves were collected from the agricultural fields of S.V.Veterinary College, Tirupati, A.P, India, during February-March.2010. The taxonomic identity of the plant is confirmed by Prof. N. Yasodamma, Department of Botany, Sri Venkateswara University, Tirupati and the voucher specimen KSS.711 was preserved in the herbarium Department of Botany as per the standard method [8]. Fresh leaves were thoroughly washed and then dried under shade for about 3 days. The dried plant parts were ground well into a fine powder in a mixer grinder and sieved to give particle size of 50-150mm. The powder was stored in air sealed polythene bags at room temperature until further use.

Preparation of aqueous and organic solvent extracts

Dried leaf powder (70 g) was soaked in water, filtered after 72 hr. Extract was dried on water bath. And also powder (40 g) was extracted using each 200 ml of methanol, ethanol and chloroform in a soxhlet apparatus respectively. The extracts were concentrated on rotavapour, dried and stored at 4^{0}C in refrigerator until further use.

Antioxidant assay

Antioxidant activity of plant extracts was determined *in vitro* methods such as, the DPPH free radical scavenging assay and reducing ability methods. All the assays were carried out in triplicates and average values were considered.

DPPH Radical Scavenging Assay [9, 10]

The free radical scavenging capacity of the hot water, methanolic, alcoholic and chloroform extracts against 2, 2-Diphenyl-1-picryl hydrazyl radicals were determined by UV- visible spectrophotometer at 517nm. Radical scavenging activity was measured by a slightly modified method using 62.5, 125, 250, 500 and 1000 µg/ml. 1 ml of each extract was placed in a test tube, and 3ml of methanol was added followed by 0.5 ml of 1 Mm DPPH in methanol. Control sample with ascorbic acid was prepared containing the same volume without any extract. A blank solution was prepared containing the same amount of methanol and DPPH. The radical scavenging activity was calculated using the following formula:

$$\% \text{ inhibition} = \{[A_b - A_a]/A_b\} \times 100$$

Where A_b is the absorption of the blank sample and A_a is the absorption of the extract.

Assay of reducing ability [11]

1ml of a leaf extract (100 - 500 mg/l) was mixed with 2.5 ml potassium ferricyanide [$K_3Fe (CN_6)$] (10g/l), then the mixture was incubated at 50^0 C for 20 minutes. 2.5 ml of trichloroacetic acid (100mg/l) was added to the mixture. This was then centrifuged at 3000 rpm for 10 min. Finally, 2.5 ml of the supernatant was mixed with 2.5 ml of distilled water and 0.5 ml $Fecl_3$ (1g/L) and absorbance measured at 700nm in UV-visible spectrophotometer. Ascorbic acid was used as a standard and phosphate buffer used as blank solution. The absorbance of the final reaction mixture of three parallel experiments was expressed as mean with standard deviation. The increased absorbance of the reaction mixture indicates a stronger reducing ability. The extract concentration providing 0.5 of absorbance (EC_{50}) was calculated from the graph of absorbance at 700 nm.

Determination of Total phenolic content

Total Phenolic contents were determined by the Folin-ciocalteau method [12, 13]. 0.5 ml of (1 mg/ml) each extracts were mixed with folin-ciocalteu reagent (5 ml, 1:10 diluted with distilled water) for five minutes and aqueous Na_2Co_3 (4 ml, 1M) were then added. The mixture was allowed to stand for 15 min and the phenols were determined by calorimetric method at 765nm. The standard curve was prepared from 1 to 10 µg/ml solutions of gallic acid in methanol: water (50:50, v/v). Total phenol values are expressed in terms of gallic acid equivalent (mg/g of dry mass), which is a common reference compound.

Determination of Total flavonoid content

Calorimetric aluminium chloride method was used for flavonoid determination [14, 15]. 0.5ml (1 mg/ml) of each extract was mixed with 1.5 ml of methanol, 0.1ml of 10% aluminium chloride, 0.1ml of 1M potassium acetate, and 2.8ml of distilled water were left at room temperature for 30 minutes. The absorbance of the reaction mixture was measured at 415nm with UV visible spectrophotometer. Total flavonoid content was calculated as quercetin calibration curve at 50 to 500 µg/ml in methanol.

RESULTS

DPPH activity: **(Table- I)**

The free radical scavenging activity of *S.chamaelea* leaf aqueous, methanol, ethanol and chloroform extracts IC_{50} values (the concentration required to inhibit radical formation by 50%) were 100.90µg/ml, 519.47µg/ml, 1065.65µg/ml and 1403.80µg/ml respectively. The standard Ascorbic acid IC_{50} value is 48.36µg/ml. Hence *S.chamaelea* leaf aqueous extract is nearly equal to that of ascorbic acid than other extracts.

Table I

Absorbancy at 517nm of S.chamaelea leaf extracts DPPH Free Radicals.

Conc (µg/ml)	Aqueous	Methanol	Ethanol	Chloroform	Ascorbic acid
62.5	0.574±0.002**	0.638±0.000**	0.635±0.002**	0.680±0.004**	0.514±0.00**
125	0.362±0.002**	0.534±0.001**	0.586±0.002**	0.622±0.002**	0.419±0.00**
250	0.262±0.002**	0.422±0.000**	0.539±0.000**	0.586±0.002**	0.188±0.00**
500	0.249±0.001**	0.362±0.001**	0.489±0.004**	0.497±0.038**	0.164±0.00**
1000	0.212±0.001**	0.294±0.011**	0.436±0.002**	0.492±0.001**	0.078±0.00**
IC_{50} Values in (µg/ml)	100.90	519.47	1065.65	1403.80	48.36

All the data are expressed as mean ± SEM: **indicate p<0.01 as compared to control ascorbic acid (One –way ANOVA followed by Dunnett's test).

Reducing ability: (Table: II)

The aqueous extract showed very high reducing ability when compared to other extracts. The EC_{50} values (the concentration at which 50% of the effect is achieved) with leaf aqueous, methanol, ethanol and chloroform extracts were 175μg/ml, 200μg/ml, 200μg/ml and 220μg/ml respectively. The EC_{50} value of ascorbic acid was 185μg/ml. Hence EC_{50} value of leaf aqueous extract at 175μg/ml is considerd to be very effective to that of the standard control drug.

Table II

Reducing ability at 700nm absorbance of Sebastiania chamaelea leaf extracts.

Conc.(μg/ml)	Aqueous	Methanol	Ethanol	Chloroform	Ascorbic acid
100	0.393±0.004**	0.364±0.007**	0.399±0.014**	0.278±0.021**	0.575±0.029
200	0.849±0.013*	0.601±0.002	0.498±0.011**	0.386±0.027**	0.756±0.023
300	0.850±0.040	0.818±0.016	0.617±0.029**	0.566±0.014**	1.039±0.115
400	1.051±0.023	0.969±0.006**	0.741±0.012**	0.704±0.031**	1.092±0.020
500	1.329±0.022*	1.227±0.028**	0.865±0.026**	0.792±0.004**	1.421±0.018
Ec_{50} values in (μg/ml)	175	200	200	220	185

All the data are expressed as mean ± SEM: ** indicate p<0.01 and * indicate p<0.05 as compared to control Ascorbic acid (One –way ANOVA followed by Dunnett s test).

***Total phenolic compounds and flavonoids* (Fig- I, II, III)**

Total phenolic compounds of *S.chamaelea* was determined calorimetrically according to Folin-Ciocalteu procedure and calculated as gallic acid equivalent to reference standard curve ($y = 0.070x + 0.124$, $r^2 = 0.988$). The total phenolic content of aqueous, methanol, ethanol and chloroform extracts are 235±5.0, 193.3±5.0, 170±10.0, 41.66±8.33 μg/ml respectively. When compared to other extracts, aqueous extract shows highest amount of phenolic compounds. Total flavonoid content was determined spectrometrically and calculated as Quercetin equivalent as reference to standard curve ($y = 0.001x – 0.066$). The total flavonoid content of aqueous, methanol, ethanol and chloroform extracts are 210±0.0, 180±0.0, 170±0.0 and 120±0.0 μg/ml respectively. Hence aqueous extract shows high quantity of flavonoids and phenolic compounds when compared to the other extracts.

Figure I

Standard graph for Gallic acid: Phenolic content

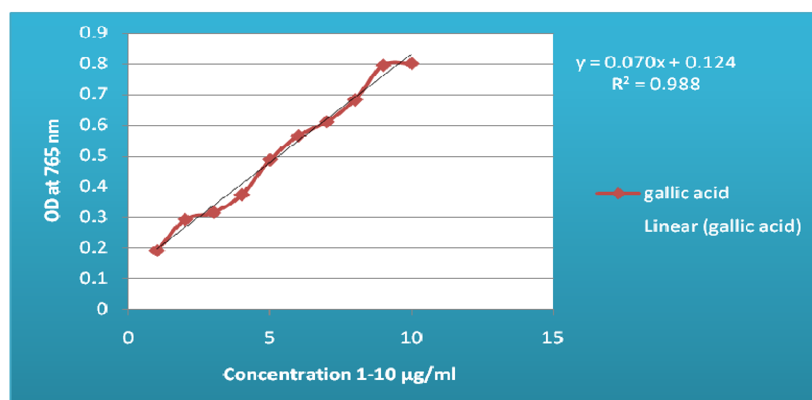


Figure II
Standard graph for Quercetin: Flavonoids

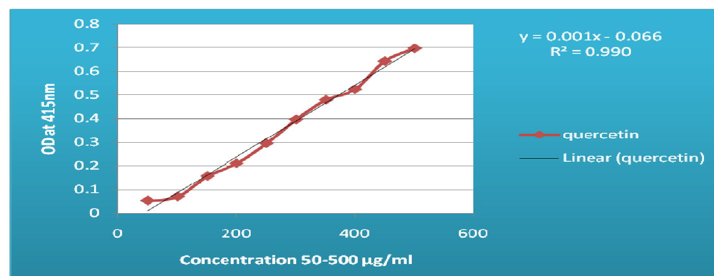
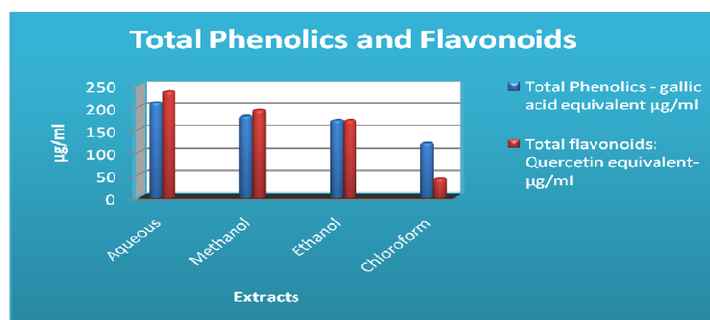


Figure III
Total phenolic and Flavonoid compounds



DISCUSSION

S.chamaelea leaf aqueous extracts antioxidant activity and the total phenolic and flavonoid content is compared with the other species of *Euphorbiaceae*. Methanol extracts of *Acalypha indica* with 81.6%, and *Mallotus philippensis* 91.33% of DPPH scavenging activity [16]. Total phenolic content of *Aporosa lindleyana* 31.20 mg/100 g of gallic acid equivalent and the flavonoid content 203.10µg/ml quercetin equivalent, antioxidant activity with LC_{50} 19.91 mg/100 g of ascorbic acid [17]. *Excoecaria agallocha* DPPH scavenging assays IC_{50} 189.27 µg/ml and the reducing ability increased dose-dependently [18]. Total phenolic content of *Ricinus communis* whole plant n-Butanol extract yielded 547 mg GAE/g shows 83.1% of DPPH scavenging activity [19].Methanol extracts of *J. curcas* stem bark against *Invitro* DPPH and nitric oxide Scavenging activity revealed significant action in leaf and latex followed by root and stem [20, 21] . Chloroform, methanol and ethanol extracts of root bark is effective radical scavenging activity [22]. Crude methanol leaf extracts possessed free radical scavenging activity with IC50 of 90.3 µg/ml while the standard Quercetin 50.71 µg/ml [23]. Ethanol extracts of root scavenging activity IC50 is 0.521 µg/ml is the strongest followed by stem, leaves and nodes [24] ; methanolic extracts are more efficient antioxidants [25].There is a correlation between the amount of Phenolic compounds present and percentage of inhibition of DPPH Radical Scavenging activity [26]. *Euphorbia hirta* hot water and crude extracts antioxidant potential using phospho-molybdenum complex and Ferric Reducing Power assay (FRAP) with 185 µg/ml to that of Ascorbic acid and 398 µ mol Fe II equivalent per gram. The crude extracts revealed significant free radical scavenging activity at

247μmol [27]. *E. thymifolia* is showing effective antioxidant activity with 82.93% at 62.5 - 1000 μg/L to that of 91-100% of Gallic acid equivalent and phenolic compound about 7.46mg [28]. *Acalypha monostachya* methanol extracts contain a variety of phenolic compounds as benzoic acid, flavone, flavanol which possesses pronounced antioxidant activity with IC50 3.45 μg/ml with 43.4% Gallic acid equivalent [29].

CONCLUSION

It is proved that *S.chamaelea* leaf aqueous extract has high phenolic contents and so correlated the antioxidant activity than other extracts. It is also correlated to that of the other *Euphorbiaceae* species. Further studies on the *S.chamaelea* aqueous extract is required to isolate and identify the bioactive compounds responsible for antioxidant activity.

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