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QUANTITATIVE ANALYSIS OF TOTAL PHENOLIC AND FLAVONOID COMPOUNDS OF ETHANOLIC EXTRACTS OF STEM AND ROOT OF *HYBANTHUS ENNEASPERMUS* (L.) F. MUELL

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ABSTRACT

The importance of antioxidant constituents in maintenance of health attracted research in plant based antioxidants. Presence of secondary metabolites like flavonoids and phenolic compounds and antibacterial activity leads to the evaluation of antioxidant activity of extracts. In the present study *H. enneapsermus* stem and root were made into fine powder and extracts were prepared using ethanol solvent. Total Phenol content was estimated using Folin-Ciocalteau method and Total flavonoid content was estimated using Aluminium Chloride method. The results were tabulated.

KEYWORDS: *Hybanthus enneaspermus* stem, Total Phenols, Total Flavonoids.

INTRODUCTION

The use of plant and its products has a long history that began with folk medicine and through the years has been incorporated into traditional and allopathic medicine. Since antiquity, many plants species reported to have pharmacological properties as they are known to posses various secondary metabolites like glycosides, saponins, flavonoids, steroids, tannins, alkaloids, terpenes which is therefore, should be utilized to combat the disease causing pathogens **(**Kamali and Amir, 2010; Lalitha *et al*., 2010; Hussain *et al*., 2011).

Correlation between the phyto chemicals and the bioactivity of plant is desirable to know the synthesis of compounds with specific activities to cure various health ailments and to treat diseases as well (Pandey *et al*., 2013). Polyphenols can accept an electron to form relatively suitable phenoxyl radicals, thereby disrupting chain oxidation reaction in cellular components (Kehrer and Smith, 1994). Flavonoids are able to chelate free radicals immediately by donating a hydrogen atom or by single-electron transfer (Procházková *et al*., 2011).

Phenolics and Flavonoids are ubiquitous secondary metabolities in Plant Kingdom. Phenolics comprise a large group of biologically active compound from simple phenol molecules to polymeric structures with molecular mass above 30000 Da (Dreosti, 2000). Flavonoids are hydroxylated phenolic substances and are known to be synthesized by plants in response to microbial infection (Dixon *et al*., 1983).

Phenolics possess a wide spectrum of biochemical activities such as anti-oxidant, anti-mutagenic, anti-carcinogenic, as well as ability to modify the gene express. Many studies confirm significant relationship between the high dietary intake of flavonoids and the reduction of cardiovascular and carcinogenic risks (Cook and Samman, 1996; Tapiero *et al*., 2002; Nakamura *et al*., 2003).

Flavonoids are widely distributed secondary metabolites with antioxidant and antiradical properties (Augustin, 2005). A number of data showed that the presence of phenolics in foods in important for oxidative stability and anti-microbial protection. Flavonoids are one of non – nutritive chemical present in plants exhibit various biological effects such as anti – inflammatory, anti-hepatotoxic and anti-ulcer actions. They also exhibited anti-coagulant, anti-hyperlipidase, anti-nephtitic, vasodilative effects and human immune deficiency virus inhibition (Wong *et al*., 2000; Meenakshi *et al.*, 2009).

Phenols and flavonoids content responsible for the medicinal value of herbal plants. the study is aims to determine the total phenol and total flavonoid content of stem and root of the *Hybanthus enneaspermus*.

MATERIALS AND METHODS

The fresh plant material of *H. enneaspermus* was collected from Kings' Institute, Chennai. The stem and 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. Preparation of plant extracts were done using combination methods used by Lu and Foo, (2001) and Pizzale *et al.* (2002). About 10g of dried fine powder of *Hybanthus enneaspermus* stem and root material were extracted with 50 ml acetone, ethanol (75%), chloroform, petroleum ether and aqueous extract using Ultra Turax mixer (13,000 rpm) for 1 minute and soaked overnight at room temperature. Using Buchner Funnel the sample was then filtered through Whatman No.1 paper. The filtered solution was evaporated under vacuum in a rota-evaporator at 40^0C to a constant weight and dissolved in respective solvents. The concentrated extracts were stored in airtight container in refrigerator below 10^0C.

a) Estimation of total phenol content

The total phenol content was estimated by Folin-Ciocalteau colorimetric method according to Aiyegroro and Okoh (2010) with slight modification. One ml of plant extract was added to 2.5ml of 10% Folin-Ciocalteu reagent and 2ml of 2% solution of Na2CO3. The reactive mixture was incubated for 15 minutes at room temperature. The absorbance of the sample was measured at 765nm. Gallic acid was used as standard (1mg/ml). All the tests were performed in triplicates. The results were determined from the standard curve and were expressed as gallic acid equivalent (mg/g of extracted compound.

b) Estimation of total flavonoid content

The total flavonoid content was determined by Aluminium Chloride colorimetric method reported by (Winky and Salatino, 1998) with some modification. One ml of the flower extract was mixed with 1.5ml of ethanol, 0.1ml of potassium acetate, 0.1ml of 10% AlCl3 and 2.8 ml distilled water was added sequentially. This mixture was allowed to incubate at room temperature for 30 minutes. The absorbance was recorded at 415 nm. Quercetin was used as standard. Different concentration of standard quercetin was prepared and the absorbance of various concentrations of quercetin was plotted for a standard graph. The flavonoid content was expressed as mg quercetin equivalent /g of sample.

RESULTS AND DISCUSSION

The total phenols and flavonoids of *H. enneapsermus* stem and root were shown in table. Total phenolic content in stem and root was 18.497±0.976 and 15.459±0.787 and total flavonoid content is 2.321±0.787 and 1.671±0.487 respectively. The phenol and flavonoid contents are more in stem compared to root.

Consumption of fruits and vegetables is one of the nutritional recommendations that help to maintain human health (Lichtenstein *et al*., 2006; Halliwell, 2012) because our diet provides a huge amount of plant-derived phenolic compounds with antioxidant activity that helps to counter oxidative stress in our body (Hertog *et al*., 1993; Halliwell, 2012). Literature showed that there are direct relationships between the type and the concentration of the active substances (volatile oils and phenolic compounds) from plants and their antimicrobial action (Dellavalle *et al*., 2011). These compounds are synthesized and used by plants as means of defense against biotic and abiotic factors (Buțu *et al*., 2013). Defense mechanisms include physical and chemical defense barriers that prevent the microbial attack by using both the preformed compounds, as well as those induced by the defense response (Martínez, 2012). The plant defense response is mainly due to the compounds that belong to the secondary metabolites group, which are classified according to the chemical characteristics (alkaloids and phenols, polyphenols, tannins), plant origin and the biosynthetic origin (terpenoids, polyketides, phenilpropanoids) (Castillo *et al*., 2012).

Phenolic compounds are widely distributed natural antioxidants in plants (Skerget *et al*., 2005). Naturally occurring plant phenols and flavonoids possess a broad range of pharmacological activities such as antioxidant, antimutagenic, antimicrobial, antiulcer, antiarthritic, anti-cancer and protein kinase inhibition (Marinova *et al*., 2005; Sulaiman and Balachandran, 2012). The brain is more vulnerable to oxidative stress due to imbalance between reactive oxygen radicals and antioxidant defense system of our body (Hotta *et al*., 2002) and the free radicals have been found to play a major role in several human diseases, including, brain disorders (Haramoto *et al*., 2008).

Exhaustive pharmacological work has been conducted where polyphenols have been reported to possess strong action on CNS (Fernandez *et al*., 2006; Viola *et al*., 1995; Saaby *et al*., 2009). The present study revealed the total phenol and flavonoid content of stem and root of *Hybanthus enneapsermus*. The obtained data may give the clear view about its bioactivity of the plant.

Table: Total phenol and total flavonoid content of stem and root of *H. enneaspermus.*

S. NO	SAMPLE	Total Phenolic content (mg GAE/ g dry sample)	Total Flavonoid content (mg QE/g dry sample)
1	*Hybanthus enneapsermus* stem	18.497±0.976	2.321±0.787
2	*Hybanthus enenaspermus* root	15.459±0.787	1.671±0.487

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