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Phytochemical analysis of the leaves of *Tabernaemontana Corymbosa* using HPLC

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Abstract

The study was aimed at quantifying a few phenolic compounds and sterols present in the leaves of *Tabernaemontana corymbosa* using HPLC. The HPLC estimation of the phytoconstituents was performed using Shimadzu High Performance Liquid Chromatographic system. The flow rate was maintained at 1 ml/min and the detection was at 254 nm. The results of the HPLC analysis of the leaf has shown peaks at retention times (metes) of 2.86 for Gallic acid, 3.49 for rutin, 4.13 for umbelliferone,7.16 for stigmasterol and 4.48 for sitosterol which were similar to that of the standards. The present study establishes the presence of phenolic compounds and sterols which are important plant constituents and could be responsible for the various activities exhibited and reported for the plant.

Keywords: *Tabernaemontana corymbosa*, HPLC, Phenolic acids, Sterols

Contents

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1. Introduction

Tabernaemontana is one of the genera commonly used in the Indian and Chinese traditional system of medicine[1,2]. The various species belonging to the genus *Tabernaemontana* are *T. bufalina, T.crispa, T. divaricata, T. pandacaqui, T. pauciflora* and *T. rostrata*. *Tabernaemontana corymbosa* is a plant of importance of this genus and belongs to the family Apocynaceae .It is widely distributed in Asia, America, Africa and Australia[3,4]. It is a multi branched evergreen shrub 3-4 meters high, the leaves are 5-12cm long, glossy, dark green leaves arranged opposite. Flowers are white in color with 5 petals. The plant has been reported to posses rejuvenating, neurotonic, antiviral, anti fungal, anti-inflammatory, antipyretic and Cardiovascular activities [5-10]. Review of literature reports the presence of alkoloids like voafinine, voalenine, vallesamine, Conodurine, conoduramine, and ervahanine; flavonoids, steroids, phenolic acids and enzymes [11-14]. Phenolic compounds are an important class of phytoconstituents as they are beneficial to health and hence analysis of this category of compounds has attracted

considerable attention. The present study was carried out to investigate the presence and amount of certain phenolic compounds and sterols which would help to develop the plant chemical profile.

2. Materials and methods

Plant material

The plant material was collected from the botanical gardens of Krupanidhi Institutions. The material was dried, powered and stored in air tight containers until further use.

Chemicals: The chemicals required for extraction and HPLC were obtained from SD fine chemicals and Qualigens.

Extraction: 200 grams of the powdered drug was subjected to Soxhlet extraction using methanol. The extract was the dried under vacuum.

Preliminary phytochemical analysis: The methanolic extract was subjected to preliminary phytochemical analysis using standard methods.

HPLC analysis: The HPLC estimation of the phytoconstituents was performed Shimadzu High Performance Liquid Chromatographic system which was equipped with LC-10ATVP pump, a rheodyne injector, SPD M10AVP Photo Diode Array Detector in combination with CLASS-VP 6.12 SP5 integration software. Chromatographic analysis was carried out in isocratic conditions using a C-18 reverse phase column C18 € ODS at 25°C.Running conditions included: injection volume 20 •l, the mobile phase used was MeOH-Phosphate buffer(0.005M) in the ratio 70:30.The flow rate was adjusted to 1 ml/min and the detection was done at 254nm.The samples to be estimated were filtered through ultra membrane filters (pore size 0.45 micro,Merck) before injection. Gallic acid, rutin, umbelliferone, sitosterol and stigmasterol were used as standards. The phytoconstituents present in the sample were identified by comparing the chromatographic peaks with the retention time of the standards. The HPLC analysis of the sample was as per the above protocol and the chromatograms were recorded.

The estimated amount of the marker compound in the extract was calculated using the formula:

$$A = \frac{X \grave{o}\ Y\ \grave{o}\ P}{Z \grave{o}\ S}$$

X = concentration of the standard marker injected.
Z = area given by the standard compound.
Y = area given by the marker compound in the sample profile.
S = sample concentration with respect to the drug taken.
P = purity of the marker compound taken.
A = percentage of marker compound in the drug.

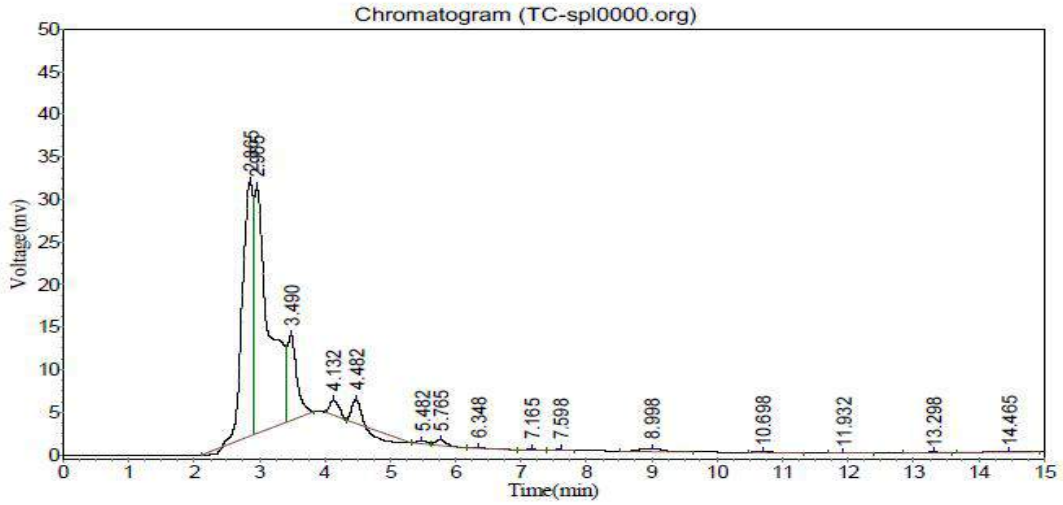

Figure 1. HPLC chromatogram for methanolic extract of leaves for Gallic acid, rutin, umbelliferone, sitosterol and stigmasterol

Table 1. showing different characters with readings

Peak No.	Identity(PDA Conf)	Ret Time	Height	Area	Conc
1	Gallic acid	2.865	29973.072	333274.063	35.35
2	rutin	3.490	9994.719	102998.461	10.91
3	Umbelliferone	4.132	1746.615	17985.850	1.90
4.	sitosterol	4.482	2885.356	4919.600	0.52
5.	stigma sterol	7.165	59.019	891.228	0.19

3. Results and Discussion

The preliminary phytochemical analysis revealed the presence of alkaloids, glycosides, phenolic compounds, carbohydrates and proteins. Phenolic acids have been associated with medicinal uses for centuries and were reported as the most efficient and therapeutically important plant substances[15,16]. These compounds have been determined using various techniques like HPLC, HPLC/MS, UV spectrophotometric, fluorimetric detection, capillary zone electrophoresis and thin-layer chromatography [17, 18].

HPLC analysis is the most popularly uses method for analysis of compounds as it is high sensitivity and selectivity. It is an efficient technique and essential for the standardization of plant-based drugs and identification of plant material. Peaks were observed in the methanolic extract in the similar retention time corresponding to the values as observed in the HPLC of the standards gallic acid, rutin, umbelliferone, sitosterol and stigmasterol. HPLC analysis of the leaf has shown peaks at retention times (mts) of 2.86 for gallic acid, 3.49 for rutin, 4.13 for umbelliferone,7.16 for stigmasterol and 4.48 for sitosterol similar to that of the standards. Hence, confirming the presence of these compounds in the methanolic extracts. Since HPLC analysis is one of the important methods for phytochemical characterization this study establishes the presence of phenolic compounds that are important phytochemicals which could be responsible for the various activities exhibited and reported for the plant.

4. Conclusion

The present study was carried out to investigate the phytoconstituents of the leaves of the plant under investigation. Preliminary phytochemical analysis revealed the presence of alkaloids, glycosides and phenolic compounds, while the HPLC analysis has confirmed the presence of Gallic acid, rutin, umbelliferone stigmasterol and sitosterol which could be responsible for the activities exhibited by the plant. Further studies on the plant constituents will give an insight to the chemical profile of the plant.

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