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Research Article

ANTIOXIDANT ACTIVITY OF LEAF EXTRACTS OF *HEMIDESMUS INDICUS* (L.) R. BR. (ASCLEPIADACEAE)

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ABSTRACT

Objective: A number of Indian medicinal plants have been used for thousands of years in a traditional system of medicine. *Hemidesmus indicus* is an important member of the Asclepiadaceae family. It is an endemic to the southern Western Ghats, India. The aim of the study was to investigate the free radical scavenging activity of *H. indicus*.

Methods: The aqueous and methanol leaf extracts of *H. indicus* were assayed for radical scavenging activity, using the stable free radical 2,2-diphenyl-1-picryl-hydrazyl-hydrate and 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid).

Results: The results revealed that the IC_{50} values of aqueous extract of *H. indicus* were found to be higher than that of the other solvent extracts. The free radical scavenging activity of the plant extracts may be due to the presence of phytoconstituents.

Conclusion: In all the methods, the aqueous extract has exhibited the good scavenging activity and this arises that the plant has a potential antioxidant agent.

Keywords: *Hemidesmus indicus*, Asclepiadaceae, Antioxidant.

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INTRODUCTION

In India, traditional communities like tribal and rural population are frequently using the crude extract of local plants for medicinal and other purposes. Crude extracts and medicines manufactured on the principles of natural compounds even by pharmaceutical companies may lead to large-scale exposure of humans to natural products. The first step toward this goal in the biological and phytochemical screening of plant extracts from traditional preparations used in popular medicine [1,2]. With ever-increasing population pressure and foot depletion of natural resources, it has become extremely important to diversify the present day agriculture to meet various human needs [3]. The observed interest in the search for alternative/additional food and feed ingredients is of paramount importance mainly for two reasons, one is the low production of oilseeds and grains and another is the stiff competition between man and livestock industry for existing food and feed materials [4]. The ethnic people use a wide variety of wild plants and plant products as their food. India has one of the largest concentrations of tribal population in the world. The forest plays a key role in the economy as well as daily needs of the tribes. In times of scarcity when the staple food is in short, of supply tribal's collected many types of wild roots and tubers to supplement their meager food available at home [5].

The commercial development of plants as sources of antioxidants to enhance health and food preservation of current interest [6]. Epidemiological studies have suggested positive associations between the consumption of phenolic-rich foods or beverages and the prevention of diseases [7]. Antioxidants, which scavenge active oxygen species (free radicals) are found in a variety of foodstuffs and are commonly referred to as scavengers. Many antioxidants are placed based and play an important role in protecting plants that are exposed to strong sunlight and live under severe oxygen stress. Antioxidants also play an important role in human health because the biological defense mechanisms cannot operate under severe oxygen stress. Pigmentation from excess melanin can cause the appearance of spots and freckles on the skin. Plants are a rich source of antioxidant, which evinced through many reports on medicinal plants with antioxidant potential [8]. Almost all the sources for such phytochemical studies are from published or unpublished ethnobotanical knowledge [9].

2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) free radical method is an antioxidant assay based on electron-transfer that produces a violet solution in ethanol. This free radical, stable at room temperature, is reduced in the presence of an antioxidant molecule, giving rise to a colorless ethanol solution. The use of the DPPH assay provides an easy and rapid way to evaluate antioxidants by spectrophotometry, so it can be useful to assess various products at a time. Due to the lack of evidence about which solution can be more effective as an antioxidant or even if there are other solutions with equal or more capacity to eliminate free radicals from dental surfaces after bleaching procedures, the purpose of this study was to evaluate the antioxidant activity of several agents proposed for reversion of problems caused by bleaching procedures using the DPPH free radical assay [10].

METHODS

Collection of plant

Hemidesmus indicus (L.) R. Br. belongs to the family Asclepiadaceae was collected from a natural forest of Mettupalayam, Coimbatore, Tamil Nadu, India. The specimen was identified by Dr. M. Murugesan, Scientist B, Botanical Survey of India, Shillong. A voucher specimen was deposited in the Department of Botany, Kongunadu Arts and Science College, Coimbatore (KASC/BOT/0012), Tamil Nadu, India. The plants were thoroughly washed in running tap water before being shade-dried for 20 days.

Preparation of plant extracts

About 50 g of powdered plant materials were extracted in soxtron apparatus for 1-2 hrs, sequentially with the alcoholic solvents, *viz.,*

petroleum ether, chloroform, methanol, and water. Then, the extract was evaporated to dryness using a vacuum rotary evaporator and stored in vials kept in 4°C for further use.

DPPH free radical scavenging activity

The DPPH scavenging activity of this plant tuber extract was measured according to the method of Blois [11]. IC_{50} values of the extracts and concentration of the extracts necessary to decrease the initial concentration of DPPH by 50% were calculated.

2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{\bullet+}$) cation radical scavenging activity

The total antioxidant activity of the samples was measured by $ABTS^{\bullet+}$ radical cation decolorization assay according to the method of Re *et al.* [12]. In this improved version, ABTS the oxidant is generated by per sulfate oxidation of $ABTS^{2-}$. $ABTS^{\bullet+}$ radical cation ($ABTS^{\bullet+}$) was produced by reacting $ABTS^{\bullet+}$ solution (7 mm) with 2.45 mm ammonium per sulfate, and the mixture was allowed to stand in the dark at room temperature for 12-16 hrs before use. For this study different concentration that range from 25 to 100 µg/ml of aqueous extract was added to 0.3 ml of $ABTS^{\bullet+}$ solution and the final volume was made up with aqueous to 1 ml. The absorbance was read at 734 nm, and the percentage of inhibition was calculated.

Statistical analysis

The data were subjected to a one-way analysis of variance, and the significance of the difference between the means was determined by Duncan's multiple range test ($p<0.05$) using statistics (Stat Soft Inc., OK, USA). Values expressed are means of three replicate determinations ± standard deviation.

RESULTS AND DISCUSSION

DPPH radical scavenging activity

The result showed the dose response, radical scavenging activities observed in the MeOH and water extracts of whole plans of *H. indicus* (Table 1). Both extracts, there was an increase in the $DPPH^{\bullet}$ radical scavenging activity with increasing extract concentration. The results of $DPPH^{\bullet}$ radical scavenging activity of *H. indicus* extracts along with the reference standards. Concentration of the sample necessary to decrease the initial concentration of $DPPH^{\bullet}$ by 50% (IC_{50}) under the experimental condition was calculated. Therefore, a lower value of IC_{50} indicates the highest antioxidant activity of 51.92±11.19 and 70.10±8.28 µg/ml, respectively (Table 1). Similarly, reported Samydurai and Thangapandian [13] the *Decalepis hamiltonii* (Asclepiadaceae) aqueous root extract showed good antioxidant activity could be used in the prevention of free radical diseases and general health tonic. The generation of free radicals can bring about thousands of reactions and thus cause extensive tissue damage. Lipids, proteins, and DNA are all susceptible to attack by free radicals [14]. According to Houghton *et al*. [15], free radical reactions are involved in the pathology of many diseases such as Alzheimer's disease, cancer, and inflammation.

The whole plant extracts of *H. indicus* showed a good radical scavenging activity as indicated by their IC_{50} values, which were significantly different from that of the butylated hydroxytoluene standard (Table 1). The results suggest the likely presence of assuring compounds that are possibly aggressive in their radical scavenging activities.

Table 1: $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ free radical scavenging activities of leaf extracts of *H. indicus*

Concentration of sample (mg/ml)	Inhibition of DPPH		$ABTS^{\bullet+}$ (µmol/g)	
	Methanol	Aqueous	Methanol	Aqueous
25	34.97	59.09	13040.9	4819.5
50	48.70	65.86	12494.2	2632.5
75	62.05	74.67	8491.5	1725.3
100	61.96	80.79	6409.1	1306.1
IC_{50} (µg/ml)	51.92	70.10	11108.9	2720.1

DPPH: 2,2-diphenyl-1-picryl-hydrazyl-hydrate,
ABTS: 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)

Total antioxidant activity by $ABTS^{\bullet+}$

$ABTS^{\bullet+}$ cation radical scavenging activity decolorization assay applicable to both lipophilic and hydrophilic antioxidants, including flavonoids, hydroxycinnamates, carotenoids, and plasma antioxidants. The preformed radical monocation of 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{\bullet+}$) is generated by oxidation of $ABTS^{\bullet+}$ with potassium persulfate and is reduced in the presence of such hydrogen donating antioxidants. The activity of the testing sample extract is expressed as a micro molar equivalent of butylated hydroxytoluene. The effect of methanol and aqueous extract of *Hemidesmus hamiltonii* on $ABTS^{\bullet+}$ cation radical scavenging activity is shown in Table 1, and the plant extract revealed that the higher total antioxidant activity of 11108.9 and 2720.1 µmol/g. Polyphenols are the major plant compounds with antioxidant activity, although they are not the only ones. The antioxidant activity of phenolic compounds is reported to be mainly due to their redox properties [16,17], which can play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides.

CONCLUSION

Antioxidant-rich plant extracts serve as sources of nutraceuticals that alleviate the oxidative stress and therefore prevent or slow down the degenerative diseases. An effort has been made to explore the antioxidant properties of the leaf extract *H. indicus*. This indicates the potential of the extracts, as a source of natural antioxidants or nutraceuticals with potential application to reduce oxidative stress with consequent health benefits. Aqueous extract of *H. indicus* was found to be higher than that of the other solvent extracts. The free radical scavenging activity of the plant extracts may be due to the presence of phytoconstituents present in the plant.

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