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ANTIMICROBIAL ACTIVITY AND POWDER MICROSCOPY OF THE FLOWERS OF *TABERNAEMONTANA DIVARICATA* R. BR.

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ABSTRACT

Tabernaemontana divaricata R.Br. is a medicinal shrub belongs to the family Apocynaceae. The medicinal properties of its leaves and flowers are well known to the traditional medicine. The flowers are useful to cure burning sensation, ophthalmic and dermatopathic diseases. However, the antibacterial effect of its flower is not yet unravelled. Therefore, the present investigation was mainly focused on the antibacterial activity of the flowers of *Tabernaemontana divaricata*. For validating the taxonomic description of flower, powder microscopic study was also carried out. In the study, different flower extracts were tested against standard bacterial strains by disc diffusion method. Microscopic character of the flower powder was studied by trinocular microscope. The results of the study indicated that only the methanolic extract of the flower was effective to inhibit the growth of *Staphylococcus aureus* and *Escherichia coli*. The different flower extracts were ineffective against *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Serratia marcescens*. Powder microscopy revealed that the flower has light fragrant spirally thickened xylem vessels, prismatic crystals of calcium oxalate, trichomes, small starch grains and pollen grains. The study concludes that the flower extract of *Tabernaemontana divaricata* has significant antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*. Therefore, the flower extract in methanol can be taken for the purification of active principle. The various flower characteristics obtained from the powder microscopy can be used as a taxonomic tool for identifying the flower and plant species.

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INTRODUCTION

For centuries, the world's folk medicine uses herbal and natural products in a large scale because of the fact that the pharmacologically active compounds in them provide protection against microbial and insect attack [1, 2]. Naturally occurring antimicrobial compounds have enormous therapeutic potentials and advantages than similar such synthetic compounds. Synthetic antimicrobials are often found to have many biological side effects. However, plant based compounds are believed to have only limited side effects because of the fact that the cellular and molecular mechanism of plant and animal cells are similar in many ways.

Many of the presently available effective modern drugs are initially used in crude form in traditional or folk medicine [3]. Because of various reasons, the drug resistance potentials of both animal and plant pathogenic micro organisms are increasing tremendously [4, 5]. As an alternative to the modern drugs, attention has been given to the isolation of anti microbial drugs from plants [6, 7]. Screening of plants for detecting their antimicrobial property is the first step for the discovery of a new antimicrobial drug.

Tabernaemontana divaricata R.Br. (Synonym: *Ervatamia coronaria*) belongs to Apocynaceae family. In India, it is mainly cultivated as an ornamental plant and it grows wild in hedges and shady forests [8, 9]. However, it is a medicinally important evergreen shrub having various curative properties. In the folk medicine, it has been used for anti-infection, anti-inflammation, analgesic effect, anti-tumor effect, antioxidative effect and the effect in neuronal activity [10]. Ash of stem is used for ophthalmic diseases, root for tooth ache and milky latex with coconut oil for headache. The flowers are cooling, fragrant and are useful for curing burning sensation, ophthalmitis and dermatopathy [8]. Flowers contain α - amyryin acetate, β - amyryin acetate, lupeol β -sitosterol, stigmasterol and several other alkaloids [8, 11-12].

Eventhough, the various medicinal properties of the flowers of *T. divaricata* are well recognized, its role in antimicrobial therapy is not yet well studied. Therefore, the present study was carried out for detecting the antimicrobial potency of the flower extracts of *Tabernaemontana divaricata* against the selected bacterial strains.

The present day taxonomic description of the flowers of *T. divaricata* is based on the traditional taxonomic principles. In modern taxonomy, one of the most important taxonomic tools for describing a plant or plant part is the powder microscopy. Hence, powder microscopic observation of the flower was also carried out for incorporating and amplifying the taxonomic identification of the flowers of *T. divaricata*.

MATERIALS AND METHODS

Fresh flowers were collected from the Medicinal Garden of Sreedhareeyam Ayurvedic Research & Development Institute, Koothattukulam, Kerala, India. Voucher herbarium specimen was prepared and deposited in Sreedhareeyam herbarium. The authentic identification of the plant material was done by Dr. Jomy Augustine, St. Thomas College, Pala-Kerala, India.

Antimicrobial studies

Preparation of the crude extract:

Fresh flowers were brought to the lab and washed first with tap water and then with saline water followed by distilled water, cut into several small pieces, dried under sunlight and powdered in a mixer grinder. 250 grams of the dried powder was serially extracted with 500 ml each of petroleum ether, methanol and water using a Soxhlet extractor. The extract was concentrated by vacuum evaporation using a rotary evaporator and the concentrate was stocked in a refrigerator at 4°C and used for antibacterial assay. From the stock, aliquot was taken and re-dissolved in the respective solvents to get a final concentration equivalent to 20 mg of the dried flower per disc.

Bacterial strains:

Standard MTCC strains purchased from Institute of Microbial Technology, Chandigarh (IMTECH), India were used for the study. The strains were, *Staphylococcus aureus*, sub species *aureus* (MTCC 96), *Escherichia coli* (MTCC 443), *Pseudomonas aeruginosa* (MTCC 741), *Serratia marcescens* (MTCC 97), and *Klebsiella pneumoniae*, sub species *pneumoniae* (MTCC 109).

Culture media:

The dehydrated Muller Hinton Agar (MHA) medium purchased from Hi-media Laboratories Pvt. Ltd. Mumbai, India was used. The medium was rehydrated, sterilized in an autoclave and was poured into sterilized petri dishes and allowed to set. The plates were stored at 4-10°C in refrigerator. Before inoculation, the surface of the petri plate was dried in an incubator.

Antibacterial Assay by Disc Diffusion Method:

The antibacterial activity was tested by Disc Diffusion Method [13]. The dried plate was inoculated by the bacterial strains uniformly over the surface using a sterile cotton swab. A sterile 5 mm Whatmann No.1 filter paper loaded with appropriate extract was placed on the surface of the inoculum and gently pressed by a sterile forceps. Control discs made up of each solvent like petroleum ether, methanol and water were also placed in the medium. The plates were incubated at 37°C for 16 to 20 hrs. The zone of inhibition of bacterial growth around the disc was measured in millimeters. The experiment was repeated for three times and the average value was recorded.

Powder microscopy

Fresh flowers were collected and washed thoroughly with water to remove dust, soil particles or any other unwanted adherent foreign material; air dried at room temperature under shade for a few days. The dried flowers were powdered and passed through 355 μ m IS sieve. The powder so obtained was used for the preparation of various temporary slides with Safranin, Phloroglucinol, Potassium iodide, Chloral hydrate and Glycerin. The slides were observed under the Magnus Trinocular microscope at 10X followed by 40X magnification and important microscopic characteristics were captured.

RESULTS AND DISCUSSION

Antimicrobial studies

The results of the antibacterial activity of the flower extracts of *Tabernaemontana divaricata* against standard strains are given in Table 1 and the powder microscopic observation is given in Figure 1.

Table 1. Antibacterial activity of flower extracts of *Tabernaemontana divaricata* against standard strains

Sl. No.	Bacterial Strains	Zone of inhibition of crude extracts in mm		
		1*	2*	3*
1	<i>Staphylococcus aureus</i>	-	++	-
2	<i>Pseudomonas aeruginosa</i>	-	-	-
3	<i>Escherichia coli</i>	-	++	-
4	<i>Serratia marcescens</i>	-	-	-
5	<i>Klebsiella pneumoniae</i>	-	-	-

1* Petroleum ether extract, 2* Methanolic extract, 3* Aqueous extract.
+:-zone of inhibition \leq 10 mm, ++:-zone of inhibition 10 – 15 mm, +++:- zone of inhibition \geq 15mm.

Control experiment with solvent alone did not show any inhibitory zone. Hence, it can be concluded that the solvent has no antibacterial activity. Nevertheless, the methanolic flower extract of *T. divaricata* was active against *Staphylococcus aureus* and *Escherichia coli*. In contrast, the petroleum ether extract and aqueous extract were non-active against *Pseudomonas aeruginosa*, *Serratia marcescens* and *Klebsiella pneumoniae*. The methanolic extract showed an inhibition zone of 10-15mm against the *Staphylococcus aureus* and *Escherichia coli*. The zone of inhibition within this range is satisfactory for further antimicrobial studies.

Powder microscopy

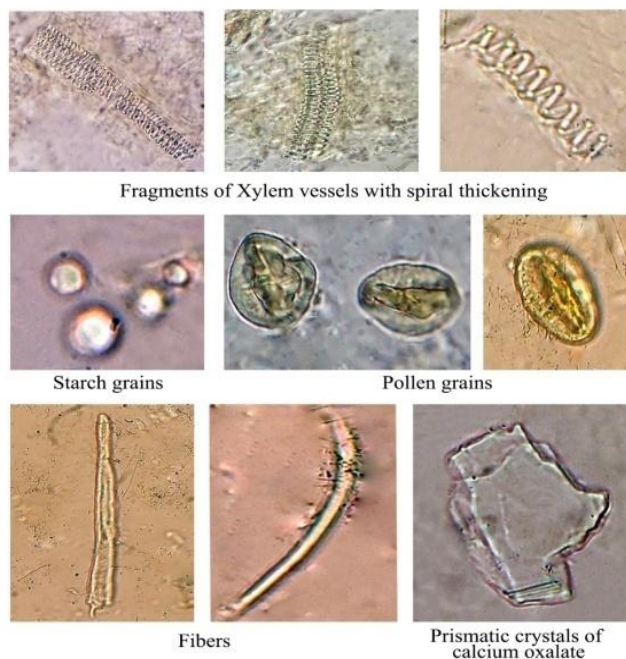


Figure 1. Powder microscopic analysis of flowers of *Tabernaemontana divaricata*

The powder microscopy of the dried flowers of the *T. divaricata* showed the following observations.

- 1) The powder appeared as yellowish brown in colour
- 2) Fragments of spirally thickened xylem vessels
- 3) Prismatic crystals of calcium oxalate, trichomes, small starch grains and pollen grains.

Tabernaemontana divaricata is a well known plant for its medicinal properties. In the last few decades, a good number of research studies have been made on this plant. The leaves and flowers are especially noted for their pharmacological properties. Studies suggest that its leaves are excellent source for antioxidant, anti infection, analgesic and anti cancer therapy [10, 14]. Leaves also possess antidiabetic, antifungal and antibacterial properties [15-17]. The flowers are identified as effective agents against anxiety [18]. The flowers are also possessing anticancer and anticonvulsant properties [19, 20]. However, studies relating the antibacterial

activities of its flowers are limited. In this context, the present study is significant and suggests that the methanolic extract of flower of *T. divaricata* has some antibacterial principle and is active against Gram⁺ve *Staphylococcus aureus* and Gram⁻ve *Escherichia coli*. In modern taxonomy, powder microscopy is one of the major taxonomic tools for the authentic identification of plant. Powder characteristics observed from the flowers of *T. divaricata* is useful for the authentic identification of this plant species.

CONCLUSION

Tabernaemontana divaricata is a thoroughly studied medicinal plant and possessing an array of medicinal properties, especially in their leaves and flowers. Nevertheless, the antibacterial effect of its flowers has not yet been well studied. Therefore, the present study was focused on the screening of its flowers to detect the antibacterial effect and also for detecting the powder microscopic characteristics of the flower for the taxonomic evaluation of the flowers. Antibacterial assay was made by standard procedure using MTCC strains. Study revealed that the flower extract in methanol alone is effective to inhibit the growth of *Staphylococcus aureus* and *Escherichia coli*. The other tested strains were resistant to the various flower extracts. Powder microscopic study revealed that the flower has light fragrant spirally thickened xylem vessels, prismatic crystals of calcium oxalate, trichomes, small starch grains and pollen grains. Therefore, it can be concluded that the methanolic extract of flower of *Tabernaemontana divaricata* possesses significant antibacterial property against *Staphylococcus aureus* and *Escherichia coli*. Since it is only a preliminary investigation, isolation and detailed pharmacological studies of the active principle are recommended for developing a new antibiotic from the flower extract of *Tabernaemontana divaricata*.

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Authors' Statements

Conflict of Interests

Here by the authors of this article declare that there is no conflict of interest on the matters given in this paper.

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