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Evaluation of Phenolic Content and Antioxidant Potency in Various Parts of *Cassia auriculata* L.: A Traditionally Valued Plant

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Abstract: Presence of polyphenolic content in various part of the plant exhibit wide pharmacological activities including antioxidant activity. The present study was designed to evaluate the phenolic contents (total phenols, flavonoid and tannins) and antioxidant properties of ethanolic extracts of flower, leaf, pod, bark and root obtained from *Cassia auriculata*. Ethanolic extracts of various parts of *C. auriculata* obtained by sonication extraction techniques are studied for their phenolic contents and DPPH (2,2-diphenyl-1-picrylhydrazine) radical scavenging assay as well as total antioxidant assays using UV visible spectrophotometer. Among the various parts of the plant studied, bark showed significant content of phenolics, flavonoids and tannins followed by the root, leaf, flower and pod. Even bark extract exhibited highest antioxidant capacity in DPPH assay followed by root, leaf, flower and pod with a value of 766.7, 679.3, 644.9, 572.5 and 474.7 mg vitamin C equivalent antioxidant capacity (mg VCEAC)/sample, respectively. In addition, mg VCEAC values obtained from the total antioxidant assay was in the increasing order of bark>root>leaf>flower>pod. Moreover, a strong correlation was also found between phenolic contents and antioxidant values indicating their influence in the found antioxidant activity, hence the bark extract can be employed as an ideal candidate for herbal based pharmaceutical product. Results of the present study also emphasize variation in the chemical composition as well as biological activity ensuring the importance of proper selection of particular part of the plant to evaluate their therapeutic potency.

Key words: *C. auriculata*, phenolic variation, VCEAC, antioxidant potency, herbal base drug

INTRODUCTION

Free radicals are produced during the normal metabolism and are neutralised by the endogenous antioxidant enzymes such as superoxide dismutase, glutathione peroxidase and catalase. However, during tissue damage and pathological conditions an increased production of Reactive Oxygen Species (ROS) creating an imbalance between their biosynthesis and their removal by antioxidant systems. This may leads to ageing, cardiovascular diseases, cancer, atherosclerosis, diabetes mellitus, hypertension, Alzheimer's and Parkinson's diseases (Finkel and Holbrook, 2000; Gutteridge and Halliwell, 2000). Use of synthetic antioxidants, such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) are restricted due to their toxic properties and unwanted side effects (Cornwell *et al.*, 1998). However, the innate body antioxidant defence systems may be supported by the daily consumption of fruits, vegetables, nutraceuticals and medicines rich in antioxidative compounds. Most of the authors correlated

the antioxidant properties of the plant materials with the presence of the phenolic components (Stankovic *et al.*, 2012). Phenolic compounds such as phenolic acids, flavonoids, tannins, catechins and anthocyanins are the secondary plant metabolites serves as natural antioxidants through their redox properties, radical scavenging ability and metal ion chelating properties (Halliwell *et al.*, 2005).

Cassia auriculata also known as Tanner's Cassia was found to be one of the traditionally valued plants which have also been included in Ayurvedic formulation used for the management of diabetes (Gogte, 2000). In addition, many medicinal, nutraceuticals and cosmetic applications of this plant have been reported throughout the world. The whole part of this plant is valuable in traditional medicine for the treatment of ulcer, leprosy, liver diseases, asthma, renal disorders, chronic fever, herpetic eruptions, rheumatism, gout, leucorrhoea and skin disorders (Kumar *et al.*, 2002; Vedavathy *et al.*, 1997; Joshi, 2000; Siva and Krishnamurthy, 2005). The infusion prepared from the leaves of this plant is used as a cooling

drink, tea prepared from the flower of this plant is used in the management of diabetes, seeds are also used in diabetes and ophthalmia (Kirthikar and Basu, 1965). Traditionally, roots are used in urinary discharges, skin diseases, asthma, tumors and diabetes and also as astringent and cooling alternatives (Samy and Ignacimuthu, 2000). Scientific investigation of leaves of *C. auriculata* revealed the antibacterial, antioxidant (Anushia *et al.*, 2009; Thulasi and Amsaveni, 2011), antidiabetic (Gupta *et al.*, 2009a, b, 2010), hepatoprotective (Kumar *et al.*, 2002), anthelmintic (Kainsa *et al.*, 2012) and antipyretic activities (Vedavathy and Rao, 1991). Even flowers were also reported to possess antibacterial (Doshi *et al.*, 2011a; Thulasi and Amsaveni, 2011), antioxidant (Kumaran and Karunakaran, 2007), antidiabetic (Pari and Latha, 2002a, b) activities along with the inhibition of lipid peroxidation (Latha and Pari, 2003). However, roots are reported for their beneficial antibacterial activity (Doshi *et al.*, 2011b).

Various chemical constituents were reported from this plant. Phytochemical screening of this plant revealed the presence of reported the presence of flavonoids, tannins, saponins, alkaloids, phenols, proanthocyanidins and β -sitosterol (Gupta *et al.*, 2009a; Rastogi and Mehrotra, 1998). Several bioactive constituents such as oleanolic acid (Senthilkumar and Reetha, 2011), kaempferol-3-O-rutinoside, kaempferol, quercetin, luteolin (Juan-Badaturge *et al.*, 2010) were reported from leaves. Whereas, quercetin, (+) catechin, (-) epigallo catechin, (-) epigallocatechin gallate, (-) epicatechin gallate, (-) gallocatechin gallate, (-) catechin gallate and caffeine were reported from the flowers (Jyothi *et al.*, 2012). Even from bark (-)-auriculacacidin (5,2',4'-trihydroxyflavan-3,4-diol) has been isolated (Theresa *et al.*, 1968).

Many author reported the presence of phenolics in the plant is mainly responsible for the found wide pharmacological activities. Literature survey of *C. auriculata* revealed the extensive study only regarding flowers and leaves part whereas, studies about other parts are lacking. Very few data regarding the phenolics content and antioxidant activity of extracts obtained from different parts of the *C. auriculata* are available. Hence, the present study was designed to evaluate and compare the phenolics contents as well as antioxidant activities of extracts from flower, pod, leaf, bark and root.

MATERIALS AND METHODS

Chemicals: The 2,2-diphenyl-1-picrylhydrazyl (DPPH), Folin-Ciocalteu reagent, vanillin, sodium carbonate, sodium nitrite, ammonium molybdate, sodium phosphate, aluminium chloride hexahydrate, ascorbic acid, gallic acid

and catechin were purchased from Sigma Chemicals, Germany. All the other solvents used in this study were of analytical grade and purchased from Fishcers Scientific.

Plant material: Fresh plant material was collected from the fields of B.G. Nagara, Mandya, Karnataka, India and identified by a botanist (Dr. Santhana, Natural Remedies Pvt Ltd, Bangalore). The collected fresh flowers, leaves, pods, stem and root were then washed with running water to remove the dirt and homogenized in a kitchen blender prior to use it for the extraction.

Preparation of extracts: Twenty-five grams of each parts of plant are mixed with 250 mL of ethanol (80%, v/v) separately in a beaker and covered with aluminum foil. These homogenized plant materials were then sonicated in a sonicator (42 kHz, 135 W; Branson Ultrasonic Corporation, USA) for 15 min in the dark at room temperature. The ultrasonic treatment was performed using a Branson 5510 ultrasonic cleaner. The solvent surface in the beaker was kept at the level of the water in the ultrasonic bath and the water in the sonicator is circulated and regulated at constant room temperature to avoid a rise in water temperature caused by the ultrasonics. After the initial extraction, the sample residues were then submitted again for 15 min of sonication twice with 150 mL of 80% ethanol and the respective liquid extracts were collected. These liquid extracts were then filtered and final volume is made up to 600 mL with the extracting solvent and kept at 4°C in air-tight containers until further analysis.

Determination of total phenolics content (TPC): Total amount of phenolics present in each samples were determined using the Folin-Ciocalteu method described by Singleton and Rossi (1965). To an aliquot of sample (400 μ L), 2 mL of Folin-Ciocalteu reagent (pre-diluted with water 1:10, v/v) was added followed by the addition of 1.6 mL sodium carbonate (7.5%) after 6 minutes of time interval. The reaction mixture was vortexed and allowed to stand for 60 min at room temperature in the dark. Absorbance of the developed color was measured at 760 nm using UV-visible spectrophotometer (Shimadzu, UV-1700, Kyoto, Japan). Gallic acid with various concentrations (10-100 μ g mL⁻¹) was used to prepare a calibration curve and the total phenolic contents were expressed in terms of mg gallic acid equivalent (mg GAE) per 400 μ L of each sample. All the measurements were done in triplicates.

Determination of total flavonoids content (TFC): The total amount of flavonoids present in each extracts was determined by spectrophotometric method described by

Sakanaka *et al.* (2005). Five hundred microlitres of each plant extract as well as various concentration of (+)-catechin standard solution (10-140 $\mu\text{g mL}^{-1}$) was mixed with 2.5 mL of distilled water in a test tube, followed by addition of 150 μL of sodium nitrite solution (5%). After 6 min of incubation time, 300 μL of aluminium chloride solution (10%) was added and the mixture was allowed to stand for 5 min before the addition of 1 mL of 1 M sodium hydroxide. Finally the mixture was brought to a final volume of 5.0 mL with distilled water and mixed well. The absorbance of the pink color formed due to the presence of flavonoid was measured immediately at 510 nm using a UV-visible spectrophotometer. Flavonoid contents present in the plant samples were expressed in terms of mg catechin equivalent (mg CAE) per 500 μL of each sample.

Determination of total tannins content (TTC): Total tannins present in each plant samples were determined by vanillin-HCl method (Burns, 1971). Briefly, to a 500 μL of sample 4.5 mL of vanillin-HCl reagent mixture (4% vanillin in methanol and 8% concentrated HCl, 1:1) was added followed by vortexing. After 20 min of incubation, absorbance of the pink color developed was measured at 500 nm using UV-visible spectrophotometer. Catechin standards at various concentrations (20-280 $\mu\text{g mL}^{-1}$) was used to prepare the calibration curve and the total tannins content in the sample was expressed in terms of mg catechin equivalents (mg CAE) per 500 μL of each samples.

Determination of DPPH radical scavenging activity: The DPPH radical scavenging ability of plant extracts and gallic acid was assessed using the method described by Brand-Williams *et al.* (1995) with slight modifications. Briefly, to 0.2 mL of plant extract solution 3.8 mL of DPPH solution (Absorbance intensity of 1.100 ± 0.20 in methanol) was added. This solution was further incubated in the dark for 30 min at room temperature. The decrease in the intensity of the colour in terms of absorbance was measured at 517 nm against a blank consisting of 0.2 mL of ethanol and 3.8 mL of DPPH solution. The vitamin C calibration curve was prepared by plotting the percentage inhibition of vitamin C at various concentrations (20-200 $\mu\text{g mL}^{-1}$). The percentage inhibition was calculated using the below formula:

$$\text{Percentage inhibition} = \frac{A_0 - A_s}{A_0} \times 100$$

where, A_0 is the absorbance of control and A_s is the absorbance of sample.

All the results were expressed as milligrams Vitamin-C Equivalent Antioxidant Capacity (VCEAC) per 200 μL of extract. All the measurements were done in triplicate.

Determination of total antioxidant activity (TAO): The colorimetric method described by Prieto *et al.* (1999) was used to measure the antioxidant capacity of various plant extracts of *C. auriculata*. This method is based on the reduction of Mo(VI) by antioxidants to Mo(V) a green color complex in acidic condition. Briefly, to a known aliquot of the sample solution (0.4 mL) taken in a vial, 4 mL of the freshly prepared reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) was added and incubated in a water bath at 95°C for 90 min. After the samples were cooled to room temperature ($25 \pm 1^\circ\text{C}$), the absorbance was measured at 695 nm against a blank. The blank solution contained 4 mL of reagent solution and 0.4 mL of methanol. The calibration curve was prepared by using a standard solution of ascorbic acid ($5-100 \mu\text{g mL}^{-1}$) and the antioxidant activity was expressed as milligrams Vitamin C Equivalent Antioxidant Capacity (VCEAC) per 400 μL of extract.

Statistical analysis: The results of the present study are presented as Mean \pm SD ($n = 3$). Analysis of variance was performed (one way ANOVA) and the significant differences between the mean values were determined by Tukey's pairwise test, at a level of significance of $p < 0.05$. Statistical analyses were carried out using SPSS 19 (SPSS Inc. USA).

RESULTS AND DISCUSSION

Phytoconstituents present in plant samples will be leached out by using proper solvent as well as the extraction techniques. In the present study alcohol was used as an extracting solvent as it was found to be a good solvent for the extraction of polyphenols with high penetration ability and also it is safe for human consumption (Metivier *et al.*, 1980). Extraction technique also influences the amount of phytoconstituents which in turn influences the pharmacological activities. Also, one more parameter considered during the selection of extraction technique is the extraction time and solvent consumption. Recent studies regarding the comparison of extraction techniques revealed that sonication is one of the simple and fastest methods of extraction with the ability to extract and retain maximum amount of antioxidants in comparison with the other available conventional extraction techniques (Wang *et al.*, 2008;

Annegowda *et al.*, 2012). In addition cavitation effect of sonication causes efficient tissue disruption and good penetration of solvent into the solvent matrix of the plant material (Mason *et al.*, 1996). Hence, we employed sonication extraction technique in this study to obtain the solvent extracts from various parts of *C. auriculata*.

Total phenolic, flavonoid and tannin contents: High molecular weight compounds such as phenolic and their related compounds have significant ability to quench free radicals. Their efficacy mainly depends on the number of aromatic rings as well as nature of hydroxyl group substitution (Hagerman *et al.*, 1998). Results of the Total Phenolic Content (TPC) in different parts of *C. auriculata* are represented in Fig. 1. The phenolic content was ranged between 392.4±8.5 to 636.8±13.3 mg GAE per 400 µL sample. Figure 1 also revealed the presence of significant amount of phenolic content among various parts of the plant with bark possessing highest amount of total phenolic content followed by root, leaf, flower and pod. Flavonoids and related polyphenols are found to exhibit antioxidant activity along with other beneficial pharmacological activities which necessitates their determination in the samples. The amount flavonoid in various parts of *C. auriculata* determined by aluminium chloride method is shown in Fig. 2. The concentration of flavonoids in various parts was ranged between 326.9±15.0 to 2675.0±24.8 mg CAE per 500 µL sample. Similar trend of TPC was found with results of total flavonoid content with bark containing significant amount of TFC followed by root, leaf, flower and pod. In addition, the found TFC content was significantly different from each other. Figure 3 indicates the Total Tannin Content (TTC) present in various parts of *C. auriculata*. TTC values were also expressed in terms of catechin equivalents (mg CAE per 500 µL sample). Among the various parts of the plant evaluated, bark extract possessed significant amount of total content and pod extract contained least amount. The order of tannin content present in various parts of the plant was in the order of bark>root>leaf>flower>pod with TTC values of 615.8±1.9, 810±18.1, 1077.0±2.6, 1146.6±3.7, 2097.4±13.2 mg CAE per 500 µL sample, respectively.

Results of the evaluation of phenolic content among flower, pod, leaf, bark and root extracts of *C. auriculata* clearly indicated the presence of significantly higher amount of phenolics in bark and lesser amount in pods. In accordance with our results, Khan *et al.* (2013) reported the presence of higher amount of total phenolics and flavonoids in bark followed by root, leaf and fruits of *Morus alba* and also in the same study the amount of flavonoid determined was higher than that of total phenolic content.

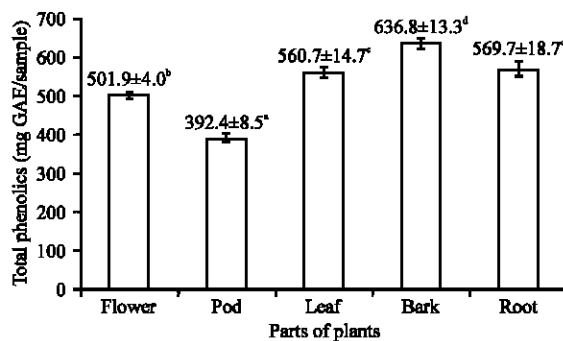


Fig. 1: Total phenolics content values of extract of *C. auriculata*. The results are Mean±SD (n = 3). Values followed by different alphabets in the superscript indicate significant (p<0.05) difference among the samples

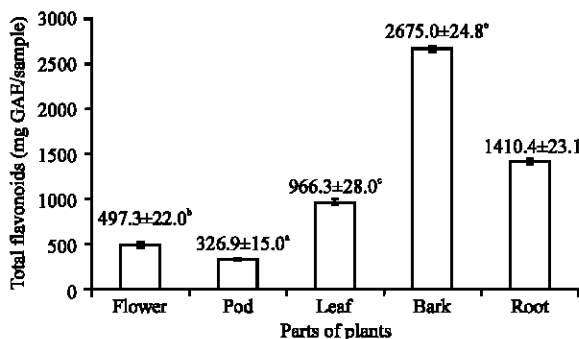


Fig. 2: Total flavonoid content values of extract of *C. auriculata*. The results are Mean±SD (n = 3). Values followed by different alphabets in the superscript indicate significant (p<0.05) difference among the samples

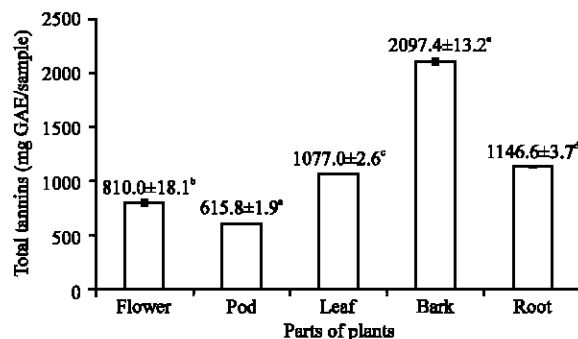


Fig. 3: Total tannin content values of extract of *C. auriculata*. The results are Mean±SD (n = 3). Values followed by different alphabets in the superscript indicate significant (p<0.05) difference among the samples

Antioxidant activity of various parts of *C. auriculata*:

Polyphenolic compounds such as phenolic acids, flavonoids, anthraquinones and tannins found in the plant samples are considered to be the major contributors of the antioxidant activity exhibited by various medicinal plants. Hence, in the present study all the extracted plant samples were studied for their antioxidant efficacy using two different antioxidant activities to assess the free radical scavenging and reducing properties of the plant extracts and gallic acid. Antioxidant values of each sample were expressed in terms of mg VCEAC as vitamin C is found to be one of the potent natural antioxidant with good anti-carcinogenic activity and present in our regular diet (Lee *et al.*, 2002; Annegowda *et al.*, 2010). Hence, expressing the antioxidant activity in terms of vitamin C equivalent will be ideal, more meaningful and descriptive as the final result provides a direct comparison of antioxidant activity of sample with vitamin C (Kim *et al.*, 2002).

Numerous antioxidant assays designed to measure antioxidant potency of samples among these DPPH radical scavenging assay is very convenient assay widely used for screening large number of samples of varying polarity. DPPH is a stable synthetic radical with purple color which in the presence of antioxidant get reduced due to the donation of hydrogen atom to free radical resulting in the loss of purple color and thereby inhibiting the propagation of lipid peroxidation (Lugasi *et al.*, 1999). DPPH radical scavenging potency of various samples of *C. auriculata* is summarized in Fig. 4. It is evident from the figure that, among all the samples screened bark extract significantly inhibited the DPPH radical followed by root, leaves, flower and pod with the antioxidant value of 766.7±10.4, 679.3±12.1, 644.9±17.0, 572.5±16.7 and 474.7±19.2 mg VCEAC per 200 µL of samples, respectively. Though the entire sample efficiently inhibited DPPH radicals, the antioxidant values of these samples were significantly lesser than the pure standard gallic acid (1350.2±19.4 mg VCEAC). It is also evident from the Fig. 4 that extract with higher phenolic content exhibited stronger DPPH radical scavenging ability. Correlation study also revealed a strong correlation between DPPH assay results and TPC ($R^2 = 0.978$), TFC ($R^2 = 0.859$) as well as TTC ($R^2 = 0.838$) indicating the influence of phenolics for the displayed antioxidant activity.

The sample which exhibit high activity in one method may not show similar result in all other methods which necessitates the use of few other methods to evaluate the efficacy of the same sample. Hence, phosphomolybdenum assay was carried out to determine the reducing ability of plant samples as well as gallic acid and the results of this

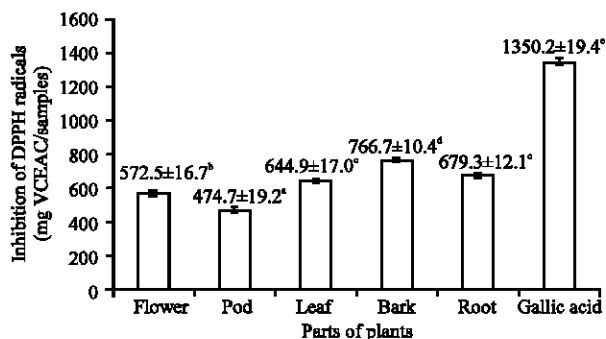


Fig. 4: DPPH radical scavenging values of extract of *C. auriculata*. The results are Mean±SD (n = 3). Values followed by different alphabets in the superscript indicate significant (p<0.05) difference among the samples

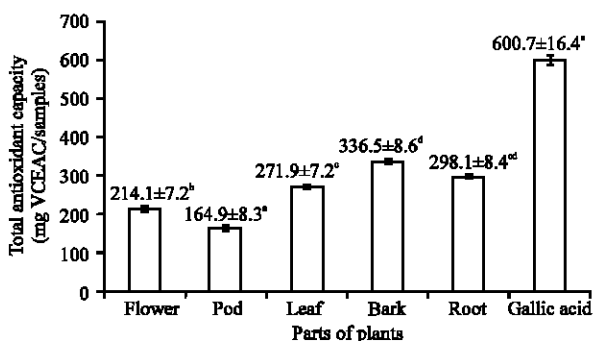


Fig. 5: Total antioxidant values of extract of *C. auriculata*. The results are Mean±SD (n = 3). Values followed by different alphabets in the superscript indicate significant (p<0.05) difference among the samples

assay were illustrated in Fig. 5. It is evident from the figure that the trend of DPPH assay is also observed in this assay among the plant samples studied with bark possessing significant antioxidant value followed by root, leaf, flower and pod. The antioxidant values were ranged between 164.9±8.3 to 336.5±8.6 mg VCEAC per 400 µL of sample. A strong correlation was also observed between total antioxidant value and TPC ($R^2 = 0.959$), TFC ($R^2 = 0.830$) as well as moderate correlation with TTC ($R^2 = 0.786$). In agreement with our results. Stankovic *et al.* (2012) reported the similar variation in phenolic content as well as antioxidant activity in various parts of *Teucrium polium* and also mentioned that extracts with higher phenolic content possess significant antioxidant activity. Current finding of this study is in consistent with the previous literature reports by

Khan *et al.* (2013) who reported the highest antioxidant activity in the stem bark followed by root bark, fruit and leaf of *Morus alba*. But the present results are in contrary to the findings of Doshi *et al.* (2011c) who reported the highest antioxidant activity in flowers followed by leaves and roots of *Cassia auriculata* which might be due to the use of dry plant materials as well as the soxhlet extraction method. Hence, the found antioxidant activities of phenolic rich plant extract might be attributed to phenolic components redox properties, which allow them to act as reducing agents, hydrogen donors and free radical scavengers, as well as due to their metal chelating abilities (Vladimir-Knezevic *et al.*, 2011).

CONCLUSION

Scientific investigation of natural resources for their therapeutic, nutraceutical and food value is gaining more importance. In regards to this, the result of the present study revealed the variation of phenolic components among the various parts of the same plant with bark possessing highest phenolic content among all the samples of *C. auriculata*. From the results of antioxidant activity it was concluded that among the various parts of *C. auriculata*, bark found to possess significant antioxidant efficacy in comparison with other parts. It might be due to the presence of highest phenolic content as there is a strong correlation found between phenolic contents and antioxidant values. Since flower as well leaves of *C. auriculata* were only scientifically exploited by researchers, results of this study provide the opportunity for researchers to utilize the other part of the plant for their scientific investigations. In addition, for the *C. auriculata* pod, root and bark phenolic contents as well as antioxidant capacities were reported for the first time.

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