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RESEARCH ARTICLE

IN VITRO ANTIOXIDANT ACTIVITY OF HYDROCOTYLE CONFERTA WIGHT (APIACEAE) - AN ENDANGERED PLANT SPECIES IN SOUTHERN WESTERN GHATS

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ABSTRACT

Hydrocotyle conferta Wight is an endangered plant species in Southern Western Ghats. Methanolic whole plant extract of *H. conferta* study was investigated for various DPPH, ABTS, hydroxyl radical scavenging, reducing power and ferrous ion chelating assays. The results of DPPH activity showed that the extract at the dose of 50µg/ml has exhibited in 89.15±0.16 inhibition with an IC50 value of 34.21±0.52mg/ml. The highest ABTS scavenging activity showed 762.63±0.53 trolox equivalence in µMol/g extract at the dose of 50µg/ml extract, Hydroxyl radical scavenging effect of the methanolic extract at the concentration of 50µg/ml was found to be 69.16±0.31. The reducing power assay showed the 0.79 absorption at 720 nm extract at the dose of 50µg/ml, and metal chelating activity at concentrations of 50µg/ml extract showed 67.52 ±0.03 suggested. It is that antioxidant activity of crude methanolic extract could be used as a source of natural antioxidants of *H. conferta* and needs further stud to bring new natural products into pharmaceutical industries.

INTRODUCTION

Antioxidants are substances that may protect your cells against those effects of free radicals. Free radicals can damage cells, and may play a role in heart disease, cancer and other diseases. Studies suggest that a diet high in antioxidants from fruits and vegetables is associated with a lower risk of cancer, cardiovascular disease, Parkinson's disease and Alzheimer's disease (Singh *et al*., 2013). Living systems have specific pathways to overcome these repair mechanisms and fail to keep pace with such deleterious effects. Natural antioxidants such as flavonoids, phenolics, tannins and terpenoids are found in various plants (Jayaprakasha *et al*., 2002). *H. conferta* is a prostrate herb belongs to the family Apiaceae, distributed in a small geographical region in southern western ghats. Therefore the present study was executed to know its efficacy as natural antioxidants.

MATERIALS AND METHODS

Preparation of plant sample

H. conferta plant was collected at a remote village of Kodanadu, the Nilgiris, the Western Ghats, Southern India, India. The plant was identified and authenticated with the help of voucher specimen in Botanical Survey of India, Southern circle, Coimbatore.The plant material was dried in shade after washing with cold water and then powdered using pulveriser and passed through sieve.

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About 100 g of dried plant powder was extracted with petroleum ether using soxhlet apparatus for 12-15 hours. The petroleum ether was evaporated from the extract and then the residue was re-extracted with methanol. After evaporation of methanol, the residue filtered and stored at 4 ^{0}C in refrigerator for further biological studies. DPPH radical scavenging activity: The scavenging effect of extract on DPPH radicals was determined according to the method of Shimada *et al.,* (1992). Various concentrations of the sample (4ml) were mixed with 1ml of methanolic solution containing DPPH radicals, resulting in the final concentration of DPPH being 0.2mM. The mixture was shaken vigorously and left to stand for 30min and the absorbance was measured at 517nm.

$$\% \text{ Inhibition} = (A_0 - A_1)/ A_0 \times 100$$

where A0 was the absorbance of the control and A1 was the absorbance of the sample.

ABTS radical cation scavenging activity: It was performed with slight modifications as described by Re *et al.,* (1999). The ABTS□+ cation radicals were produced by the reaction between 7mM ABTS in water and 2.45 mM potassium persulfate and stored in the dark at room temperature for 12 h. prior to use, The same solution was diluted with ethanol to get an absorbance of 0.700 ± 0.025 at 734nm. Free radical scavenging activity was assessed by mixing 10 µl of test sample with 1.0 ml of ABTS working standard in a microcuvette. The absorbance was measured exactly after 6min.

$$\% \text{ Inhibition} = (A_0 - A_1)/ A_0 \times 100$$

where A0 was the absorbance of the control and A1 was the absorbance of the sample.

Hydroxyl radical scavenging assay: Hydroxyl radical scavenging activity of plant extract was assay by the method of (Smirnoff and Cumbes, 1989). The reaction mixture 3.0 ml contained 1.0 ml of 1.5 mM FeSO4, 0.7ml of 6mM hydrogen peroxide, 0.3 ml of 20 mM sodium salicylate and various concentrations of the extract. After incubation period of 1 hour at 37°C, the absorbance of the hydroxylated salicylate complex was measured at 562nm.

$$\% \text{ Inhibition} = (A_0 - A_1)/ A_0 \times 100$$

where A0 is absorbance of the control (without extract) and A1 is the absorbance in the presence of the extract, A2 is the absorbance without sodium salicylate.

Reducing power: It was performed according to the method of Oyaizu (1996). Various concentrations of methanolic extracts (10, 20, 30, 40 and 50µg/ml) of the study plant sample was mixed with 1ml of 200 mM sodium phosphate buffer (pH 6.6) and 1ml of 1% potassium ferriccyanide followed by incubation at 500 C for 20 minutes. After that 1ml of 10% trichloroacetic acid, was added and centrifuged at 3000 rpm for 10 minutes. Then, the supernatant was mixed with 2 ml of distilled water and 0.5 ml of 1% ferric chloride. After incubation of 10 minutes, the absorbance was measured at 700 nm.

Ferrous ion chelating: The chelating of ferrous ions was estimated in methanolic extracts of *H.conferta* using the method of Dinis *et al.,* (1994). The various concentrations of methanolic extracts (10, 20, 30, 40 and 50 µg/ml) were mixed with 100µl of 2 mM ferrous sulphate solution and 300µl of 5mM ferrozine. After 10 minutes of incubation period, the absorbance of the reaction mixture was measured at 562 nm against blank. Here, ethylene diammine tetra acetate (EDTA) was used as standard. All the tests were performed in triplicate and per cent inhibition was calculated using the flowing formula

% of inhibition = Abscntrl-Abstest X 100 /Abscntrl

RESULTS AND DISCUSSION

The effects of methanolic extract of *H. conferta* was evaluated for its antioxidant activity on different *in vitro* models like DPPH, ABTS scavenging and Hydroxyl radical scavenging assays various levels. The result showed that DPPH radical scavenging activity has exhibited potent scavenging activity at various concentrations of the extract (Table 1). The methanolic extract of *H. conferta* at the dose of 50µg/ml exhibited 89.15±0.16 inhibition and with an IC50 value of 34.21±0.52mg/ml. the methanoli extract had scavenging activity on DPPH radical in a dose depended manner.

Table 1. DPPH radical-scavenging activity at various concentrations of methanolic extract of *H. conferta*

S. No	Concentrations of sample (µg/ml)	% inhibition ± SD	IC_{50} value (µg/ml)
1.	10	19.65±0.36	
2.	20	34.16±0.76	
3.	30	46.21±0.28	34.21±0.52
4.	40	73.12±0.14	
5.	50	89.15±0.16	

Table 2. ABTS radical scavenging activity at various concentrations of methanolic extract of *H. conferta*

S. No	Concentrations of sample (µg/ml)	ABTS radical scavenging activity*
1.	10	193.51±0.28
2.	20	251.27±0.09
3.	30	344.81±0.48
4.	40	464.76±0.27
5.	50	762.63±0.53

*Values expressed as Trolox equivalence in µ Mol/g extract

Table 2 depicts ABTS radical scavenging activity at the different concentrations of methanolic extract of *H. conferta.* Per cent of ABTS radical scavenging activity was increased with increasing concentration. The highest activity was found to be 762.63±0.53 trolox equivalence in µ Mol/g extract at the dose of 50µg/ml. In the present study, the hydroxyl radical scavenging effect of the extract at the concentration of 10µg/ml was found to be 21.22% and at the concentration of 50µg/ml was found to be 69.16%. The IC50 value was found to be 39.33±0.28µg/ml (Table 3).

Table 3. Hydroxyl radical-scavenging activity at various concentrations of methanolic extract of *H. conferta*

S. No	Concentrations of sample (µg/ml)	% inhibition ± SD	IC_{50} value (µg/ml)
1.	10	21.22±0.16	
2.	20	29.03± 0.22	
3.	30	41.96± 0.08	
4.	40	58.13±0.16	39.33±0.28
5.	50	69.16±0.31	

Table 4. Reducing power at various concentrations of methanolic extract of *H. conferta*

S. No	Concentrations of sample (µg/ml)	Absorbance at 700nm
1.	10	0.32
2.	20	0.53
3.	30	0.57
4.	40	0.71
5.	50	0.79

Table 5. Chelating effects on ferrous ions/ Metal chelating activity at various concentrations of methanolic extract of *H. conferta*

S. No	Concentrations of sample (µg/ml)	Ferrous ion chelating activity*
1.	10	24.52±0.53
2.	20	31.21±0.40
3.	30	49.02±0.19
4.	40	54.23±0.50
5.	50	67.52±0.03

DPPH is a free radical phytochemical compounds that has been widely used to determine the free radical-scavenging ability of different samples (Amarowicz *et al*., 2002). Fangchinoline and cepharanthine isolated from *S. rotunda* performed at different *in vitro* antioxidant assays, including 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) free radical scavenging activity (Gulcin *et al.,* 2010). The observed antioxidant activity of extract may be due to the neutralization of free radical (DPPH), either transfer of hydrogen atom or by transfer of an electron (Naik *et al*., 2003). It is reported that antioxidant properties of water and ethanol extracts of *Pimpinella anisum,* (Apiaceae) seed was previously evaluated using various antioxidant tests (Gulcin *et al*., 2003). The Apiaceae species were used for food, spices and in traditional medicine since long in the world. The antioxidant effects of some Apiaceae species have been reported previously

by some authors (Coruh *et al*., 2007). And other species *Trachyspermum ammi, Coriandrum sativum, Anethum sowa, Foeniculum vulgare* and *Cuminum cyminum* were also reported (Patel and Jasrai, 2015). Reducing power activity is often used to study ability of natural antioxidant to donate electron (Yildirim *et al*., 2000, Dorman *et al*., 2003). Various reports have exposed that there is a direct correlation between antioxidant activities and reducing power of certain plant extracts (Duh, 1998; Duh *et al*., 1999; Yildirim *et al.,* 2000). The reducing power activity of methanolic extracts of *H. conferta* increased consistently with the increase in the volume of extract from 10 µg to 50µg (Table 4). Iron is essential for life because it is required for oxygen transport, respiration and activity of many enzymes. However, it is an extremely reactive metal and catalyzes oxidative changes in lipids, proteins and other cellular components (Smith *et al*., 1992). The metal chelating ability of the methanolic extracts was measured by the formation of ferrous ionferrozine complex. Ferrozine combines with ferrous ions forming a red coloured complex absorbs at 562n m (Yamaguchi *et al.,* 2000). The chelating agents which forms σ bond with a metal are effective as secondary antioxidants which reduce the redox potential there by stabilizing the oxidized form of the metal ion (Duh *et al*., 1999). Iron binding capacity of the methanolic extract of *H. conferta* at 50µg/ml was 67.52±0.03 µg/ml and showed it potent activity (Table 5). All the extracts of nine Malaysian vegetables of *Curcuma domestica, Kaempferia galanga, Piper betel, Piper sarmentosum, Polygonum minus, Cosmos caudatus, Centella asiatica*, *Hydrocotyle bonariensis* and *Barringtonia racemosa* were screened for their antioxidant properties (Sumazian *et al*., 2010).

Conclusion

The results confirmed that the methanolic whole plant extract of *H. conferta* has proton-donating ability and it could serve as free radical inhibitors or scavengers, acting probably as primary antioxidants. In the present study supports to the further pharmacological experiments could be carried out for the discovery of novel drug.

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