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In vitro antioxidant potential of methanol extract of the medicinal plant, *Acacia caesia* (L.) Willd

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ABSTRACT

Objective: The *in vitro* antioxidant capability of methanolic leaf extract of the medicinal plant, *Acacia caesia* (L.) Willd (Mimosaceae) was evaluated by different assaying. **Methods:** The *in vitro* antioxidant activity was evaluated for total antioxidant, 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging, hydroxyl radical scavenging and metal chelating activities. Ascorbic acid and butylated hydroxy toluene (BHT) were kept as standards. **Results:** IC50 values observed for DPPH, hydroxyl radical scavenging and metal chelating assays were 109, 177 and 295 μg/ml respectively. **Conclusions:** The results clearly indicate that the methanolic leaf extract of the study species is effective in scavenging free radicals and has the potential to be powerful antioxidant.

1. Introduction

Free radicals generated in the human body may increase the risk of chronic diseases like cancer, cardio vascular diseases, etc. These free radicals are usually produced through aerobic respiration. Although the human body produces antioxidant enzymes to neutralize free radicals[1], a diet rich in edible antioxidants is recommended to assist the human body to protect itself from food borne free radicals.

The putative protective effects of antioxidants against these deleterious oxidation-induced injuries have received increasing attention in recent times, especially within biological, medical, nutritional and agrochemical areas. Among dietary antioxidants, phenolic compounds, the secondary metabolites from plants are the most abundant natural antioxidants[2], which act as reducing agents, hydrogen donators, free radicals scavengers and singlet oxygen quenchers and therefore, as cell saviors[3].

Acacia caesia (L.) Willd. is an armed woody straggling shrub of medicinal importance belongs to the family, Mimosaceae, distributed widely in the foot hills of the Western Ghats around the altitude of 500ms above msl. For its medicinal properties, the traditional healers of western districts of Tamil Nadu (Tirupur, Coimbatore, Erode and Dindugal) prescribed the species for various ailments. The leaves are used as vegetable and in the treatment of asthma, skin diseases[4,5], menstrual disorder[6-9] and scabies[10]. Due to these values, it is exploited severely by the local public in western districts of Tamilnadu and so it becomes a rare sighted species in the natural habitats of Western Ghats[11]. Based on the traditional knowledge of medicinal system, the present study was carried out to evaluate the antioxidant activity of methanol leaf extract of *A. ceasia*.

2. Materials and Methods

2.1. Plant material and extraction

To know the medicinal importance, the shade dried leaf part of the study species was made into a fine powder of 40 mesh

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size using the pulverizer. Following that, 100 g of the powder was filled in the filter paper and successively extracted using 500 mL methanol using the soxhlet extractor for 8 – 10 hours[12]. Then the extract was filtered through Whatman No.1 filter paper to remove all undissolved matter, including cellular materials and other constituents that are insoluble in the extraction solvent.

2.2. Chemicals

All the chemicals used in the work were purchased from HI-MEDIA Pvt. Ltd, Bombay. The chemicals used were of analytical grade.

2.3. Determination of antioxidant activity

The antioxidant activity was evaluated by four ways which are as follows;

2.4. Total antioxidant activity

The total antioxidant activity of the sample was analyzed by phosphomolybdenum method[13]. Two mg/mL of sample was taken and mixed with 1 mL of reagent solution (0.6 M sulfuric acid, 28mM sodium phosphate and 4mM ammonium molybdate) followed by incubation at 95 ℃ for 90 min. Absorbance of the sample was measured at 635 nm both for sample and the standard, ascorbic acid. The total antioxidant activity was expressed as ascorbic acid equivalents (mg/g of sample).

2.5. Free radical scavenging activity (DPPH method)

The scavenging activity for DPPH free radicals was measured according to the procedure described by Blios *et al*[14]. Methanol solution of the sample extract at various concentrations (50, 100, 150, 200 and 250 μg/mL) was added separately to each 5 mL of 0.1 mM methanolic solution of DPPH and allowed to stand for 20 min at 27℃. After incubation, the absorbance of each solution was determined at 517 nm using spectrophotometer. BHT (butylated hydroxy toluene) was used as standard. The corresponding blank reading was also taken and DPPH radical scavenging activity was calculated by using the following formula:

$$\text{DPPH radical scavenging activity (\%)} = \frac{\text{Control OD} - \text{Sample OD}}{\text{Control OD}} \times 100$$

IC_{50} value is the concentration of the sample required to scavenge the 50% DPPH free radical. It has been determined by using the software SPSS v.16.

2.6. Hydroxyl radical scavenging activity

Hydroxyl radical scavenging activity was measured as per the method of Klein *et al*[15]. Various concentrations of the extracts viz., 50, 100, 150, 200, 250 and 300 μg/mL of *A. caesia* were added separately with 1 mL of iron- EDTA solution (0.13 % ferrous ammonium sulfate and 0.26% EDTA), 0.5 mL of EDTA (ethylene diamine tetraacetic acid) solution (0.018 %) and 1 mL of DMSO (dimethyl sulfoxide) (0.85% v/v in 0.1 M phosphate buffer, pH – 7.4). The reaction was initiated by adding 0.5 mL of ascorbic acid (0.22%) and incubated at 80 – 90℃ for 15 minutes in a water bath. After incubation, the reaction was terminated by the addition of 1 mL of ice – cold TCA (tricarboxylic acid) (17.5% w/v). Three mL of Nash reagent (75 g of ammonium acetate, 3 mL of glacial acetic acid and 2 mL of acetyl acetone were mixed and raised to 1L with distilled water) was added and left at room temperature for 15 min. The reaction mixture without sample was used as control. The intensity of the colour formed was measured spectrophotometrically at 412 nm against reagent blank. BHT was used as standard. The hydroxyl radical scavenging activity (HRSA) (%) was calculated by using the following formula:

HRSA (%) = 1–(differences in absorbance of sample/difference in absorbance of blank)× 100

2.7. Metal chelating activity

The chelating of ferrous ions by various extracts of *A. caesia* was estimated by the method described by Dinis *et al*[16]. Various concentrations of the extracts viz., 50, 100, 150, 200, 250 and 300 μg/mL of *A. caesia* were added with 1 mL of 2mM FeCl2 seperately. The reaction was initiated by the addition of 5mM ferrozine (1mL). Absorbance was measured at 562nm after 10min. BHT was used as standard.

$$\text{Chelating activity (\%)} = \frac{\text{Control OD} - \text{Sample OD}}{\text{Control OD}} \times 100$$

3. Results

3.1. Total antioxidant assay

The total antioxidant activity was determined to be 42.18 mg/g of ascorbic acid equivalents for the methanol leaf extracts of *A. caesia* which is comparable to that of the standard, ascorbic acid (30.12 mg/g) (Table 1).

Table 1
Total antioxidant* and DPPH radical scavenging activities of Acacia caesia.

DPPH radical scavenging activity		
Sample concentration (μg/ml)	Percentage activity	IC50 (μg/ml)
50	38.89e±0.40	109
100	44.44d±0.82	
150	50.00c±0.49	
200	61.11b±1.63	
250	72.22a±0.65	

Values are expressed as mean±SD. (n=6).
Values within the column not sharing common superscript letters (a–e) differ significantly
at P<0.05 by DMRT.
* Total antioxidant activity for the sample concentration, 2 mg/ml is 42.18 ±0.67 (mg/g).

3.2. DPPH radical scavenging activity

To evaluate the antioxidant activity of the methanol leaf extract, the radical scavenging capacity based on DPPH assay was determined and the results are shown in Table 1 for the species, *A. caesia*. The percentage of scavenging effect on the DPPH radical was increased with the increase in the concentrations of the extract from 50 −250 μg/mL. The percentage of inhibition of the DPPH radical was varying from 38.89% (in 50 μg/mL of the extract to 72.22% (in 250 μg/mL of extract). The IC50 value of the methanolic leaf extract of this species was determined to be 109 μg/mL. The extracts in all concentrations showed higher percentage of inhibition of free radicals than the standard drug, BHT (50% − 36.16, 100% − 42.12, 150% − 49.32, 200% − 52.02 and 250% − 57.10).

3.3. Hydroxyl radical scavenging activity

The results of antioxidant activity of the leaf extract of *A. caesia* based on hydroxyl radical scavenging activity are presented in Table 2. The scavenging activity was determined to be increased with the increase in the concentration of extract from 50 to 300 μg/mL. The percentage of inhibition of the hydroxyl radical was varying from 42.66% (in 50 μg/mL of extract) to 51.04% (in 300 μg/mL extract). The IC_{50} value of the methanolic leaf extract of the study species was 177 μg/mL. All the concentrations of sample extracts showed higher percentage of inhibition of hydroxyl radicals than the respective concentration of standard drug, BHT (50% − 18.08, 100% − 30.17, 150% − 38.21, 200% − 45.12, 250% − 46.25 and 300% − 52.35).

Table 2
Hydroxyl radical scavenging activity of methanol leaf extract of Acacia caesia.

Hydroxyl radical scavenging activity		
Sample concentration (μg/ml)	Percentage activity	IC50 (μg/ml)
50	42.66e±0.82	
100	43.36cd±0.49	
150	44.76cd±0.65	177
200	45.45bc±0.82	
250	47.55b±0.33	
300	51.04a±0.41	

Values are expressed as mean±SD. (n=6).
Values within the column not sharing common superscript letters (a–f) differ significantly at P<0.05 by DMRT.

3.4. Metal chelating activity

The results of antioxidant activity of the leaf extract of *A. caesia* based on metal chelating activity are given in Table 3. As observed in DPPH and hydroxyl radical scavenging assays, the percentage of metal chelating activity was determined to be sample concentration dependent and it was increasing with the increase in the concentration of extract from 50 to 300 μg/mL. The percentage of inhibition of the metal chelation was varying from 35.53 % (in 50 μg/mL of extract) to 50.41% (in 300 μg/mL extract). The IC_{50} value of the methanolic leaf extract of the study species was 295 μg/mL. The percentage of inhibition of free radicals by various concentrations of sample was more or less equal to that of the respective concentration of standard drug, BHT (50% − 32.42, 100% − 40.51, 150% − 43.61, 200% − 47.43, 250% − 54.12 and 300% − 52.35).

Table 3
Metal chelating activity of methanol leaf extract of Acacia caesia.

Metal chelating activity		
Sample concentration (μg/ml)	Percentage activity	IC50 (μg/ml)
50	35.53c±0.65	
100	37.19c±0.49	
150	38.01c±0.33	295
200	41.32b±0.41	
250	43.80b±1.63	
300	50.41a±0.82	

Values are expressed as mean±SD. (n=6).
Values within the column not sharing common superscript letters (a–f) differ significantly at P<0.05 by DMRT

4. Discussion

The free radicals are chemical species which contains one or more unpaired electrons. They are highly unstable and cause damage to other molecules by extracting electrons from them in order to attain stability. Radical scavenging activities are very important due to the deleterious role of free radicals in biological systems. In this study, it is evident that the extract of the study species, *Acacia caesia* possess effective antioxidant activity (Tables 1–3).

In vitro antioxidant activity of the methanolic leaf extract of *A. caesia* was investigated in the present study by total antioxidant, DPPH, hydroxyl radical scavenging and metal chelating assays. It is probably due to the presence of respective phytochemicals like flavonoids, phenolics etc in these species[17, 18]. The main function of the antioxidants is to neutralize the free radicals, which routinely produced in the biological system. Reactive oxygen species (ROS) readily combine and oxidize biomolecules such as carbohydrates, proteins and lipids and thus making them inactive with subsequent damage to cells, tissues and organs[19, 20]. The total antioxidant activity of the methanolic leaf extracts of *A. caesia* which is comparable to that of the standard, ascorbic acid. Sangeetha *et al*[21] observed similar type of results in the plant species, Sphaeranthus indicus, an Asteraceae member.

DPPH is a stable free radical and accepts an electron or

hydrogen radical to become a stable diamagnetic molecule. Antioxidant on interaction with DPPH, transfer electron or hydrogen atom to DPPH and thus neutralizing its free radical character and convert it to 1 − 1 diphenyl − 2 − picryl hydrazine and the degree of discoloration indicates the scavenging activity of the drug[22]. The decrease in absorbance of DPPH radical caused by antioxidants is due to the reaction between antioxidant molecules and radical progress which results in the scavenging of the radical by hydrogen donation. It is visually noticeable as a change in colour from purple to yellow. Hence, DPPH is usually used as a substance to evaluate the antioxidant activity[23, 24]. In the present study, the extracts had significant scavenging effect on the DPPH radical which was increasing with the increase in the concentration of the sample from 50 − 250 μg/mL. Similar trend of DPPH free radical scavenging activity was reported for the species, Lippia alba[25] and Languas galangal[26].

The present study shows the abilities of the extracts to inhibit hydroxyl radical mediated deoxyribose degradation in a concentration dependent manner. The extracts had significant scavenging effects on the hydroxyl radical, which was increasing with the increase in concentrations from 50 − 300 μg/mL. The methanolic leaf extracts of *A. caesia* possessed higher hydroxyl radical scavenging activity than that of the standard drug. Singh *et al*[27] and Patil *et al*[28] observed similar type of hydroxyl radical scavenging activity for the plants, Piper nigram and Gmelina arborea respectively. The reducing properties of the plant extracts are generally associated with the presence of reductones, which have been shown to exert antioxidant action by breaking the free radical chain by donating a hydrogen atom[29- 33].

The presence of transition metal ions in a biological system could catalyse the Haber-Weiss and Fenton type reactions, resulting in generation of hydroxyl radicals (OH). However, these transition metal ions could form chelates with the antioxidants, which results in the suppression of OH generation and inhibition of peroxidation processes of biological molecules[34]. The extracts had significant metal ion scavenging effects, which was increasing with the increase in the concentrations of the extract from 50 − 300 μg/mL. The methanolic leaf extracts of *A. caesia* possessed higher metal ion scavenging activity than that of the standard. It may be due to the chelating agents, which form sigma bonds with the metal and effective as secondary antioxidants because they reduce the redox potential, thereby the oxidized form of the metal ion[35]. Similar trend of metal ion scavenging activity was observed in the species, Cyperus rotundus[36], Melothria maderaspatana[37] and Leucas ciliate[38].

In the present study, it is found that methanolic extract of *A. caesia* showed concentration dependent free radical scavenging activity and this antioxidant effect may be due to the higher content of alkaloids, flavonoids, steroids, glycosides and saponins, highly responsible secondary metabolite for antioxidant activities[39]. Thus, the *Acacia caesia* leaf extract as promising natural sources of antioxidants can be used in nutritional or pharmaceutical fields for the prevention of free-radical-mediated diseases. However, pharmacognostical studies are suggested to confirm the antioxidant ability before going for commercialization.

Conflict of interest statement

We declare that we have no conflict of interest.

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