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Evaluation of *in vitro* antioxidant activity in the traditional medicinal shrub of western districts of Tamilnadu, India, *Acalypha fruticosa* Forssk. (Euphorbiaceae)

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ABSTRACT

Objective: To evaluate the antioxidant capability of the methanolic leaf extract of *Acalypha fruticosa*. **Methods:** *In vitro* antioxidant activity was evaluated by studying 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity, hydroxyl radical scavenging activity and metal chelating activity. Butylated hydroxy toluene was used as standard for all the three experiments. **Results:** IC_{50} values observed for DPPH radical scavenging and hydroxyl radical scavenging activities were determined to be 92 and 290 μg/mL, respectively and for metal chelating activity it was again 287 μg/mL as observed for hydroxyl radical scavenging activity. **Conclusions:** The results clearly indicate that methanolic leaf extract of the study species is effective in scavenging free radicals and has the potential to be a powerful antioxidant.

1. Introduction

Free radicals have been implicated in the causation of several problems like asthma, cancer, cardiovascular disease, cataract, diabetes, gastrointestinal inflammatory disease, liver disease, muscular degeneration and other inflammatory process[1]. Reactive oxygen species (ROS) are continuously produced during cell metabolism and under normal conditions, they are scavenged and converted to nonreactive species by different intracellular enzymatic and non-enzymatic antioxidant system[2].

Over production or an ineffective elimination of ROS may induce oxidative stress and cause damage to all types of biomolecules such as proteins, lipids and nucleic acids[3]. Antioxidants may act as free radical scavengers, reducing agents, chelating agents for transition metals, quenchers of singlet oxygen molecules and or activators of antioxidative defense enzyme system to suppress the radical damages in biological systems[4,5]. Antioxidants thus play an important role in the protection of human body against damage by reactive oxygen species[6,7]. Therefore, inhibition of free radical-induced oxidative damage by supplementation of antioxidants has become an attractive therapeutic strategy for reducing the risk of these diseases. In recent years, it has been investigated that many plant species are serving as source of antioxidants and received therapeutic significance[8-10].

Acalypha fruticosa (*A. fruticosa*) (Euphorbiaceae), one such shrubby plant species, generally distributed in the southern Western Ghats of India upto 1 80 0m above msl[11] is traditionally prescribed by the local medical practitioners in the western districts of Tamil Nadu, India (Coimbatore, Tirupur, Erode and Dindugal) for various ailments. The leaves are prescribed for digestive troubles such as dyspepsia, colic and diarrhea and even to treat cholera. Leaves are used to treat burns and sometimes used for bee stings[12]. The whole plant is used to cure cough, cold and headache. The leaf infusion is used as vulnerary and in the treatment of ophthalmia. In addition, the plant is administered to treat the diseases like jaundice, fever, and even as an antidote[13]. However, information pertaining to the antioxidant properties of *A. fruticosa* is meagre. However in the present study, the possible antioxidant activity of this

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plant was investigated in detail by employing three different *in vitro* antioxidant models.

2. Materials and methods

2.1. Plant material and extraction

To know the medicinal importance, the shade dried leaves of the study species were made into a fine powder of 40 mesh size using the pulverizer separately. 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 h[14]. 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 methods which are as follows:

2.3.1. Free radical scavenging activity (DPPH method)

The scavenging activity for DPPH free radicals was measured according to the procedure described by Blios *et al*[15]. Methanol extract of the sample 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. Tannic acid 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.

2.3.2. Hydroxyl radical scavenging activity

Hydroxyl radical scavenging activity was measured by Klein *et al*[16]. Various concentrations of the extracts *viz*., 50, 100, 150, 200, 250 and 300 μg/mL of *A. fruticosa* 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 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. Tannic acid 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

2.3.3. Metal chelating activity

The chelating of ferrous ions by various extracts of *A. fruticosa* was estimated by the method described by Dinis *et al*[17]. Various concentrations of the extracts *viz*., 50, 100, 150, 200, 250 and 300 μg/mL of *A. fruticosa* were added with 1 mL of 2mM $FeCl_2$ separately. The reaction was initiated by the addition of 5 mM ferrozine (1 mL). Absorbance was measured at 562nm after 10min.

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

3. Results

The level of free radical scavenging activity through DPPH method, hydroxyl radical scavenging activity and metal chelating activity of methanol leaf extract of *A. fruiticosa* is shown in Table 1.

3.1. Free radical scavenging activity (DPPH method)

The percentage of scavenging effect on the DPPH radical was increased with the increase in the concentration of leaf extract from 50-250 μg/mL. The percentage of inhibition was varying from 40% in 50 μg/mL of the extract to 75% in 250 μg/mL of extract. The IC_{50} value of the methanolic leaf extract of this species was calculated to be 92 μg/mL while for the standard butylated hydroxy toluene (BHT) it was 30.14 μg/mL

3.2. Hydroxyl radical scavenging activity

The hydroxyl radical scavenging activity was determined to be increased with the increase in the concentration of leaf extract from 50 to 300 μg/mL. The percentage of inhibition of the hydroxyl radical was varying from 38.80% in 50 μg/

Table 1 .
In vitro antioxidant activity of the methanol leaf extract of the species, *A. fruticosa* (mean±SD, *n*=6)..

Sample concentration (μg/mL	DPPH radical scavenging activity (%)	Hydroxyl radical scavenging activity(%)	Metal chelating activity(%)
50	$40.00^{e}\pm 0.40$	$38.80^{def}\pm 0.81$	$32.23^{ef}\pm 0.81$
100	$50.00^{d}\pm 0.81$	$39.55^{cde}\pm 1.63$	$33.88^{de}\pm 1.63$
150	$55.00^{c}\pm 1.63$	$41.04^{cde}\pm 0.03$	$36.36^{cde}\pm 0.81$
200	$65.00^{b}\pm 1.22$	$41.79^{bcd}\pm 0.81$	$38.01^{cd}\pm 0.46$
250	$75.00^{a}\pm 0.81$	$44.02^{bc}\pm 1.63$	$41.32^{b}\pm 1.63$
300	–	$50.74^{a}\pm 0.81$	$50.41^{a}\pm 1.63$

Values within the column not sharing common superscript letters (a–e) differ significantly at $P<0.05$ by DMRT.

mL of extract to 50.74% in 300 μg/mL of leaf extract. The IC_{50} value of the methanolic leaf extract of the study species was found to be 290 μg/mL and it was far higher than that of the standard drug, BHT (28.69 μg/mL).

3.3. Metal chelating activity

As observed in DPPH and hydroxyl radical scavenging assays, the percentage of metal chelating activity was determined to be increased with the increase in the concentration of leaf extract from 50 to 300 μg/mL. The percentage of inhibition of the metal chelation was varying from 32.23% by 50 μg/mL of extract to 50.41% by 300 μg/mL extract. The IC_{50} value of the methanolic leaf extract of the study species was 287 μg/mL. Similar to the observation in other two antioxidant studies, the IC_{50} value of the extract was higher than the standard, BHT (21.81 μg/mL)

4. Discussion

Free radicals and other reactive species are thought to play an important role in many human diseases. Radical scavenging activities are very important due to the deleterious role of free radicals in biological systems. Many secondary metabolites which include flavonoids, phenolic compounds etc serve as sources of antioxidants and do scavenging activity[18,19]. In this study, it is evident that the extract of the study species, *A. fruticosa* possess effective antioxidant activity. This feature perhaps due to the presence of respective phytochemicals like flavonoids, phenolics etc in this species[20].

In vitro antioxidant activity of the methanol extract of *A. fruticosa* was investigated in the present study by DPPH, hydroxyl radical scavenging and metal chelating assays. These methods have proven the effectiveness of the extracts in comparison to that of the reference standard antioxidant, BHT. 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 1–1diphenyl–2–picryl hydrazine and the degree of discoloration indicates the scavenging activity of the drug[21]. 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[22, 23]. In the present study, the scavenging effect of different concentrations of the extracts on the DPPH radical is illustrated. The extracts had significant scavenging effect on the DPPH radical, which was generally significantly increasing with the increase in the concentration from 50–250 μg/mL.

The present study shows the ability of the leaf extract to inhibit hydroxyl radical mediated deoxyribose degradation in a concentration dependent manner. The extract had significant scavenging effects on the hydroxyl radical, which increased with the increase in the concentrations from 50–300 μg/mL.

The presence of transition metal ions in a biological system could catalyse the Haber–Weiss and Fenton type reactions, resulting in the 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[24]. The metal ion scavenging effect was increasing with the increase in the concentration of the extracts from 50–300 μg/mL. The high metal ion scavenging activity of the methonolic leaf extract of the study species is probably 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[25].

The results of the present study indicate that the methanol leaf extract of *A. fruticosa* has significant antioxidant activities which is comparable to that of the standard drug like BHT. It may be due to the higher content of flavonoids, a highly responsible secondary metabolite for antioxidant activities[20]. Despite the higher IC_{50} values of all the three studied antioxidant assays than the standard drug, the leaf extract may be considered as important source of material for the scavenging of radicals as its obtained IC_{50} value is most worthy. Many reports have been available that the antioxidant properties in Euphorbiaceae members are mainly due to the presence of high content of secondary metabolite, flavonoids of different types[26–28]. Thus, the *A. fruticosa* leaf extract as promising natural sources of antioxidants can be used in nutritional or pharmaceutical

fields for the prevention of free-radical-mediated diseases.

Conflict of interest statement

We declare that we have no conflict of interest.

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