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Evaluation of antioxidant activity of the root and leaf extracts of *Hemidesmus indicus*

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ABSTRACT

Free radicals or highly reactive oxygen species are capable of inducing oxidative damage to human body. Antioxidants are the compounds which terminate the attack of reactive species and reduce the risk of diseases. *Hemidesmus indicus* is used in treatment of various disorders in humans. The study was conducted to determine the antioxidant properties of *Hemidesmus indicus* leaf and root extracts in methanol. The antioxidant activity of this medicinal plant was evaluated by measuring reducing ability, free radical scavenging activity by DPPH and ABTS. *Hemidesmus indicus* roots and leaves exhibited significant antioxidant values. The methanolic crude extracts of whole leaf powder of *Hemidesmus indicus* exhibited significantly higher antioxidant activity than the root of *Hemidesmus indicus*. It can be concluded from the study that *Hemidesmus indicus* can be used in treatment of disorders caused by free radical damage.

Key Words: *Hemieismus indicus* DPPH, free radical scavenging activity, antioxidant activity, reducing ability.

INTRODUCTION

Oxidation is essential for the production of energy in biological system. During the normal course of producing energy, free radicals are generated. Though free radicals, radical derivatives and non-radical reactive species are useful during oxidation but hazardous to living organisms at high concentration and damage all major cellular constituents in our body (Aruna Sindhe et al, 2013). In this scenario, the medicinal properties of plants have been investigated in several countries with a focus on identifying key phytochemicals with potential therapeutic effects. The important active phytochemical founding plant are alkaloids, tannins, flavonoids, phenols, proteins and carbohydrates (Okwu, 2001; Zoe Merculieff et al, 2014). Phytochemicals are widely used to treat many oxidative stress induced diseases such as cancer, diabetes etc., which are associated with ROS that damage cellular biomolecules. Medicinal plants, spices are often rich in phenols, flavonoids, proteins are known to have potent antioxidant properties that can prevent oxidative stress and provide health benefits (Tapiero et al, 2002; Saeed et al, 2012).

Sarsaparilla is an important drug used in ayurveda, endowed with many medicinal properties. Many plants are known as sarsaparilla in different countries. *Hemidesmus indicus* (L.) R.Br. var. *indicus* is referred to as Indian Sarsaparilla and is known as Sariva in Ayurveda. In addition to the typical variety *Hemidesmus indicus* var. *indicus*, one more taxonomic variety, *Hemidesmus indicus* (L.) R. Br. var. *pubescens* (W. & A.) Hk. f. is found in South India and is often substituted for *H. indicus* var. *indicus* (Yoganarasimhan et al, 2000). European sarsaparilla is derived from *Smilax china* L. and Australian sarsaparilla from *Smilax glycyphila* Sm.(Trease et al, 2009; Cox et al, 2005). The roots of *H. indicus* var. *indicus* possesses antipyretic, antidiarrheal, astringent, diaphoretic, diuretic, refrigerant, tonic properties etc. It is used in the treatment of biliousness, blood disorders, dysentery, respiratory disorders, skin diseases, syphilis, fever, leprosy, leucoderma, leucorrhoea, itching, bronchitis, asthma, eye diseases, kidney stones and for epileptic fits in children (Longman, 1996). The phytoconstituents, β- sitosterol and tannins have been reported in *H. indicus* var. *pubescens* (Austin et al, 2002). Based on the lack of anti-oxidant properties on this plant this work has been undertaken to study the antioxidant potential of leaves and roots of *H. indicus.*

MATERIALS AND METHODS

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Sample collection

The plant *H. indicus* roots and leaves were collected from authentic source, Telangana State, India. The collected roots and leaves were cleaned thoroughly with double distilled water and dried under the shade. Once the drying process is complete, the dried roots and leaves were ground to powder, stored for further studies.

Preparation of Extract

The roots and leaves of *H. indicus* were cleaned, shade dried and powdered. The powdered material (500g) was successively extracted in a soxhlet apparatus using methanol solvent. The extract was filtered and concentrated to dryness under reduced pressure to yield methanol crude. The yielded methanol crude extract stored at -10^{0}C.

DPPH radical scavenging assay

The ability of *H. indicus* extract/ascorbic acid to scavenge 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical was measured by the reported method Shimada et al. (1992). The addition of 0.1 mM DPPH solution in various concentrations (50, 100, 150, 200 and 250 µg/mL) of plant root and leaves extract/ascorbic acid in the presence of Tris–HCl buffer (50 mM, pH 7.4) resulted in decreased absorbance, which was measured at 517 nm. A mixture of methanol and extract served as the blank. The per cent inhibition was calculated by measuring the absorbance of extract/ascorbic acid treated samples against the blank. The IC_{50} values for the extracts were calculated and compared with the standard reference compound ascorbic acid.

Reducing power assay

The reducing power was determined based on the ability of the antioxidant to form a coloured complex with potassium ferric cyanide, TCA and $FeCl_3$. It was measured by a modified method (Jayaprakash et al, 2001) in which the extract of *H. indicus* leaves and roots (1 mL) at various concentrations (200, 300, 400, 500 and 600 µg/ml) were mixed with potassium ferric cyanide and phosphate buffer (pH 6.6) and incubated at 50^{0}C for 20 min. Then, TCA (10%) was added and centrifuged at 3000 rpm for 10 min. The supernatant was removed and mixed with $FeCl_3$ (0.1%). The absorbance was then measured at 700 nm. A higher absorbance of the reaction mixture indicates a higher reducing power.

Free radical scavenging activity (ABTS*$^+$)

The total antioxidant activity of the samples was measured by [2, 2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)] ABTS•+ radical cation decolorization assay according to themethod of Re et al. (1999). ABTS•+ was produced by reacting 7mM ABTS+ aqueous solution with 2.4mM potassium persulfate in the dark for 12-16 hours at room temperature. The radical was stable in this form for more than two days when stored in the dark at room temperature. Prior to assay, this solution was diluted in ethanol (about 1:89 v/v) and equilibrated at 30^{0}C to give an absorbance of 0.7000±0.02 at 734 nm. Then, 2ml of diluted ABTS•+ solution was added to the sample concentration at 20µl (1mg/ml). After 30 minutes of incubation at room temperature, the absorbance was recorded at 734nm and percentage of inhibition was calculated. Trolox was used as a reference standard. Triplicates were performed.

Statistical analysis

The results are expressed as the mean ± standard error of the mean (SEM). The IC50 values were calculated and compared by paired *t* tests. $p < 0.05$ was considered significant.

RESULTS AND DISCUSSION

Free radical is a molecule with an unpaired electron and is involved in bacterial and parasitic infections, lung damage, inflammation, reperfusion injury, cardiovascular disorders, atherosclerosis, aging and neoplastic diseases (Roy et al., 1994). They are also involved in autoimmune disorder like rheumatoid arthritis etc. (Rao et al., 2004). Our results demonstrated that the methanolic extracts of leaf and root of *H. indica* possess free radical scavenging activity in vitro models like DPPH, HPSA and reducing power activity assays.

Free Radical Scavenging Activity-DPPH:

DPPH is one of the free radicals widely used for testing preliminary radical scavenging activity of the plant extract (Bhuiyan et al., 2009). Scavenging of DPPH• radical is related to the inhibition of lipid peroxidation (Rekka and Kourounakis 1991). DPPH• is usually used as a substance to evaluate the antioxidant activity (Tara Chand et al., 2012). Antioxidants either transfer an electron or a hydrogen atom to DPPH•, thus neutralizing its free radical character (Pan et al., 2008). DPPH• test, which is based on the ability of DPPH•, a stable free radical, to decolorize in the presence of antioxidants, is a direct and reliable method for determining radical scavenging action (Raquibul Hasan et al., 2009). The DPPH• assay has been largely used as a quick, reliable and reproducible parameter to search the in vitro general antioxidant acitivity of pure compounds as well as plant extracts (Koleva et al., 2002). The reducing capacity of compounds could serve as indicator of potential antioxidant property (Meir et al., 1995). In the present study, the percentage of scavenging effect on the DPPH• radical was concomitantly increased with the increase in the concentration of both leaf and root methanolic extracts from 50 to 250 µg/ml. The percentage of inhibition was existing from 59.91 at 50µg/ml to 102.36 at 300 µg/ml for leaf extract and for root extracts, they were 28.27 at 50µg/ml and 101.72 at 250 µg/ml (Table-1). From the results it is known that the species, *H. indicus* possess hydrogen donating capabilities for methanolic leaf extract and does scavenging free radicals. Furthermore,

it was noticed that the leaf extract has more pronounced scavenging activity than that of the standard, BHT (Table-1).

Reducing power activity

Reducing power activity is often used to evaluate the ability of natural antioxidant to donate electron (Yildirim et al., 2000, Dorman et al., 2003). Many reports have revealed 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 leaf and root extracts of H. radicata increased consistently with the increase in the volume of extract from 200µg to 600µg for leaf and 300µg to 700µg for root. When compared with the root extract (0.433 at 700µg/ml), leaf extract showed higher absorbance (1.092 at 600µg/ml). It is known further that the reducing power activity of leaf extract was far higher than the standard, ascorbic acid (Table-2).

Free radical scavenging activity (ABTS•+)

The decolorization of the ABTS•+, through measuring the reduction of the radical cation as the percentage inhibition of absorbance at 734nm (Re et al., 1999). ABTS•+ was generated by incubating ABTS•+ chromophore through the reaction (Wolfenden et al., 1982). The presence of specific chemical compounds in the extracts of H. radicata may inhibit the potassium persulfate activity and hence reduced the production of ABTS•+. This study reports that the methanolic leaf extract of H. radicata has highest antioxidant activity (2706.73 µmol/g) than that of its root counterpart (2028.37µmol/g) (Table-3).

Table-3. ABTS activity of methanolic leaf and root extracts of *H. indicus*

Sample	Total antioxidant activity (umol TE/g extract
Leaf	3205.89 ± 2.31
Root	2529.3 ± 3.21

Total antioxidant activity (µmol equivalent trolox).
Values were performed in triplicates and represented as mean ± SD.
Mean values followed by different superscript in a column are significantly different (p<0.05).

CONCLUSION

Searching plant sources may bring new natural products into pharmaceutical, cosmetic and food production. An in vitro antioxidant study provides scientific evidence to prove the traditional claims to the Asteraceae member, *H. indicus.* On the basis of the results obtained in the present study, it was concluded that the methanolic leaf and root extracts of this species possess significant antioxidant activity. Presence of adequate amount of phenol and flavonoid compounds may account for this fact. So these findings of present study suggest that this plant have a potential source of natural antioxidant. Further studies are warranted for the isolation and characterization of antioxidant compounds, and also in vivo studies are needed for understanding their mechanism of action as antioxidants.

Conflict of Interests

Table-1. Free radical scavenging activity (DPPH) of methanolic leaf and root extracts of *H. indicus*

S.No	Sample concentration (µg/ml)	% of inhibition		
		Leaf Extract	Root Extract	Standard (BHT)
1	50	59.21±0.56	28.4a ± 0.23	41.24a ± 0.30
2	100	95.87b ± 0.36	61.52b + 0.37	47.21b + 0.37
3	150	98.25c ± 0.61	80.4c ± 0.46	54.39c ± 0.34
4	200	101.3d ± 0.50	100.36d ± 0.46	47.16d ± 0.40
5	250	102.98c ± 035	101.44c ± 0.32	62.1c ± 0.25

BHT was used as reference standard.
Values were performed in triplicates and represented as mean ± SD.
Mean values followed by different superscript in a column are significantly different (p<0.05).

Table-2. Reducing power activity of methanolic leaf and root extracts of *H. indicus.*

S.No	Sample concentration (µg/ml)	Absorbance at 700 nm		
		Leaf Extract	Root Extract	Standard (Ascorbic acid)
1	200	0.72a ± 0.43	0.21a ± 0.02	0.44a ± 0.05
2	300	0.76b ± 0.03	0.22a + 0.01	0.69c + 0.04
3	400	0.83c ± 0.01	0.27b ± 0.02	0.91d ± 0.03
4	500	1.10dc ± 0.01	0.41c ± 0.02	1.01c ± 0.02
5	600	1.43c ± 0.59	0.49d ± 0.02	1.14f ± 0.04

Ascorbic acid was used as reference standard.
Values were performed in triplicates and represented as mean ± SD.
Mean values followed by different superscript in a column are significantly different (p<0.05).

Authors declare that there is no conflict of interests regarding the publication of this paper.

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