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In vitro antioxidant activity of ethyl acetate and methanolic extracts of *Hedyotis umbellata* linn

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ABSTRACT

In this study, the antioxidant activity of ethyl acetate and methanolic extracts of *Hedyotis umbellata* was evaluated by various antioxidant assays, including total antioxidant, free radical scavenging, and reducing power method. The various antioxidant activities were compared to standard ascorbic acid. From the results, it is suggested that the ethyl acetate and methonolic extracts of aerial parts of *H.umbellata* having strong antioxidant activity in all the tested methods. In addition to the anti oxidant activities of these extracts their chemical constituents were also found. Based on the results, it is concluded that the antioxidant activities may be due to the presence of phenolic compounds and flavonoids in the extracts.

KEYWORDS

Hedyotis umbellata;
Rubiaceae;
Ascorbic acid;
Antioxidant activity;
In vitro.

1. INTRODUCTION

Oxidative stress, induced by oxygen radicals, is believed to be a primary factor in various degenerative diseases such as cancer[1], atherosclerosis[2], gastric ulcer[3] and other conditions[4]. Recently, interest has increased considerably in finding naturally occurring antioxidants for use in foods or medicinal materials to replace synthetic antioxidants, which are being restricted due to their side effects such as carcinogenicity[5].

In the search of plants as a source of antioxidants, some medicinal plants and fruits have been extensively studied for their antioxidant activity and radical scavenging in the last few decades[6]. The plants of the genus Hedyotis (Rubiaceae) are widely distributed in most tropical and subtropical countries and have long been extensively used in folk medicine in India and most other countries for the treatment of a broad spectrum of disease, such as diabetes, various liver diseases, and expectorant and in the treatment of asthma[7].

This *Hedyotis umbellata* species are very common in Tamilnadu, and to our knowledge, this species has not previously been investigated chemically & pharmacologically.

2. EXPERIMENTAL

2.1. Plant material and chemicals

The fresh aerial parts of the plant (Rubiaceae) was collected from Tirunelveli (district), Tamilnadu, India, in the month of june 2007, and it was authenticated by Dr. D. Stephen, Department of Botany, The American college, Madurai, Tamilnadu, India. A voucher speci-

men is deposited in the department of pharmacognosy, A.K.College of pharmacy, Tamilnadu India. All the chemicals were purchased from S.D. Fine chemicals and all the solvents used were of A.R grade.

2.2. Preparation of plant extracts

The collected aerial parts were cleaned, dried under shade and pulverized into coarse powder. The powder was defatted with petroleum ether. One part of the defatted powder was macerated with ethyl acetate and another part of the defatted powder was macerated with methanol for 10 days at room temperature, and filtered. The filtrate was concentrated, and evaporated to dryness.

2.3. Phytochemical studies

All the extracts were subjected for phytochemical study[8].

2.4. DPPH radical scavenging activity

The free radical scavenging activity of the extracts, based on the scavenging activity of the stable 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical, was determined by the method described by Braca et al[9]. Plant extract (0.1 ml) was added to 3 ml of 0.004% methanolic solution of DPPH. Absorbance at 517 nm was determined after 30 min, and the percentage inhibition activity was calculated from $[(A_0-A_1)/A_0] \times 100$, where A_0 is the absorbance of the control and A_1 is the absorbance of the extract / standard.

2.5. Determination of total antioxidant capacity

The total antioxidant capacity of the extracts was evaluated by the phosphomolybdenum method according to the procedure of Prieto, Pineda, and Aguilar[10]. The assay is based on the reduction of Mo (VI)-Mo (V) by the extracts and subsequent formation of a green phosphate/Mo (V) complex at acid pH. 0.3 ml of extract was combined with 3 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes containing the reaction mixture were incubated at 95^0C for 90 min. Then the absorbance of the solution was measured at 695 nm using a spectrophotometer. Methanol (0.3 ml) in the place of extract is used as the blank. The total antioxidant capacity is expressed as the number of equivalents of ascorbic acid.

2.6. Reducing power determination

The reducing power of ethyl acetate and methanolic extracts were determined according to the method of Oyaizu[11]. Different amounts of ethyl acetate and methanolic extracts (50-250µg) in 1ml of methanol were mixed with phosphate buffer (2.5ml, 0.2mol/L, pH 6.6) and potassium ferricyanide $[K_3Fe(CN)_6]$ (2.5 ml, 1%). The mixture was incubated at 50^0C for 20 min. A portion (2.5ml) of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged (650 × g at room temperature) for 10 min. The upper layer of solution (2.5ml) was mixed with distilled water (2.5ml) and $FeCl_3$ (0.5ml, 0.1%), and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power.

2.7. Statistical analysis

All the values are expressed as mean ± S.E.M. of three parallel measurements. The data were analyzed statistically by student's t-test.

3. RESULTS AND DISCUSSIONS

3.1. Phytochemical study

Both the extracts were subjected for phytochemical study. Ethyl acetate extracts showed the presence of phenolic compounds, proteins, amino acids and flavonoids. The methanolic extracts showed the presence of alkaloids, glycosides, carbohydrates and flavonoids.

3.2. DPPH radical scavenging activity

DPPH is usually used as a reagent to evaluate free radical scavenging activity of antioxidants[11]. DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule[12]. The reduction capability of DPPH radical is determined by the decrease in absorbance at 517 nm induced by antioxidants. Ascorbic acid is used as standard. The extracts are able to reduce the stable radical DPPH to the yellow-colored diphenylpicrylhydrazine. A 200µg/ml of ethyl acetate, methanolic and ascorbic acid exhibits 74.58%, 82.46% and 94.30% inhibition respectively. The experimental data of this plant reveal that all these extracts are likely to have the effect of scavenging free radical. From the TABLE 1 it's observed that a dose-response relationship is found in the DPPH radi-

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TABLE 1: Scavenging effect of ethyl acetate and methanolic extracts of aerial parts of *Hedyotis umbellata* against 1, 1-diphenyl-2-picrylhydrazyl radical

S. no	Concentrations (µg/ml)	% Inhibition		
		Ascorbic acid	Ethyl acetate extract	Methanolic extract
1.	50	61.94±0.001	46.54±0.001	57.32± 0.002
2.	100	69.48±0.004	58.64± 0.004	63.44± 0.001
3.	150	73.21±0.02	65.41± 0.03	68.72± 0.005
4.	200	94.30±0.03	74.58±0.01	82.46± 0.07
5.	250	94.30±0.01	74.58±0.002	82.46± 0.01

Results are mean ± S.E.M.of three parallel measurements

TABLE 2: Total antioxidant capacity of ethyl acetate and methanolic extracts of aerial parts of *Hedyotis umbellata*

S. no	Extracts	Yield (%)	Total antioxidant capacity equivalent to ascorbic acid mg/g plant extract
1.	Ethyl acetate	10.08	26.12 ± 0.146
2.	Methanol	14.21	30.46 ± 0.109

Results are mean ± S.E.M.of three parallel measurements

TABLE 3: Reducing power determination of ethyl acetate and methanolic extracts of aerial parts of *Hedyotis umbellata*

S. no.	Concentrations (µg/ml)	Absorbance		
		Ascorbic acid	Ethyl acetate extract	Methanolic extract
1.	50	0.08±0.033	0.033±0.05	0.022±0.01
2.	100	0.083±0.02	0.0355±0.04	0.023±0.02
3.	150	0.12±0.007	0.04±0.03	0.032±0.001
4.	200	0.224±0.07	0.0455±0.004	0.041±0.003
5.	250	0.246±0.01	0.0625±0.002	0.044±0.01

Results are mean ± S.E.M.of three parallel measurements

cal scavenging activity; the activity increases as the concentration increased for each extracts. It has been found that cysteine, glutathione, ascorbic acid, tocopherol, flavonoids, tannins, and aromatic amines (p-phenylene diamine p-amino phenol, etc.), reduce and decolorize DPPH by their hydrogen donating ability[13]. Phenolic compounds and flavonoids of the *H.umbellata* extracts are probably involved in their antiradical activity.

3.3. Total antioxidant capacity

The yield (%) of ethyl acetate and methanolic extracts of *H.umbellata* and their total antioxidant capacity are given in TABLE 2. Total antioxidant capacity of *H.umbellata* is expressed as the number of equivalents of ascorbic acid. The phosphomolybdenum method was based on the reduction of Mo (VI)-Mo (V) by the antioxidant compound and the formation of a green phosphate Mo (V) complex with a maximal absorption at 695 nm. The assay is successfully used to quantify vitamin E in seeds and, being simple and independent of other antioxidant measurements commonly employed, it was decided to extend its application to plant extracts[10]. It is a quantitative one, since the antioxidant activity was expressed as the number of equivalents of ascorbic acid. The total antioxidant capacity of methanolic extracts is more, than the ethyl acetate extracts.

3.4. Reducing power determination.

TABLE 3 shows the reductive capability of two extracts compared to ascorbic acid; it has been investigated from the Fe^{3+}-Fe^{2+} transformation in the presence of extract samples using the method followed by Oyaizu[11]. Earlier authors have observed a direct correlation between antioxidant activity and reducing power of certain plant extracts[14, 15]. The antioxidant activity of antioxidants has been attributed by various mechanisms, among which some of them are prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, reductive capacity and radical scavenging[16]. Like the antioxidant activity, the reducing power of ethyl acetate and methanolic extracts increases with increasing volume of sample.

4. CONCLUSIONS

The results from various free radicals scavenging systems reveal that the *Hedyotis umbellata* has significant antioxidant activity, which can be attributed due to the presence of various chemical components. Further studies are warranted for the isolation and identification of individual phenolic and flavonoids compounds and also in vivo studies are needed for better understanding their mechanism of action as an antioxidant.

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