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Research Article

QUALITATIVE AND QUANTITATIVE ANALYSIS USING AERIAL PARTS FROM DOUBLE FLOWER VARIETY OF *TABERNEMONTANA DIVARICATA*

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ABSTRACT

Qualitative and quantitative phytochemical analyses were done using aerial parts of double flower variety of *Tabernaemontana divaricata*. Qualitative phytochemical analysis was performed to identify the presence of various phytoconstituents with standard procedures. Total phenols, total flavonoids and total protein were determined by folin-ciocaltaeu method, aluminium chloride colorimetric method and Lowry's method. Phytochemical screening exhibits the occurrence of alkaloids, flavonoids, terpenoids, cardiac glycosides, saponins, tannins, carbohydrates and protein. In quantitative analysis, elevated level of phenols, flavonoids and protein were identified in leaves, flowers and stem. The present study concluded that *Tabernaemontana divaricata* plant has the ability to cure a variety of diseases with antioxidant capacity because of the presence of phytochemicals in it.

KEYWORDS: *Tabernaemontana divaricata*, Phytochemicals, Qualitative analysis, Quantitative analysis**.**

INTRODUCTION

Nature is a warehouse for various plants with medicinal properties[1]. Many varieties of medicinal plants are distributed worldwide but now only 80% of people trust conventional medicines made from plants for their primary care of health [2]. It can be used to treat all diseases because of its least or no side effects in the human being[3]. Medicinal importance of the plants depends on the presence of some phytochemical constituents. These phytochemical substances in the medicinal plants can generate physiological consequences in the human body system [4, 5]. It can be derived from diverse parts of the plants like flowers, leaves, stem and root to exploit against disorders. There are two forms of phytochemicals. Primary metabolites are amino acids, proteins, sugars and chlorophyll, secondary metabolites comprises of phenols, alkaloids, flavonoids, terpenoids, tannins, saponins and glycosides [6].

Tabernaemontana divaricata is a medicinal as fine as ornamental plant belonging to the family of Apocynaceae shown in fig. 1. In various languages, it is known as Crepe jasmine (English), Chandani (Hindi), Nandivardhanamu (Telugu), Nandibatlu (Kannadam), Kutampale (Malayalam) and Nandiyavattai (Tamil) [7, 8, 9]. Flowers are milky white in colour, cooling and fragrant. It can be utilized to heal dermatopathy, flaming sense and ophthalmic problems [10]. The leaves, flowers and stem of the plant are shown in the fig. 2, fig. 3 and fig. 4 respectively. They have been utilized habitually for the healing of ulcers and rheumatism [11]. Root's flavour is bitter and squashing of root reduces tooth ache. It is used to avert inflammation when combine with water in sores [12]. *T.divaricata* possesses extensive series of useful actions like anti-infection, anti-inflammation, anti-cancer, astringent, analgesic, antioxidant[13,14], anxiolytic, anti diabetic and anticonvulsant [15- 17].

MATERIALS AND METHODS

Extract preparation

Flowers, Leaves and Stem were collected from the plant then washed, shade dried and powdered. For the preparation of ethanol extract, 30g of each powdered materials had been extracted with 200ml absolute ethanol using soxhlet apparatus for 24 hours. The solvent was dispersed under vacuum and dried extracts used for further analysis. Each extracts were mixed with suitable amount of respective solvent at the time of usage. This hot percolation method was

done in the department of biotechnology, Marudupandiyar college, Thanjavur.

Qualitative Phytochemical screening

Several chemical tests to ascertain different phytochemicals are follows [18-22]

Alkaloids

Alkaloids were identified through Mayer's test. In the test, the extracted residues were dissolved in 2N Hydrochloric acid. The mixture was filtered and the filtrate treated with a few drops of Mayer's reagent. Formation of cream precipitate indicates the presence of alkaloids.

Flavonoids

Using alkaline reagent test, flavonoids were detected. During this test, extracts treated with few drops of NaOH solution. After addition of dilute hydrochloric acid, formed intense yellow colour, which becomes colourless, indicates the presence of flavonoids.

Steroids

Libermann Burchard test was used for the identification of steroids. Within the test, few drops of acetic anhydride was added to the extract, boiled and cooled. After cooling, concentrated H_2SO_4 also added to the sides of the tube. At the junction of two layers a brown ring formation followed by upper layer turns into green reveals the presence of steroids.

Terpenoids

Terpenoids were found by means of salkowski test. In this test, extracts treated with little drops of Conc. H_2SO_4 leads to yellow coloured lower layer formation shows the existence of terpenoids.

Glycosides

During the examination of glycosides, extracts added with 5ml of dilute H_2SO_4 on water bath. Filtrates neutralize with 0.1ml of 5% NaOH. 0.1ml of Fehling's solution A and B added until it becomes alkaline and keep in water bath for 2minutes. Development of red precipitate illustrates the occurrence of glycosides.

Cardiac Glycosides

In this experiment, small amount of extracts dissolved in 1 ml of glacial acetic acid containing 1 drop of ferric chloride solution and 1ml of Conc. sulphuric acid. Appearance of brown ring between the interface reveals the presence of cardioids.

Saponins

Using foam test, saponins were detected. In the examination, little amount of extracts shake with water. If foam produced persists for 10minutes shows the presence of saponins.

Tannins

Tannins were identified through ferric chloride test. Within this test, small amount of extracts dissolve in water separately. When extracts treated with 5% dilute ferric chloride leads to the appearance of blue colour if hydrolysable tannins are present. Condensed tannins are identified by the existence of green colour.

Phenols

During the time for the analysis of phenols, small volume of extracts dissolved in alcohol and alcoholic ferric chloride added to the above mixtures. Violet colour formation leads to the presence of phenols.

Carbohydrates

Carbohydrates were found via Molisch's test. In this test, distilled water was used to dissolve extracts and filtered. Few drops of alcoholic α-naphthol solution and few drops of conc. H_2So_4 added to the extracts containing test tubes resulted in purple to violet colour ring at the junction of two layers reveals the occurrence of carbohydrates.

Protein

Using Biuret test, protein was ascertained. In this examination, with 2ml of extracts, 1ml of 40% NaOH and 2 drops of 1% Copper sulphate ($Cuso_4$) were added. Appearance of violet colour shows the existence of protein.

Quantitative analysis

Phenols, Flavonoids and Protein were measured by means of diverse methods. They are as follows [23-25]

Total Phenols

Total phenols was analysed using Folin-Ciocaltaeu method. In 200 µl of Samples, 1ml of folin- ciocalteu reagent and 0.8 ml of sodium carbonate (7.5%) was added. The tubes stand for 30 min. Absorption measured at 765 nm. Gallic acid was used as standard. The total phenolic content expressed as gallic acid equivalents (GAE) in milligram per gram extract.

Total flavonoids

Total flavonoids was estimated by aluminium chloride colorimetric method. In this test, extracts (50µl) added to 4950 µl of distilled water and mixed with 0.3 ml of 5 % $NaNO_2$. This mixture incubated for 5 min at room temperature and 0.3 ml of 10 % $AlCl_3$ was added. After 6

minutes of incubation, 2 ml of 1M NaOH was combined followed by the addition of 2.4 ml distilled water. Absorbance taken at 510 nm. Quercetin was used as standard.

Total Protein

Total protein was calculated via Lowry's method. In this procedure, with different concentrations of standards, 0.1 ml of extracts was added in the separate tubes and volume made up to 1ml with distilled water. In all the tubes, 5 ml of alkaline copper sulphate reagent was mixed and left at room temperature for 10 min. Then 0.5 ml of folin - ciocalteau reagent was dissolved and incubated at room temperature for 20 min. The colour developed read at 660 nm and the protein concentration expressed as mg per gm extract.

RESULTS AND DISCUSSION

The result of the present study made known the presence of flavonoids, terpenoids, phenols, tannins, carbohydrates and protein in all parts. But alkaloids and steroids were absent only in flowers. In stem, Cardiac glycosides, steroids and saponins were not present. It is specified in Table 1.

Quantitative analysis from ethanol extract of *T.divaricata* revealed that the rich amount of flavonoids and protein present in leavesl and flowers when compared to stem. As well as in leaves increased level of phenols were observed when compared to stem and flowers. Total flavonoids was assessed as 19.6 milligram quercetin equivalents/g (mg QE/g), 15.4 mg QE/g and 7.1 mg QE/g in leaves, flowers and stem. Likewise in leaves, flowers and stem, total phenols was estimated as 47.1milligram gallic acid equivalents/g (mg GAE/g), 6.2 mg GAE/g and 5.4 mg GAE/g respectively. Moreover total protein level was deliberated. It is denoted in Table 2.

Table 1: Results of Qualitative Phytochemical screening

Phytoconstituents	Flowers	Stem	Leaves
Alkaloids	-	+	+
Flavonoids	+	+	+
Steroids	-	-	+
Terpenoids	+	+	+
Cardiac glycosides	+	-	+
Carbohydrates	+	+	+
Protein	+	+	+
Saponins	+	-	+
Tannins	+	+	+
Phenols	+	+	+

Table 2: Quantitative analysis of ethanol extract of *Tabernaemontana divaricata*

Plant parts	Total Phenols (mg GAE/g)	Total Flavonoids (mg QE/g)	Total protein (mg/g)
Flowers	6.2	15.4	2
Leaves	47.1	19.6	18
Stem	5.4	7.1	1.8

Plants produce a miscellaneous group of secondary metabolites with antioxidant capacity. Antioxidants block the action of free radicals which have been concerned in the pathogenesis of numerous diseases[26-28]. Due to existence of phenolic compounds and flavonoids, plant holds antioxidant activity on human fitness. Phenols, flavonoids and tannins are act as antioxidant compounds which play a role as free radical scavengers. Flavonoids are a set of polyphenolic compounds and exploit the inhibition of oxidative and hydrolytic enzymes[29-31]. Tannins also accelerate the remedy for lesions in addition to irritated mucous membranes[32]. Terpenoids, as vitamins, act as regulators of metabolism and play a protective role as antioxidants along with it acquires antimicrobial, antiallergic and antiinflammatory activity[33]. Saponins seize the unique possession of precipitating then coagulating red blood cells[34, 35]. Steroids aid in normalizing the immune response as well as hold cholesterol-reducing properties[36]. Alkaloids have been related with many remedial uses also with their cytotoxic capacity[37]. According to numerous reports, glycosides retain the ability to lower the blood pressure. in addition to this it provides resistance mechanism against many insects and microorganisms[38,39].

CONCLUSION

The present study concluded that the presence of primary and secondary metabolites in the aerial parts (stem, leaves and flowers) of *T.divaricata* with standard procedures. Based on our result this plant may be used for the production of new drugs, because of its phytochemical constituents which is involved in antioxidant activity for curing several ailments. These might be advantageous to make use of this plant for biomedical relevance in pharmaceutical companies by reason of assured class of phytocompounds. In future, this work can be extended to predict further biochemical compounds availability in this plant by adapting numerous chromatographic techniques.

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PLANT PHOTOGRAPHS

Fig. 1: Double Flower Variety of *Tabernaemontana divaricata*

Fig. 2: Leaves of *T. divaricata*

Fig. 3: Flower of *T. divaricata*

Fig. 4: Stem of *T. divaricata*