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PRELIMINARY PHYTOCHEMICAL ANALYSIS OF *TABERNAEMONTANA ALTERNIFOLIA*

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ABSTRACT

Medicinal plants are an important source of phytochemicals that offers traditional medicinal treatment of various ailments. Phytomedicinal constituents can be derived from any part of the plant like bark, leaves, fruits, flowers, roots, seeds etc. The herbal medicines are becoming more popular because of its inability to cause side effects and cheaper than synthetic drugs. Phytochemical is chemical compounds that occur naturally in plants. Phytochemistry deals with the chemical structure, biosynthesis, metabolism, natural distribution and biological function. Phytochemical studies have attracted the attention of plant scientists due to the development of new and sophisticated techniques. This can be sorted by their chemical class, bio synthetic origin and functional groups into primary and secondary metabolites. The study and analysis of chemical constituents of plants are desirable, not only for the discovery of therapeutic agents, but also for disclosing new resources of such chemical substances. Therefore it is of interest to investigate the phytochemical analysis of aqueous chloroform, acetone, aqueous ethanolic, ethyl acetate and aqueous extracts *Tabernaemontana alternifolia*.

KEYWORDS: ***Tabernaemontana alternifolia*, Phytochemistry, Secondary metabolites, Aqueous Chloroform, Acetone, Ethyl acetate.**

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INTRODUCTION

India is known as the "Emporium of Medicinal plants" due to occurrence of several thousands of medicinal plants in the different bioclimatic zones. The traditional heritage of India include many time tested medicinal plants and drugs for various diseases. The demand for Ayurvedic, herbal drugs and phytomedicines is increasing day by day globally [1]. Most cultures from ancient times to the present day have used plants as a source of medicine. *Tabernaemontana alternifolia* is a species of plant in the Apocynaceae (Dogbane) family. It is endemic to India. The name *Tabernaemontana heyneana* Wall. is often used for this species, and a proposal was made to conserve that name so that it would be used instead of T. alternifolia. Arguments made for conservation were that the type of the species was not a specimen but an illustration, and that the plant has opposite leaves, not alternate leaves as *alternifolia* implies [2]. However, the proposal was rejected, and the correct name of this species is the original name, *T. alternifolia* [3]. The aerial parts of the plant such as leaves, seeds, flowers, stems were used for the study. Therefore it is of interest to investigate the phytochemical analysis of aqueous chloroform, acetone, aqueous ethanolic, ethyl acetate and aqueous extracts *Tabernaemontana alternifolia*. All parts of *T. alternifolia* are used as local medicines. Whole plant including roots and leaves contains alkaloids which are found to be active against lymphocytic leukemia [4]. A compound like camptothecin is isolated from the plant. This compound is widely used against various forms of cancer [5]. The leaves, flowers, seeds and stems of *T. alternifolia* are used in the treatment of various diseases like cancer, diarrhoea, syphilis etc.

MATERIALS AND METHODS

Collection of samples

The medicinal plants used for the experiment were aerial parts of *Tabernaemontana alternifolia* collected from Tambaram, Chennai, TamilNadu. The plant *Tabernaemontana alternifolia* were obtained, identified and authenticated from the chief botanist, Tamilnadu Aromatic Medicinal plant Corporation Ltd., Chennai, India.

Preparation of extracts

100 grams of dried aerial parts of *Tabernaemontana alternifolia* was packed in five separate round bottom flask for sample extraction using solvents namely , Chloroform(70%)-Water(30%) mixture, Acetone, Ethyl Alcohol(70%)-Water(30%) mixture, Ethyl Acetate, and Water. The extraction was conducted by 150 ml of the each solvent mixture for a period of 24 hours. At the end of the extraction the respective solvents were concentrated under reduced pressure and kept it in water bath (at 50°c). Now the extracted experimental solutions were stored in refrigerator.

Phytochemical analysis

The extracts prepared were analyzed for the presence of alkaloids, saponins, tannins, steroids, flavonoids, anthraquinones, cardiac glycosides and reducing sugars based on the protocols available in the literature [6][7][8].

Test for alkaloids

The extract of dry aerial parts were powdered of each solvent was evaporated to dryness in boiling water bath. The residues were dissolved in 2 N Hydrochloric acids. The mixture was filtered and the filtrate was divided into three equal portions. One portion was treated with a few drops of Mayer's reagent, one portion was treated with equal amount of Dragondorff's reagent and the third portion was treated with equal amount of Wagner's reagent respectively. The appearance of creamish precipitate, the orange precipitate and brown precipitate indicated the presence of respective alkaloids [9].

Test for saponins
About 0.5 g of the aerial plant parts extract was vigorously shaken with water in a test tube and then heated to boil. Frothing was observed which was taken as a preliminary evidence for the presence of the saponins.

Test for tannins
About 0.5 g of aerial parts of the plant extract was added in 10 ml of water in a test tube and filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or blue-black coloration [10].

Test for steroids
2 ml of acetic anhydride was added to 2 ml of aerial parts of the plant extract of each sample along with 2 ml sulphuric acid. The colour changed from violet to blue or green in some samples indicating the presence of steroids.

Test for flavonoids
2 ml of extract solution was treated with 1.5 ml of 50% methanol solution. The solution was warmed and metal magnesium was added. To this solution few drops of conc. Hydrochloric acid was added and the red colour was observed for flavonoids and orange colour for flavones [11].

Test for anthraquinones
About 0.5 g of extract was taken in a dry test tube and 5 ml of chloroform was added and shaken for 5 min. The extract was filtered and the filtrate shaken with equal volume of 10% of ammonia solution. A pink violet or red colour in the ammonical layer indicates the presence of anthraquinones [11].

Test for cardiac glycosides
0.2 g of extract was dissolved in 1 ml of glacial acetic acid containing 1 drop of ferric chloride solution. This was then under layered with 1ml of concentrated sulphuric acid. A brown ring obtained at the interface indicated the presence of a deoxysugar characteristic of cardioids [11].

Test for Proteins
To 2ml of protein solution 1ml of 40% NaOH solution and 1 to 2 drops of 1% CuSO4 solution was added. A violet colour indicated the presence of peptide linkage of the molecule [11].

Test for Amino Acids
To 2 ml of sample was added to 2 ml of Ninhydrin reagent and kept in water bath for 20 minutes. Appearance of purple colour indicated the presence of amino acids in the sample [11].

Test for Tri-Terpenoids
5ml of each extract was added to 2ml of chloroform and 3ml of con. H2SO4 to form a monolayer of reddish brown coloration of the interface was showed to form positive result for the tri-terpenoids [11].

Test for Reducing Sugar
To 2 ml of extract 2 drops of Molisch's reagent was added and shaken well. 2ml of conc. H2SO4 was added on the sides of the test tube. A reddish violet ring appeared at the junction of two layers immediately indicated the presence of carbohydrates [11].

RESULTS

Table 1

shows the phytochemical analysis of aqueous chloroformic, acetone, aqueous ethanolic, ethyl acetate and aqueous extracts of Tabernaemontana alternifolia.

S.NO.	Phytoconstituents	Aqueous Chloroform Extract	Acetone Extract	Aqueous Ethyl Alcohol Extract	Ethyl Acetate Extract	Aqueous Extract
1	Flavonoids	--	++	--	++	--
2	Alkaloids	++	--	--	++	--
3	Tri-terpenoids	--	--	--	--	--
4	Saponins	++	--	--	--	++
5	Tannins	++	--	--	++	--
6	Reducing sugar	++	--	--	--	--
7	Amino acids	--	--	--	--	--
8	Anthraquinones	++	--	++	--	--
9	Steroids	--	--	--	--	++
10	Proteins	--	--	--	--	--
11	Cardiac glycosides	++	--	--	--	--

"++": Positive
"--" :Negative

Table 1 shows the phytochemical constituents of aqueous chloroformic, acetone, aqueous ethanolic, ethyl acetate and aqueous extracts of *Tabernaemontana alternifolia.* The phytochemical screening of the crude extract revealed the presence of flavonoids in acetone and ethyl acetate extract, whereas the tri-terpenoids were absent in all the extracts. The tannins are present in aqueous chloroform and ethyl acetate extracts, whereas absent in remaining all extracts. The saponins are present in aqueous chloroform, aqueous extract and remaining shows negative result. In the case of amino acids and proteins shows negative result. Anthraquinones are positive in the extracts of aqueous chloroform and aqueous ethyl alcohol, whereas acetone, ethyl acetate and aqueous extract show negative. In steroid analysis aqueous extract shows positive, where as remaining show negative. In the case of alkaloids, present in aqueous chloroform and ethyl acetate, whereas absent in acetone, aqueous ethyl alcohol and aqueous extracts. In the case of cardiac glycosides and reducing sugars were present in aqueous chloroform extracts but remaining all the extracts got negative results.

DISCUSSION

The result indicates the presence of numerous phytochemicals, including reducing sugars, flavonoids, cardiac glycosides, alkaloids, saponins, tannins, terpenoids and anthraquinones in the aqueous chloroformic, acetone, aqueous ethanolic, ethyl acetate and aqueous extracts of *Tabernaemontana alternifolia.* Ethanol was used which has a wide range of solubility in both polar and non-polar region [12]. Tannins are one of the major constituents of the plants which may have antidiarrheal potential [13][14], and leaves of *Tabernaemontana alternifolia* also contain tannins which is been confirmed from the positive phytochemical test. Scientists all over the world are concentrating on the herbal medicines to boost immune cells of the body against cancer. Medicinal herbs are also significant source of synthetic and herbal drugs. Herbal system of medicine has been practiced for thousands of years [15].

CONCLUSION

The phytochemical screening carried out with *Tabernaemontana alternifolia* has revealed the presence of many metabolites which intern contributes to phytochemical and pharmacological activity. Not much has been

reported with *Tabernaemontana alternifolia*. The present study portrays that the phytochemicals in *Tabernaemontana alternifolia* may contribute in many significant ways for various studies in a truthful manner to the various activities of the plant.

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