PHARMACOGNOSTIC AND ANTIMICROBIAL STUDIES OF THE LEAVES OF *TABERNAEMONTANA DIVARICATA* R.BR.

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Summary

Tabernaemontana divaricata R.Br. or *Ervatamia coronaria* is a glabrous, evergreen dichotomously branched shrub belonging to the family Apocynaceae. *Tabernaemontana divaricata* possess a wide range of therapeutic activities like alexipharmic, emmenogogue, astringent, anticancer, hepatoprotective, aphrodisiac, digestible, purgative,antibacterial etc. In present investigation, the detailed pharmacognostic study of *Tabernaemontana divaricata* leaf is carried out to lay down the standards which could be useful in future experimental studies. The study includes macroscopy, microscopy, preliminary phytochemical screening and physicochemical evaluation. Simultaneously antimicrobial activity of various leaf extracts viz petroleum ether,chloroform,methanol,alcohol, and aqueous from the leaves of *Tabernaemontana divaricata* was studied against various Gram positive and Gram negative bacteria. By agar well diffusion method, various extracts showed moderate inhibitory effect against both species of bacteria. The Anti microbial activity of all the extracts were compared (125 µg/disc) with the standard antibiotic Ampicillin (10µg/disc).

KEY-WORDS: *Tabernaemontana divaricata,* Apocynaceae, Extracts, Antimicrobial, Ampicillin

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Introduction

In the developing world the trend has been changed from synthetic to natural herbal medicine. They show minimum or no side effects and are considered to be safe. India is one of the world's twelve leading biodiversity centers with the presence of over 45,000 different plant species. Of these about 15,000 to 20,000 plants have good medicinal properties. (1) Generally herbal formulations involve use of fresh or dried plant parts. Correct knowledge of such crude drugs is very important aspect in preparation, safety and efficacy of the herbal product. Pharmacognosy is a simple and reliable tool, by which complete information of the crude drug can be obtained(2,3,4).

It is generally considered that compounds produced naturally rather than synthetically ,will be biodegraded more easily and therefore more environmentally acceptable. Thus natural antioxidants ,antibacterials,cytotoxic ,fungicidal ,anti viral agents and nutrients have gained popularity in recent years. In recent years ,multiple drug resistance in both human and plant pathogenic microorganism have been developed due to the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases.(5,6). In order to find new therapeutic agents ,plants that have antimicrobial activity have attracted attention.(7,8,9)

Tabernaemontana divaricata (family Apocynaceae), commonly known as chandani (Hindi), is a medicinal plant and utilizable species. Different varities of this plant are available. In different languages it is commonly called as Wax flower(Eng.), Nandivriksha (Sanskrit), Nandibatlu (Kannad), Nandivardhanamu (Telagu), Chandani or Sugandabala (Hindi), Kutampale (Malayalam) (5,6,7). It is a shrub or small tree, usually glabrous, found in the Konkan, North Kanara, Western ghats in malabar, throught north india and Travencore upto 3000 ft. 6 leaves of *Tabernaemontana divaricata* contain indole alkaloids stapfinine,(10,11) dimeric indole alkaloids - conophyline and conophyllidine (12). a minor alkaloid-voaharine (13). Flowers of *E. coronaria contains* α- amyrin acetate, β- amyrin acetate, lupeol β–sitosterol and stigmasterol , a flavone , apigenin ,Four indole alkaloids harmine, heyneanine, voacristine and apparicine , phenolic acids namely salicylic acid, syringic acid and vanillic acid.Stems of *E. Coronaria* contains bis indole alkaloid 19,20-dihydro ervatanine A, other alkaloids coronidine, heyneanine, voacristine, voacamine, descarbomethoxy voacamine and five phenolic acids namely vanillic, gentisic, syrengic, α-hydroxy benzoic and salicylic acid. (14). Root bark of *T. divaricata* contains α- amyrin acetate, lupeol acetate, α- amyrin, lupeol, cycloartenol, β-sitosterol, campesterol, benzoic acid, aurantiamide acetate, coronaridine, coronaridine hydroxyindolenine, ibogamine, 5-hydroxy-6-oxocoronaridine, 5-oxo- coronaridine, 6-oxocoronaridine, (±) 19-hydroxycoronaridine and 3- oxocoronaridine and voacamine (15).

In literature details of morphology,phytoconstituents, medicinal properties and uses of *Tabernaemontana divaricata* is very sparse therefore, in present study pharmacognostic standards of the leaves of *Tabernaemontana divaricata* are studied. These standards are of upmost importance not only in finding out genuity, but also in detection of adulterants in marketed drug and as well in formulation.Simultaneously the antimicrobial properties have also been investigated using various leaf extracts as it is not been dealt in depth earlier.

Material and methods

Pharmacognostic Studies

The leaves of *Tabernaemontana divaricata* were collected in January, 2009, from Bhopal, M.P., India. The plant was identified and authenticated by Dr. D.V.Amla,Deputy Director,national botanical research institute,Lucknow, India and a voucher No.Tit/NBRI/CIF/141/2009 specimen was deposited in Department of Pharmacognosy and Phytochemistry,TIT-Pharmacy.The leaves were dried in shade and stored at 25^0C. It was powdered, passed through sieve no.40 and stored in air tight bottles. An exhaustive Pharmacognosy was carried out using standard methodology (16-27).

Macroscopic study of leaf

Leaves are simple, opposite, color is glossy green above and paler beneath. acuminate apex, , wavy margine and Symmetrical base. They are 9 - 15 cm in length and 4 cm – 6 cm in width narrowed towards the base. Lanceolate to ovate –lanceolate in shape Venation is , petioled 5 – 6 cm long. Taste is bitter.The various physicochemical parameters were determined using standard procedures given in the literature and the results are mentioned in **table -1**

TABLE .1 PHYSICOCHEMICAL EVALUATIONS

Extractive Value	
Alcohol soluble extractive	10.4 %W/W
Water soluble extractive	28.8 %W/W
Petroleum-ether soluble	1.6 %W/W
Chloroform soluble extractive	20 %W/W
Loss on drying	4.02 %W/W
Ash Values	
Total ash	11.66 %W/W
Water soluble ash value	2.8 %W/W
Acid-insoluble ash value	0.66 %W/W

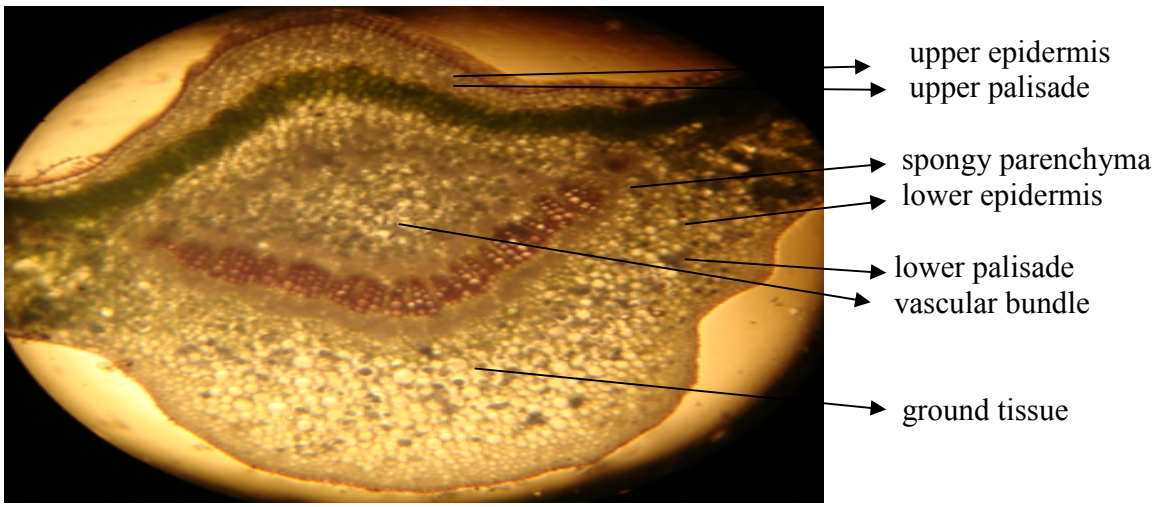

fig.1: T.S. of leaf

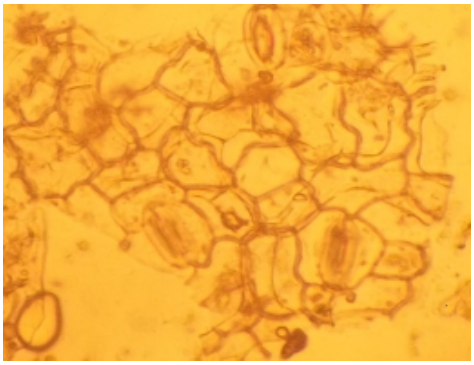

Fig.2: lower epidermal cells

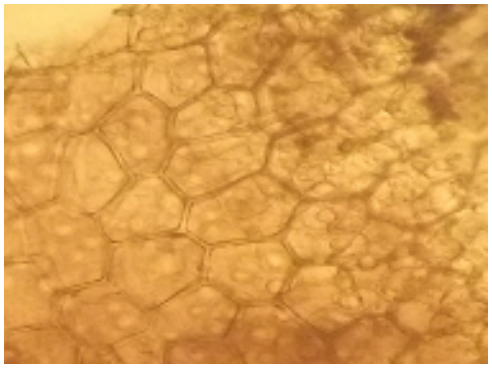

Fig.3: upper epidermal cells

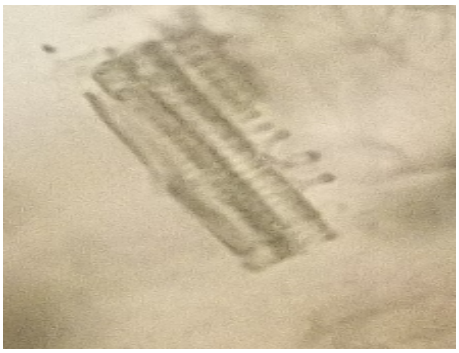

Fig. 4:vessels of meristele

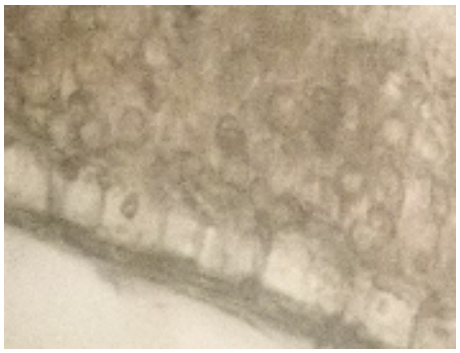

Fig. 5:transversely cut fragment of lamina

Microscopy

Transverse section of *Tabernaemontana divaricata* leaf showed following features-

Powder study of the Chandani leaves powder

Colour : Brown in color
Parenchymetous cell : Oval shape parenchymatous cells are present
Starch grain : Round starch grains are present

Extraction

Sun dried powdered leaves of *Tabernaemontana divaricata* (1.5 kg) was extracted successively in order of increasing polarity of solvents viz; Petroleum ether,chloroform,methanol,alcohol and water.Each extract was concentrated to a small volume and allowed to dry.After drying ,the respective extracts were weighed and packed in the suitable container and kept in the refrigerator for further use.various preliminary phytochemical tests were performed in order to determine the presence or absence of phytoconstituents.The results for preliminary phytochemical evaluation are depicted in **table -2.**

TABLE. 2. PRELIMINARY PHYTO-CHEMICAL SCREENING.

Sr. No.	Phytochemical Nature	Chandani (95%v/v Alcohol extract)	Petroleum ether extract	Methanolic Extract	Chloroform extratct	Water extract
1	Acidic compound	-	-	-	-	-
2	Aleurone grains	-	-	-	-	-
3	Alkaloids	+	+	+	+	+
4	Proteins & Amino acids	+	+	+	+	+
5	Carbohydrates	-	-	-	-	-
6	Flavonoids	+	+	+	+	-
7	Phenols	+	+	+	+	+
8	Glycosides	+	+	+	+	+
9	Saponins	+	+	+	+	+
10	Tannis	-	+	+	+	+
11	Steroids	+	+	+	+	-
12	Triterpenoids	+	+	+	+	-
13	Fixed oils & Fats	+	+	+	+	-

(+) = present (-) = absent

Antimicrobial Activity

Modified Kirby - Bauer method(28) was used for evaluation of anti microbial activity of various leaf extracts.The anti microbial activity was tested by disc diffusion method.The Bacterial culture were grown in Mueller Hinton agar by transferring 25ml. of the media into pre sterilized petridish and allowed to solidify at room temp. for 1hr. Suspension of organism (10^6cell/ml.)

were spread into nutrient agar.The sterile filter paper disc (6 mm.) impregnated with 10 µl of test samples (leaf extracts) were placed into agar surface at equidistant and petri dishes with bacterial cultures were incubated at 37° C for 24 hrs.The paper disc of standard antibiotic Ampicillin (10µg/disc) was also incorporated in the petri dishes.The inhibition zone diameter (IZD) was measured by deducting the disc diameter.The anti microbial activity of test compound is shown in **table-3**

Table-3 Antimicrobial activity of leaf extracts

S.No.	Leaf extracts	Diameter of zone inhibition(mm.)			
		B. sub	S. aur	E. coli	P. aer
1.	Petroleum Ether	14	13	16	12
2.	Chloroform	16	14	17	13
3.	Methanol	15	13	16	12
4.	Alcohol	17	16	17	14
5.	Aqueous	14	13	14	12
6.	Ampicillin	14	15	14	12

Results and discussion

The pharmacognostic standards for the leaves of *Tabernaemontana divaricata* are laid down for the first time in this study. Morphological and anatomical studies of the leaves will enable to identify the crude drug. The information obtained from preliminary phytochemical screening will be useful in finding out the genuity of the drug. Ash values,extractive values can be used as reliable aid for detecting adulteration. These simple but reliable standards will be useful to a lay person in using the drug as a home remedy. Also the manufacturers can utilize them for identification and selection of the raw material for drug production. Among the leaf extracts Chloroform and alcoholic extract showed more inhibitory potential against both gram positive and negative bacteria when compared to standard drug Ampicillin at 10µg level hence leaf extracts of *Tabernaemontana divaricata* can replace safely to standard synthetic antimicrobial agents.

References

1. Gokhale SB: Textbook of Pharmacognosy. Nirali Prakashan, 1979.
2. Mukherjee PK: Quality Control of Herbal Drugs-An Approach to evaluation of Botanicals. Business Horizons Pharmaceutical Publishers, 2002.
3. Raghunathan K and Mitra R: Pharmacognosy of indigenous plants. Central council for research in ayurveda and siddha, 1982.

4. Trease GE and Evans WC: Pharmacognosy. Harcourt brace & Co. Asia, Pvt. Ltd., W.B. Saunders Company Ltd., 15th Ed. 2002.
5. Kirtikar KR and Basu BD: Indian Medicinal Plants. Periodical Experts, Vol. II, 1975: 1052-53.
6. Nadkarni KM: Indian Materia medica. Popular book depot Bombay, Vol. I, 1954: 516-18.
7. Sharma P and Mehta PM: In Dravyaguna vignyan. (The Chowkhamba Vidyabhawan, Varansi) Part II & III, 1969: 586.
8. Chopra RN, Nayar SI and Chopra IC: In Glossary of Indian medicinal plants. P.I.D., 1956: 238.
9. Indian Medicinal Plants. A Compendium of 500 Species, Vaidyaratnam PS, Varrier's Arya Vaidyasala. Kottakkal. Orient Longman, 1980: 225-9.
10. Atta - Ur - Rahman, Anjum M, Nader D, Stapfinine, an Indole alkaloid from *E. coronaria.* Phytochemistry 1986; 25 (7): 1781 - 1782.ervatinine.
11. Atta – Ur - Rahman, Anjum M, Nader D, Ervatinine, an Indole alkaloid from *E. coronaria.* Phytochemistry 1985; 24 (10): 2473 –2474.
12. Kam TS, Loh KY, Wei C, Conophylline and Conophyllidine: New dimeric alkaloids from *T. divaricata*. J Nat Prod 1993; 56: 1865 –1871.
13. Kam TS, Loh KY, Lin LH, Loong WL, Chuah CH, Wei C, New alkaloids from the leaves of *T. divaricata.* Tetrahedron Letters 1992; 33: 969 – 972.
14. Henriques AT, Melo AA, Moreno PRH, Ene LL, Henriques JAP, Schapoval EES, *Ervatamia coronaria* : Chemical constituents and some pharmacological activites. J of Ethnopharmacology 1996; 50: 19 – 55.
15. Kamesh R, Randhir SK, Satya PP, New alkaloids from *T. divaricata*. Phytochemistry 1980; 19: 1209-1212.
16. Anonymous, The Ayurvedic Pharmacopoeia of India. Government of India, Ministery of Health & Family Welfare, Published by The Controller of Publications, Civil Lines, New Delhi, Vol. I, 2001.
17. Brain KR and Turner TD: Practical evaluation of Phytopharmaceuticals. Wright Scientechnica, Bristol 1975.
18. Chase CR and Pratt R: J Am Pharm Ass (Sci.Ed.) 1949; 38:324-331.
19. Karnick CR: Pharmacopoeial standards of herbal plants. Sri Saguru publication, 1994:124.
20. Khandelwal KR: Practical Pharmacognosy. Nirali Prakashan, Edition 5, 1998.
21. Khedkar PV: Pharmacognostic studies in some marketed crude drugs. A thesis submitted to University of Mumbai for the degree of M.Sc. 2000.
22. Kokate CK: Practical Pharmacognosy. Vallabh Prakashan, 1999.
23. Kokate CK, Purohit AP and Gokhale SB: Pharmacognosy. Nirali Prakashan, Edition XII, 1999.
24. Kokoski CJ, Kokoski RJ and Salma FJ: J Am Pharm Ass, 1958; 10:715-717.
25. Merina B; Natural Product Radiance 2004; 3: 349- 350.
26. Nandhakumar J, Nandhakumar J, Sethumathi PP, Malini A, Sengottuvelu, Duraisamy R, Karthikeyan D and Sivakumar TJ: Health Sci, 2007; 53:655-663.
27. Wallis TE: Practical Pharmacognosy. J & A Churchill Ltd., London, EditionV, 1984.
28. Bauer AW, Kriby WMH, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardised single disc method. Amer J Clin Pathol 1966,493-496