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Research Article

Qualitative Phytochemical Screening and *In vitro* Antioxidant Activity of *Hybanthus enneaspermus*

Anupa M P[1], Chinju S[1], Murugan M[2*]

[1]*Department of Biotechnology, Noorul Islam College of Arts and Science, Kumaracoil -629 180, Tamil Nadu, India*
[2]*Centre for Biological Science, Noorul Islam Centre for Higher Education, Kumaracoil -629 180, Tamil Nadu, India*

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ABSTRACT

The present study was aimed to determine the phytochemical constituents and antioxidant activity of different extracts of *Hybanthus enneaspermus* (L). Dried plant leaf was extracted with different solvents such as hexane, chloroform, ethyl acetate, ethanol and distilled water. Phytochemical screening was performed with standard protocols and this study showed the presence of flavonoids, terpenoids, tannins, phenol and saponin. Also the ethanol and aqueous extract contained more phytochemicals. Antioxidant activity of the ethanol extract was determined by DPPH assay, reducing power and hydrogen peroxide radical scavenging assay. The results showed that, the maximum radical scavenging activity in DPPH activity 24.32% and the standard was 45.41% at 60 mg/ml of concentration. The reducing power assay exhibited maximum absorbance of 1.038 at 60 mg/ml of concentration and in the hydrogen peroxide radical scavenging activity, the percentage of inhibition was 35.11%. Thus the ethanolic leaf extract of *H. enneaspermus* has a significant antioxidant activity and used as a better source of natural antioxidants which might be helpful in preventing the progress of oxidative stress.

Keywords: *Hybanthus enneaspermus*, phytochemicals, antioxidant activity

INTRODUCTION

Medicinal plants are an important part of natural wealth, serve as important therapeutic agents as well as valuable raw materials for manufacturing numerous traditional and modern medicines[1]. Phytomedicine applies scientific research and the highest proficient standards to the practice of herbal medicine[2]. Plant based drugs remain an important source of therapeutic agents because of the availability, relatively cheaper cost and non-toxic nature when compared to modern medicine and they have received considerable attention in recent years due to their diverse pharmacological properties[3]. The medicine plants contained a wide range of chemical substances (called as phytochemicals) that can be used to treat chronic as well as infectious diseases[4]. Phytochemicals are chemical compounds that occur naturally in plants. They are classified into two groups, such as primary metabolites and secondary metabolites. Primary metabolites (ethanol, lactic acid and aminoacids) are involved in growth, development and reproduction of the organism[5]. Secondary metabolites (alkaloids, antibiotics, naphthalene, nucleosides, phenazines, quionolines, terpenoids, peptides and growth factors) are found in a smaller range of plants, serving a more specific function[6]. Secondary metabolites often play an important role in plant defense against herbivory and other interspecies defenses. Humans use secondary metabolites as medicines, flavorings and recreational drugs[7].

Hybanthus is a genus of the family violacea, *H. enneaspermus* is a small perennial herb with a woody base and numerous diffuse or ascending branches, growing 10-20 cm tall. The plant is used as an aphrodisiac, demulcent, tonic, diuretic, anti-convulsant and antimalarial and used to treat urinary infections, diarrhoea, leucorrhoea, dysuria, inflammation and male sterility[8]. The plant is used to treat ailments such as, urinary calculi, painful dysentery, vomiting, burning sensation, blood troubles, asthma, epilepsy and breast tone[9]. This present study aimed to isolate the phytochemical constituents present in the plant of *Hybanthu enneaspermus* and study of its antioxidant activity.

MATERIAL AND METHODS

Plant sample

The fresh plant material of *H. enneaspermus* was collected from Thingal Nagar, Kanyakumari District, Tamil Nadu. It was washed thoroughly, dried completely at room temperature and made powder using mixer grinder.

Preparation of plant extract

The successive extraction of powered material was carried out with different solvents such as hexane, chloroform, ethyl acetate, ethanol and aqueous using soxhlet apparatus. The extracts were then concentrated by evaporating the solvent under reduced pressure and kept at 4˚C until use.

Qualitative phytochemical screening

The plant extracts were qualitatively analysed for the presence of different phytochemical constituents by standard protocols[5,10].

Detection of Flavonoids

Ferric chloride test: 2 ml of plant extract was treated with

**Author for Correspondence*

Table 1: Phytochemical constituents of *H.enneaspermus* leaf extracts

Tests/ Solvents	Hexane	Chloroform	Ethyl acetate	Ethanol	Aqueous
Flavonoids	-	-	-	+	+
Terpenoids	-	+	-	+	+
Tannins	-	+	+	+	+
Phenols	-	-	+	-	-
Saponins	-	-	-	+	+

'+' presence of compounds; '-' absence of compounds

few drops of $FeCl_3$ solution. The formation of blackish red colour indicating the presence of flavonoids.

Detection of Terpenoids

Salkowski test: To 1 ml of plant extract, 2 ml of chloroform and 3 ml of Concentrated H_2SO_4 were added. A reddish brown coloration of the interface indicates the presence of terpenoids.

Detection of Tannins

To 1 ml of plant extract, few drops of 1% $FeCl_3$ solution were added. The appearance of blue, black, green or blue green precipitate indicates the presence of tannins.

Detection of Phenols

Ferric Chloride Test: To 1ml of plant extract, 3ml of distilled H_2O was added. Then few drops of neutral 5% $FeCl_3$ solution were added. A dark green colour indicates the presence of phenolics.

Detection of Saponins

Foam test: About 2 ml of distilled water and 1ml of plant extract were mixed and shaken vigorously. A stable persistent forth indicates the presence of saponins.

In-vitro antioxidant activity

The antioxidant activities of the *H.enneaspermus* extracts were determined by various methods viz. DPPH assay, reducing power assay and hydrogen peroxide radical scavenging activity.

DPPH assay

The free radical scavenging activity was determined by using 1, 1-Diphenyl-2-picryl-hydrazyl (DPPH), protocol of Gadow *et al.*[11] 0.1 mM solution of DPPH in methanol was prepared and 1 ml of this solution was added to 3 ml of sample extract at different concentration (20, 40, 60 mg/ml). Also, the blank was prepared by adding plant extracts. Thirty minutes later, the absorbance was measured at 517 nm. DPPH solution in 3 ml of methanol.

Reducing power assay

Different concentrations of plant extracts (20, 40, 60 mg/ml) were mixed with 2.5 ml of phosphate buffer (200 mM, pH 6.6) in the test tubes. 100mg of ascorbic acid was dissolved in 1ml methanol and this was taken in the same concentration of test tubes in another five tubes. 2.5 ml of 1 % potassium ferricyanide was added to all the test tubes and boiled for 20 min at 50°C. After cooling to room temperature, 2.5 ml of 10% trichloroacetic acid were added to the mixtures, followed by centrifuged at 2000 rpm for 10 minutes. The upper layer (5 ml) was mixed with 5 ml of distilled water and 1 ml of 0.1 % ferric chloride and the absorbance of the resultant solution were measured at 700 nm[12].

Hydrogen peroxide radical scavenging activity

40mM hydrogen peroxide solution was prepared in phosphate buffer (pH 7.4). The concentration of hydrogen peroxide was determined by absorption at 230 nm using a spectrophotometer. The plant extracts at different concentrations (20, 40, 60 mg/ml) in distilled water were added to hydrogen peroxide solution. After 10 min the absorbance was read at 230 nm against a blank solution containing phosphate buffer without hydrogen peroxide.

RESULT AND DISCUSSION

Qualitative phytochemical screening

Phytochemical analysis was carried out in the leaf extracts of *H. enneaspermus* with different solvents such as hexane, chloroform, ethyl acetate, ethanol and distilled water. The results confirmed that, the phytochemical constituents such as flavonoids, terpenoids, tannin, phenol and saponin (Table 1). In this investigation, the ethanol and aqueous extracts showed positive result for four test and hexane showed none of positive. The present study was correlated with the findings of Anand *et al.*[13] reported that the preliminary phytochemical screening of the leaf extracts of H. *enneaspermus* confirmed the presence of phytochemical compounds such as terpenoids, phenols, saponins, flavonoids and tannins.

The medicinal worth of plants lies in some chemical substances that have a specific physiological action on the human body. Different phytochemicals have been found to possess a broad range of activities, which may help in protection against persistent diseases[14]. Flavonoids are present in the form of polyphenolic compounds that have potent antimicrobial[15], anti-inflammatory[16] activity. They prevent oxidative cell damage and also have strong anticancer activity[17]. Terpenoids are the myriad compounds used by humans in the food and pharmaceuticals[18]. Phenols are largest group of plant metabolites, which have many biological properties such as antiapoptosis, antiageing, anticarcinogen, anti-inflammation and cell proliferating activities[19]. Tannins have astringent properties, which accelerate the healing of wounds and inflamed mucous membrane due to their physiological activities such as anti-oxidant, antimicrobial and anti-inflammatory properties[20]. Saponins have traditionally used as foaming and surface active agents, which help in controlling cardiovascular diseases and in controlling cholesterol in humans[21]. Also have a wide range of medicinal applications[22].

In-vitro antioxidant activity

DPPH assay

In the present work, DPPH radicals were evaluated to determine the free radical scavenging capacity of *H. enneaspermus* leaf extracts and ascorbic acid was used as standard. It was observed that, higher scavenging activity of the plant extracts was observed at higher concentration

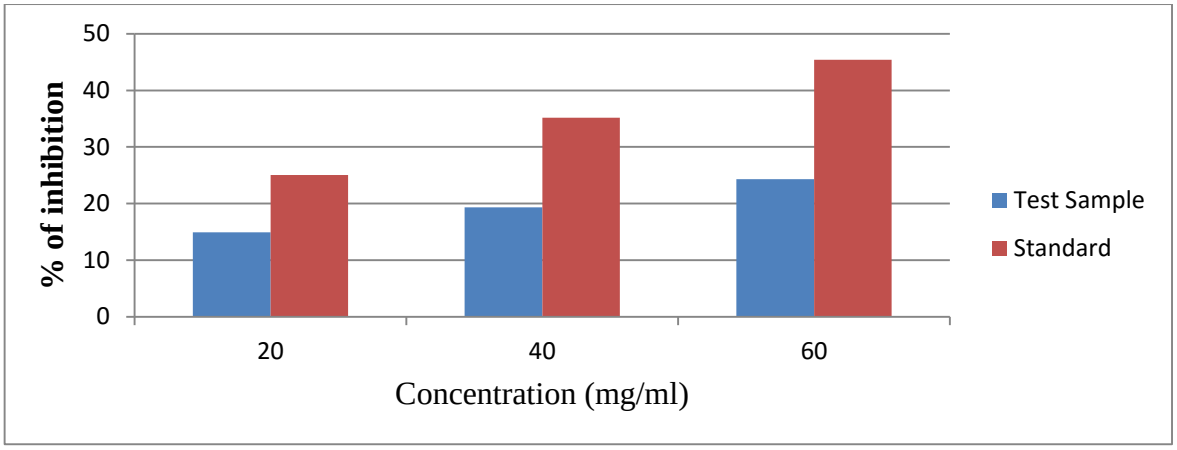

Chart 1: DPPH radical scavenging activity of *H. enneaspermus* (L)

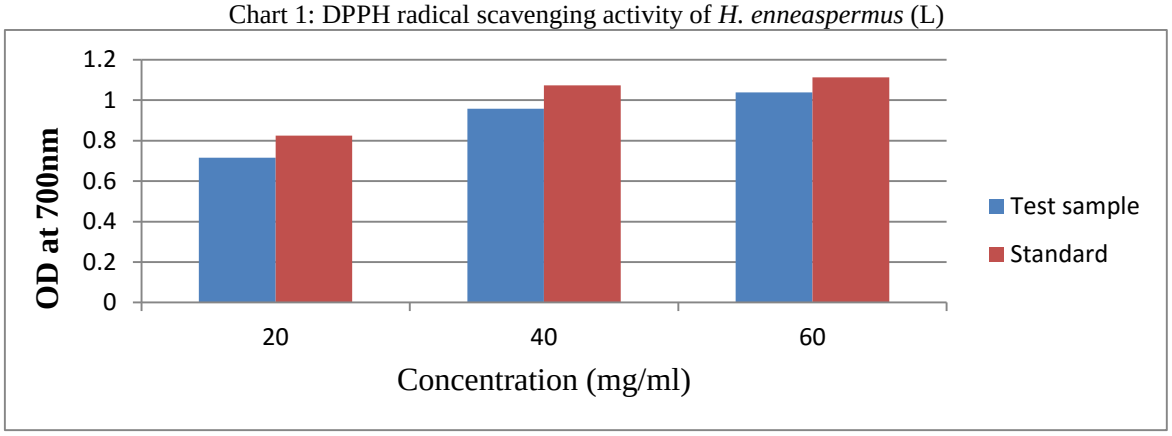

Chart 2: Reducing power assay of *H. enneaspermus* (L)

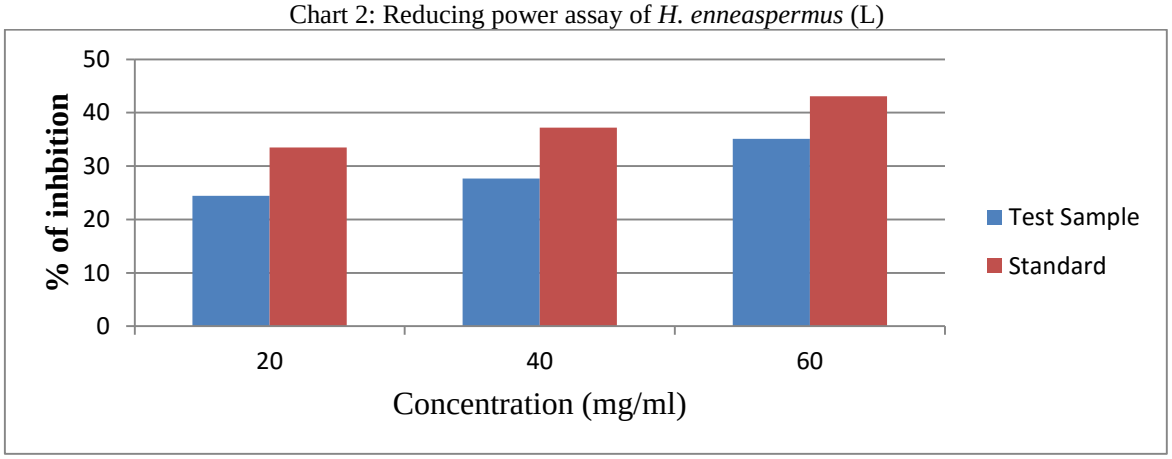

Chart 3: Hydrogen peroxide radical scavenging activity of *H. enneaspermus* (L)

of plant extract. At a concentration of 60 mg/ml, it was found to be 24.32%, and the same concentration the ascorbic acid result was 45.41 % of scavenging activity (Chart 1).

Reducing power assay

Ferric reducing power was determined as an indication of antioxidant activity. Antioxidants turned a coloured complex of potassium ferric cyanide in the presence of trichloro acetic acid and ferric chloride. Increase in the absorbance of the reaction mixture suggested an increase in the reducing power. In this study, the ethanolic leaf extract of *H. enneaspermus* exhibited a maximum absorbance of 1.038 at a concentration of 60 mg/ml. The activity of the extract was found to increase in a dose dependent manner. It possessed the significant activity compared with that of standard ascorbic acid where the absorbance was found to be 1.112 (Chart 2).

Hydrogen peroxide radical scavenging activity

The absorbance was found to be increased when plant dose increases. The ethanolic leaf extract of *H.enneaspermus* exhibited a percentage of inhibition 35.11 % at a maximum concentration of 60 mg/ml respectively. It possessed significant activity comparable with that of the standard drug ascorbic acid, when the percentage of inhibition was found to be 43.09 % (Chart 3).

In the present study was correlated with the findings of Dab *et al.*[23] reported that, the DPPH, nitric oxide and total antioxidant activity of *H.enneaspermus* was increased with

the percentage of inhibition at the concentration 500 µg/ml. The study was also compared with the findings of Setty *et al.*[24] reported that, the alcoholic and aqueous extracts *H.enneaspermus* showed significant free radical scavenging effect on reducing power assay and DPPH. The ethanolic leaf extract of *H. enneaspermus* exhibited potent antioxidant activity because of the presence of various phytochemical constituents. The presence of flavonoids and tannins likely to be responsible for the free radical scavenging effects observed and phenolics are a major group of compounds that act as primary oxidants or free radical scavengers[25].

CONCLUSION

The presence of phyto-constituents makes the plant useful for treating different ailments. In the present study, we have found that most of biologically active phytochemical were present in ethanolic leaf extract of *H.enneaspermus.* Since, the ethanolic leaf extract of the plant contained more constituents and it was found to beneficial for further investigation.

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