



PHYSICO CHEMICAL PARAMETERS, PHYTOCHEMICAL ANALYSIS, ACID AND BASIC RADICALS ANALYSIS IN THE LEAF EXTRACT OF *HYBANTHUS ENNEASPERMUS* (L.) F. MUELL

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ABSTRACT

Objective: The objective of the present study is to analysis the physico-chemical characteristics, phytochemicals and inorganic nutrients present in the leaf of *Hybanthus enneaspermus*.

Methods: The plants in which the bioactive compounds are present in high concentration are known as medicinal plants. Herbal medicine may contain many trace elements, it is necessary to understand about the physico- chemical characters of medicinal plants. In the present study was the leaf extract of *Hybanthus enneaspermus* was subjected to physico-chemical characters such as PH, moisture content, total ash, acid soluble ash and acid-basic radicals by standard methods. Phytochemical analysis and inorganic compounds are analyzed by following standard protocol.

Results: The pH of solution was found to be 5.04 ± 0.122 . Percent moisture content was $32.45 \pm 0.365\%$. Low moisture content could prevent the sample from microbial infection during storage (Pandey *et al.*, 2012). The ash values like total ash and acid insoluble ash value were found to be $5.20\% \pm 0.253$ and $2.85 \pm 0.435\%$ respectively. The phytochemical and inorganic compounds present in *Hybanthus enneaspermus* leaf were tabulated. The obtained results of present study will provide data about the physico- chemical characters and minerals present in *H. enneaspermus*

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INTRODUCTION

Plant kingdom which provides by the basic necessity of food, clothes and shelter to the human beings. The use medicinal plants for curing diseases are one of the traditional practices in India which later become popular throughout the world. Peoples believes that organic drugs are better to improves immune system and less harm than synthetic medicines [1,2]. Fresh and dry parts of plants are used in ayurvedic system. Generally herbal formulations involve the use of fresh or dried plant parts [3]. Understanding the application of crude drugs is very important phase in treatment of using medicinal plants. The bio application of crude extracts can be determined by systemic standard analysis to ensure the particular dose of the medicinal plant [4,5]. Analysis of pH, moisture content, total ash and acid soluble ash plays important role in characterization of native medicinal plants [6].

According to World Health Organization, many modern drugs are discovered from the medicine plants which are reservoirs of potentially useful chemical compounds [7]. Phytochemicals are naturally occurring defense chemicals in plants present in

various parts of plants such as leaves, roots, seeds, stems and barks etc [8]. Several research groups have also reported such studies in screening plant parts for phytochemicals all over the world [9-13].

Extraction of more number of phytochemicals from plants is mainly depends on the type of solvent used for extraction procedure [14]. Therefore it is necessary to try different types of solvents for extraction procedure [15].

Most of the people especially in India, consumes vegetarian food includes cereals and nuts to improve their health [16]. Improvement of nutritional quality of our food supply, especially with respect to essential nutrient minerals, such as magnesium, iron and zinc, could be an important goal of vegetable crops [17].

Living organism needs appropriate level of organic and inorganic compounds such as water, carbohydrates, proteins, vitamins, minerals and fats for their activities. Many numbers of essential minerals are present in the plants which can be taken by other animals and humans [18]. The plants obtain these minerals from environment which enhance the growth of the plants and also play other unknown benefits [19]. The mineral composition of the plants is influenced by geographic factors, climate, soil minerals, seasonal changes, and

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phenological changes [20]. Intake of balance minerals helps to maintain health and improper mineral content may leads to abnormalities.

Nutrition quality of foods determined by the concentration of minerals present in it and also plays important role in prevention of many diseases whereas some can be toxic (e.g. Lead, Mercury, Cadmium and Aluminium) [21].

Several works have been reported regarding the trace elements of medicinal plants by many researchers [22-24]. Many data are available about the mineral content of the medicinal and aromatic plants [25]. Many works was proved that the deficiency of minerals lead to abnormalities in human [26].

Imbalance mineral content may leads to number of deficiencies and disorders. Low level of calcium, magnesium, iron and zinc leads to breast cancer [27]. Low level if zinc causes lung cancer [28]. This minerals are also plays vital role in nutrition, metabolic processes and enzyme reaction [2].

Hybanthus enneaspermus, a traditional medicinal herb belonging to the family Violaceae is distributed in the tropical and subtropical regions of the world. Its leaf is known among the Yoruba tribe in Nigeria as 'Abiwere' (meaning leaf that makes delivery painless, trouble-free or fast). Several studies found it to have antibacterial effects [29], anti-inflammatory, anti-arthritis [30,31] and hypoglycemic [32].

MATERIALS AND METHODS

Collection and Processing of plant material

Fresh whole plant of *Hybanthus enneaspermus* were collected at Guru Nanak College campus, Velachery, Chennai. The collected sample was washed well to remove sand and dust and leaves were separated. The separated leaves were shade dried and made into fine powder using mixer grinder and powder used for further analysis.

Preparation of leaf extract

Five grams of fine powder of *Hybanthus enneaspermus*(L.) leaf was extracted with ethanol. The solution was plugged with cotton wool and then kept on a rotary shaker at 190-220 rpm for 24 hours. After that the supernatants were collected and the solvents were evaporated to make the final volume and stored at 4°C in air tight containers [33].

Analysis of Physico - chemical properties

The leaf powder of *Hybanthus enneaspermus* was analyzed to report the physico - chemical characteristics such as pH, moisture content, percentage of total ash content and acid soluble ash by following standard protocols.

Determination of pH

One gram of leaf powder was placed in a 100ml volumetric flask and made upto 100 ml by adding distilled water. The solution was sonicated for 10 minutes and pH was measured by digital pH meter [4].

Determination of Moisture content

The fresh leaves were collected and subjected to drying in an oven at 105° C for 24 hrs. The moisture content of the sample was evaporated to get constant weight of sample [5]. The dried samples were cooled and weighed. Moisture content was calculated using formula.

$$\text{Moisture content (\%)} = \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Fresh weight}} \times 100$$

Determination of total ash

Five gram of dry leaf powder was taken separately in pre weighed crucibles and placed over a tripod stand and ignited slowly over Bunsen flame, till no fumes were evolved. The crucibles were then transferred to Muffle's furnace at a temperature of 550 °C ± 5 for hours, till the black carbon particle turns into white color. Then the crucibles were cooled and weighed [36].

$$\text{Ash value} = \frac{\text{Initial Weight} - \text{Final Weight}}{\text{Initial Weight}} \times 100$$

Determination of acid - insoluble ash

The total ash was washed from the crucible transferred into 100 ml beaker using 25 ml of 2 N HCl. The solution was boiled for 5 min over a Bunsen burner and filtered through an ashless filter paper (Whatman No: 42). The residue was washed with hot water twice, ignited to ash, cooled in desiccators and weighed [36]. The obtained material was weighed and the acid insoluble ash was calculated.

$$\text{Acid insoluble ash (\%)} = \frac{A \times 100}{100 - M}$$

Where,

- A= Acid insoluble ash present by weight (g)
- M=Percentage of moisture in sample (%)

Qualitative Phytochemical Analysis of Plant Material

The Phytochemical investigation of collected plant was carried out according to the standard protocols [37, 38].

Determination of acid and basic radicals:

The acid and basic radicals like chlorides, nitrate, sulphide, chromate, phosphates, sulphates, carbonate, aluminium, arsenic, ammonium, mercury, copper, calcium, ferric, ferrous, lead, zinc in the leaf of *H. enneaspermus* was analyzed by standard method [39].

RESULT AND DISCUSSION

Physicochemical parameters of the leaf powder of *H. enneaspermus* are represented Table 1. The pH of solution was found to be 5.04 ± 0.122. Percent moisture content was 32.45 ± 0.365%. Low moisture content could prevent the sample from microbial infection during storage. The ash values like total ash and acid insoluble ash value were found to be 5.20% ± 0.253 and 2.85 ± 0.435% respectively. Determination of ash value is one of important step to know the quality and purity of sample [40].

Table 1 Determination of Physico - chemical analysis of leaf extract of *Hybanthus enneaspermus*

S.No	Physiochemical Parameters	Mean ± SD (%W/W)
1.	pH	5.04 ± 0.122
2.	Moisture content	32.45 ± 0.365%
3.	Total ash	5.20% ± 0.253%
4.	Acid insoluble ash	2.85 ± 0.435%

All analyses were mean of triplicate measurements ± standard deviation.

Biologically active compounds are chemical in nature they have potential to cure various diseases [41]. *H. enneaspermus*

(L.) leaf was extracted with five different solvents namely ethanol, aqueous, acetone, petroleum ether and chloroform. The results are presented in the Table 2. The result revealed that the leaf of *H. enneaspermus* contains tannin, flavonoids, terpenoids, phenols, steroids, quinones, cardio glycosides, coumarins and beta cyanins.

Table 2 Analysis of inorganic acid radicals in the leaf extract of *Hybanthus enneaspermus*

S.NO	Acid radicals	Inference
1	Carbonate	Negative
2	Chloride	Negative
3	Chromate	Negative
4	Nitrate	Negative
5	Phosphate	Positive
6	Sulphate	Negative
7	Sulphide	Negative

+ = Positive, - = Negative.

Anand *et al.* (2012) [42] and Anupa *et al.*, 2016 [43] have reported that the preliminary phytochemical screening of the plant extracts of *H. enneaspermus*. Mohanapriya *et al.*, (2016) [44] reported that the flower of *Hybanthus enneaspermus* also rich in phytochemicals.

The present findings agreed with the findings of Dab and Ragavan (2014)[45] where it has been reported that the leaf extract of *H.enneaspermus* present alkaloids, flavonoids, phenols, steroids, tannins, glycosides and terpenoids. Compared to aqueous, chloroform and acetone, the ethanolic extract revealed more number of phytochemicals.

The plant extract positive for steroids which are very important compounds for antibacterial properties [46]. Phenols are the largest group of plant metabolites, which have many therapeutic benefits such as antioxidant, anti-ageing, anti-inflammation, anti-apoptosis and anti-cancer activities [47]. Tannins have astringent property, which hasten the healing of wounds due to their anti-oxidant and anti-inflammatory properties [48]. Tannins are well known for its antiseptic activity and antiviral activity [49,50]. Flavonoids are present in the form of polyphenolic compounds that have potent anti-oxidant, anti-bacterial [51,52], anti-inflammatory and anti-cancer activity properties [53-55].

Coumarins are known to possess antitumor, antibacterial and anti helminthic properties [56]. Further terpenoids are active against bacteria [57-59] and cardiac glycosides used in treatment of cardiac arrest [60-61]. Alkaloids tend to be organic and natural ingredients that have nitrogen, and alkaloids have reported to exert analgesic, antispasmodic and antibacterial activities [62,63]. Steroids are used for arthritis, allergy, menstrual cycle regulation and increase fertility in women [64].

The inorganic trace elements of acid and basic radicals have been shown in the table 3 and 4. The plants needs more amount of potassium for important metabolism like photosynthesis, protein synthesis and activation of enzymes. In human the potassium actively participates in the regulation of cardiac rhythm [65,66].

The minerals like calcium copper and magnesium are considered as medically important elements [67]. Copper is a vital mineral that plays an important role in the production of hemoglobin, myelin, collagen and melanin [26].

Table 3 Phytochemical screening of the plant *h. Enneaspermus* using different solvents

S. No	Phytochemicals	Leaf extracts of <i>Hybanthusenneaspermus</i>				
		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.	Cardio Glycosides	+	+	-	+	±
11.	Coumarins	+	++	+	-	-
12.	Betacyanin	+	+	+	-	-

(++ = strong positive, + = positive, - = negative, ± = semi positive)

Table 4 Analysis of inorganic basic radicals in the leaf extract of *Hybanthusenneaspermus*

S.No	Basic radicals	Inference
1	Aluminum	Negative
2	Ammonium	Negative
3	Calcium	Positive
4	Copper	Positive
5	Ferric	Negative
6	Ferrous	Negative
7	Lead	Negative
8	Mercury	Negative
9	Zinc	Positive
10	Silver	Negative
11	Potassium	Positive
12	Magnesium	Positive

Zinc is responsible for sperm production, fetus development and proper function of immune response [68]. Zinc deficiency can lead to loss of appetite, growth retardation, weakness and even stagnation of sexual growth particularly in children [69]. Zinc has important roles in the cholesterol metabolism as well as heart diseases. Zinc activates formation of chlorophyll, enzymes and plays a role in formation of auxin, chloroplasts and starch [70].

Magnesium is an important component of chlorophyll in plants. It activates many enzymes involved in photosynthesis, respiration and is involved in the synthesis of DNA and RNA in plants. Magnesium is absorbed in the intestine and then transported through the blood to cells and tissues of humans. It is also involves in formation teeth, bones and enzyme cofactor [70]. Calcium is essential in bone structure and function and zinc regulates various enzyme functions [71].

CONCLUSION

The physico- chemical analysis such as pH, moisture content, total ash and acid insoluble ash value shows the quality of plants. The present study revealed the phytochemical composition of *Hybanthusenneaspermus* leaves. The results vary in different solvents because of its polarity. The different solvents were used in this present research to extract different types of phytochemicals and among all the solvents ethanol was able to extract large number of compounds. The data obtained from this study can be used to discover novel herbal drugs to cure various diseases of animals as well as human beings. From the present study it can be concluded that these plants contains important trace elements and the percentage of all the trace elements within permissible level which directly

influence the quality of secondary metabolites. Further studies are needed in this direction to explore more pharmacological actions of the plant.

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