*Plant Archives* Vol. 24, No. 2, 2024 pp. 1979-1982 e-ISSN:2581-6063 (online), ISSN:0972-5210

Plant Archives

Journal homepage: http://www.plantarchives.org

DOI Url : https://doi.org/10.51470/PLANTARCHIVES.2024.v24.no.2.284

# *TABERNAEMONTANA CORYMBOSE*: INSIGHTS THERAPEUTIC PLATFORM THROUGH PHYTOCHEMICAL AND BIOCHEMICAL ANALYSIS

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(Date of Receiving-28-03-2024; Date of Acceptance-18-06-2024)

**ABSTRACT**

Preliminary phytochemical analysis has been performed in aqueous and ethanolic extracts of *T. corymbose* (Stem, Leaf and Flower). The various phytochemical constituents like protein and amino acids, carbohydrates including reducing sugar, glycosides, tannin, phenol and saponin are present in all the extracts. All the phytochemicals are abundantly found in flower extract (aqueous and ethanolic) followed by in leaf extract and in stem extract, respectively. This phytochemical screening is more prominent in ethanolic (organic) extract as compared to aqueous extract as bioactive compound are organic in nature and soluble in organic solvent. Highest α and β amylase activities were found in flower extract while phosphatase activity was observed in stem extract. Flowers of *T. corymbose* were found to be enrich with protein and reducing sugar whereas stem and leaves were moderately enriched.

***Key words :*** Phytochemical, Enzymes, *T. corymbose*.

## Introduction

*Tabernaemontana corymbose* belonging to the family Apocynaceae, commonly known as Chandni is an ornamental and evergreen shrub. It is mostly found in tropical region of India. Deep research has been done on numerous species belonging to the genus *Tabernaemontana* and utilization for their board spectrum of biological activities. At present, about 100 -150 species related to this genus have been found all over the world (Silveira *et al*., 2017). *T. corymbose* is the most prominent species in tropical region of India. The medicinal importance of this species is mainly related to its phytochemical composition. Proteins, sugar and cholorophyll are included in primary metabolites. They are essential for growth and survival of plants. Biosynthetically derived substances from primary metabolites like flavonoids, phenolic compounds, alkaloids, steroids, tannins, saponins, terpenoid are secondary metabolites. Different parts of *T. corymbose* are used as poultice, boiled juice, decoctions and infusions for treatment against ulceration, fracture, post-natal recovery, syphilis, fever, tumours and orchitis in Malaysia, China, Thailand and Bangladesh. Numerous bioactive constituents have been obtained from medicinal herbs such as: flavonoids, phenolic compounds, alkaloids, steroids, tannins, saponins, catechins, among others; these chemical constituents are responsible for a particular pharmacological action on the human body (Gill *et al*., 2012; Das *et al*., 2012).

## Materials and Methods

### Collection of plant material

The leaves, stem and flowers of. *T. corymbose* were collected from the Botanical Garden, Department of Botany, D.A.V. (PG) College, Muzaffarnagar, U.P., India. The different plant parts were washed with running tap water and subjected to crude extract preparation at room temperature for further studies.

### Preparation of crude extract

The leaves, stem and flowers of the plant *T. Corymbosa* were used for crude extract preparations (A&B) separately for phytochemical and biochemical

analysis. Extracts were kept at 4°C for the further analysis.

A) 10 g (fresh weight) of different parts of *T. corymbosa* were homogenized in 3-5 folds of aqueous and organic solvent (50%, ethanol) in pestle and mortar at room temperature. The extracts were filtered through sterilised whattmaan filter paper and filtrate was centrifuged at 10000 rpm at $4^0$C. The clear supernatant was used as crude extract for phytochemical analysis.

B) 10 g (fresh weight) of different parts of *T. corymbosa* were homogenized in 3-5 folds of 50mM of different buffers with 0.04% beta mercaptoethanol (acetate buffer; pH 5.0; phosphate buffer; pH 7.0 and tris buffer; pH 7.6) in pestle and mortar along with a pinch of acid washed sand. The extracts were filtered through sterilised four layered cheese cloth and filtrate was centrifuged at 10000 rpm at $4^0$C. The clear supernatant was used as crude extract for biochemical analysis. Unless stated, all operations were carried out at 0-4 $^o$C.

**Phytochemical screening of different parts of *T. corymbosa***

Phytochemical tests were carried out in the aqueous and organic extract of *T. corymbosa* using standard methods to identify the phytochemical constituents as described by Sofowara (1993), Trease and Evans (1989), Omoya and Akharaiyi (2012), Jyothiprabha and Venkatachalam (2016), Harborne and Williams (2000).

**Screening for steroids**

1 ml of extract was dissolved in 10 ml of chloroform and equal volume of concentrated sulphuric acid was added by the sides of the test tube without disturbing the contents. The upper layer turns red and sulphuric acid layer showed yellow with green fluorescence. This indicates the presence of steroids.

**Screening for tannins**

5 ml each of the extracts were stirred separately with 100 ml distilled water and filtered. One ml ferric chloride reagent was added to the filtrate. A blue-black or blue green precipitate was an indication of the presence of tannins.

**Screening for terpenoids**

5 ml of extract was taken in a test tube and 2 ml of chloroform was added to it followed by the addition of 3 ml of concentrated sulphuric acid. Formation of reddish-brown layer at the junction of two solutions confirms the presence of terpenoids.

**Screening for flavonoids**

A pinch of zinc dust was added to 2 ml of extract followed by the addition of 1 ml concentrated HCl. Appearance of pink colour indicate the presence of flavonoids

**Screening for saponins**

5 ml each of the extracts were mixed with distilled water and shaken separately in a test tube. Frothing, which persists on warm heating was taken as preliminary evidence for the presence of the saponins.

**Screening for glycosides**

5 ml extract was mixed thoroughly with 1 ml of glacial acetic acid and 1 ml of 5% $FeCl_3$ solution in a test tube. 1ml of concentrated sulphuric acid was added to the above reaction mixture carefully along the side of testtube. Development of green-blue colouration shows the presence of glycosides (Kellar-Kiliani test).

**Screening of phenols**

Few drops of 10% lead acetate solution were added to 5ml of test solution. Formation of white precipitates indicates the presence of phenol in the test solution.

**Protein estimation**

Protein concentration (mg/ml) was quantified according to Lowry *et al*. (1951). BSA was used as a standard protein (20-200 µg).

**Reducing sugar estimation**

The concentration of reducing sugar was determined by Di nitro Salicylic (DNS) method using D- glucose as standard reducing sugar (200-2000 µg).

**Enzyme assay**

- **Amylase activity :** α and β amylase activities were determined by DNS Method using Starch and Maltose (1%, w/v) as a substrate, respectively. The method involves the incubation of crude enzyme extract with 100mM phosphate buffer (pH 7.0) and specified substrate at $30^ï$C for 30 minutes. The reaction was terminated by adding DNS reagent after which the absorbance was taken at 540 nm.
- **Phosphatase activity :** Acid and alkaline activities were estimated by a modified method of Bowers and McCom (1996). It involves the incubation of crude enzyme extract with 100mM buffer (acetate buffer, pH-5.0 - acid phosphatase; tris buffer, pH-7.6 -alkaline phosphatase) and 100mM para nitro phenyl phosphate (pNPP) at $30^0$C for 30 minutes. The reaction was terminated by adding 0.1 N NaOH and there after the absorbance was taken at 430 nm.

One unit of enzyme activity was defined as the amount of enzyme which liberates 1µmole of product (amylase – reducing sugar; phosphatase – pNP, para nitro phenol)/ minute/ ml under standard assay conditions. The calibration curve were prepared using D – Glucose for amylase and pNPP for phosphatase.

## Results and Discussion

In the present investigation, preliminary phytochemical analysis has been performed in different extracts of *T. corymbose* (different parts), which showed the presence of various phytochemical constituents like protein and amino acids, carbohydrates including reducing sugar, glycosides, tannin, phenol and saponin (Table 1). While the terpenoids and flavonoids are absent in stem extracts of *T. corymbose*. Steroids are found to be absent in all studied parts of *the T. corymbose*. All the phytochemicals are abundantly found in flower extract (aqueous and ethanolic) followed by in leaf extract and in stem extract, respectively. This phytochemical screening is more prominent in ethanolic (organic)extract as compared to aqueous extract as bioactive compound are organic in nature and soluble in organic solvent.

The presence of phytochemicals as such; flavonoid, alkaloid, tannin showed cytotoxic effect (Chowdhury *et al*., 2017). Additionally, cholesterol-lowering, as well as

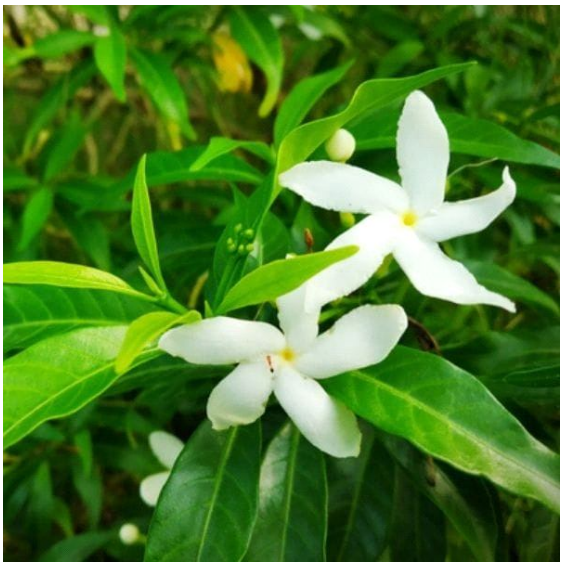

**Fig. 1 :** *Tabernaemontana corymbose* Plant.

cytotoxic qualities, anti-bacterial, anti-viral properties are credited to the presence of saponin (Bailly and Vergoten, 2020). Tannin shows an anticancer property that is perceptible from its inhibitory activity towards growth (Mazni, ho Yin, Azizul and Nurdin, 2016) The phenolic compound, tannin, terpenoid, flavonoids possess an ant-helmintic property so the plant Zanthoxylum, Acorus could be used to treat stomach problems (Nath and Yadav, 2016).

**Table 1 :** Qualitative Phytochemical and biochemical screening of different parts of *T. corymbose* in aqueous and ethanolic extract.

| S. no. | Name of bioactive compound | Leaf | | Stem | | Flower | |
|---|---|---|---|---|---|---|---|
| | | aq | Et | aq | et | Aq | et |
| 1 | Glycosides | ++ | ++ | + | ++ | +++ | +++ |
| 2 | Tannin | + | ++ | + | ++ | +++ | +++ |
| 3 | Terpenoids | ++ | +++ | - | - | - | ++ |
| 4 | Steroids | - | - | - | - | - | - |
| 5 | Phenols | ++ | +++ | + | ++ | + | ++ |
| 6 | Saponin | +++ | +++ | +++ | +++ | +++ | +++ |
| 7 | Flavonoids | - | ++ | - | - | +++ | +++ |

aq= aqueous extract, et=ethanolic extract, + = presence, - = absence.

**Table 2 :** Quantitative estimation of biomolecules in different parts of *T. corymbosa*.

| S. no. | Concentration and activity of biomolecules / mL | Stem | Leaf | Flower |
|---|---|---|---|---|
| 1 | Total protein (mg/mL) | 2.8 | 3.6 | 6.64 |
| 2 | Reducing sugar (mg/mL) | 4.45 | 7.52 | 9.72 |
| 3 | α- enzyme (U/mL) | 23.33 | 43.33 | 58.67 |
| 4 | β- enzyme (U/mL) | 62.0 | 46.0 | 54.67 |
| 5 | Acid phosphatase (U/mL) | 0.54 | 0.55 | 0.23 |
| 6 | Alkaline phosphatase (U/mL) | 0.09 | 0.015 | 0.0084 |

In biochemical screening, plenty of primary metabolites like proteins and carbohydrates (reducing sugar) were observed in flower extract while least amount in stem. Plant derived digestive enzymes are effective over a broad range of pH levels (3-9), which is highly well matched with the human gastrointestinal environment. Different enzymes were found to be active in different parts (leaf, stem and flower) of plant (Table 2). α and β amylase activity was profusely detected in flower extract followed by in leaf and stem extract, respectively. It indicates that flower extract may be

compatible with supporting comprehensive digestive health (Muhammad Sarwar Khan, 2018) involving carbohydrate metabolism. Medicinal plants are the rich source of enzymes or bioactive natural products, which could be used as enzyme modulators for the management of various disorders (Omar *et al.*, 2019), Phosphatase (acid and alkaline) enzymes were found to be more active in stem, i.e., capable to hydrolyse the phophoesters. So, stem extract can be used to regulate the metabolic pathways involving reversible phosphorylation. Leaf extract also contains moderate quantity of phytochemical and biochemical constituents so it can be used for above purpose in fact, leaves can be obtained in appreciable amount at any time as compared to flowers and stem for preparing extract.

## Conclusion

From the study, it could be concluded that plants are a great source of phytochemicals that could be utilized in curing various ailments. Tannin, terpenoids, flavonoid, steroid, saponin proteins and amino-acid, carbohydrates including reducing sugar, glycosides, phenols were the phytoconstituents present abundantly in test plant. This phytochemical screening test may be helpful in the screening of bioactive compound and eventually may provide a therapeutic platform to develop new drugs.

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