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Research Article

EVALUATION OF ANTIBACTERIAL ACTIVITY OF STUDY OF LEAVES OF *TABERNAEMONTANA DIVARICATA* (L)

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ABSTRACT
Phytochemical analysis of the dried leaves of *Tabernaemontana divaricata* (L). (Apocynaceae) indicated the presence of a steroids,tannins, saponins, gums and reducing sugar. The pharmacological interest of these compounds, coupled with the use of this plant in traditional medicine prompted the authors to check for its possible antibacterial activity. The extracts (ethanol, petroleum ether, diethyl ether, methanol and aqueous) were found to 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*. The zone of inhibition was observed with almost all bacteria with some exceptions. Minimum inhibitory concentrations of the extracts were found to be significant. The obtained results provide a support for the use of this plant in traditional medicine and its further investigation.
Key words: Antibacterial activity, Apocynaceae, Minimum inhibitory concentration, Phytochemical analysis, *Tabernaemontana divaricata*

INTRODUCTION

Some medical plants have been used for a wide variety of purposes such as food preservation, pharmaceutical, alternative medicine, and natural therapies for many thousands of years. It is generally considered that compounds produced naturally, rather than synthetically, will be biodegraded more easily and therefore be more environmentally acceptable. Thus, natural antioxidants, antibacterial, cytotoxic, antiviral, fungicidal agents and nutrients have gained popularity in recent years, and their use and positive image among consumers are spreading. In recent years, multiple drug resistance in both human and plant pathogenic microorganisms have been developed due to the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases[1, 2]. In order to find new therapeutic agents, plants that have antimicrobial activity have attracted attention[3-5]

Tabernaemontana divaricata (L). (Apocynaceae) (synonym-Tabernaemontana coronaria, Ervatamiacoronaria), commonly known as Togor, Dudhphul in Bangladesh and Wax flower, Crepe flower, Crepe jasmine in India , is an evergreen shrub to 6 feet (1.8 m) distributed in Coast forests of Bengal, Myanmar, mangrove forests of China and Japan[6]. Plants used in Thai traditional rejuvenating and neurotonic remedies[7]. *T.divaricata* extract inhibits neuronal acetylcholinesterase activity in rats[8]. Although, a number of chemical investigations have been performed and some constituents have been reported as alkaloids, carotenoids, flavonoids, glycosides, lipids, triterpines, polyphenols, saponins, etc.[9-11]. It is used in the Indian subcontinent as a folk medicine for the treatment of variety of ailments.

Two bisindole alkaloids has Isolated from the plant, 19,20-dihydrotabernamine and 19,20-dihydroervahanine A and this alkaloids are well-known acetylcholinesterase inhibitor[12]. Again new alkaloids (voaharine **1** and conophylline **2)** have isolated from the leaves of *Tabernaemontana divaricata*[13] .

Therefore, the present study was designed to investigate the antibacterial activity of the whole plant of *T. divaricata* in order to examine the pharmacological basis of the use of the plant in folk medicine for the treatment of infectious diseases.

MATERIALS AND METHODS

Plant material collection and Reference standards: The leaves of *Tabernaemontana divaricata* were

collected from the Dighinala Thana, Khagrachari, Bangladesh in August 2009, and were taxonomically identified by experts at the Bangladesh National Herbarium (accession number: 39566). The dried plant material was grinded into fine powder and the total mass was divided into two halves. One fraction was extracted by hot percolation method with ethanol in soxhlet extractor for 72 hrs. And another fraction was subjected to sequential extraction using solvents of increasing polarity viz. petroleum ether, diethyl ether, methanol and distilled water in Soxhlet extractor. Each solvent extraction step was carried out for 24 hrs and after extraction the extracts were concentrated by evaporation and stored at 4°C for further study. Azithromycin and tetracycline was used as standard drugs.

Bacteria and growth medium: Ten bacterial species; five of gram positive - *Staphylococcus saprophyticus*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Staphylococcus pyogenes* and *Streptococcus agalactiae* and five of gram negative - *Salmonella typhi*, *Escherichia coli*, *Shigella boydii*, *Shigella dysenteriae* and *Pseudomonas aeruginosa* were used as test organisms obtained from Bangladesh Council of Scientific and Industrial Research. These organisms were cultured on Sabouraud dextrose agar at 30°C for 24hrs and the stock culture was maintained at 4°C and then sub-cultured as needed.

Antibacterial sensitivity assay by disc diffusion method: Antibacterial activity of the extracts was tested using disc diffusion method against the bacterial strains. In the agar diffusion method, wells were cut in seeded agar and the test sample was then introduced directly into these wells. After incubation the diameter of the clear zone around the well were measured and compared against zones of inhibition produced by solutions of known concentrations of standard antibiotics. Measured amount of the test samples were dissolved in definite volumes of solvent to give solutions of known concentration (µg/ml). Then sterile filter paper discs were impregnated with known amount of test substances using micropipette and dried. Standard antibiotic discs and discs on which the solvent (used to dissolve the samples) was adsorbed and dried and the discs were used as positive and negative control, respectively. These discs were then placed in petridishes (120mm in diameter) containing a suitable agar medium seeded with the test organisms using sterile transfer loop for anti-microbial screening . The plates were then kept at 4^0C for facilitating maximum diffusion. The test material diffuses from the discs to the surrounding medium. The plates were then kept in an incubator (37°C) for 12-18hrs to allow the growth of the microorganisms. The antibacterial activity of the test agent was determined by measuring the diameter of the zone of inhibition in term of millimeter. The experiments were carried out three times and the mean of the reading were recorded *divaricata*[14] .

Determination of minimum inhibitory concentration: MIC was determined by micro-dilution method using serially diluted (2 folds) plant extracts according to the National Committee for Clinical Laboratory Standards[15]. MIC values of the extracts were determined by dilution of the extracts of various concentrations of 512, 256, 128, 64, 32, 16, 8, 4, 2µg/ml respectively. Equal volume of each extract and nutrient broth were mixed in a test tube. Specifically 0.1ml of standardized inoculums (1-$2x10^7$cfu/ml) was added in each tube. The tubes were incubated aerobically at 37^oC for 18hrs. Three control tubes were maintained for each test batch. These included antibiotic control (tube containing extract and growth media without inoculum), organism control (tube containing the growth medium and the inoculum) and negative control (tube containing the growth medium only). The lowest concentration (highest dilution) of the extract that produced no visible bacterial growth (no turbidity) when compared with the control tubes were regarded as MIC

RESULTS AND DISCUSSION

In this study the antimicrobial activity of the extracts (ethanolic, petroleum etheric, diethyl etheric, methanolic and aqueous) of *Tabernaemontana divaricata* L. was assayed to determine the zones of inhibition and MIC against those bacteria.

Antibacterial sensitivity assay by disc diffusion method: As shown in table 1, the ethanolic extract of the plant had great *in vitro* potential of antimicrobial activities against 9 species of the 10 strains tested. In this study, the antimicrobial activity of the extracts at two different concentrations of 250 and 500µl/discs were compared with those of positive control such as azithromycin and tetracycline at the dose of 30µg/disc. The data obtained from the disc diffusion method (table 1) indicated that the extract displayed a variable degree of antimicrobial activity on different tested strains. The inhibitory effect increased with increase of the extract concentration from 250 to 500µg/disc. Gram-positive *E. faecalis* was the most sensitive strain with the strongest inhibition zones (28-34mm) followed by *S. dysenteriae* (23-32mm), *P. aeruginosa* (17-25mm), *E. coli* (22-24mm) *S. pyogenes* (18-21mm) *S. saprophyticus* (14-23mm) *S. aureus* (17-21mm) *and S. agalactiae* (14-

17mm). But in case of gram-negative bacteria, *S. typhi* it displayed no zone of inhibition.

From the table 1, it was observed that the petroleum etheric extract of the plant showed different degree of antimicrobial activity against *S. aureus* (15-23 mm), *S. pyogenes* (12-19mm), *S. agalactiae* (15-18mm), *E. coli* (22-24mm) and *P. aeruginosa* (14-19mm) among the 10 bacteria species tested. The data indicated that the extract displayed a variable degree of antimicrobial activity on different tested strains. But the bacterial species *S. saprophyticus*, *E. faecalis*, *S. typhii*, *S. boydii* and *S. dysenteriaei* showed no susceptibility to petroleum etheric extract.

The diethyl etheric extract of the plant showed antimicrobial activity against all of the 10 bacterial strains tested (table 1). The extract produced different degree of zones of inhibition against the bacteria. Highest activity was observed against *E. faecalis* (23-29mm) followed by *E. colii* (25-28mm), *S. pyogenes* (24-28mm), *S. boydii* (17-26mm), *S. aureus* (23-25mm), *S. agalactiae* (19-25mm), *S. dysenteriae* (19 -24), *S. saprophyticus* (16-23mm), *P. aeruginosa* (14-23mm) *S. typhi* (15-21mm). The data indicated that the extract displayed a variable degree of antimicrobial activity on different tested strains.

The methnolic extract produced similar activity of ethanolic extract. As like ethanolic extract methanolic extract showed no zone of inhibition against *S. typhi*. The highest zone of inhibition was observed against *E. coli* (26-29mm) followed by *E. faecalis* (26-27mm), *S. pyogenes* (25-27mm), *S. saprophyticus* and *S. boydii* (23-25mm), *S. aureus* (23-24mm), *S. agalactiae* (13-19mm) and *S. dysenteriae* (16-21mm) and *P. aeruginosa* (8-12mm). *P. aeruginosa* showed the lowest susceptibility (table 1).

From the table 1, the aqueous extract of the plant showed antimicrobial activity against *S. saprophyticus* (16-19mm), *S. aureus* (21-25mm), *E. faecalis* (20-23mm), *S. pyogenes* (17-19mm), *E. coli* (16-22mm) *S. dysenteriaei* among the 10 bacteria species tested. The data indicated that the extract displayed a variable degree of antimicrobial activity on different tested strains. The highest activity was against *S. aureus* and the lowest was against *S. saprophyticus*. But he extract showed no zone of inhibition against *S. agalactiae*, *S. typhii*, *S. boydii* and *P. aeruginosa*.

Gram-negative bacteria exhibited low susceptibility to the extract which is in accordance with the fact that those have high level of intrinsic resistance to virtually all known antimicrobials and antibiotics due to a combination of a very restrictive outer membrane barrier, and it is highly resistant even to synthetic drugs[16] .

Determination of Minimum inhibitory concentration (MIC): The minimum inhibitory concentration (MIC) values obtained for extracts against the bacterial strains varied from one solvent extract to the other (table 2). For instance, MIC values of 32, 16, 16, 128, 4, 8, 16, 4 and 32µg/ml were obtained for ethanolic extract Tabernaemontana divaricata against *S. saprophyticus, S. aureus, E. faecalis, S. pyogenes, S. agalactiae, E. coli, S. boydii, S. dysenteriae and P. aeruginosa respectively* while MIC values of 16, 32, 32 , 16, 128µg/ml were against *S. aureus, S. pyogenes, S. agalactiae, E. coli, and P. aeruginosa* respectively in petroleum ether extract. *The growth of S. saprophyticus, E. faecalis, S. typhi , S. boydii, S. dysenteriae* were observed even at he highest dose 512µg/ml. Diethyl ether showed the highest activity against all of the bacteria. The MICs were 8, 4, 8, 8, 4, 8, 8, 4, 4 and 4µg/ml against the bacteria *S. saprophyticus, S. aureus, E. faecalis, S. pyogenes, S. agalactiae, S. typhi, E. coli, S. boydii, S. dysenteriae and P. aeruginosa* respectively. The methnolic extract showed no inhibition of growth against *S. typhi* and the MIC for *S. saprophyticus, S. aureus, E. faecalis, S. pyogenes, S. agalactiae, E. coli, S. boydii, S. dysenteriae and P. aeruginosa* were 32, 16, 8, 32, 8, 16, 8, 4 and 4µg/ml respectively. MIC values of 32, 32, 32, 64, 32, and 16µg/ml were against S. *saprophyticus, S. aureus, E. faecalis, S. pyogenes, E. coli, S. boydii, S. dysenteriae and P. aeruginosa* species.

The plant extracts were bacteriostatic at lower concentrations and bactericidal at higher concentrations as released by MIC values shown in table 2. The medicinal plants constitute an effective source of both traditional and modern medicines. Herbal medicines have been shown to have genuine utility and about 80% of rural population depends on its primary health care. Over the years, the world health organization advocated that countries should interact with traditional medicines with a view to identifying and exploiting aspects that provide safe and effective remedies for ailments of both microbial and non-microbial origin. The results of present study indicated that six medicinal plants commonly used by traditional medical practitioners to cure liver damage were active against bacterial strains.

Among the crude extracts of *Tabernaemontana divaricata* L. diethyl ether extract was highly active against all the 10 bacteria species used but the petroleum ether and aqueous extract showed lower activity and produced no inhibition against *S. saprophyticus, E. faecalis, S. typhi, S. boydii and S. dysenteriae*. The

investigations further showed that both ethanolic and methanolic extracts were inactive against bacterial species *S. typhi*. But against all other species they produced almost similar MIC values.

Our results therefore tend to support the traditional claim that these medicinal plants are preferably extracted in ethanol.

CONCLUSION

The present results therefore offer a scientific basis for traditional use of the plant *Tabernaemontana divaricata* L. against infection by burns or wounds. But *in vivo* studies on these medicinal plants are necessary and should seek to determine toxicity of active constituents, their side effects, serum-attainable levels, pharmacokinetic properties and diffusion in different body sites. The antibacterial activity could be enhanced if active components are purified and adequate dosage determined for proper administration. It goes a long way in curbing administration of inappropriate concentration, a common practice among many traditional medicines practitioners. This represents a preliminary report on the antibacterial activity of the medicinal plant *Tabernaemontana divaricata* L. in Bangladesh. And for rational use of the traditional plant it requires further scientific study as necessary on it.

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Table 1: Zone of inhibition of different extracts against the test bacteria

Sample	Dose (µg/disc)	Zones of inhibition (mm)									
		S. saprophyticus	*S. aureus*	*E. faecalis*	*S. pyogenes*	*S. agalactiae*	*S. typhi*	*E. coli*	*S. boydii*	*S. dysenteriae*	*P. aeruginosa*
Azithromycin	30	18	28	22	26	24	15	28	20	20	15
Tetracycline	30	28	24	26	26	28	28	26	24	22	11
Ethanolic extract	250	14	17	28	18	14	nd	22	16	23	17
	500	23	21	34	21	17	nd	24	19	32	25
Petroleum etheric extract	250	nd	15	nd	12	15	nd	22	nd	nd	14
	500	nd	23	nd	19	18	nd	24	nd	nd	19
Diethyl etheric extract	250	16	23	23	24	19	15	25	17	19	14
	500	23	25	29	28	25	21	28	26	24	23
Methanolic extract	250	23	23	26	25	13	nd	26	23	16	8
	500	25	24	27	27	19	nd	29	25	21	12
Aqueous extract	250	16	21	20	17	nd	nd	16	nd	18	nd
	500	19	25	23	19	nd	nd	25	nd	22	nd

nd = not detected

Table 2: MIC of different extracts against the test bacteria

Sample	MIC (µgm/ml)									
	S. saprophyticus	*S. aureus*	*E. faecalis*	*S. pyogenes*	*S. agalactae*	*S. typhi*	*E. coli*	*S. boydii*	*S. dysenteriae*	*P. aeruginosa*
Ethanolic extract	32	16	16	128	4	nd	8	16	4	32
Petroleum etheric extract	nd	16	nd	32	32	nd	16	nd	nd	128
Diethyl etheric extract	8	4	8	8	4	8	8	4	4	4
Methanolic extract	32	16	8	32	8	nd	16	8	4	4
Aqueous extract	32	32	32	64	-	nd	32	nd	16	nd

nd = not detected

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