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Research Article

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Anthelmintic and Antioxidant Activity Study of Aerial Parts of Hedyotis Corymbosa

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Abstract This study was conducted to investigate phytochemical constituents, anthelmintic and antioxidant activity of aerial parts of *Hedyotis corymbosa*. Phytochemical constituents were determined by qualitative chemical tests and carbohydrate, glycoside and flavonoid were found in the methanolic and ether extracts of the aerial parts. In vitro anthelmintic activity was carried out against Pheretima posthuma. The parameters like: time of paralysis and time of death were determined by using the extract at the concentrations of 10, 25 and 50 mg/ml. The extract exhibited significant anthelmintic activity at highest concentration of 50 mg/ml as compared with piperazine citrate (10 mg/ml) as standard reference and distilled water as control. For antioxidant properties- total phenols, total flavonoids, total antioxidants, DPPH free radical scavenging capacity, cupric reducing antioxidant capacity (CUPRAC) and reducing power of the extracts of the sample were assessed. From the antioxidant study it was found that the plant has poor antioxidant activity.

Keywords *Hedyotis corymbosa*, Methanolic and Ether Extract, Anthelmintic, Antioxidant Activity

Introduction

Helminth infections in human are one of the most common parasitic infections disturbing a huge population around the world. During the past decade there have been major efforts to plan, implement, and sustain measures for reducing the burden of human disease that accompanies helminth infections [1]. A wide variety of anthelmintics are used for the treatment of helminths in animals and in human. However, majority of gastro-intestinal helminthes becomes resistant to currently available anthelmintic drugs. Moreover synthetic drugs used in helminthiasis treatment have some potential side effects. Hence there is an increasing demand towards natural anthelmintics [2-4].
There is an emerging interest in the use of naturally occurring anti-oxidant for the preservation of food and in the management of number of patho-physiological condition, most of which involve free radical damage. Recent reports indicated that there is an inverse relationship between the dietary intake of anti-oxidant rich food and incidence of human diseases.
Hedyotis corymbosa belongs to the family of Rubiaceae. It has been used in the indigenous system of medicine for the treatment of various ailments. Leaves are used in fever and liver diseases, Traditionally it is also used in treatment of tonsilitis, pharyngitis, bronchitis, pneumonia, mump, appendicitis, hepatitis, cholecystitis, pelvic inflammation, urinary tract infections, boils, ulcers, cancer, lymphosarcoma, breast cancer, rectum fibrosarcoma. We in these studies aimed to investigate the anthelmintic and antioxidant activity of the aerial parts of the *Hedyotis*

corymbosa using different *in vitro* methods in order to evaluate a relationship of the anthelmintic and antioxidant activity with the phytochemical constituents.

Materials and Methods

Chemicals: DPPH (1, 1-diphenyl, 2-picrylhydrazyl), Ferric chloride, Gallic acid and Quercetin were obtained from Sigma Chemical Co. USA. Ascorbic acid and Aluminium chloride were obtained from SD Fine Chem. Ltd. India. Folin - ciocalteu reagent, Methanol, Sodium carbonate, Potassium acetate, Concentrated H_2SO_4, Sodium phosphate, Ammonium molybdate, Cupric chloride ($CuCl_2.2H_2O$), Potassium ferricyanide, Trichloroacetic acid were purchased from Merck, Germany.

Plant Material

For this present investigation the *Hedyotis corymbosa* was collected from Jahangirnagar University, Savar, Dhaka and was identified at the Bangladesh National Herbarium, Mirpur, Dhaka where the Voucher specimen no: 35344 has been deposited. Immediately after collection the whole plant was thoroughly washed with water. Then the aerial parts of the plant were dried under shade for 2 days and were ground to coarse powder with a mechanical grinder. The powdered plant was extracted individually with methanol and ether in a Soxhlet apparatus. The mixture was filtered and the filtrate was concentrated in Rotaevaporator to yield semisolid mass. The extracts were preserved in refrigerator till further use.

Experimental Procedure

All the following experiments were repeated two times and the results were averaged.

Phytochemical Screening

The presences of different chemical constituents were identified by the characteristic color changes using standard procedures. Freshly prepared crude extracts of *Hedyotis corymbosa* were qualitatively tested for the presence of chemical constituents using the following reagents and chemicals: Carbohydrates with Molisch's reagents, glycoside with Fehling's solutions alkaloids with Dragendorff's reagent, flavonoids with the use of Mg and HCl; tannins with ferric chloride and potassium dichromate solutions and saponins by the ability to produce stable foam after shaking and reducing sugars with Benedict's reagent and color changes was observed in respective cases [5].

Anthelmintic Assay

The anthelmintic activity was evaluated according to the method of Ajayieoba E. O. et al. [6] with minor modifications. In this study adult Earthworm (*Pheretima posthuma*) was used due to its anatomical and resemblance with the intestinal roundworm parasites of human being [7]. Three groups of equal sized earthworms consisting of six earthworms in each group were released in 50ml of sample with desired concentrations, (10, 25 and 50 mg/ml). Group of earthworms in saline solution was used as Control group and Group of earthworms in Piperazine citrate (10mg/ml) was used as Reference. Observations were made for the time taken to paralysis and death of individual worms. Paralysis as said to occur when no movement of any sort could be observed except the worms was shaken vigorously. Time for death of worms was recorded after death and was conformed when the worms neither moved when shaken vigorously nor when dipped in warm water at 50 o C.

Determination of Total Phenolic Content

Total phenolic content of the plant extracts was determined according to the method described by Yu et al. [8]. 1 ml each of Plant extracts (200µg/ml) was mixed with 5 ml of Folin-ciocalteu (Diluted 10 fold) reagent solution into a test tube. Then 4 ml of 20% Sodium carbonate solution was added and shaken thoroughly. The mixture was then allowed to stands for 1 hour. The absorbance was measured at 765 nm using a UV-VIS spectrophotometer against blank. Then the concentration of the total phenol content was determined as mg of Gallic acid equivalent using an equation that was obtained from standard Gallic acid graph.

Determination of Flavonoid Content

Flavonoid content of the plant extracts was determined according to the method described by Kumaran and Karunakaran [9]. 1.0 ml each of plant extracts (200µg/ml) was taken in test tube and 3 ml of methanol was added into the test tube. Then 200 µl of 10% aluminium chloride solution and 200 µ1 of 1M potassium acetate solution were added to the mixtures in the test tube. Then 5.6 ml of distilled water was added into the test tube and was incubated for 30 minutes at room temperature to complete reaction. Then the Standard (Quercetin) solutions of different concentration were prepared at the same manner. The absorbance of the solutions was measured at 415 nm using a UV-VIS spectrophotometer against blank. Then the concentration of the Flavonoid content was determined as mg of Quercetin equivalent using an equation that was obtained from standard Quercetin graph.

Determination of Total Antioxidant Capacity

Total antioxidant capacity of the plant extract was determined using the method described by Prieto et al. [10]. The assay is based on the reduction of Mo (vi) to Mo(v) by the extract and formation of a green Phosphate/ Mo(v) complex at acid pH. 300µl of extract solution was taken in a test tube and 3 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) was added into the test tube. The test tube was incubated at 95 °C for 90 minutes to complete reaction. The Standard (Ascorbic Acid) solutions of different concentration were prepared at the same manner. Then the absorbance of the solutions was measured at 695 nm using a UV-VIS spectrophotometer against blank after cooling to room temperature. For the preparation of blank solution 300µl Methanol was used instead of sample. The antioxidant capacity was expressed as the number of gram equavalents of Ascorbic acid.

DPPH Radical Scavenging Assay

DPPH free radical scavenging activity of the plant extract was determined following the method described by Braca et al. [11]. Free radical scavenging activity of the plant extracts was determined using stable free radical 1, 1-diphenyl-2-picrylhydrazyl (DPPH). Plant extract (0.1 ml) was added to 3ml of a 0.004% methanol solution of DPPH. Absorbance at 517nm was determined after 30 min using a UV-VIS spectrophotometer and the percentage inhibition activity was calculated from [(A0–A1)/A0] x100, where A0 is the absorbance of the control, and A1 is the absorbance of the extract/ standard. The inhibition curves were prepared and IC50 values were obtained.

Cupric Reducing Antioxidant Capacity (CUPRAC)

Cupric reducing antioxidant capacity of the plant extracts was determined following the method described by Resat et al. [12]. 500µl each of plant extracts (5µg/µl) was taken in a test tube and 1.0 ml of 0.01 M $CuCl_2$-$2H_2O$ solution and 1.0 ml of ammonium acetate buffer, pH 7.0 was added into the test tube. Then 1.0 ml of 0.0075 M of neocaproin solution and 600 µl of distilled water was added to adjust the final volume 4.1 ml. After 1 hour the absorbance of the solution was measured at 450 nm using a UV-VIS spectrophotometer. The molar absorptivitiy of the CUPRAC method was obtained from the slope of the calibration line of Standard Ascorbic acid.

Reducing Power Capacity Assessment

Reducing power of the extracts was evaluated following the method described by Oyaizu [13]. Different concentrations of the plant extracts (125, 250, 500, and 1000 µg/ml) in 1 ml of distilled water were taken in different test tubes. Then 2.5 ml of Phosphate buffer (0.2 M, Ph 6.6) and 2.5 ml of 1% w/v potassium ferricyanide [$K_3Fe(CN)_6$] solution were mixed. Then the mixture was incubated at 50 °C for 2 minutes. After incubation 2.5 ml of 10% Trichloro Acetic Acid solution was added and was Centrifuged at 3000 rpm for 10 min. 5 ml of the upper layer solution was mixed with 5 ml of distilled water and 1 ml of Ferric chloride ($FeCl_3$) solution (1%w/v) and the absorbance was measured at 700 nm using a UV-VIS spectrophotometer against a blank (Phosphate buffer). The reducing power of the extract of linearly proportional to the concentration of the sample. Ascorbic acid was taken as reference standard.

Results and Discussion

Phytochemical Screening

The results of phytochemical screening of the crude extracts reveal the presence of carbohydrates; glycoside and flavonoids (Table 1).

Table 1: Results of chemical group test of the crude extract of *Hedyotis corymbosa*

Name of test	Results
Test for carbohydrates	Positive (+)
Test for reducing sugar	Negative (-)
Test for glycosides	Positive (+)
Tests for alkaloids	Negative (-)
Test for saponins	Negative (-)
Test for flavonoids	Positive (+)
Test for tannins	Negative (-)

Anthelmintic Activity Evaluation

The methanolic and ether extracts of the aerial parts of *H. corymbosa* showed significant (Maximum activity given at the concentration of 50 mg/ml) anthelmintic activity. The activity increased with the increase of concentration (10 to 50mg/ml) of the test sample. Observations of the experiment are given in the table 2.

Table 2: Anthelmintic activity of standard drug and sample

Treatment	Concentration (mg/ml)	Time for paralysis (minute)	Time for death (minute)
Saline water (Control)	10	No paralysis	No death observed
Piperazine citrate (Standard)	10	24 ± 0.87	38 ± 0.63
Methanolic fraction	10	69.25	> 90
	25	32.12	60.49
	50	15.24	25.61
Ether fraction	10	75.86	> 90
	25	39.75	64.72
	50	20.31	30.25

Total Phenol Content

The Phenol content of the *H. corymbosa* was determined by using the calibration curve of Gallic acid and the value was expressed as Gallic acid equivalent (GE) which was 21.74±4.37 and 13.04±5.64 mg/gm for methanolic and ether extracts respectively.

Total Flavonoid Content

Flavonoids are a large clsss of benzo-pyrone derivatives, ubiquitous in plants and exhibit antioxidant activity. Flavonoids content of the *H. corymbosa* was expressed as Quercetin equivalent (QE) and the value was 56.58±3.25 and 67.04±4.33 mg/gm for methanolic and ether extracts respectively.

Total Antioxidant Capacity

Total antioxidant capacity of the *H. corymbosa* was calculated using the standard curve of ascorbic acid was expressed as ascorbic acid equivalents (AAE) per gram of plant extract. The value was 15.00±5.34 and 31.31±4.72 mg/gm for methanolic and ether extracts respectively.

DPPH Radical Scavenging Activity

The plant extract demonstrated H donor function and showed dose dependent free radical scavenging activity. The percentage inhibition is shown in fig. 1. The extracts possessed DPPH radical scavenging activity (IC_{50}= 277.36 and 252.46 for methanolic and ether extracts respectively) compared with standard Ascorbic acid (IC_{50}= 8.81)

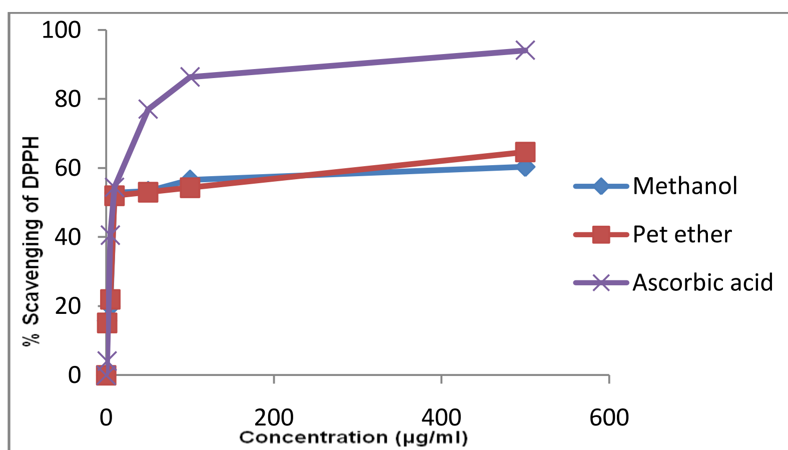

Figure 1: DPPH radical scavenging activity of the methanolic and ether extracts of H. corymbosa.

Cupric Reducing Antioxidant Capacity (CUPRAC)

Reduction of Cu^{2+} ions was found to rise with increasing concentrations of the different extractives of the aerial parts of *H. corymbosa*. All extracts produced a dose dependent reduction of Cu^{2+} in a way similar to the standard antioxidant ascorbic acid. Methanolic extract showed weak to moderate Cu^{2+} ion reducing capacity. However, ether fraction showed highest Cu^{2+} ion reducing capacity (fig: 2).

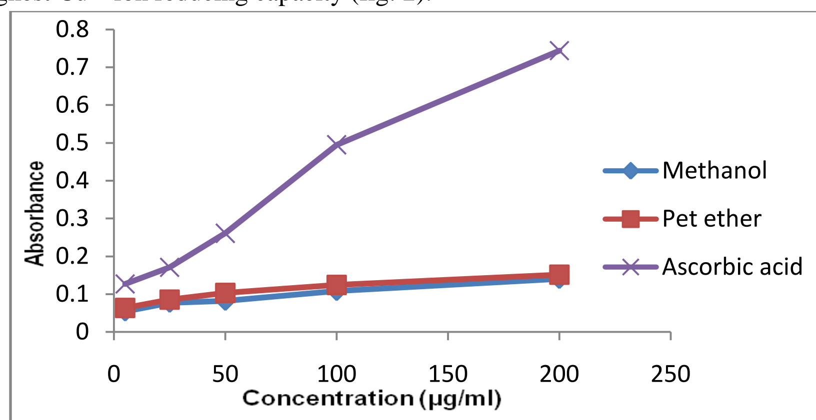

Figure 2: Cupric Reducing Antioxidant Capacity (CUPRAC) of the methanolic and ether extracts of H. corymbosa

Reducing Power Capacity

The extracts showed significant reducing power activity as compared to standard Ascorbic acid and the absorbance was proportionally increased with the increasing concentration of the extracts which is shown in the fig: 3.

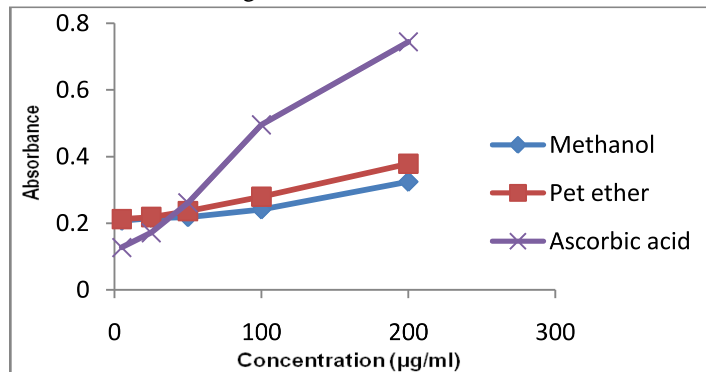

Figure 3: Reducing power of the methanolic and ether extracts of H. corymbosa

Discussion

Preliminary phytochemical screening of crude extract of *H. corymbosa* revealed the presence of various bioactive components like flavonoids, glycoside and carbohydrate (Table 1).

Our investigational result presented above demonstrated that methanolic and ether extracts of the aerial parts of *H. corymbosa* can cause paralysis as well as death of worms at a time comparable to reference standard drug especially at the concentration of 50mg/ml in dose dependent manner. Standard drug Piperazine citrate cause a flaccid paralysis on the warm that result in expulsion of the worm by peristalsis. Piperazine citrate, by increasing chloride ion conductance of worm muscle membrane produces hyperpolarisation and reduced excitability that leads to muscle relaxation and flaccid paralysis [14]. Polyphenolic compounds shown anthelmintic activity [15]. Another possible anthelmintic effect of tannins is that they can bind to free protein in the gastrointestinal tract of host animal or glycoprotein on the cuticle of the parasite and cause death [16].

Phenolic compounds and flavonoids have been reported to be associated with antioxidative action in biological systems, acting as scavengers of singlet oxygen and free radicals [17]. Among the numerous naturally occurring antioxidants; ascorbic acid, carotenoids and phenolic compounds are more effective. They are known to inhibit lipid peroxidation, to scavenge free radicals and active oxygen species by propagating a reaction cycle and to chelate heavy metal ions. The antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play an important role in adsorbing and neutralising free radicals, quenching singlet and triplet oxygen, or decomposing peroxides. Total antioxidant capacity is expressed as the number of equivalents of ascorbic acid. The phosphomolybdenum method is based on the reduction of Mo (VI) to Mo (V) by the antioxidant compound and the formation of a green phosphate/Mo (V) complex with a maximal absorption at 695 nm.

Antioxidant effect often correlates with reductive activity. In the reducing power assay, the presence of antioxidants in the samples results in the reduction of the ferric cyanide complex to the ferrous form which can be monitored by measuring the formation of Pearl's Prussian blue at 700 nm. The increased absorbance at 700 nm indicates an increase in reducing power of samples.

Conclusion

The present study suggests that the plant extracts have significant anthelmintic activity but poor antioxidant activity. Since a variety of constituents are known from the extracts studied, it becomes difficult to ascribe the anthelmintic properties selectively to any one group of constituents without further studies which are beyond the scope of this paper. Thus, further extensive investigations are needed to be carried out using higher animal models to evaluate anthelmintic activity.

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