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Evaluation of in Vitro Antimicrobial Activity of Flower Extract of *Tabernaemontana divaricata* against Oral Pathogens

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Original Research Article

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Abstract: *Tabernaemontana divaricata* is commonly used for the treatment of various illnesses. In the present study crude flower extracts were made using four different solvents such as water, methanol, ethanol and chloroform and were subjected to preliminary phytochemical analysis .Well diffusion method was used for screening of the antimicrobial activity against selected oral pathogens such as *Streptococus mutans, Streptococcus salivarius, Streptococcus mitis and Lactobacillus acidophilus*. Results showed the presence various phytochemicals including alkaloids, tannins, reducing sugars, saponin, steroids and triterpenoids and proteins. Among these extracts, methanol extract showed significant antibacterial activity against most tested bacteria. The weakest activity was seen in the acetone extracts.
Keywords: *Tabernaemontana divaricata*, oral pathogens, well diffusion method.

INTRODUCTION

Dental cavities and periodontal diseases are the most common health problems effecting people of all age groups in the both developing as well as developed countries. Even after significant development in the field of dentistry, dental problems still continues [1]. Oral cavity consists of different types of microorganisms of which some are pathogenic and are precursors of dental problems. Pathogens mainly involved in the dental decay includes *S.mutans, S.salivarius, S.mitis* and *L.acidophilus* [2]. These bacteria settle onto the surface of the teeth and cause damage to the teeth by production of acids from dietary carbohydrates.

Many chemicals and synthetic drugs have proven to be effective in the prevention of these diseases, but they also have marked side effects such as brown staining of the teeth, tongue, transient impairment of taste perception, toxic effects on connective tissues, dryness and soreness of oral cavity, allergic reactions in patients, and oral desquamation in children. Today's date use of herbal medications or plant drugs in the treatment of various ailments became a common practice.

Tabernaemontana divaricata, commonly known as pinwheel flower belongs to the family Apocynaceae, is a beautifully shaped evergreen shrub which blooms in spring but flowers may appear sporadically throughout the year .It is widely distributed throughout India and other parts of the South East Asia. Alkaloids and non-alkaloid constituents such as terpenoids, steroids, flavonoids, phenyl propanoids, phenolic acids and enzymes from the leaves, stems, and roots have been reported. In folklore practice, it is used to treat fever and diarrhea [3]. The plant is also used as a tonic to the brain, liver, and spleen. It is reported to have various pharmacological actions such as antinociceptive, antioxidant, anti-inflammatory and reversible acetyl cholinesterase inhibition activities [4-5].

The crude extracts of the plant also showed potent antibacterial activity against various pathogens such as *Staphylococcus aureus, Lacto bacillus, Proteus vulgaris, Enterobacter aerogenes*. In the present study we have evaluated the antimicrobial activity of various crude flower extracts of *Tabernaemontana divaricata* against oral pathogens.

METHODS

Preparation of the extract

Flowers of T. divaricata were washed thoroughly with distilled water and dried in shade for a week and ground using a mixer to coarse powder. The powder was used for preparing the aqueous extract. 1 g of flower powder was boiled in 10 ml of deionized water for 10 minutes. It was cooled and filtered through Whatman No. 1 filter paper, and the filtrate was stored at 4°C until further use.

Remaining extracts were prepared by adding 20 grams of pulverized samples into 100 ml of solvents such as methanol, ethanol and acetone. The samples were

preserved in the dark away from the light for four days with intermittent shaking. The resultant extracts were passed through filter paper and filtrate was concentrated in an oven at 50° ± 1 °C for 1 h and stored at 4^0C until further use. All solvents used were of analytical reagent grade and obtained from Sigma Chemical Co.

Test microorganisms of interest

Oral pathogens such as *Streptococus mutans, Streptococcus salivarius, Streptococcus mitis* and *Lactobacillus acidophilus* were used for the present study. These organisms were isolated from clinical samples collected from patients of the Malla Reddy Institute of Dental Sciences, Telangana, India. The organisms were identified and confirmed by our clinical microbiologist.

Antibacterial activity [6]

Antibacterial activity was carried out using the well diffusion method on Mueller Hinton Agar (MHA) plates. The test cultures were swabbed on the top of the solidified media and allowed to dry for 10 minutes. The wells were loaded with the extracts and left for 30 minutes at room temperature for compound diffusion. Positive as well as negative control was also used. The plates were incubated for 24 hrs at 37°C, and the zone of inhibition was measured in millimeters (mm).

Determination of MIC (minimum inhibitory concentration)

Broth dilution method was followed for the determination of minimum inhibitory concentration of the extract. Fresh amount of the nutrient broth was prepared and sterilized by autoclaving.20 ml of the sterilized nutrient broth was transferred to the test tubes. Measured amount of the extract was added in to the test tubes containing nutrient broth in such as way that the final concentration per ml was 0 (control), 5,15,25,50 and 100mg. Loopful of test microorganism was incorporated into the test tubes and incubated at 37^0C for 24hrs. After completion of the incubation period, the tubes were checked for the growth. Growth in the liquid cultures was seen in the form of turbidity. Tubes showing growth was denoted by '+' and '-' for absence of growth. Concentration at which there is complete absence of growth was taken as MBC whereas the concentration at which growth start decreasing was taken as MIC.

Preliminary phytochemical screening [7]

The crude extracts were tested for the presence of the phytochemicals by the methods described by S. Sadasivam *et al*.

RESULTS AND DISCUSSION

Qualitative tests of plant extracts of T. divaricata L. were performed to detect the presence of various phytochemicals including Alkaloids, Tannins, Reducing sugars, Saponin, Steroids and triterpenoids and Proteins. Bioactive compounds like Steroids, Alkaloids, Tannins, Reducing sugars, proteins were present in the solvent extracts (Table 1). Alkaloids and tannins were present abundantly in almost all the extracts. Ethanol, methanol and aqueous extracts were found to contain various phytochemicals as compared to acetone extract of plants.

Table-1: Preliminary phytochemical screening

	Tabernaemontana divaricata L.			
	Ethanol	Methanol	Acetone	Aqueous
Alkaloids	++	++	+	++
Carbohydrates	_	_	+	++
Glycosides	++	++	_	_
Tannins	++	++	+	++
Phenols	++	++	+	++
Steroid	+	+	-	+
Tri-terpenoid	-	-	+	+
Saponin	+	+	_	+
Protein	+	+	+	+
Reducing sugars	+	+	+	+

(++) Indicates moderate, (+) Mild, (-) Absent.

Different solvent extracts of *Tabernaemontana divaricata* L. were tested against oral pathogens including *S.mutans, S.salivarius, S.mitis* and *L.acidophilus.* The antimicrobial activity was determined by agar well diffusion method and the zone of inhibition was recorded. Ethanol, methanol and aqueous extracts of T. divaricata L. showed effective antibacterial activity against all the bacteria with highest activity against *L.acidophilus*. T. divaricata L. was found effective against almost all the bacteria. Acetone extract of the plants showed weak antibacterial activity whereas methanol extracts showed the highest.

Table-2: Determination of MIC of various flower extracts of *Tabernaemontana divaricata* against different bacteria

Name of the bacteria	Growth in nutrient broth containing different concentration of extract in mg/ml							
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
	Ethanol extract		Methanol Extract		Acetone extract		Aqueous Extract	
Streptococcus mutans	10	25	10	25	25	100	15	50
Streptococcus sobrinus	10	25	10	25	25	100	15	50
Lactobacillus acidophilus	5	25	5	25	15	100	10	50

The antimicrobial activity of flower extracts of *Tabernaemontana divaricata* showed a significant level of antimicrobial activity against all the microbes studied but the concentration required for the impact varied. Among the four bacterial strains significant antimicrobial activity was observed on *Lactobacillus acidophilus* over all other organisms studied.

In the studies carried out by Radhika B on the antibacterial activity of Tabernaemontana divaricate flowers using chloroform and methanol as solvents on *Staphylococcus aureus, Lacto bacillus, Proteus vulgaris, Enterobacter aerogenes,* methanol extract showed significant antibacterial activity against most tested bacteria [8]. The most susceptible microorganism was found to be *Proteus vulgaris* followed by *Staphylococcus aureus*. In another study, among various extracts only the methanolic extract of the flower was effective to inhibit the growth of *Neisseria mucosa, Streptococcus pneumonia, Mycobacterium kansasii, Salmonella typhimurium, Staphylococcus aureus* and *Escherichia coli*. Both the results comply with our studies [9]. Rahman Md *et al*. on his studies showed that leaves extracts (ethanol, petroleum ether, diethyl ether, methanol and aqueous) possess maximum potency against infectious pathogens *Staphylococcus saprophyticus, Staphylococcus aureus, Enterococcus facealis, Staphylococcus pyogenes, Streptococcus agalactae, Salmonella typhi, Escherichia coli, Shigella boydii, Shigella dysenteriae* and *Pseudomonas aeruginosa* [10].

lAny antimicrobial agent is considered effective, if it produces zone of inhibition of about 2 mm or more. In the present study, the minimum zone of inhibitions obtained were 6mm and 7mm respectively. The study results concluded that the flower extract of *Tabernaemontana divaricata* showed best antimicrobial property against the oral pathogens.

Antimicrobial susceptibility test could be used to identify the effectiveness of extracts against bacterial infections caused by pathogens. The plant extracts had bactericidal effect at higher and bacteriostatic at lower concentrations respectively (Table 2). 80% of rural people utilized herbal medicines in their life cycle as primary healthcare. In our normal life, antimicrobial substances could be used for the treatment of bacterial infections at different time intervals. Because of huge costs and side effects of some synthetic drugs, there was always need for other products as natural medicines from plants. In our present study, extracts of Tabernaemontana divaricate showed potent antimicrobial activity against the oral pathogens.

Further work is needed to isolate the active principle from the plant extract and to carry out pharmaceutical studies. It is hoped that this study would lead to the establishment of some compounds that could be used to formulate new and more potent antimicrobial drugs of natural origin in the treatment of dental diseases. Further studies can be conducted in future to assess the safety levels of the plant.

Table-3: Antibiotic activity of the crude extracts

	Ethanol	Methanol	Acetone	Aqueous	Standard antibiotic
S.mutans	16	16mm	7mm	12mm	17mm
S.salivarius	20	21	6	13	22
S.mitis	21	22	7	14	23
L.acidophilus	24	26	9	17	26

CONCLUSION

In conclusion, we evaluated the antimicrobial activities of different extracts of *Tabernaemontana divaricata* against oral bacteria. And the extracts showed the strongest antimicrobial activity against all of the bacteria tested; notably, this is the first report of the inhibitory effect of *Tabernaemontana divaricata* on oral bacteria. The antimicrobial activity of the extracts could be enhanced if active components are purified and adequate dosage determined for proper administration. Therefore, these results suggest that plant based medicines with proven antimicrobial effects, such as *Tabernaemontana divaricata*, may be useful for the treatment of dental diseases.

REFERENCES

1. Silk, H. (2014). Diseases of the mouth. *Primary care: Clinics in office practice*, *41*(1), 75-90.
2. Hardie, J. M. (1992). Oral microbiology: current concepts in the microbiology of dental caries and

periodontal disease. *British Dental Journal*, *172*(7), 271.

3. Latha, K. P., Latha, M. S., & Vagdevi, H. M. (2012). Comparative studies on anthelmintic activity of Mangifera indica L. Var. Thotapuri and Mangifera indica L. Var. Neelam root crude extracts. *International Journal of Phytopharmacy*, *2*(1), 21-24.
4. Gopinath, S. M., Suneetha, T. B., Mruganka, V. D., & Ananda, S. (2011). Evaluation of antibacterial activity of *Tabernaemontana divaricata* (L.) leaves against the causative organisms of bovine mastitis. *Int J Res Phytochem Pharmacol*, *1*(4), 211-213.
5. Poornima, K., Krishnan, R., Aswathi, K. V., & Gopalakrishnan, V. K. (2012). Toxicological evaluation of ethanolic extract of Tabernaemontana coronaria (L) R. Br. *Asian Pacific Journal of Tropical Disease*, *2*, S679-S684.
6. Nayak, B., & Patel, K. (2010). Pharmacognostic studies of the Jatropha curcas leaves. *methodology*, *1*, 6-18.
7. Sadasivam, S., & Manickam, A. (1992). *Biochemical methods for agricultural sciences*. Wiley Eastern Limited.
8. Kalappurayil, T. M., & Joseph, B. P. (2017). A review of pharmacognostical studies on Moringa oleifera lam. flowers. *Pharmacognosy Journal*, *9*(1).
9. Sumathi, R., & Devipriya, S. (2016). Antibiogram of Candida albicans using *Tabernaemontana divaricata* leaf extracts by AWD assay. *Int. J. Curr. Res. Biol. Med*, *1*(2), 6-13.
10. Ashikur, R. M., Hasanuzzaman, M. D., Mofizur, R. M., Zahan, S. I., & Muhuri, R. S. (2011). Evaluation of antibacterial activity of study of leaves of *Tabernaemontana divaricata* (L.). *Int. Res. J. Pharm*, *2*, 123-127.