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QUALITATIVE ANALYSIS OF PHYTOCHEMICALS AND *IN VITRO* EVALUATION OF DPPH RADICAL SCAVENGING ACTIVITY OF *HYBANTHUS ENNEASPERMUS* (L.) F. MUELL. ROOT EXTRACT

Jayanthi K., Kavitha K., Jayanthi, J. and Ragunathan M. G.*

Department of Advanced Zoology and Biotechnology, Guru Nanak College, Chennai- 6000 042, Tamil Nadu, India.

***Corresponding Author: Dr. Ragunathan M. G.**
Department of Advanced Zoology and Biotechnology, Guru Nanak College, Chennai- 6000 042, Tamil Nadu, India.

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ABSTRACT
Free radicals are toxic and harmful for humans. It is generated by cell metabolism in body and external factors like smoking, radiation, pollution and drugs. Over production of free radicals leads to auto immune diseases, cell death, cataract, cancer and heart attack. Medicinal plants are important source phytochemicals which are responsible for natural anti-oxidants. In the present study *Hybanthus enneaspermus* plant was analyzed for its phytochemicals and anti-oxidant activity. Five different solvents were used to prepare root extract of *H. enneaspermus* and the results were tabulated.

KEYWORDS: DPPH assay, Phytochemicals, Antioxidants.

INTRODUCTION

Asia has rich in herbals, medicinal plants and aromatic plants and its used to treat diseases since old time. India is one of the flora rich community where the ayurvedic medicines popular among everyone. New medicines are developed form medicinal plants and it gets more attention. Modern medicines (Allopathy) are causes many harmful side effects which made people to think about alternate medicine. (Sherikar and Mahanthesh, 2015).

Hybanthus enneaspermus (Linn) F.Muell belongs to Violaceae family, known as rathna purush in Ayurveda and 'orithazthamarai' in tamil. This plant is widely present in the tropical regions in the world. It is small herbs grows about 10-25 cm in height (Ibrahim *et al*., 2008). The plant is rich in bioactive compound which used to treat anti-convulsant (Kirtikar and Basu, 1991), diarrhoea, dysuria, sterility in male and male sterility and diabetes (Yoganarasimhan, 2000). In some part of India the plant is used to treat diabetes and which is also having anti-oxidant property and free radical scavenging activity (Das *et al*., 2004). The flower of the plant is also rich in phytochemicals (Mohana priya *et al.*, 2016).

A characteristic of plant life is the production of a many number of natural compounds, often called secondary metabolites. Phytochemicals are basically divided in two groups that are primary and secondary metabolites based on the function in plant metabolism. Secondary metabolites consists phenols, flavonoids, tannins, alkaloids, steroid and so on (Kumar *et al*., 2009).

Reactive oxygen species (ROS) are class of highly reactive molecules derived from the metabolism of oxygen. Recent studies revealed that the plant antioxidant are involved in treatment of many diseases (Frie, 1995; Halliwell, 1997; Liu, 2003). Quick production of free radicals leads to chronic illness like cancer, neural disorders, ageing and diabetics (Hyun *et al*., 2006; Sas *et al*., 2007). Plant based anti oxidants and its health benefits have gained more attention recently (Goveas and Asha, 2013). They have multiple functions in cell metabolism mainly defense against oxidation which produce free radicals in foods, chemicals and in living systems (Szabo *et al*., 2007).

Antioxidants have the ability of protecting organisms from damage caused by free radical-induced oxidative stress (Li, 1999). The evaluation of the antioxidant activity of plant is also necessary because of their nutraceuticals effects. Antioxidants are compounds that delay or inhibit the oxidation of lipids and reduce the risk of certain diseases like cancer (Damintoti *et al*., 2005; Punitha *et al*., 2005).

There are many synthetic anti-oxidants but they have side effects (Ito *et al*., 1983). Consumption of plants based phytochemicals are helps to maintain sufficient amount of antioxidant (Halliwell, 1996). Recently it is considered as important work to determine of natural

antioxidant and free radicals scavenging ability of medicinal plant (Jayaprakash and Rao, 2000; Nishaa *et al*., 2012) Natural antioxidants play a vital role in antioxidant defense mechanism in the biological system and acts as free radical scavenger (Jeyapragsh*et al*., 2016). There is lack of information regardingthe phytochemistry of the root of *H. enneaspermus* and the present study is aimed to evaluate the phytochemicals and free radical scavenging activity of the particular sample.

MATERIALS AND METHODS

a) Preparation of plant extract

The fresh plant material of *H. enneaspermus* was collected from Kings' Institute, Guindy, Chennai. The root of the plant was separated manually and shade dried. After drying, the plant materials were ground well using mechanical blender into fine powder and transferred into airtight containers separately.

About 10g of dried fine powder of *Hybanthus enneaspermus* root was extracted with 30 ml water, acetone, ethanol (75%), chloroform and petroleum ether using motor and pestle and soaked overnight at room temperature. The sample was filtered through Whatman No.1 filter paper and evaporated under vacuum in a rota-evaporator at 30^0 C to get constant weight then dissolved in respective solvents. The concentrated solutions were stored at 15^0C for further use (Lu and Foo, 2001; Pizzale *et al.*, 2002).

b) Qualitative analysis of Phytochemical Analysis

The plant extracts were qualitatively analyzed for the presence of different phytochemical constituents by standard protocols (Sofowora, 1993).

c) Quantitative Analysis of Antioxidant Activity

The free radicals quenching ability of sample was determined by DPPH analysis. To 3 ml of methanol, 100µl of plant sample was mixed followed by addition of 200µl of DPPH. The mixture was incubated in dark condition for 30 minutes. The mixture of same amount of methanol and DPPH was served as blank and absorbance was noted. At every five minutes interval the absorbance of samples were measured using colorimeter at 517 nm. Subsequently, at every 5 minutes interval, the absorption maxima of the solution were measured using a UV double beam spectra scan (Chemito, India) at 517 nm. The free radical scavenging activity of samples were compared with synthetic standard Butylated Hydroxy Toluene (BHT) (Xu and Chang, 2007).

CALCULATION

$$\text{Inhibition (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

RESULTS AND DISCUSSION

The results of present study revealed the phytochemicals and antioxidant potential of *Hybanthus enneaspermus* root was investigated using the standard methods. The phytochemical analysis and antioxidant activity of root is tabulated for all the five extracts (Tables 1 and 2). Out of all the solvents ethanol extract shows more phytochemicals and shows higher percentage of scavenging activity. The anti-oxidant activity of *Hybanthus enneaspermus* is (75.563±0.543). These results have proven the effectiveness of the plant sample compared to synthetic standard anti-oxidant BHT. This assay performed based on the decolorize ability of DPPH in addition of antioxidants. When DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolorized, which can be quantitatively measured from the changes in absorbance (Sherikar and Mahanthesh, 2015).

Several workers have reported the analgesic, antispasmodic and antibacterial properties of alkaloids (Antherden, 1969; Stray, 1998; Okwu, and M. Okwu, 2004). Steroids compounds possess antimicrobial activity and consider as essential compound because of their structure similarities with sex hormones (Okwu, 2001; Epand *et al*., 2007).

Phenols are involved in many biological activities such as apoptosis, anti-aging, anti-carcinogen, anti-inflammation, anti-atherosclerosis and cardiovascular protection. The growth of many fungi, yeasts, bacteria and viruses can be inhibited by tannins (Han *et al*, 2007).

Excess amount of free radicals are produced due to stress and exposure of radiation damage to the cells and inhibits normal cell functions (Halliwell and Gutteridge, 1993; Jorgensen *et al*., 1998). Anti-oxidants through their radical scavenging ability have proved relevant for the effective management of these diseases and the protection of various cells from mutagens. Anti-oxidants are used as medications to treat various forms of brain injury (Yamaguchi *et al*., 1998; Nwaguikpe*et al*., 2014). The present study reports on the phytochemicals and anti-oxidant potential of various extracts of the *H. enneaspermus* root.

TABLE 1: PHYTOCHEMICAL SCREENING OF THE PLANT *H. ENNEASPERMUS* ROOT USING DIFFERENT SOLVENTS

S.no	Phytochemicals	Extracts of *Hybanthus enneaspermus* root				
		Aqueous	Ethanol	Acetone	Chloroform	Petroleum Ether
1.	Tannins	+	++	_	_	_
2.	Saponins	_	_	_	_	-
3.	Alkaloids	+	+	_	_	_
4.	Terpenoids	+	+	_	_	_
5.	Phenols	+	++	_	_	_
6.	Flavonoids	+	+	_	_	_
7.	Steroids	+	+	_	_	_
8.	Quinones	+	+	_	_	_
9.	Glycosides	_	_	_	_	_
10.	CardioGlycosides	+	+	_	_	_
11.	Coumarins	_	+	_	_	_
12.	Betacyanin	±	+	_	_	_

(++ = Highly positive, + = positive, ± = semi positive and - = negative,)

TABLE 2: FREE RADICALS SCAVENGING ACTIVITY OF *HYBANTHUS ENNEASPERMUS* ROOT EXTRACTS.

	TIME (Minutes) Activity in %						
SAMPLES	**0**	**5**	**10**	**15**	**20**	**25**	**30**
Standard (BHT)	87.448±0.678	88.624±0.675	92.659±0.986	95.711±0.676	96.705±0.327	97.167±0.438	98.302±0.478
Aqueous	65.546±0.432	65.979±0.578	66.143±0.772	68.176±0.476	69.247±0.437	71.486±0.632	72.124±0.653
Ethanol*	64.187±0.361	65.186±0.587	67.398±0.487	68.286±0.752	70.548±0.619	73.358±0.789	75.563±0.543
Acetone	56.608±0.697	57.298±0.255	59.875±0.875	59.957±0.698	60.287±0.597	61.876±0.756	62.321±0.753
Chloroform	45.297±0.475	46.896±0.567	48.674±0.896	48.978±0.357	49.485±0.986	50.097±0.897	52.635±0.563
Petroleum ether**	43.695±0.979	44.989±0.865	45.907±0.975	46.460±0.876	46.857±0.977	47.767±0.767	47.742±0.867

All analyses were mean of triplicate measurements ± standard deviation.
Standard vs Ethanol *P<0.001, Standard vs Petroleum **P> 0.01. *P level of significance, **P level of insignificance

CONCLUSION

The present study showed that the phyto constituents and anti-oxidant activities of the extracts of *H. enneaspermus* root. Phytochemical screening of aqueous, ethanol, acetone, chloroform and petroleum ether extracts revealed the presence flavonoids, phenols**,** quinones, cardio glycosides, tannins, terpenoids, steroids, alkaloids, coumarins and betacyanin by positive reaction with the respective test reagent. Phytochemical screening showed that maximum presence of phytoconstituentsin ethanol extract and showed anti-oxidant potential.

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