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Phytochemical Screening of *Hybanthus enneaspermus* (Linn) F. Muell.

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ABSTRACT:

Screening of phytochemicals is a precious stair in the detection of bioactive principles present in unique medicinal plant and may lead to novel drug discovery. In the present study, phytoconstituents of *Hybanthus enneaspermus* (Linn) F. Muell. was identified. Screening of the plants were performed using standard methods in methanol, ethanol, petroleum ether, chloroform and aqueous extracts and resulted in the detection of the presence of carbohydrates, proteins, aminoacids, alkaloids, flavonoids, glycosides, steroids, saponins, phenols, tannins, triterpenoids, anthraquinone, and phytosterols. Steroids were present higher quantity compare with other bioactive compounds. Saponins are absent in all the extracts. The presence of these phytochemicals can be correlated with medicinal potential of these plants. Further studies are needed with these plants to consider their pharmacological potentials, isolate, characterize and elucidate the structures of the bioactive compounds responsible for their activities and other medicinal values.

Keywords: *Hybanthus enneaspermus* (Linn) F. Muell., phytochemical screening, solvent, extracts.

INTRODUCTION:

Medicinal plants are an important part of natural wealth, serve as essential therapeutic agents as well as valuable raw materials for manufacturing several traditional and modern medicines (Talalay, 2001). Medicinal plants act as chemical factories, which are capable of synthesising unlimited range of highly complex and active phytochemicals (Newman and Cragg, 2007).

Phytochemicals are chemical compounds that occur naturally in plants. They are classified into two groups, such as primary and secondary metabolites. Primary metabolites (ethanol, lactic acid and aminoacids) are concerned in growth, development and reproduction of the organism. Secondary metabolites are chemical components produced by plants; and their function in growth, photosynthesis, replication and different primary essential approaches are not known yet. Such phytochemicals have been screened and reported by many researchers (Sampath *et al*., 2016; Krupashree *et al*., 2018). Approximately 119 pure chemicals has been extracted from higher plants and are used in medicine throughout the world. 10% of our main

drugs now include phytochemicals still extracted directly from higher plants. Bio-active components of plants like phenol, saponins, alkaloids, amino acids, and flavonoids, which are possessing organic activities together with anti- cancer, anti-fungal, and anti-inflammatory activities.

Hybanthus enneaspermus (Linn) F. Muell is a Violaceae family recognized as Sthalakamala in Ayurveda which is distributed in the tropical and subtropical areas in the world. It is a herbal plant used for medicinal purpose. It is additionally called as “hump back flower" and they are moreover called as green violet (L) F. Muell. A small, suffrutescent perennial herb 15-30cm high, with many diffuse or ascending branches, glabrous or more or less pubescent stem sparingly branched with woody base and spreading erect branches. The plant is reported to possess tonic, diuretic and demulcent properties. The root sandals are employed for the bowel complaints of children. The leaves and tender stalks are demulcent and used as a decoction or electuary. The fruits and leaves are used as antidotes for scorpion stings and cobra bites by the Yanadi tribes (Reddy *et al*., 1989; Sudarsanam and Sivaprasad, 1995). In the existing investigation, efforts are made to screen the phytochemical compounds of different solvent extracts of *Hybanthus enneaspermus* (Linn) F.Muell. for better results.

Sampath *et al*. (2016) carried out a study to apprehend the phytochemical nature of the *Hybanthus enneaspermus* (Linn.) F. Muell. entire plant. It was grind to coarse and successively extracted with solvents of increasing polarity in a soxhlet extractor, extracts has been subjected to qualitative evaluation and revealed many phytochemical compounds. The present study of Krupashree *et al*. (2018) investigated *Hybanthus enneaspermus* (Linn.) F. Muell. for the presence of phytochemicals of therapeutic significance. The results revealed the presence of different phytochemicals in various extracts.

MATERIALS AND METHODS:

The plant chosen for the study *Hybanthus enneaspermus* (Linn.) F. Muell. was gathered from Nattaalam, Kanyakumari District, Tamilnadu. The plant was identified taxonomically. Fresh plant parts were washed thoroughly 2-3 times with running tap water and then with sterile water. Then it was shade-dried, powdered and used for extraction.

Fig.1. *Hybanthus enneaspermus* (Linn.) F. Muell.

Preparation of aqueous leaf extracts:

The collected plant parts (10 g) have been washed and the adhering dirt's had been removed. Then it was cut into small pieces and shade dried. The powder of plant materials are macerated separately with 25 ml of sterile distilled water using pestle and mortar. The macerate was first filtered through four layer of muslin cloth and then filtrate was centrifuged at 8,000 rpm for 15 min at room temperature. Supernatant was filtered through Whatmann No.1 filter paper and heat sterilized at 120°C for 30 min. The extract was preserved aseptically in a brown bottle at 4°C until further use (Sukanya *et al.*, 2009).

Preparation of solvent extracts:

The plant parts were washed with clean water and air dried for 5 days. The dried leaves were stored in sealed and labeled containers for use. 20 gms of the plant parts were suspended in 120 ml of 98% ethanol and left for 24 hours. Thereafter, the suspensions were filtered into sterile containers separately using Whatmann No.1 filter paper. The extracts were allowed to dry at a temperature of 40^{o}C into powder. The powder of the extracts obtained were stored in sealed bottles and kept in a refrigerator at 4^{o}C until further use as per the method followed by Akerele *et al.* (2008).

Phytochemical analysis:

Preliminary phytochemical tests for the identification of carbohydrates, protein, aminoacid, glycosides, steroids, saponins, tannins, alkaloids, flavonoids, phenols, terpenoids, anthraquinone and phytosterols were carried out for all the extracts.

Test for Carbohydrates (Brain & Turner, 1975)

Benedict's test: To 0.5 ml of test drug about 0.5 ml of Benedict's reagent is added. The mixture is heated on a boiling water bath for 2 minutes. A characteristic coloured precipitate indicates the presence of sugar.

Glycosides (Ansari, 2006)

Keller-Killiani Test: To 2 ml of the extract, glacial acetic acid, one drop 5% $FeCl_3$ and conc. H_2SO_4 was added. Reddish brown colour appeared at junction of two liquid layers and upper layer turned bluish green indicating the presence of glycosides.

Steroids (IP, 1996)

Salkowski Test: To 2 ml of extract, 2 ml of chloroform and 2 ml of conc. H_2SO_4 was added. The solution was shaken well. As a result chloroform layer turned red and acid layer showed greenish yellow fluorescence.

Alkaloids (Ansari, 2006)

The extract was evaporated in a test tube. To the residue dilute HCL was added, shaken well and filtered.

Mayer's Test: To the 2-3 ml of filtrate Mayer's reagent was added. Formation of yellow precipitate showed the presence of alkaloids.

Flavanoids(Kokate, 1994)

Shinoda Test: To the extract, 5 ml of 95% ethanol and few drops of concentrated hydrochloric acid was added. To this solution 0.5 gm of magnesium turnings were added. Pink colouration indicated the presence of flavanoids.

Tannins (Mukherjee, 2002)

Lead Acetate Test: On addition of lead acetate solution to the extract white precipitate appeared.

Saponin (Ansari, 2006)

Foam Test: Drug extract was shaken vigorously with water. No persistent foam was formed.

Protein (Ansari, 2006)

Biuret test: With 3 ml of test solution, few drops of 4% NaOH and 1% CuSO4 solution were added. The tubes were observed for violet or pink colour formation.

AminoAcid (Evans, 1996)

Ninhydrin test: About 0.5 mg of extract was taken and two drops of freshly prepared 0.2% Ninhydrin reagent was added and heated. The appearance of pink or purple colour indicates that the presence of proteins, peptides or amino acids.

Phenol (Mukherjee, 2002)

Ferric chloride test: The extract was diluted to 5 ml with distilled water. To that a few drop of neutral 5% ferric chloride solution was added. A dark green color indicates the presences of phenolic compounds.

Test for Glycosides (Horbone, 1984)

0.5 mg of extract was dissolved in 1 ml of water and then aqueous NaOH solution was added. Formation of yellow color indicates the presence of glycosides.

Test for Triterpenoids (Horbone, 1984)

To the test solution 2ml chloroform was added with few drops of conc. Sulphuric acid (3ml) at the side of the test tube. An interface with a reddish brown coloration is formed if terpenoids constituent is present.

Test for Anthroquinones (Evans, 1996)

Borntrager s Test: About five ml of the extract was boiled with 10% HCl for few minutes in a water bath. It was filtered and allowed to cool. Equal volume of CHCl3 was added to the filtrate. Few drops of 10% NH3 were added to the mixture and heated. Formation of pink colour indicates that the presence anthroquinones.

Test for Phytosterols (Evans, 1996)
Salkowski's Test: The plant extract was mixed with chloroform and filtered. The filtrate is treated with 5-6 drops of conc. Sulphuric acid carefully and shaken gently, allowed to stand. A golden yellow colour indicates the presence of triterpenes (phytosterol).

RESULTS AND DISCUSSION

The phytochemical evaluation of *Hybanthus enneaspermus* (Linn) F. Muell. plant is proven in Table.1. In *Hybanthus enneaspermus* (Linn) F. Muell. maximum number of compounds have been noticed in ethanol and methanol extract. Flavonoids and phytosterols were the phytochemical compounds present in chloroform extract. The petroleum ether extract showed the compounds like steroids and anthraquinones. Aqueous extracts confirmed the presence of carbohydrates, flavonoids and phytosterols.

Table.1. Phytochemical analysis of *Hybanthus enneaspermus* (Linn) F. Muell extracts

Phytochemicals	Ethanol	Methanol	Petroleum Ether	Aqueous	Chloroform
Carbohydrates	+++	+++	-	+	+
Proteins	++	+++	-	-	-
Aminoacids	+++	++	-	-	-
Alkaloids	++	++	-	-	-
Flavonoids	++	+++	-	++	-
Glycosides	+	+	-	-	-
Steroids	+	++	++	-	++
Saponins	-	-	-	-	-
Phenols	++	+++	-	-	++
Tannins	+++	+++	-	-	-
Triterpenoids	+	++	-	-	-
Anthraquinone	++	++	+	-	-
Phytosterols	+	++	-	+	-

Note: (+++ denotes very high, ++ denotes moderate, + denotes low, – denotes absence of compounds)

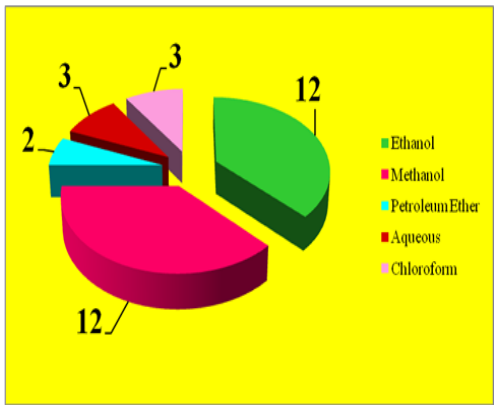
The preliminary phytochemical screening of all the extracts confirmed the presence of carbohydrate, protein, aminoacid, alkaloids, flavonoids, glycosides, steroids, phenols, tannins, terpenoids, anthraquinone and phytosterols (Table.1). The presence of maximum number of compounds were recorded in ethanol and methanol extracts whereas minimum number of compounds were recorded in aqueous, chloroform and petroleum ether extracts.

Many researchers have laboured on the phytochemical components of *Hybanthus enneaspermus* (Linn) F. Muell. and found the compounds such as phenols, alkaloids, flavonoids, steroid, glycosides, tannins etc. (Anand and Gokulakrishnan, 2012, Anupa *et al*., 2016, Patel *et al*., 2011). Anand and Gokulakrishnan, (2012) states that the extract of this plant includes alkaloids and flavonoids, which are having biological activities. Flavonoids are present in the form of polyphenolic compounds that have potent antimicrobial, anti-inflammatory activity. They prevent oxidative cell damage and in addition have strong anticancer activity. Terpenoids are the myriad compounds used by humans in the food and pharmaceuticals. Phenols are largest group of plant metabolites, which have many biological properties such as antiapoptosis, antiageing, anticarcinogen, anti- inflammation and cell proliferating activities. 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. Raju *et al*. (2016) has reported that the isolated alkaloids and their synthetic

derivatives are used as basic medicinal agents for their analgesic, antispasmodic and bactericidal effects.

Steroids had been used as allergy, arthritis and coronary failure therapy, control in menstrual cycle and increasing women fertility and tannins are reported to possess anti-irritant, anti-secretolytic, anti-phlogistic, antimicrobial and anti-parasitic effects. Flavonoids and tannins are phenolic compounds and plant phenolics are a major group of compounds that act as primary antioxidants or free radical scavengers. Phenolic compounds such as flavonoids, phenolic acids and tannins are widely distributed in plants which have gained much attention, due to their antioxidant activities and free radical-scavenging abilities, which potentially have beneficial implications for human health. Generally, secondary metabolites and nutrients confirmed the pharmacological and ethno medicinal values of the plants. Most bounded polyphenols are condensed tannins and comprising up to 50% of the dry weight of the leaves which are easily bound to proteins and carbohydrates ensuring in reduction in digestibility of these macromolecules. Harborne (1984) stated that the phenolic compounds are majorly involved in antioxidant and antitumour activity. As well as, the quantitative determination of flavonoids and glycosides played.

Fig.1. Phytochemicals present in the selected solvent of *Hybanthus enneaspermus* (Linn) F. Muell extracts



Hence the present study revealed that *Hybanthus enneaspermus* (Linn) F. Muell. has quite a number of pharmacognostic values. The outcomes of these research might thus serve as a base for proper identification, collection and investigation of the *Hybanthus enneaspermus* (Linn) F. Muell. In conclusion the parameters which are mentioned here can be regarded as special adequate to recognize and decide the accuracy of this drug in pharmaceutical industry.

SUMMARY AND CONCLUSION:

The phytochemical analysis revealed the presence of all the compounds analyzed except saponins. Ethanol and methanol extract confirmed the presence of more components whereas petroleum ether, chloroform and aqueous extracts exposed few compounds. There is a need to advance research for the development and characterization of new natural drugs with the aid of better screening strategies from plants and other natural sources. In the development of traditional medicines and further investigation needs to elute novel active compounds from the *Hybanthus enneaspermus* (Linn) F. Muell. which may be created a new way to treat many incurable diseases.

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