Efficacy of different solvent extracts of *Vitex trifolia* L. and *aristolochia indica* L. for potential antibacterial activity

Murugan M and Mohan V R

Ethnopharmacology Unit, Research Department of Botany,
V.O.Chidambaram College, Tuticorin-628008, Tamil Nadu, India
vrmohan_2005@yahoo.com

ABSTRACT

Two medicinal plants namely *Vitex trifolia* and *Aristolochia indica* were screened for potential antibacterial activity against *Staphylococcus aureus, Klebsiella pneumoniae, Bacillus subtilis*, *Escherichia coli, Salmonella typhi and Pseudomonas aeruginosa.* The antibacterial activity was determined in petroleum ether, benzene, acetone, methanol and aqueous extracts using disc diffusion method. The benzene extract of *vitex trifolia* leaf showed highest inhibition against *Bacillus subtilis,* whereas, methanol extract of *Aristolochia indica* leaf exhibited maximum inhibition against *Staphylococcus aureus.* Phytochemical screening revealed the presence of alkaloids, flavonoids, saponins, phenolics, tannins and terpenoids. The results of these studies revealed most valuable information and also support the continued sustainable use of these plants in traditional systems of medicine.

Keywords: *Vitex trifolia, Aristolochia indica,* antibacterial activity, disc diffusion method.

INTRODUCTION

According to World Health Organization (WHO) more than 80% of the world's population relies on traditional medicine for their primary healthcare needs. Use of herbal medicines in Asia represents a long history of human interactions with the environment. Plants used in traditional medicine contain a wide range of ingredients that can be used to treat chronic as well as infectious diseases. A vast knowledge of how to use the plants against different illnesses may be expected to have accumulated in areas where the use of plants is still of great importance (Diallo *et al*, 1999).

Traditionally used medicinal plants produce a variety of compounds of known therapeutic properties (Bruneton, 1995). The substances that can inhibit pathogens and have little toxicity to host cells are considered candidates for developing new antimicrobial drugs. In recent years, antimicrobial properties of Indian medicinal plants have been increasingly reported (Ahmad *et al*, 1998: Ahmad and Beg, 2001; Sashikumar *et al*, 2003; Chendurpandy *et al*, 2010) However, a majority of traditionally used Indian medicinal plants have not yet bean systematically screened against various microbial pathogens. With this background, the present study was carried out to evaluate the antibacterial potential of leaf and stem of *Vitex trifolia* L. and *Aristolochia indica* L.

MATERIALS AND METHODS

Collection of plant materials

The leaf and stem materials of *Vitex trifolia* L. and *Aristolochia indica* L.were collected from the well grown plants in Grizzled Giant Squirrel Wildlife Sanctuary, Western Ghats, Srivilliputhur, Tamil Nadu. They were shade dried at room temperature for 10-15 days.

Extraction of plant material

Various organic solvents were used for the extraction of bioactive compounds. The leaf and stem powders (10g) of *Vitex trifolia* and *Aristolochia indica* were first extracted with petroleum ether for defatting in a Soxhlet apparatus. The defatted powdered sample of *Vitex trifolia* and *Aristolochia indica* were dried and successfully extracted with petroleum ether, benzene, acetone, methanol and then water in a Soxhlet apparatus. The extracts obtained were completely evaporated by using vacuum rotary evaporator. The concentrated extracts were subjected to qualitative test for the identification of various phytochemical constituents as per standard procedures (Brindha *et al*, 1981, Anonymous 1996, Lala, 1993*).* The concentrated extracts were used for antibacterial activity.

Microorganisms

Bacterial strains of *Staphylococcus aureus* (MTCC 96), *Klebsiella pneumoniae* (MTCC 109), *Bacillus subtilis* (MTCC 441), *Escherichia coli* (MTCC 424), *Pseudomonas aeruginosa* (MTCC 443) and

Salmonella typhi (MTCC 531) were procured from microbial type culture collection, Chandigarh. The bacteria were incubated on a nutrient agar-slant (stationary cultures) for 48h at 37°C followed by inoculation in Muller Hinton Agar (MHA) medium.

Antibacterial assay

Antibacterial activity was demonstrated using a modification of the method originally described by (Bauer *et al.* 1966) which is widely used for the antibacterial susceptibility testin (Barry and Thornsberry 1985). A loopful bacterium was taken from the stock culture and dissolved in 0.1ml of saline. All the tests were done by placing the disc (6mm diameter) impregnated with (20µl) various crude solvent extracts on the Muller Hinton Agar surface previously inoculated with 10ml of MHA liquid medium with Gram positive and Gram negative bacteria. Respective solvents without plant extracts served as negative control. Standard antibiotics of chloramphenicol and tetracycline were used as reference or positive control. Plates were incubated at 37°C for 24 hours.

Table 1: Preliminary phytochemical screening of leaf and stem extracts of *Vitex trifolia.*

Presence/absence of bioactive compounds	Name of the extracts									
	Petroleum ether		Benzene		Acetone		Methanol		Water	
	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem
Alkaloids	-	-	+	+	-	-	+	+	-	-
Anthraquinones	-	-	+	-	-	-	-	-	-	-
Catechin	-	-	-	-	-	-	-	-	-	-
Coumarin	-	-	+	+	-	-	+	-	+	-
Flavonoids	-	-	+	+	+	+	+	+	+	-
Phenols	-	-	+	+	-	-	+	+	-	+
Quinones	-	-	-	-	-	-	-	-	-	-
Saponins	+	+	-	-	-	-	+	+	-	+
Steroids	+	+	+	+	+	+	-	-	-	-
Sugar	+	+	+	+	-	+	+	+	-	+
Tannins	-	-	+	+	+	-	+	+	+	-
Terpenoids	-	-	+	+	+	+	+	+	-	-
Xanthoprotein	-	-	-	-	-	-	+	+	-	-

+ denotes: presence; - denotes: absence

Table 2: Preliminary phytochemical screening of leaf and stem extracts of *Aristolochia indica.*

Presence/absence of bioactive compounds	Name of the extracts									
	Petroleum ether		Benzene		Acetone		Methanol		Water	
	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem
Alkaloids	-	-	-	+	-	-	+	+	-	-
Anthraquinones	+	+	+	+	-	-	+	+	-	-
Catechin	-	-	-	-	-	-	-	+	-	-
Coumarin	-	-	+	+	+	-	-	-	+	-
Flavonoids	+	-	+	-	+	+	+	+	+	+
Phenols	+	+	+	+	-	-	+	+	+	+
Quinones	-	-	+	-	-	-	-	-	-	-
Saponins	-	-	+	+	-	-	+	+	+	+
Steroids	-	-	+	+	-	-	-	-	-	-
Sugar	-	-	-	-	-	-	+	+	-	+
Tannins	+	-	+	+	+	-	+	+	+	+
Terpenoids	+	+	+	+	+	+	+	+	-	+
Xanthoprotein	+	+	+	+	-	-	+	+	+	+

+ denotes: presence; - denotes: absence

After the incubation period, the diameter of the inhibition zone around the plant extracts saturated discs were measured and also compared with the diameter of inhibition zone of commercial standard antibiotic discs.

RESULTS AND DISCUSSION

The preliminary phytochemical study of the methanol extracts of leaf and stem of *Vitex trifolia* revealed the presence of alkaloids, flavonoids, phenols, saponins, sugar, tannins, terpenoids and xanthoprotein (Table 1) whereas, the methanol extract of leaf and stem of *Aritolochia indica* revealed the presence of alkaloids, anthraquinones, flavonoids, phenols, saponins, sugar, tannins, terpenoids and xanthoprotein (Table 2).

Table 3: Antibacterial activity of leaf and stem bark of *Vitex trifolia* and *Aristolochia indica.*

Name of the extract	Plant Name	Plant part & (Antibiotic)	Zone of inhibitor (mm)					
			S.aureus	*K.pneumoniae*	*B.subtilis*	*E.coli*	*S. typhi*	*P. aeruginosa*
Petroleum ether	*Vitex trifolia*	L	2	3	1	2	1	2
		S	1	1	-	3	2	2
		T	8	9	8	8	8	8
		C	9	9	8	9	9	9
	Aristolochia indica.	L	3	1	3	2	2	1
		S	1	3	2	2	2	2
		T	9	8	8	8	8	9
		C	9	9	8	9	9	9
Benzene	*Vitex trifolia*	L	-	5	6	-	3	1
		S	2	1	4	1	3	2
		T	8	8	9	8	9	9
		C	8	8	8	8	8	9
	Aristolochia indica.	L	4	-	5	1	3	4
		S	1	4	2	-	2	3
		T	9	8	8	9	9	9
		C	9	9	9	9	9	9
Acetone	*Vitex trifolia*	L	2	5	-	3	-	4
		S	3	2	3	3	-	1
		T	8	9	9	9	9	8
		C	9	9	9	9	9	9
	Aristolochia indica.	L	2	5	4	3	4	-
		S	3	2	3	1	1	-
		T	9	8	8	9	9	9
		C	9	9	9	8	9	9
Methanol	*Vitex trifolia*	L	1	3	5	2	4	2
		S	5	1	4	2	1	-
		T	8	8	9	8	9	8
		C	9	9	8	8	9	9
	Aristolochia indica.	L	6	1	4	2	2	3
		S	2	2	3	1	3	-
		T	8	9	8	9	9	9
		C	9	9	8	9	9	9
Water	*Vitex trifolia*	L	-	1	3	2	-	1
		S	-	2	-	1	3	-
		T	8	8	8	8	8	8
		C	9	9	9	9	8	8
	Aristolochia indica.	L	-	3	-	-	2	1
		S	-	-	2	1	-	-
		T	8	9	8	9	9	9
		C	9	9	9	9	8	9

L- leaf **S- Stem** **T- Tetracycline** **C- Chloramphenicol**

The antibacterial activities of the different extracts obtained from the plants under study by the disc diffusion method are shown in Table 3. All the extracts have exhibited different degrees of antibacterial activity. Petroleum ether extracts of *Vitex trifolia* leaf showed activity against all the tested pathogens, whereas, stem extract failed to inhibit *Bacillus subtilis.* Benzene extract of *V. trifolia* stem exhibited activity against all the tested pathogenic bacteria, whereas, leaf extract did not inhibit the growth of *Staphylococcus aureus* and *Escherichia coli.* Acetone extract of *V. trifolia* leaf inhibited the growth of all the tested pathogens except *Bacillus subtilis* and *Salmonella typhi.* Similarly, stem extracts did not inhibit the growth of *Salmonella typhi.* Methanol extract of leaf and stem of *V. trifolia* exhibited activity against all the tested pathogens. The benzene extract of leaf of *V. trifolia* showed the highest inhibition zone observed against *Bacillus subtilis.*

Petroleum ether, acetone and methanol extracts of *Aristolochia indica* leaf and stem showed antibacterial activity against all the tested pathogens. Benzene extract of *A. indica* leaf failed to inhibit the growth of *K. pneumoniae,* whereas*,* stem extract did not inhibit the growth of *Escherichia coli.* Methanol extract of *A. indica* leaf exhibited maximum inhibition against *S. aureus.*

Aqueous extract of both the plants were observed least inhibition against all the tested pathogens. Where it has no activity against *S. aureus* in leaf and stem extract of both plants presently investigated. Antibacterial activity was comparable with that of standard antibacterial agent tetracycline and chloramphenicol against the organisms tested.

Herbal drugs contain unique constituents which differ from one herb to another, hence the type and extent of their medicinal property also differs. (Le, 1989; Evans, 1996). Solubility of each constituent in an herb is very specific to different solvents used in the extraction process. Hence, chemical nature as well as the pharmacological activity of herbal extracts will be different (Kirtikar and Basu, 1999).

These results indicated that, the different extracts of the two plants under study exhibited antibacterial activity and among the various extracts, benzene, and methanol extracts have shown better activity as compared to other extracts. The phytochemical analysis of the different extracts from the leaf and stem of both the plants revealed the presence of important phytochemicals. Further work on the types of phytoconstituents and purification of individual groups of bioactive components can reveal the exact potential of the plant to inhibit several pathogenic microbes.

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