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Research Article

Phytochemical and Comparative Antioxidant Evaluation by DPPH and Reducing Power Assay of *Hybanthus Enneaspermus*

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Abstract

The objective of the present study was to evaluate the phytochemical constitution and antioxidant activity of extract of *Hybanthus enneaspermus*. Phytochemical analysis was performed to estimate tannins, flavonoids and alkaloids. Antioxidant activity was performed using DPPH and reducing power assay. The phytochemical screening revealed that the extract contain terpenes, flavonoids and alkaloids. Antioxidant activity was performed due to presence of phenolic compounds; flavonoids which may possess significant antioxidant potential. The results suggested that *Hybanthus enneaspermus* has promising antioxidant activity and could serve as potential source of natural antioxidants.

Keywords: Hybanthus enneaspermus; Antioxidant activity; Flavonoids; DPPH and reducing power assay

Introduction

Free radicals are chemical species contains unpaired electrons which makes them highly reactive towards biological molecules. Free radicals are produced in the human body due to the various detoxification processes [1]. Ultraviolet light, radiation and metabolic pro-cesses can induce the production of free radicals. Free radicals can react with protein, lipid and DNA and may cause tissue destruction which leads various diseases. Antioxidants are the compounds which reduce free radical activities and thus used for the treatment of many diseases induced by oxidative destruction of tissue. Antioxidants neutralize or scavenge reactive free radical species by hydrogen donation [2]. Medicinal plants contain phenolic compounds; tannins, flavonoids are known to have antioxidant property [3]. The antioxi-dant activity of phenolics is principally due to their redox properties, which allow them to act as reducing agents and hydrogen donors. Phenolic compounds play an important in the treatment of cancer, heart diseases and ageing [4].

Hybanthus enneaspermus (Linn) F. Mull is a *violaceae* family plant distributed in the tropical and sub tropical regions, 15-30 cm in height [5], used to treat diarrhea, urinary tract infections and diabetes. Chemically it possess many bioactive components such as phenol, alkaloids and flavanoids [6], due to the presence of phenolics compounds present investigation was carried out which involve phytochem-ical screening and estimation of antioxidant activity of plant.

Materials and Methods

Plant was collected in the month of December. The collected plants were open-air-dried under the shade, pulverized in to a moderately coarse powder and stored for further use.

Preparation of Plant Extract

Powdered plant material was extracted with methanol. After 24 hours the supernatant was collected by filtration and the solvent was evaporated to make the crude extract. The residues obtained were stored in airtight bottles in a refrigerator for further use.

Preliminary Phytochemical Screening

The methanolic extract of plant was subjected to different chemical tests for the detection of different phytoconstituents using standard procedures [7,8].

Test for Tannins

1 ml of the sample was taken in a test tube and then 1 ml of 0.008 M Potassium ferricyanide was added. 1 ml of 0.02 M Ferric chloride containing 0.1 N HCl was added and observed for blue-black coloration.

Test for Flavonoids

5 ml of dilute ammonia solution was added to a portion of the crude extract followed by addition of concentrated H_2SO_4. A yellow coloration observed in extract for flavonoids. The yellow coloration disappeared on standing.

Test for Steroids

2 ml of acetic anhydride was added to 0.5 ml crude extract of plant sample with 2 ml H_2S0_4. The colour changed from violet to blue or green in samples indicates the presence of steroids.

Test for Alkaloids

Crude extract was mixed with 2 ml of Wagner's reagent. Reddish brown colored precipitate was observed for the presence of alkaloids.

Test for Terpenoids

5 ml of extract was mixed with 2 ml of chloroform and 3 ml of concentrated H_2S0_4 was carefully added to form a layer. A reddish brown coloration of the inter face was observed for the presence of terpenoids.

Determination of Total Phenolic Content

Total phenolic content of the methanolic extract of plant was determined by standard method [9] with little modifications, using tan-nic acid as a standard phenolic compound. The extract was diluted with distilled water to a known concentration in order to obtain the readings within the standard curve range of 0.0 to 600 µg of tannic acid/ml. 250 µl of diluted extract or tannic acid solution was mixed with 1 ml of distilled water in a test tube followed by the addition of 250 µl of Folin - Ciocalteu reagent. The sample was mixed well and then allowed to for 5 min at room temperature in order to allow complete reaction with the Folin-Ciocalteu reagent. Then, 2.5 ml of 7 % sodium carbonate aqueous solution was added and the final volume was made up to 6 ml with distilled water. The absorbance of the resulting blue colour solution was measured at 760 nm using spectrophotometer after incubating the sample for 90 min.

In Vitro Antioxidant Activity

DPPH Radical Scavenging Assay

The DPPH radical scavenging method was used to evaluate the antioxidant property. The antioxidant activity was compared with that of the natural antioxidant, ascorbic acid. The antioxidant activity of was expressed in terms of IC_{50} and was calculated [10]. 1.5 ml of 0.1 mM DPPH solution was mixed with 1.5 ml of various concentrations (10 to 500 µg/ml) of extract. The mixture was shaken vigorously and incubated at room temperature for 30 min in the dark. The reduction of the DPPH free radical was measured by reading the absorbance at 517 nm by a spectrophotometer. The solution without any extract and with DPPH and methanol was used as control. Inhibition of DPPH free radical in percentage was calculated by the formula:

$$\text{Inhibition (\%)} = [(A_{control}-A_{test})/A_{control}] \times 100$$

Where; $A_{control}$ is the absorbance of the control (L-Ascorbic acid)

A_{test} is the absorbance of reaction mixture sample (in the presence of sample).

Determination of Reducing Property

The reducing power of the plant extract was determined by a slightly modified method [11]. 1 ml of each plant extract concentra-tion (0.1, 0.5 and 1 mg/ml) was mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (2.5 ml, 1 %). The mixtures were then incubated at 50°C for 20 min. Aliquots (2.5 ml) of trichloroacetic acid (10 %) was added, then centrifuged for 10 min at. The upper layer of the solutions (2.5 ml) was mixed separately with distilled water (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1 %), and the absorbance levels were measured at 700 nm using a spectrophotometer. Methanol was used instead of the extract as a control. BHT (Butylated Hydroxy Toluene) was used as positive control.

Results and Discussion

The result of the preliminary phytochemical screening was carried out on the plant extract and revealed the presence of alkaloids, flavonoids, tannins and terpenoids as showed in Table 1. Total Phenolic Content was also estimated by using modified Folin-Ciocalteu calorimetric method. Tannic acid was taken as standard. Total phenolic content in plant extract was found to be 19.71 %. Antioxidant activity was measured using DPPH and Reducing power assay. Generally DPPH scavenging activity increased with increasing phenolic components such as flavonoids, phenolic acids, and phenolic diterpenes. These phenolic components possess many hydroxyl groups including dihydroxy group which have very strong radical scavenging effect and antioxidant power. The dose response curve of DPPH radical scavenging activity of extract of plant was observed, when compared with standard ascorbic acid and shown in Figure 1. Potent antioxidant activity was observed in dose dependent manner and presence of polyphenolic groups can be attributed to the radical scav-enging activity. Reducing power assay was also performed to establish prominent antioxidant activity of plant material. In this assay, the yellow colour of the test solution changes to green and blue. Increased absorbance of the reaction mixture indicated increased reducing power of the extract. The reducing power of extract increased with its concentration. Result revealed that extract possess potent antioxi-dant property since it reduces free radicals promptly.

Phytochemicals	Present/Absent
Tannins	+
Flavonoids	+
Steroids	+
Alkaloids	+
Terpenoids	+

Table 1: Results of Phytochemical Analysis.

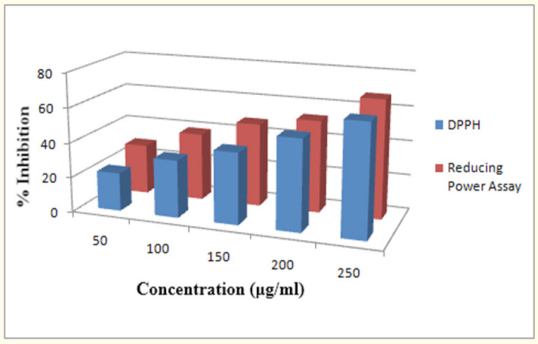

Figure 1: Comparison of DPPH and Reducing Power Assay of Extract.

Conclusion

Phytochemical screening of plant extract revealed the presence of flavonoids, terpenoids and alkaloids. Results obtained in this investigation indicated that extract possess potent antioxidant activity due to the presence of phenolics. The finding of this study suggests that this plant can be used as potential source of natural antioxidant for the treatment of oxidative stress related degenerative diseases. Further investigation on the isolation and characterization of the antioxidant constituents is also required.

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