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INVITRO ANTIOXIDANT AND FREE RADICAL SCAVENGING PROPERTIES OF FLOWER EXTRACT OF *CASSIA AURICULATA*, LINN (CAESALPINIACEAE)

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ABSTRACT

The present study aims at evaluating the antioxidant and free radical scavenging potential of aqueous extract of flowers of *C.auriculata*, Linn belonging to the family Caesalpiniaceae. It is a common plant in Asia, profoundly used in Ayurvedic medicine as a tonic, astringent and as a remedy for diabetes, conjunctivitis and ophthalmia. It is one of the principle constituents of "Avaarai panchaga chooranam"- an Indian herbal formulation used in the treatment of diabetes to control the blood sugar level. Free radicals are known to play an important role in origin of life and biological evolution implicating their beneficial effects on the organism. Many researchers suggest that the methanolic and ethanolic extract of flowers of *C.auriculata* has been found as a good anti-oxidant and it is the underlying mechanism of its use in diabetic mellitus. But in traditional practice it has been consumed as an edible vegetable. So the current research highlights the anti-oxidant potentials of aqueous extracts of *C.auriculata* and the results obtained were statistically significant ($p<0.01$) when compared with reference and control. This study may substantiate the traditional use of C.auriculata in its uncooked form to control diabetes mellitus.

Key words: *C.auriculata*, Caesalpiniaceae, Avaarai panchaga chooranam, Anti-oxidant activity.

INTRODUCTION

Cassia auriculata L. commonly known as tanner's cassia, also known as "avaram" in Tamil language, is a shrub belonging to the Caesalpiniaceae family (Basu and Kirtikar, 1935, Anandan *et al.*, 2011). The shrub is especially -famous for its attractive yellow flowers which are used in the treatment of skin disorders and body odour. The leaves are alternate, stipulate, paripinnate compound, very numerous, closely placed, rachis 8.8-12.5 cm long, narrowly furrowed, slender, pubescent, with an erect linear gland between the leaflets of each pair, leaflets 16-24, very shortly stalked 2-2.5 cm long 1-1.3 cm broad, slightly overlapping, oval oblong, obtuse, at both ends, mucronate, glabrous or minutely downy, dull green, paler beneath, stipules very large, reniform-rotund, produced at base on side of next petiole into a filliform point and persistent. Its flowers are irregular, bisexual, bright yellow and large (nearly 5 cm across), the pedicels glabrous and 2.5 cm long. The racemes are few-flowered, short, erect, crowded in axils of upper leaves so as to form a large terminal inflorescence (leaves except stipules are suppressed at the upper nodes).

The 5 sepals are distinct, imbricate, glabrous, concave, membranous and unequal, with the two outer ones much larger than the inner ones. The petals also number 5, are free, imbricate, crisped along the margin, bright yellow veined with orange. They are separate, with the three upper stamens barren; the ovary is superior, unilocular, with marginal ovules. The fruit is a short legume, 7.5–11 cm long, 1.5 cm broad, oblong, obtuse, tipped with long style base, flat, thin, papery, undulately crimpled, pilose, pale brown. 12-20 seeds per fruit are

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carried each in its separate cavity (Kumar *et al.,* 2002, Umadevi *et al.,* 2006, Manickam *et al.,* 2002). It is widely used in traditional medicine for rheumatism, conjunctivitis and diabetes. It has many medicinal properties. Its bark is used as an astringent, leaves and fruits anthelminthic, seeds used to treat in eye troubles and root employed in skin diseases Pari L and Latha M 2003; Kumaran *et al.,* 2007). It holds a very prestigious position in Ayurveda and Siddha systems of medicine. The plant has been reported to possess antipyretic, hepatoprotective, antidiabetic, antiperoxidative, antihyperglycemic and microbicidal activity. The flowers are used to treat urinary tract infections, diabetes and throat irritation. They are one of the constituent of polyherbal formulation 'Diasulin' in the concentration range of 40 mg/dl which is proven to have antidiabetic activity. The dried flower bud powder is used as a substitute for tea in case of diabetic patients and it is also supposed to improve the complexion in women (Anonymous 2000, Kumar *et al.,* 2003, Maneemegalai S and Naveen T 2010 and Abesundara *et al.,* 2004). The present investigation deals with evaluating the antioxidant and free radical scavenging potential of aqueous extract of flowers of *C.auriculata,*

MATERIALS AND METHODS

Plant Materials and extraction:

The flowers of *C.auriculata* were collected from the dry lands of Kancheepuram District, Tamil Nadu, Chennai and it was authenticated by a taxonomist. The voucher specimen of the sample has been kept in the department library for further references (Accession number: 2016/SRM/002). The CAFÉ (*Cassia auriculata* flower extract) was obtained by adding 80^0C of distilled water in dried power of flowers, stirred gently, cooled and filtered. The filtrate was concentrated under reduced pressure and kept under 2-8^0C in a refrigerator until further use.

Chemicals and Reagents:

DPPH, 2-deoxy-2-ribose, Napthyl ethylene diamine dihydrochloride, H_2O_2, EDTA, TCA, Sulphanilamide, Sodium nitroprusside, Orthophosphoric acid, potassium ferricyanide, ferric chloride, Ascorbic acid and Mannitol were purchased from Sigma Aldrich, USA. All other chemicals and solvents used were of HPLC and analytical grade.

Reducing power ability:

The Fe^{3+}-reducing power of extracts was determined based on the method described by Oyaizu using ascorbic acid as reference standard. 1 mL of flower extracts of *Cassia auriculata* L (20-100 µg mL^{-1}) was mixed with 2.5 mL of 1% potassium ferricyanide and 2.5 mL of phosphate buffer pH 6.6. The mixture was incubated at 50^0C for 20 minutes. 10% TCA was added to the mixture and centrifuged at 3000 rpm for 10 minutes. The supernatant was mixed with distilled water and 0.1% $FeCl_3$. Absorbance was measured at 700 nm (Benzie IF, Strain JJ 1996).

Hydroxyl radical scavenging activity:

Hydroxyl radical scavenging activity of the plant extracts were estimated by the method given by Kunchandy and Rao. The reaction mixtures containing 1 mL of flower extracts of *Cassia auriculata* L (20-100 µg mL^{-1}), 2-deoxy-2-ribose (28 mmol L^{-1}), EDTA (1.04 mmol L^{-1}), $FeCl_3$ (0.2 mmol L^{-1}) and ascorbic acid (1 mmol L^{-1}) were incubated at 37^0C, for 1 hour. The preventive effects of extracts on deoxyribose damage, imposed by hydroxyl radicals were determined colorimetrically at 532 nm against separate blank for each concentration. Mannitol was used as the reference compound (Burcu Bektaşoğlu *et al.,* 2006; Harsha Ramakrishna *et al.,* 2012).

Nitric oxide radical scavenging activity:

Nitric oxide radical scavenging abilities were assayed using Griess reaction according to method described by Green *et al.,* 1 mL of 10 mmol L^{-1} sodium nitroprusside was mixed with 1 mL of flower extracts of *Cassia auriculata* L. (20-100 µ g mL^{-1}). The mixture was incubated at 25^0C for 150 minutes. To 1 mL of incubated solution, 1 mL Griess reagent (1% sulphanilamide, 2% ortho phosphoric acid and 0.1% naphthyl ethylene diamine dihydrochloride) was added. Absorbance of the chromophore formed was measured at 546 nm and percent inhibition was calculated. Gallic acid was used as reference compound (Rozina Parul *et al.,* 2012, Fadzai Boora *et al.,* 2014 and Dharmendra Singh *et al.,* 2012).

DPPH radical scavenging assay:

Total antioxidant activity of plant extracts were estimated using stable DPPH radical scavenging assay described by Cotelle *et al.,*. To 200 µL of DPPH (100 µ mol L^{-1}in methanol), 2.8 mL of flower extracts of *Cassia auriculata* L (20-100 µg Ml^{-1}) were added. After 20 minutes the absorbance of assay mixture was read at 517 nm (Rajani Kanta *et al.,* 2013, Tailor Chandra Shekhar and Goyal Anju 2014 and Padmanabhan P, Jangle SN, 2012).

The percentage inhibition of the extracts was calculated for all the above parameters using the formula:

$$\text{Inhibition (\%)} = \frac{(\text{Absorbance of Control} - \text{Absorbance of Test}) \times 100}{\text{Absorbance of Control}}$$

Statistical Analysis

The experimental results were expressed as mean± SD of three parallel measurements. IC_{50} values were calculated by regression analysis quoting correlation coefficient. Data was evaluated by one way analysis of variance (ANOVA) followed by Dunnett's method of multiple comparisons using Graphpad Instat 3.0 software. $p<0.05$ was considered to indicate the statistical significance.

RESULTS

Table 1. Antioxidant activity of *Cassia auriculata* Linn flowers by reducing power ability

Group	Concentration (µg/ml)	% inhibition	IC_{50} µg/ml
CAFE	20 40 60 80 100	15±0.03 35±0.14 39±0.07 47±0.42 68±0.66	88±1.90
Ascorbic acid (standard)	20 40 60 80 100	64±0.32 77±1.08 82±0.20 85±0.06 87±1.14	12±0.06

Values are mean ± S.E.M. (n=3), P<0.05 when compared with control

Table 2. Antioxidant activity of *Cassia auriculata* Linn flowers By Hydroxyl scavenging (Deoxyribose degradation) assay

Group	Concentration (µg/ml)	% inhibition	IC_{50} µg/ml
CAFE	20 40 60 80 100	12.26 ± 0.14 19.34 ± 0.02 31.19 ± 0.05 42.75 ± 0.03 56.76 ± 0.04	86.13±0.12
Mannitol (standard)	20 40 60 80 100	19.79 ± 0.39 35.16 ± 0.21 57.81 ± 0.28 73.75 ± 0.23 92.26 ± 0.21	44.5±0.29

Values are mean ± S.E.M. (n=3), p<0.05 when compared with control.

Table 3. Antioxidant activity of *Cassia auriculata* Linn flowers By Nitric oxide radical scavenging activity

Group	Concentration (µg/ml)	% inhibition	IC_{50} µg/ml
CAFE	20 40 60 80 100	16.16 ± 0.11 26.21 ± 0.08 40.10 ± 0.21 52.25 ± 0.01 63.36 ± 0.20	74.04±0.10
Gallic acid (standard)	20 40 60 80 100	29.79 ± 0.33 35.10 ± 0.16 47.22 ± 0.18 63.18 z 0.41 82.42 ± 0.88	64.50±0.04

Values are mean ± S.E.M. (n=3), p<0.05 when compared with control.

Table 4. Antioxidant activity of *Cassia auriculata* Linn flowers By DPPH radical scavenging assay

Group	Concentration (µg/ml)	% inhibition	IC_{50} µg/ml
CAFE	20	18.24 ± 0.01	62.00±1.12
	40	26.11 ± 0.12	
	60	40.22 ± 0.20	
	80	62.12 ± 0.11	
	100	83.42 ± 0.16	
Ascorbic acid (standard)	20	39.27 ± 0.08	34.21±0.10
	40	55.12 ± 0.24	
	60	67.20 ± 0.12	
	80	73.12 ± 0.21	
	100	88.12 ± 0.12	

Values are mean ± S.E.M. (n=3), $p<0.05$ when compared with control.

DISCUSSION

Free radicals are known to play an important role in origin of life and biological evolution implicating their beneficial effects on the organism. The cytotoxic effect of free radicals is deleterious to mammalian cells and mediates the pathogenesis of many chronic diseases, but it is responsible for killing of pathogens by activated macrophages in the immune system (Dubovsikiy *et al.,* 2008). Antioxidants fight against free radicals by protecting us from various diseases and scavenge of reactive oxygen radicals or protect the antioxidant defense mechanism. Reactive oxygen species (ROS) are capable of damaging biological macromolecules such as DNA, carbohydrates and proteins. ROS is a collective term, which includes not only oxygen radicals (O^2 and OH^-) but also some non-radical derivatives of oxygen like H_2O_2, HOCl and ozone (O_3). If human disease is believed to be due to the imbalance between oxidative stress and antioxidant defense, it is possible to limit oxidative tissue damage and hence prevent disease progression by antioxidant defense supplements. In addition, antioxidant activity may be regarded as a fundamental property important for life (Dubovsikiy *et al.,* 2008).

The reductive capability of the CAFE was compared with Ascorbic acid (Table 1). For the measurements of the reductive ability, we investigated the Fe^{3+}-Fe^{2+} transformation in the presence of the CAFE. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. However, the antioxidant activity of antioxidants have been attributed to various mechanisms, among which are prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, reductive capacity and radical scavenging antioxidant activity(Gulcin *et al.,* 2003). The presence of reductants (antioxidants) in the CAFE causes the reduction of Fe^{3+} (ferric cyanide complex) to Fe^{2+} (ferrous form) (Amarowicz *et al.,* 2004). The reducing power of the CAFE increased with increasing concentration. In this study, yellow colour of the test solution changed to various shades of green and blue depending upon the reducing power of the extract.

Hydroxyl radicals are highly reactive biological molecules and its scavenging property may provide an important therapeutic approach against oxidative stress induced ailments. It is well established that in the absence of EDTA, Fe^{3+} directly binds with deoxyribose sugar and causes its site specific degradation due to hydroxyl radicals which are found immediately at the vicinity of the irons binding site. Hydroxyl radicals are the most reactive radicals which are produced via the Fenton's reaction in living system. Hydroxyl radicals scavenging activity was quantified by measuring inhibition of the degradation of the deoxyribose by free radicals (Guzman *et al.,* 2001). Deoxyribose levels were determined by reaction with thiobarbituric acid. The CAFE showed significant ($P<0.05$) hydroxyl radical scavenging activity at a higher rate constant than Mannitol. From our investigation, the results obtained by screening of CAFE confirmed the antioxidant activity of the plant extract.

CONCLUSION

The hypothesis of obtaining plant based medicine is beneficial to human health based on the active profile exposed through various *in vitro* assays. It can be concluded that the aqueous extract of flowers of *Cassia auriculata* showed significant antioxidant activity. Further investigations on the isolation and identification of bioactive components on the plant would help to ascertain its potency.

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