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IN VITRO EVALUATION OF FREE RADICAL SCAVENING ACTIVITY OF THE FLOWER *HYBANTHUS ENNEASPERMUS* (L.) F. MUELL. EXTRACT

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ABSTRACT

Using five different solvents the flower extracts of *H. enneaspermus* was analyzed for its antioxidant property. The results revealed that ethanolic extract showed highest antioxidant activity followed by acetone, water, petroleum ether and chloroform. Further the compounds present in the ethanolic extract were identified and quantified as flavonoids and phenols. The high antioxidant activity observed in the ethanolic extract of the flower of *Hybanthus enneaspermus* could be suggested for its use to replace synthetic drugs which are used against oxidative damage.

Key words: Free radicals, Antioxidants, *Hybanthus*.

INTRODUCTION

In recent past, there has been an increasing awareness of medicinal plants to mankind (Dahanukar *et al.*, 2000). As a result of physiological and biochemical processes in an organism the cells generates free radicals and other reactive oxygen species by-products resulting in oxidative damage to lipids, proteins and DNA. Therefore it causes many chronic diseases, such as cancer, diabetes, aging, and other degenerative diseases in humans (Oyedemi and Afolayan, 2011). Antioxidants, on the other hand, significantly prevent tissue damage that stimulates wound healing process (Fitzmaurice *et al*., 2011). There are available synthetic antioxidants like butylated hydroxy anisole (BHA), butylated hydroxy toluenes (BHT), tertiary butylated hydroquinone and Gallic acid esters, but have been suspected to cause or prompt negative health effects. Hence, strong restrictions have been placed on their application and there is a trend to substitute them with naturally occurring antioxidants. Plant are endowed with free radical scavenging molecules, such as vitamins, terpenoids, phenolic acids, lignins, flavonoids, tannins, alkaloids and other metabolites which are rich in free radical scavenging property reported by (Cai *et al*., 2003 and Sateeshkumar *et al*., 2011). The phytoantioxidant compounds are able to neutralize the excess ROS and prevention of many diseases such as atherosclerosis, cancer, cardiovascular and neurological diseases (Trumbeckaite *et al*., 2006 and Muselik *et al*., 2007).

Hybanthus enneaspermus (L.) F. Muell belongs to violaceae family known as "Orithazh thamarai" in Tamil which is distributed in tropical and sub-tropical region of the world (Kiritar and Basu, 1991; Anand and Gokulkrishnan, 2012). *Hybanthus enneaspermus* is a small erect herb in which the colour of the flowers are pink, auxiliary, solitary and zygomorphic (Retnam and De Brito, 2003). Traditionally the plant is used as an aphrodisiac and anti-infertility activity (Narayanaswamy *et al*., 2007; Senthil Kumar and Vijay Kumar, 2012; Nathiya and Senthamil Selvi, 2013), antiarthritic property (Tripathy *et al*., 2009), CNS activity (kar *et al*., 2010), cardioprotective activity (Radhika *et al*., 2011), anti-inflammatory (Tripathy *et al*., 2011), antioxidant effect (Deepika and Premalashmi, 2011; Anand and Gokulakrishnan, 2012), antiulcer and anti-secretory (Sakthi *et al*., 2012), potent regulator for membrane

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bound mitochondria (Anand *et al*., 2012), *in vitro* aldose reductase activity (Patel *et al*., 2012), hypolipidemic activity (Sateesh kumar and Kottai, 2012), *in vitro* antifungal activity (Napoleon *et al*., 2012) antimicriobial (Vijaya Bharathi *et al*., 2012), anti-allergic and analgesic activity (Thamizhmozhi *et al*., 2013), glucose utilization capacity (Dinesh *et al*., 2013).

In the literature survey a paucity of reports exist on the medicinal potential of the flower *H. enneaspermus*. Hence it was aimed to evaluate the *in vitro* free radical scavenging activity of *Hybanthus enneaspermus* flower extract using different solvents.

MATERIALS AND METHODS

Collection and authentication of the flower

The flower of *H. enneaspermus* plant was collected from Ichankadu, Kanchipuram district of Tamil Nadu and was authenticated by the Plant Anatomy Research Centre, West Tambaram, Chennai.

Preparation of flower extracts

This was done according to the combination of methods (Lu and Foo, 2001; Pizzale *et al*., 2002). The concentrated extracts were stored in airtight containers and were refrigerated below 10^0C for further analysis.

Evaluation of Antioxidant Activity by *In Vitro* Techniques

Qualitative analysis of antioxidant activity from flower extract of *H. enneaspermus*

The antioxidant activity of flower extracts of *Hybanthus enneaspermus* was determined by following the method as described by George *et al.* (1996). 100µl of flower extract of *Hybanthus enneaspermus* medicinal plant was taken in a microtitre plate. 100µl of 0.1% methanolic 1, 1– Diphenyl -2- picryl - hydrazyl (DPPH) was added over the samples. It was incubated for 30minutes in dark condition. The samples were then observed for discoloration. The colour changes from purple pink to yellow and pale pink were considered as strong and weak positive reactively. The antioxidant positive samples were subjected for further quantitative analysis.

Quantitative analysis of antioxidant activity from flower extract of *H. enneaspermus*

The antioxidant activities were determined using DPPH, (Sigma-Aldrich) as a free radical. Flower extract sample of 100µl from qualitative assay were mixed with 2.7ml of methanol. Then 200µl of 0.1% methanolic DPPH was added. The suspension was incubated for 30 minutes in dark condition. Initially, absorption of blank sample containing the same amount of methanol and DPPH solution was prepared and measured as a control (Lee *et al.*, 2003) Subsequently, at every 5 minutes interval the absorption maximum of the solution was measured using a UV double beam spectra at 517 nm. The antioxidant activity of the sample was compared with known synthetic standard of 0.16% of BHT (Butylated hydroxyl toluene).

The radical sample is calculated by the following formula,

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

Total Phenol Estimation

The Folin-Ciocalteau reagent method has been used for estimation of total phenolic extracts according to Lister and Wilson (2001) with slight modification. The results were expressed in mg of Gallic acid equivalent (GAE) per gram dry weight of flower powder.

Total Flavonoid Estimation

The aluminum chloride colorimetric method was modified from the procedure reported by Woisky and Salatino (1988) used to estimate the total flavonoid present in the sample. A standard calibration plot was generated at 415nm using known concentrations of quercetin. The concentrations of flavonoid in the test samples were calculated from the calibration plot and expressed as mg quercetin equivalent per gram of sample.

RESULTS AND DISCUSSION

The qualitative and quantitative analysis of free radical scavenging activity is represented in the Tables 1and 2 and Figure 1. Qualitative analysis results indicate that the ethanol and acetone flower extracts exhibited antioxidant property. Therefore, the control (BHT) showed strong positive which reveals it has a strong antioxidant property. Based on obtained result indicates, the ethanolic flower extract of *H. enneaspermus* was found most effective than that of other extracts. When compared with standard (BHT) ethanol extract of flower *H. enneaspermus* showed 89% inhibition of free radical scavenging activity using DPPH assay. It was followed by Acetone 81%, Aqueous 52%, petroleum ether 46% and chloroform extract 39%. Similar results were observed in petroleum ether extract of whole plant of *Ionidium suffruticosum* (Ging) based on concentration dependence using Rutin as standard (Satheeshkumar *et al*., 2011). The ethanol flower extract of *H. enneaspermus* was analyzed for total phenol and flavonoid content (Table 3 and 4). Phenol content was higher than that of flavonoid content quantified in ethanol extract. Phytochemical investigation on the whole plant of *Ionidium suffruticosum* (Ging) showed higher amount of phenol and flavonoid contents has high antioxidant property (Kottai *et al*., 2011). The biological properties of flavonoids are considered on the *in vitro* evaluation of the medicinal and nutritional value (Harborne and Williams, 2000). Many researchers stated that total phenol and flavonoid content is responsible for

antioxidant property. Similarly, the total phenolic content of plant directly correlates to their antioxidant property (Tosun *et al*., 2009). The plant, *Hybanthus enneaspermus* widely used in tradition medicine to treat variety of common and stress related disorders and plant extract was evaluated for nephroprotective activity in rats (Manjunath Setty *et al*., anon)

The results of the present study clearly indicated that the crude ethanolic flower extract of *H. enneaspermus* shows equivalent antioxidant activity when compared with the synthetic standard drug (BHT). The ethanol extract of *H. enneaspermus* was found to have high content of phenolic and flavonoids compounds which is responsible for the antioxidant activity. In the present work it is observed that the ethanol flower extract of *Hybanthus enneaspermus*, the high antioxidant activity could replace synthetic toxic antioxidants that are unsafe and harmful to human life. Hence the plant extracts and its products are the best phytomedicine that plays a vital role in preventing human diseases such as ageing, cancer, cardiovascular and other diseases caused by the free radicals.

Figure 1. Antioxidant activity of *Hybanthus enneaspermus* flower extracts

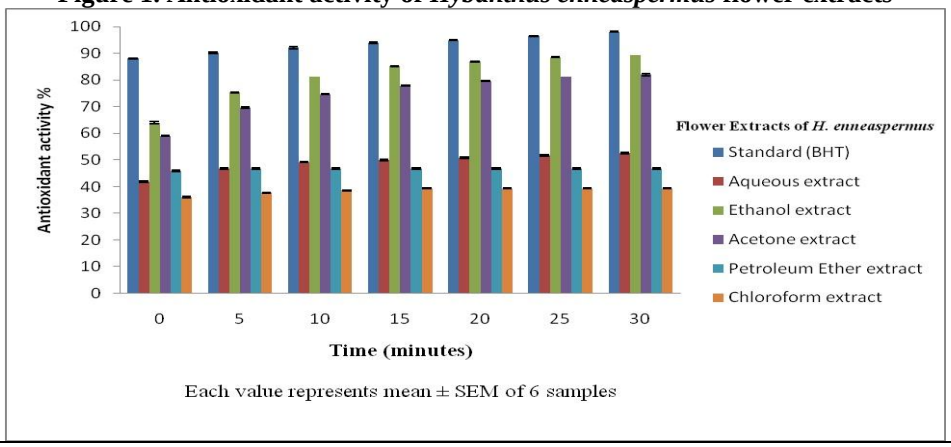

Table 1. Qualitative analysis of Antioxidant activity of *Hybanthus enneaspermus* flower extracts (++ strongly positive, + positive and - negative)

S.no	Samples	Antioxidant activity
1.	Control	-
2.	BHT (Standard)	++
3.	Aqueous	-
4.	Ethanol	+
5.	Acetone	+
6.	Petroleum ether	-
7.	chloroform	-

Table 2. Quantitative analysis of Antioxidant of *Hybanthus enneaspermus* flower extracts

	TIME (Minutes) Activity in %						
SAMPLE	**0**	**5**	**10**	**15**	**20**	**25**	**30**
Experimental Control (BHT)	88.115±0.1591	90.1933±0.3142	92.1016±0.3947	93.9383±0.1934	94.9766±0.0751	96.4466±0.2031	98.125±0.1350
Aqueous Extract	41.365±0.2660	46.2383±0.3826	49.06±0.0585	50.4433±0.2520	50.535±0.3099	51.475±0.2421	52.0266±0.2566
Ethanol Extract	63.2616±0.4081*	75.0666±0.1251*	81.035±0.0561*	85.195±0.1683*	86.1766±0.2565*	88.0283±0.2046*	89.1166±0.0896*
Acetone Extract	59.0133±0.0131	69.32±0.1875	74.24±0.1420	77.3333±0.1834	79.2333±0.1431	81.0633±0.0531	81.3983±0.3143
Pet. Ether Extract	45.5283±0.2554	46.16±0.2431	46.16±0.2431	46.16±0.2431	46.16±0.2431	46.16±0.2431	46.16±0.2431
Chloroform Extract	36.02±0.2060	37.3933±0.1662	38.2266±0.1135	39.08±0.0839	39.08±0.0839	39.08±0.0839	39.08±0.0839

Each value represents mean ± SEM of 6 samples expressed as % inhibition of free radicals Ethanol extract was compared with aqueous, acetone, pet. ether and chloroform extracts * indicates $P < 0.01$ level.

Table 3. Total Phenol Content in the *H. enneaspermus* flower exract

S.NO.	Sample	Total phenol content (mg GAE/g)
1.	Ethanol extract	10.8

Table 4. Total Flavonoid Content in the *H. enneaspermus* flower exract

S.NO.	Sample	Total flavonoid content (mg QE/g)
1.	Ethanol extract	2.625

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CONFLICT OF INTEREST:
The authors declare that they have no conflict of interest.

REFERENCES

Anand T and Gokulakrishnan K. Amelioration of carbon tetrachloride induced oxidative stress in kidney tissues by ethanolic extract of *Hybanthus enneaspermus* in Wistar rats. *International Journal of Pharmaceutical Science and Health Care*, 2(2), 2012, 62 – 70.

Anand T, Kalaiselvan A and Gokulakrishnan K. *Hybanthus enneaspermus* is a potent regulator for membrane bound enzymes in mitochondria on carbon tetrachloride induced oxidative stress in rats. *International Journal of Drug Development & Research*, 4(3), 2012, 259-262.

Cai YZ, Sun M and Corke H. Antioxidant activity of betalains from plants of the Amaranthaceae**.** *J Agric Food Chem*, 51(8), 2003, 2288-2294

Dahanukar SA, Kulkarni RA and Rege NN. Pharmacology of medicinal plants and natural products. *Indian J. of Pharmacology*, 32, 2000, S81-S118.

Deepika Thenmozhi C and Premalashmi V. Antioxidant effects of Hydroethanolic extract of *Hybanthus enneaspermus* on paracetamol induced oxidative stress in albino rats. *International Journal of Research in Pharmaceutical and Biomedical Sciences*, 2(3), 2011, 1285–1287.

Dinesh K Patel, Sairam Krishnamurthy and Hemalatha S. Evaluation of glucose utilization capacity of bioactivity guided fractions of *Hybanthus enneapermus* and *Pedalium murex* in isolated rat hemidiaphragm. *Journal of Acute Disease*, 2013, 33 – 36.

Fitzmaurice SD, Sivamani RK and Isseroff RR. Antioxidant therapies for wound healing, A clinical guide to currently commercially available products. 24, 2011, 113-126.

George, H, Teng, C. M, Wu, C.L. and Ko, F. N. (1996). Marchantin H as a natural antioxidant and free radical scavenger. *Arch.of Biochem. and Biophys*.334, pp.18- 26.

Harborne JB and Williams CA. Advances in flavonoid research since 1992. *Phytochemistry*, 55(6), 2000, 481-504.

Kar DM, Maharana L and Rout SP. CNS activity of aerial parts of *Hybanthus enneaspermus* Mull. *Pharmacologyonline*, 3, 2010, 959–981.

Kirtikar KR and Basu BD. In, Indian medicinal plants, vol. I, 2nd ed. Delhi, Periodical Experts Book Agency, 1991, 212–213.

Kottai Muthu A, Satheesh Kumar D and Manavalan R. *In vitro* Antioxidant Potential of Various Extracts from Whole Plant of *Ionidium Suffruticosum* (Ging). *International Conference on Biotechnology and Pharmaceutical Sciences*, 2011, 369- 371.

Lee SE, Hwang HJ and Ha JS. Screening of medicinal plant extracts for Antioxidant activity. *Life Sci*, 73, 2003, 167-179.

Lister E and Wilson P. Measurement of total phenolics and ABTS assay for antioxidant activity. Crop Research Instititue,Lincoln, New Zealand, 2001.

Lu Y and Foo Y. Antioxidant activities of polyphenols from sage (*Salvia officinalis*). *Food Chem*, 75, 2001, 197-202.

Manjunath Setty M, Narayanaswamy VB, Sreenivasan KK and Annie Shirwaikar (anon). Free Radical Scavenging and Nephroprotective Activity of *Hybanthus enneaspermus* (L) F. Muell. *Pharmacologyonline*, 2, 2002, 72-87.

Muselík J, García-Alonso M, Martín-López MP, Želmièka M and Rivas-Gonzalo JC. Measurement of antioxidant activity of wine catechins, procyanidins, antocyanins and piranoantocyanins**.** *Int J Mol Sci*. 8, 2007, 797-809

Napoleon Arumugam, Sasikumar Kandaswamy and Sekar M. *In-vitro* antifungal activity of *Hybanthus enneaspermus* F Muell. *International Journal of Pharmacy and Pharmaceutical Science*, 4(2), 2012, 594-596.

Narayanaswamy VB, Manjunatha Setty M, Malini S and Annie Shirwaikar. Preliminary aphrodisiac activity of *Hybanthus enneaspermus* in Rats. *Pharmacologyonline*, 1, 2007, 152-161.

Nathiya S and Senthamil Selvi R. Anti-infertility effect of *Hybanthus enneaspermus* on endosulfan induced toxicity in male rats. *Int J Med Biosci*. 2(1), 2013, 28-32.

Oyedemi SO and Afolayan AJ. Antibacterial and antioxidant activities of hydroalcoholic stem bark extract of *Schotia latifolia* Jacq. *Asian Pacific Journal of Tropical Medicine*, 2011, 952-958.

Patel DK, Kumar R, Kumar M, Sairam K and Hemalatha S. Evaluation of *in vitro* aldose reductase inhibitory potential of different fraction of *Hybanthus enneaspermus* Linn F. Muell. *Asian Pac. J. Trop. Biomed,* 2(2), 2012, 134-139.

Pizzale L, Bortolomeazzi R, Vichi S and Conte LS. Antioxidant activity of sage and oregano extracts related to their phenolic compound content. *Journal of the Science of Food and Agriculture*, 82, 2002, 1645–1651.

Radhika S, Smila KH and Muthezhilan R. Cardioprotective activity of *Hybanthus enneaspermus* (Linn.) on Isoproterenol induced rats. *Indian Journal of Fundamental and Applied Life Sciences,* 1(3), 2011, 90-97.

Retnam KR and De Britto AJ. Phytochemical analysis of a medicinal plant *Hybanthus enneaspermus* (L) F. Muell. *J. Econ. Taxon. Bot*, 27(3), 2003, 701-706.

Sakthi PS, Subudhi BB, Ramchandran S, Dhanraju MD and Kumaraswamy B. Evalutation of anti-ulcer and anti-secretory activity of *Hybanthus enneaspermus. International Journal of Pharmaceutical Research & Allied Sciences,*1(4), 2012, 85 – 83.

Sateesh KD and Kottai MA. Comparative evaluation of flavone from *Mucuna pruriens* and coumarin from *Ionidium suffruticosum* for hypolipidemic activity in rats fed with high fat diet. *Lipids in Health and Disease*, 11(126), 2012, 1 – 6.

Satheeshkumar D, Kottai Muthu and Manavalan. *In vitro* free radical scavenging activity extracts of whole plant of Ionidium suffruticosum (Ging). *Journal of Pharmacy Research*, 4(4), 2011, 976 – 977.

Senthil kumar B and Vijaya kumar J. Effect of *Ionidium suffruticosum* on Testes of Young Albino Rat. *International Journal of Pharmacy and Biological Sciences*, 2(3), 2012, 106-112.

Thamizhmozhi M, Swarnalatha S, Sakthivel P, Manigandan LS, Jayabharath A and Suresh kumar P. Anti-allergic and analgesic activity of aerial parts of *Hybanthus enneaspermus. International Research Journal of Pharmacy,* 4(6), 2013, 243-248.

Tosun M, Ercisli S, Sengul M, Ozer H and Polat T. Antioxidant properties and total phenolic content of eight *Salvia* species from Turkey. *Biol. Res*, 42, 2009, 175-181.

Tripathy S, Parchuri S and Pradhan D. Preliminary investigation of the anti-inflammatory properties of *Hybanthus enneaspermus. International Journal of Universal Pharmacy and Life Sciences,* 1(2), 2011, 239-248.

Tripathy S, Sahoo SP, Pradhan D, Sahoo S and Satapathy DK. Evaluation of anti-arthritic potential of *Hybanthus enneaspermus. African Journal of Pharmacy and Pharmacology,* 3(2), 2009, 611-614.

Trumbeckaite S, Bernatoniene J, Majiene D, Jakastas V, Savickas A and Toleikis A. The effect of flavonoids on rat heart mitochondrial function. *Biomed. Pharmacotherapy*, 60, 2006, 245-248.

Vijaya Bharathi S, Nithya TG, Sivakumar Saira Banu and Muruga Lakshmi. Screening of bioactive compounds and antimicrobial effects of Orithazh thamarai Chooranam. *International Journal of Pharm Tech Research*, 4(2), 2012, 572-575

Woisky R and Salatino A. Analysis of Propolis. Some parameter and procedures for chemical quality control. *J. Apic. Res*, 37, 1998, 99-105.