Innovare Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 7, Issue 8, 2015

Original Article

PHARMACOGNOSTICAL AND ANTIMICROBIAL STUDIES OF THE STEM OF *TABERNAEMONTANA DIVARICATA* LINN

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Received: 11 Mar 2015 Revised and Accepted: 15 Jun 2015

ABSTRACT

Objective: The aim of the present study is to evaluate the pharmacognostic and antimicrobial studies of *Tabernaemontana divaricata* Linn. Stem.

Methods: The Pharmacognostical studies were performed using standard parameters and antimicrobial studies were based on checker board and disc diffusion technique using various bacterial strains. The antifungal studies were based on the agar slant technique.

Results: The transverse section shows the presence of undifferentiated cells known as cortex in the outer region. They are followed by an extended part of cortex, which consists of 7-8 layers of Phellogen, followed by 6-7 layers of Phelloderm. The antimicrobial studies confirmed that the methanolic extract was quite effective for bacterial strains *Sh. flexneri type* BCH 995, *Shigella boydii* 8, *Sh. sonnei* NK 840, *Sh. dysenteriae* 1, *Sh. dysenteriae* 9, *Vibrio cholerae* 1023, *V. cholerae* 1341, *V. cholerae* 575, *V. cholerae* 1311, *E. coli* RH 07/12, *E. coli* 18/9, *E. coli* K88, *Enterobacter* spp AP596, *Staphylococcus aureus* ML 267, *S. aureus* MTCC 96, *Bacillus subtilis* MTCC 441, *Pseudomonas auriginosa* AP585 NLF, *Bacillus pumilus* 8241 and *Klebsiella pneumoniae.* The maximum antibacterial activity was observed against *Staphylococcus aureus* MTCC 96 (zone of inhibition at 800µg/ml is 12.17±0.124 mm), whereas minimum activity against *E. coli* RH 07/12 (zone of inhibition at 800µg/ml is 10.45±0.063 mm). It was also effective against the fungal strains *Aspergillus niger* MTCC 281*, Candida albicans* ATCC 10231 and *Penicillium chrysogenum* MTCC 2725.

Conclusion: The study of pharmacognostical features of the stem of *T. divaricata* Linn. May serves as a tool for identification and standardization of the crude drug as per WHO guidelines.

Keywords: *Tabernaemontana divaricata*, Phellogen, Physicochemical, Antimicrobial.

INTRODUCTION

The plants have been used traditionally for centuries and modern scientific studies have shown the existence of the good correlation between the traditional or folkloric application of some of the plants further strengthens the search for pharmacological active components from plants [1]. *Tabernae montana* (family Apocynaceae) is a genus of flowering plants. It is generally found in Asia, Africa, Australia, North America and South America. *Tabernaemontana divaricata* (syn. *Ervatamia coronaria*) commonly called as Pinwheel Flower and Crape Jasmine. It is a blabrous evergreen shrub, 1.8-2.4 m in height with silvery grey bark and milky latex. It bears attractive white colored flowers with five-petal and pinwheel shaped [2]. Various parts of the plant are used in the indigenous system of medicine for the treatment of skin diseases and cancer. The decoction of leaves is used as hypertensive and diuretic. The milky juice of the leaf is used for wounds due to anti inflammatory properties [3]. It is also used in diarrhoea, externally for ophthalmia, toothache and skin diseases. The root contains resin and bitter alkaloid. The root bark is antihelmintic and also chewed for the relief of toothache [4]. The most common medicinal use of crude *T. divaricata* extract involves its antimicrobial action against infectious diseases such as syphilis, leprosy, and gonorrhoea, as well as its antiparasitic action against worms, dysentery, diarrhoea, and malaria [5]. The pharmacological properties of *T. divaricata* are antioxidant, anti-infection [6], anti-ulcer [7], analgesia [8] and the enhancement of cholinergic activity in both peripheral and central nervous systems [9].

The establishment of pharmacognostic profile of the stem of *Tabernaemontana divaricata* Linn. Will assist in standardization of the plant as per WHO guidelines, which can help in the identification of plant. Considering the vast potentiality of plants as sources for antimicrobial drugs with reference to antimicrobial agents, a systematic investigation was undertaken to screen the antibacterial and antifungal potentiality of the stem extract of *T. divaricata*. The determination of the minimum inhibitory concentration of the extract against various bacterial and fungal strains will be helpful in the discovery of new antimicrobial agent.

MATERIALS AND METHODS

Plant material

The plant was collected from Greater Noida and authenticated, by Dr. K. C. Bhatt, Senior Scientist, NBPGR, Pusa Campus, New Delhi. The voucher number was NHCP/NBPGR/2011-64 and a voucher specimen is also retained in our laboratory for future references. The stem was sun dried after washing and then grinded to a coarse powder in a mechanical grinder.

Macroscopy

The stem was examined for shape, color, odor, taste etc and reported.

Microscopy

A thin section of the stem was obtained and then stained with phloroglucinol and concentrated HCl and mounted with glycerin and observed under a compound microscope [10, 11].

Powder microscopy

The dried stem was powdered with the help of the grinder. The fine powder was separately treated with chloral hydrate and glycerin to determine the presence of various tissues [10, 11].

Physico-chemical evaluation

The powdered stem was determined for Total ash, acid-insoluble and water soluble ash value, sulphated ash value and water soluble and alcohol soluble extractive value [10, 11].

Phytochemical examination

Chemical tests were employed in the preliminary phytochemical screening for various secondary metabolites [10, 11].

Method of extract preparation

The coarse powder of the stem (50 g) was extracted in a soxhlet apparatus with methanol and the solvent was removed by evaporation a heating mantle by taking care that the temperature did not rise above 60 °C. A semisolid dark viscous crude extract (yield 4.58% w/w) thus obtained was tested for its antibacterial and antifungal potentiality.

Test micro-organisms

The test bacteria used were *Shigella flexneri* type 36 NK 381,*Sh. flexneri* type 6B 999, *Sh. flexneri* type BCH 995, *Sh. boydii* 22461, *Sh. boydii* 16552, *Sh. boydii* 8, *Sh. soneii* BCH 397, *Sh. soneii* E08869, *Sh. soneii* NK 840, *Sh. soneii* BCH 937, *Sh. soneii* I, *Sh. soneii* DN3, *Sh. soneii* F11001, *Sh. soneii* NK 29, *Sh. dysenteriae* 1, *Sh. dysenteriae* 9, *V. cholerae* 1023, *V. cholerae* BD 1/81, *V. cholerae* 1341, *V. cholerae* 452, *V. cholerae* 1033, *V. cholerae* 575, *V. cholerae* 765, *V. cholerae* 1311, *V. cholerae* 756, *V. cholerae* DN6, *V. cholerae* A 26, *E. coli* AP600, *E. coli* 383, *E. coli* RH 07/12, *E. coli* 18/9, *E. coli* 597, *E. coli* 798, *E. coli* 35B, *E. coli* 306, *E. coli* K88, *E. coli* 872, *Enterobacter spp* AP596, *Salmonella typhi* Type 2, *Staphylococcus aureus* ML 267, *S. aureus* ATCC 6538, *S. aureus* MTCC 96, *S. aureus* 381, *Bacillus subtilis* MTCC 441, *B. cereus* MTCC 1305, *B. pumilus* 8241, *Pseudomonas putida* MTCC 2252, *P. auriginosa* AP585 NLF, *Klebsiella pneumoniae* and *Proteus vulgaris* AP679 NLF.

The test fungi used were *Candida albicans* ATCC 10231, *Candida albicans* 5, *Aspergillus niger* MTCC 281, *Penicillium chrysogenum* MTCC 2725, *Phaenorochaete chrysporium* MTCC 787 and *Ralstonia entropha* MTCC1255. These microbial strains included various drug resistant hospital isolates collected and characterized in Department of Pharmaceutical Technology, Jadavpur University, India. All strains were maintained on Nutrient Agar (NA) for bacteria and Sabourauds's Dextrose Agar (SDA) slants for fungi at 4°C prior to use for antibacterial and antifungal tests respectively.

Determination of minimum inhibitory concentration by serial dilution technique [12, 13]

The stem extract (stock solution) was reconstituted with a minimum amount of dimethyl sulfoxide (DMSO). This solvent did not posses any antimicrobial activity of its own. Calculated volume of this stock solution were dispensed in a series of McCartney bottles previously containing calculated volume of sterile cooled molten nutrient agar media (40-45°C) to prepare final volume of 30 ml each with dilutions of 5, 10, 25, 50, 100, 200, 400, 800 and1000 µg/ml. The stock solution was dispensed into molten SDA to prepare varying dilutions of 100, 200, 400, 800, 1500 and 2000 µg/ml, final volume of 5 ml in sterile test tubes while determining the MIC against the fungi. Then these molten media containing varying concentration of extract were poured aseptically in pre sterilized petridishes (70 mm) to give sterile nutrient agar plates with varying dilution of extract. The SDA media containing extract were kept in the inclined position for slant tubes. These plates and slant tubes were then kept in the refrigerator at 4°C for 24h to ensure uniform diffusion of extract. Then these plates and tubes were dried at 37°C for bacteria and 25°C for fungi for 2 h before inoculations. One loopful (loop diameter: 3 mm) of an overnight grown bacterial strains suspension (10^5 CFU/ml) were added in each quadrant as marked by checker board technique for bacteria and one loopful of an overnight grown fungal suspension was streaked on the SDA slant surface [12, 13]. The spotted plates were incubated at 37 °C for 24 h for bacteria and streaked slants were incubated at 25 °C for 7 d and MIC values were obtained.

Determination of zones of inhibition by disc diffusion method [12, 13]

The stock solution (each of 10 mg/ml) of both extract and ciprofloxacin was prepared. From these stock solutions two sets of four dilutions (200, 400, 800, 1000 µg/ml) each of stem extract (solvent: DMSO) and ciprofloxacin (solvent: sterile distilled water) were prepared in sterilized McCartney bottles. However, we have compared the effectiveness of griseofulvin with the extract at 1000, 1200, 1500, 2000 µg/ml in antifungal activity. Sterile nutrient agar plates and Sabourauds's dextrose agar plates were prepared and incubated at 37 °C for bacteria and 25 °C for fungi for 24 h to check for the presence of any sort of contamination. Then each sterilized agar plates were flooded with liquid culture of the strains, dried for 30 min at 37 °C for bacteria and 25 °C for fungi. The sterile whatman filter paper disc (4 mm diameter) were soaked in four different dilution of the crude extract and placed in appropriate position of the plates marked as quadrant at the back of petridishes. All the flooded plates with corresponding paper discs soaked with appropriates dilution of extract were incubated for 24 h and diameter of zone of inhibition were measured in mm. Similar procedure was adopted for the reference standard drug and corresponding zone diameters were measured and compared accordingly.

RESULTS AND DISCUSSION

Macroscopy

The following characteristics of stem were noted:

Color: Dark Green to brown

Odor: Characteristic

Taste: Acrid

Extra feature: Short and Branched

Microscopy

The transverse section of the stem shows the presence of undifferentiated cells known as the cortex in the outer region. They are followed by an extended part of the cortex, which consists of 7-8 layers of Phellogen, followed by 6-7 layers of Phelloderm.

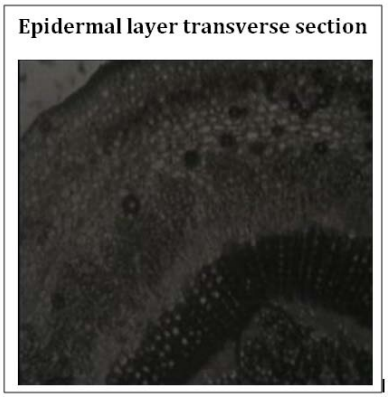

Fig. 1: Transverse section of stem of *T. divaricata* (Epidermal layer)

The pericyclic fibres are in the circular motions which are collaged, followed by thin lining. The xylems are towards the upper surface followed by phloem in a concentric manner, they are known as open vascular bundle. The central portion consists of parenchyma cells called as pith with small number of mucilage cells. They are also composed of compactly packed cells with less space; these are called as collenchyma cells.

Table 1: Physico-chemical parameters of the stem of *Tabernaemontana divaricata* Linn

S. No.	Parameter	Results (w/w)
1	Total ash value	14.50%
2	Acid insoluble ash value	5.60%
3	Moisture content	0.80%
4	Extractive value(water soluble)	2.40%
5	Extractive Value (alcohol soluble)	5.60%

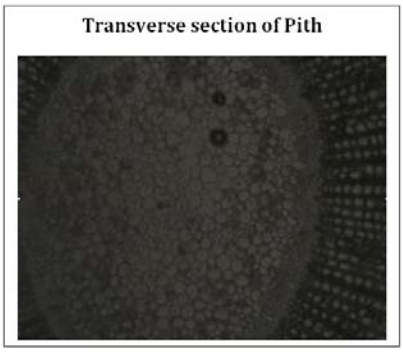

Fig. 2: Transverse section of stem of *T. divaricata* (pith region)

Physico-chemical parameters

The *T. divaricata* stem powder showed the presence of Total ash 14.50% w/w, Acid-insoluble ash 5.60 % w/w, Moisture content 0.80%, Water soluble extractive value 2.40% and Alcohol soluble extractive value 5.60%.

Preliminary phytochemical studies

The methanol extracts of *T. divaricata* showed a positive report for alkaloids, saponins, flavonoids, steroids, glycosides and tannins. The results were shown in table no. 2:

Antibacterial activity

The result in table 3 depicted the MIC values of the methanolic extract of the bark of *Tabernaemontana divaricata* Linn. Against various tested bacterial pathogens. It is evident from table 4, that the extract is highly active against *Shigella flexneri type* BCH 995, *Shigella boydii* 8, *Sh. sonnei* NK 840, *Sh. dysenteriae* 1, *Sh. dysenteriae* 9, *Vibrio cholerae* 1023, *Vibrio chloerae*1341, *V. cholerae* 575, *V. cholerae* 1311, *V. cholerae* 756, *E. coli* RH 07/12, *E. coli* 18/9, *E. coli* K88, *Enterobacter* spp AP596, *Styphylococcus aureus* ML 267, *S. aureus* MTCC 96, *S. aureus* ATCC 6538, *Bacillus subtilis* MTCC 441, *Pseudomonas aeruginosa* AP585 NLF, *Bacillus pumilis* 8241 and *Klebsiella pneumoniae*.

Among the selected strains the extract shows maximum effectiveness against *Staphylococcus aureus* MTCC96 (zone of inhibition at 800µg/ml is 12.17±0.124 mm) and minimum against *E. coli* RH 07/12 (zone of inhibition at 800µg/ml is 10.45±0.063 mm) as shown in table 4.

Table 2: Preliminary Phytochemical screening of the stem of *Tabernaemontana divaricata* Linn. using various solvents.

S. No.	Plant constituents	Petroleum ether extract	Chloroform extract	Acetic Acid extract	Acetone extract	Methanol extract	Aqueous extract	Benzene extract
1	Alkaloids	+	+	+	+	+	+	+
2	Carbohydrates	-	-	-	+	-	-	-
3	Glycosides	+	+	-	-	+	-	+
4	Saponins	-	-	-	-	+	+	-
5	Phenolic compds & Tannins	+	+	+	+	+	+	+
6	Flavonoids	+	+	-	+	+	+	-
7	Phytosteroides	+	+	+	+	+	+	+

+= Present,-= Absent

Table 3: Antibacterial Activity by Serial dilution method of methanolic extracts of the stem of *T. divaricata*

S. No.	Name of bacterial strains	Dilution of methanolic extract of stem (µg/ml)									
		0*	5	10	25	50	100	200	400	800	1000
1	*Shigella flexneri* type 36 NK 381	+	+	+	+	+	+	+	-	-	-
2	*Sh. flexneri type* BCH 995	+	+	+	+	±	±	-	-	-	-
3	*Sh. boydii* 22461	+	+	+	+	+	+	-	-	-	-
4	*Sh. boydii* 8	+	+	±	IC	3IC	-	-	-	-	-
5	*Sh. sonnei* E08869	+	+	+	+	+	+	±	-	-	-
6	*Sh. sonnei* NK 840	+	+	+	±	±	±	±	-	-	-
7	*Sh. sonnei* NK 29	+	+	+	+	+	+	-	-	-	-
8	*Sh. dysenteriae* 1	+	+	+	+	±	IC	-	-	-	-
9	*Sh. dysenteriae* 9	+	+	+	+	±	±	-	-	-	-
10	*V. cholerae* 1023	+	+	+	+	±	-	-	-	-	-
11	*V. cholerae* 1341	+	+	+	±	±	-	-	-	-	-
12	*V. cholerae* 575	+	+	+	+	+	-	-	-	-	-
13	*V. cholerae* 1311	+	+	+	±	±	-	-	-	-	-
14	*V. cholerae* 756	+	+	+	+	±	±	-	-	-	-
15	*E. coli* RH 07/12	+	+	+	IC	IC	IC	-	-	-	-
16	*E. coli* 18/9	+	+	+	-	-	-	-	-	-	-
17	*E. coli* 597	+	+	+	+	+	+	-	-	-	-
18	*E. coli* K88	+	+	±	±	±	±	±	-	-	-
19	*Enterobacter* spp AP596	+	+	+	+	±	±	±	-	-	-
20	*S. aureus* ML 267	+	+	+	-	-	-	-	-	-	-
21	*S. aureus* ATCC 6538	+	+	+	+	+	±	-	-	-	-
22	*S. aureus* MTCC 96	+	+	+	-	-	-	-	-	-	-
23	*B. subtilis* MTCC 441	+	+	+	+	±	-	-	-	-	-
24	*B. cereus* MTCC 1305	+	+	+	+	+	+	-	-	-	-
25	*Ps. aeruginosa* AP585 NLF	+	+	+	+	-	-	-	-	-	-
26	*B. pumilus* 8241	+	+	+	+	±	IC	-	-	-	-
27	*Klebsiella pneumoniae*	+	+	+	+	-	-	-	-	-	-

Number of bacterial strains=28; Number of Gram Negative Bacteria = 21; Number of Gram Positive Bacteria= 6, * = Control (without extract),±= Inhibited Growth,+= Growth,–= No Growth, IC=Isolated colonies.

Table 4: Determination of diameter of zone of inhibition (in mm) produced by the methanolic extract of the stem of *T. divaricata* and its comparison with that of Ciprofloxacin against selected sensitive bacterial strains

S. No.	Name of microorganism	Extract (µg/ml)			Ciprofloxacin (µg/ml)		
		200	400	800	200	400	800
1.	*Sh. boydii* 8	9.15±0.05	10.48±0.045	11.21±0.08	10.26±0.036	11.39±0.057	12.19±0.062
2.	*Sh. dysenteriae* 1	9.0±0.07	9.45±0.15	11.63±0.032	10.45±0.035	11.72±0.172	12.88±0.077
3.	*V. cholerae* 1341	9.23±0.125	11.21±0.086	11.97±0.169	10.69±0.02	12.42±0.062	13.1±0.082
4.	*V. cholerae* 1311	8.54±0.058	10.57±0.063	11.6±0.049	11.13±0.076	12.6±0.064	13.6±0.124
5.	*E. coli* RH 07/12	8.05±0.052	9.57±0.061	10.45±0.063	10.44±0.07	11.52±0.091	13.4±0.081
6.	*E. coli* 18/9	10.1±0.294	10.57±0.033	12.07±0.031	12.06±0.043	12.9±0.78	14.0±0.163
7.	*S. aureus* ML 267	10.13±0.07	11.07±0.07	12.47±0.169	12.48±0.017	13.55±0.108	14.58±0.0237
8.	*S. aureus* MTCC 96	10.49±0.11	11.15±0.108	12.17±0.124	12.7±0.038	14.17±0.059	15.53±0.047
9.	*Ps. aeruginosa* AP585 NLF	9.43±0.124	10.41±0.048	11.22±0.025	11.1±0.041	12.4±0.025	13.73±0.047
10.	*Klebsiella pneumoniae*	9.63±0.094	10.98±0.087	11.61±0.033	11.45±0.054	12.25±0.05	13.55±0.0707

Number of bacterial strains=10

Table 5: Determination of MIC of the stem extract of *T. divaricata* Linn. against different fungal strains

S. No.	Name of Fungi	Dilution of methanolic stem extract (µg/ml) in Sabouraud's Dextrose Agar(SDA) media							
		0*	100	200	400	800	1000	1500	2000
1	*Aspergillus niger* MTCC 281	+	+	±	-	-	-	-	-
2	*Candida albicans* 5	+	+	+	±	±	-	-	-
3	*Candida albicans* ATCC 10231	+	+	+	±	-	-	-	-
4	*Penicillium chrysogenum* MTCC 2725	+	+	+	+	-	-	-	-
5	*Phaenorochaete chrysporium* MTCC 787	+	+	+	+	±	-	-	-
6	*Ralstonia entropha* MTCC1255	+	+	+	+	±	-	-	-

Number of fungal strains=6

* = Control (without extract), ±= Inhibited Growth,+= Growth,–= No Growth, IC=Isolated colonies.

Table 6: Determination of diameter of zone of inhibition (in mm) produced stem extract of *Tabernaemontana divaricata* and its comparison with Griseofulvin against different sensitive fungal strains

S. No.	Name of Fungi	Extract (µg/ml)			Griseofulvin (µg/ml)		
		1000	1500	2000	1000	1500	2000
1.	*Aspergillus niger* MTCC 281	7.5±0.308	9.6±0.074	11.51±0.079	11.17±0.125	13.49±0.074	14.15±0.118
2.	*Candida albicans* ATCC 10231	6.07±0.056	8.64±0.082	9.2±0.127	9.02±0.192	11.11±0.096	13.03±0.163
3.	*Penicillium chrysogenum* MTCC 2725	5.68±0.087	7.9±0.071	8.59±0.074	7.95±0.111	10.54±0.065	12.15±0.112

Number of fungal strains=3

Antifungal activity

The result in table 5 and 6 showed that the stem extracts of *T. divaricata* Linn. is effective against in decreasing order: *Aspergillus niger* MTCC 281, *Candida albicans* ATCC 10231, *Penicillium chrysogenum* MTCC 2725, *Candida albicans* 5, *Phaenorochaete chrysporium* MTCC 787 and *Ralstonia entropha* MTCC1255.The maximum effectiveness was seen against *Aspergillus niger* MTCC 281 and minimum activity against *Penicillium chrysogenum* MTCC 2725 (table 6).

CONCLUSION

The study of pharmacognostical features of the stem of *T. divaricata* Linn. may serves as a tool for identification and standardization of crude drug as per WHO guidelines. The effectiveness of *T. divaricata* Linn. Stem extracts against a number of bacterial and fungal strains reveals its potentiality towards a new therapeutic agent. The active plant extracts may be further subjected to biological and pharmacological investigations for isolation of antimicrobial and therapeutically active compounds.

CONFLICT OF INTERESTS

Declared None

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