Antioxidant activity of *Cassia auriculata* Linn flowers

Varadharajan SubhadraDevi*, Ushanandhini R, Kuppusamy AsokKumar, Muthuswamy UmaMaheswari, AndichettiarThirumalaisamy Sivashanmugham, Puliyath Jagannath

Summary

The present study was carried out to evaluate the antioxidant activity of ethanolic extract of *Cassia auriculata* flowers (CAFE) (Caeselpineaceae) in various *in vitro* systems. *Cassia auriculata* found in the dry regions of India and Srilanka. In herbal medicine, it is used for the treatment of diabetes, rheumatoid arthritis, skin diseases and other soft tissue infections. Antioxidant and free radical scavenging activities of CAFE was assessed by using reducing power ability, superoxide anion radical and hydroxyl radical scavenging assays. The antioxidant activity of CAFE increased in a concentration dependent manner. The CAFE was found to scavenge the superoxide generated by hypoxanthine - xanthine oxidase. CAFE was also found to inhibit the hydroxyl radical generated by Fenton's reaction. The ethanolic extract of flowers of *Cassia auriculata* has shown effective antioxidant activity in all assay techniques. The results obtained in the present study indicate that the flowers of *Cassia auriculata* are a potential source of natural antioxidants.

Key words: Antioxidant activity, reducing power ability, superoxide radical, hydroxyl radical.

Address of the corresponding author:
Mrs. V. Subhadra Devi,
Lecturer,
Dept. of Pharmacology,
College of Pharmacy,
Sri Ramakrishna Institute of Paramedical Sciences,
Sarojini Naidu Road,
Siddhapudur,
Coimbatore - 641044,
Tamil Nadu,
India.
Mobile: + 919994284820.
Email: subhaakshay@gmail.com

Introduction

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.

Reactive oxygen species (ROS) such as $O_2^{\bullet}$, H_2O_2 and $OH^{\bullet}$ are highly toxic to cells. Cellular antioxidant enzymes and the free radical scavengers normally protect a cell from toxic effects of the ROS. When generation of the ROS overtakes the antioxidant defense of the cells, oxidative damage of the cellular macromolecules (lipids, proteins and nucleic acid) occurs, leading finally to various pathological conditions (Bandyopadhyay *et al.,* 1999). Reactive nitrogen species (RNS) are the products of normal cellular metabolism. $NO^{\bullet}$ is a small molecule that contains one unpaired electron on the antibonding ($2\pi^*_{y)}$ orbital and is, therefore, a radical. NO• is generated in biological tissues by specific nitric oxide synthases (NOSs). Overproduction of reactive nitrogen species is called nitrosative stress. This may occur when the generation of reactive nitrogen species in a system exceeds the system's ability to neutralise and eliminate them. Nitrosative stress may lead to nitrosylation reactions that can alter the structure of proteins and so inhibit their normal function (Valko *et al.,* 2006).

Cassia auriculata L. known locally as 'avaram'and 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, ulcers, leprosy, skin and liver diseases (Kirtikar and Basu, 1987). The main objective of the present study was designed to investigate the antioxidant efficacy of *Cassia auriculata* flower extract (CAFE).

Material and methods

Drugs and chemicals

Quercetin, hypoxanthine, butylated hydroxy toluene, 2-deoxy-2-robose were purchased from SRL, Mumbai. Trichloroacetic acid and thiobarbituric acid were purchased from SD Fine Ltd., Mumbai. Xanthine oxidase, ascorbic acid, nitro blue tetrazolium were purchased from Himedia Labs. Pvt. Ltd., Mumbai. All other chemicals used in the study are of analytical grade purchased from respective suppliers.

Collection of Plant material

The plant material consists of dried powdered flowers of *Cassia auriculata* belonging to the family Caeselpiniaceae. Fresh flowers of the plant were collected from Coimbatore district, Tamilnadu, India during the month of July, 2009. The plant was identified and authenticated by the Dr. G.V.S. Murthy, Joint Director, Botanical Survey of India (BSI), Tamilnadu Agricultural University Campus, Coimbatore, bearing a reference number BSI/SC/5/23/09-10/Tech-255.

Preparation of extract

The flowers of *Cassia auriculata* were collected, thoroughly dried under shade and powdered mechanically and sieved through No.20 mesh sieve. The finely powdered flowers were kept in an airtight container until the time of use. The extraction of flower was carried out by continuous hot percolation method using Soxlet apparatus. The solvent used was 95% ethanol. About 50 g of powder was extracted with 400 ml of solvent. The extract was concentrated to dryness under controlled temperature between 40-50ºC. The percentage yield of the *Cassia auriculata* flower extract (CAFE) was 18.2%.

***In vitro* methods employed in antioxidant studies**

Reducing power ability

Reducing power ability was measured by mixing 1.0 ml fractions of various concentration prepared with distilled water to 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of 1% potassium ferricyanide and incubated at 50°C for 30 min. After that 2.5 ml of trichloroacetic acid (10%) were added to the mixture and centrifuged for 10 min at 3000 rpm, 2.5 ml from the upper part were diluted with 2.5 ml water and shaken with 0.5 ml fresh 0.1% ferric chloride. The absorbance was measured at 700 nm using UV-spectrophotometer. The reference solution was prepared as above, but contained water instead of the samples. Increased absorbance of the reaction mixture indicates increased reducing power. All experiments were done in triplicate using butylated hydroxyltoluene (BHT) as positive control (Yildrim *et al.,* 2001).

Superoxide anion scavenging (NBT reduction) assay

A reaction mixture with a final volume of 3 ml per tube was prepared with 1.4 ml of 50 mM KH_2PO_4-KOH, pH 7.4 containing 1 mM EDTA, 0.5 ml of 100 μM hypoxanthine, 0.5 ml of 100 μM NBT. The reaction was started by adding 0.066 units per tube of xanthine oxidase freshly diluted in 100 μl of phosphate buffer and 0.5 ml of test extract fractions in saline. The xanthine oxidase was added last. The subsequent rate of NBT reduction was determined on the basis of spectrophotometric determinations of absorbance at 560 nm. Ascorbic acid was used as standard. The results are expressed as the percentage inhibition of NBT reduction rate with respect to the reaction mixture without test compound (saline only) (Guzman *et al.*, 2001).

Hydroxyl radical scavenging (Deoxyribose degradation) assay

The decomposing effect of extract on hydroxyl radicals was determined by the assay of malondialdehyde chromogen formation due to 2-deoxy 2-ribose degradation. The assay mixture contained in a final volume of 1ml: 100 µl of 28 mM 2-deoxy 2-ribose dissolved in phosphate buffer, pH 7.4, 500 µl of the plant extract fractions of various concentrations in buffer, 200 µl of 200 mM ferric chloride (1:1 v/v) and 1.04 mM EDTA and 100 µl of 1.0 mM hydrogen peroxide and 100 µl of 1.0 µM ascorbic acid. After incubation of the test sample at 37^{o}C for 1 h the extent of free radical damage imposed on the substrate deoxyribose was measured using thiobarbituric acid (TBA) test. Percentage inhibition of deoxyribose degradation was calculated. Quercetin was used as standard (Gomes *et al.,* 2001).

Statistical analysis

Results were statistically evaluated by analysis of variance (ANOVA) followed by Dunnets test, $P < 0.05$ was considered to be statistically significant.

Results

***In vitro* antioxidant activity**

Reducing power ability

Table 1 shows the reductive capabilities of CAFE when compared to the standard butylated hydroxy toluene (BHT). The increase in absorbance of the reaction mixture containing the extract showed increased reducing power with increase in concentration. The reducing power increased significantly ($P<0.01$) with increasing amounts of the extract. However, the activity of the CAFE was less than the standard.

Superoxide radical scavenging activity

The CAFE was found to be a scavenger of superoxide anion generated in xanthine oxidase-NBT systems (Table 2). The extract showed significant ($P<0.01$) superoxide inhibiting activity at a concentrations of 25-400 µg/ml. The IC_{50} of the CAFE was found to be 318.13 ± 0.21; where as the IC_{50} of the standard ascorbic acid is 92.46µg/ml.

Hydroxyl radical scavenging (Deoxyribose degradation) assay

The degradation of deoxyribose by Fe^{3+}-ascorbate–EDTA-H_2O_2 system was markedly decreased by CAFE indicating the significant ($P<0.01$) hydroxyl radical scavenging activity. The IC_{50} of quercetin was 24.5µg/ml where 66.13±0.12 was found for the CAFE (Table 3).

Antioxidant activity of *Cassia auriculata* Linn flowers

Table 1: Reducing power ability

Group	Concentration (µg/ml)	Absorbance at 700 nm
CAFE	50	0.065± 0.007
	100	0.126 ± 0.003
	200	0.172 ± 0.004
	400	0.233 ± 0.003
	800	0.317 ± 0.004
BHT (Standard)	50	0.244 ± 0.003
	100	0.328 ± 0.005
	200	0.488 ± 0.009
	400	0.976 ± 0.006
	800	1.534 ± 0.003

Values are mean ± S.E.M. (n=3), P<0.01 when compared with control

Table 2: Superoxide radical scavenging (NBT reduction) assay

Group	Concentration (µg/ml)	Absorbance at 560 nm	% inhibition	IC_{50} µg/ml
Control		0.2117±0.0001		
CAFE	25	0.189± 0.0002	10.46 ± 0.111	318.13 ± 0.21
	50	0.172± 0.0002	18.75 ± 0.126	
	100	0.148 ± 0.0001	29.81 ± 0.082	
	200	0.128 ± 0.0003	39.21 ± 0.147	
	400	0.092 ± 0.0003	56.41 ± 0.150	
Ascorbic acid (standard)	25	0.099 ± 0.004	33.33 ± 0.035	92.46 ± 0.54
	50	0.054 ± 0.006	63.87 ± 0.458	
	100	0.034 ± 0.003	76.78 ± 0.214	
	200	0.021 ± 0.002	85.79 ± 0.177	
	400	0.012 ± 0.002	91.82 ± 0.143	

Values are mean ± S.E.M. (n=3), p<0.01 when compared with control.

Table.3 Hydroxyl scavenging (Deoxyribose degradation) assay

Group	Concentration (µg/ml)	Absorbance at 532 nm	% inhibition	IC $_{50}$ µg/ml
Control		0.5732±.010		
CAFÉ	5	0.511 ± 0.0095	12.26 ± 0.141	66.13±0.12
	10	0.462 ± 0.0001	19.34 ± 0.028	
	20	0.394 ± 0.0002	31.19 ± 0.051	
	40	0.328 ± 0.0001	42.75 ± 0.031	
	80	0.247 ± 0.0002	56.76 ± 0.040	
Quercetin (standard)	5	0.646 ± 0.003	19.79 ± 0.393	24.5±0.29
	10	0.522± 0.001	35.16 ± 0.216	
	20	0.340 ± 0.003	57.81 ± 0.288	
	40	0.211 ± 0.004	73.75 ± 0.231	
	80	0.062 ± 0.001	92.26 ± 0.218	

Values are mean ± S.E.M. (n=3), $p<0.01$ 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 ($O_2^{\cdot}$, and $OH^{\cdot}$) 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.

The reductive capability of the CAFE was compared with BHT (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 mechanism, among which are prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, reductive capacity and radi 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) (Amarowiz *et al.,* 2004). The reducing power of the CAFE increased with increasing concentration. In this study, yellow colour of the test solution changes to various shades of green and blue depending upon the reducing power of the extract.

Superoxide anions were generated *in vitro* enzymatically by hypoxanthine / xanthine oxidase system that reduces NBT and forms a blue coloured chromophore, formazone that can be measured at 560nm (Ghosh *et al.,* 2007). Superoxide radicals generated *in vitro* by the system was determined by NBT photoreduction method. The decrease of absorbance at 560 nm with antioxidants thus indicates the consumption of superoxide anion in the reaction mixture (Gulcin *et al*., 2003). Superoxide radical is converted by SOD to hydrogen peroxide, which subsequently can produce extremely reactive hydroxyl radicals in the presence of transition metal ions such as iron and

copper or by UV photolysis. Determination of the mean rate of increase in absorbance over a 1 minute period provides a measure of the extent to which the ethanol extracts of CAFE capable of inhibiting NBT reduction.

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 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 ($P<0.01$) hydroxyl radical scavenging activity at a higher rate constant than quercetin. From our investigation, the results obtained by screening of CAFE confirmed the antioxidant activity of the plant extract.

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 ethanolic extract of flowers of *Cassia auriculata* showed significant antioxidant activity. Further investigations on the isolation and identification of Bio active components on the plant would help to ascertain its potency.

References

1. Dubovsikiy IM, Martemyanov VV, Voronlsova YL, Rantala MJ, Gryzanova, EV and Glupov VV, Effect of bacterial infection on antioxidant activity and

lipid peroxidation in the midgut of *Galleria mellonella* L. larvae. Elsevier Comparitive Biochemistry and Physiology 2008; 148: 1 – 5.

2. Bandyopadhya U, Das D and Banerjee RK, Reactive oxygen species: Oxidative damage and pathogenesis. Current Science 1999; 77: 658-666.
3. Valko **M. Rhodes C J, Izakovic Mand Mazur M, Free radicals metals and antioxidants in oxidative stress induced cancer, Chemico-Biological Interactions,2006; 1-40**
4. Kirtikar, K.R., and Basu, B.D. Indian medicinal Plants,Vol III, 2nd ed. 1987. International book distributors: Dehradun. pp.867-868.
5. Yildirin A, Oktay M, and Bilalogu V. The Antioxidant activity of the leaves of *Cyclonia vulgaris.* Turkish Journal of Medical Science 2001; 31: 23-27.
6. Guzman S, Gato A, and calleja, M. Antinflammalory, analgesic and free radical scavenging activity of the marine microalgae *Chorella stigmatophora* and *Phaeodactylum tricornutum*. Phytotherapy research 2001; 15: 224-230.
7. Gomes AJ Lunardi CN, Gonkalcz S, and Tedesco AC. The Antioxidant action of *Polypodium becuotomes* extract and kojicacid reaction with reactive oxygen species. *Brazilian Journal of medical and Biological research* 2001; 34: 1487-1494.
8. Gulcin I, Buyukokuroglu ME. Oktay M, and Kufrevioglu OI. Antioxidant and analgesic activities of turpentine of *Pinus nigra* Arn. subsp. pallsiana (Lamb) Holmboe. Journal of Ethanopharmacology 2003; 86: 51-58.
9. Amarowicz R, Pegg RB, Moghaddam PR, Barl B, and Weil JA, Free-radical scavenging capacity and antioxidant activity of selected plant species from the Canadian prairies. Food Chemistry 2004; 84: 551-562.