

Screening of Certain Ethnomedicinal Plants for Antibacterial Activity

P. Murugan, A.Rajesh^a, T. Athiperumalsami and V.R. Mohan*

Ethnopharmacology unit, Research department of Botany
V.O.Chidambaram college.Tuticorin. Tamilnadu.

^aDepartment of Botany, Annamalai University, Annamalai Nagar, Tamil Nadu.

*Corresponding Author e mail:vrmoohan_2005@yahoo.com

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Abstract

Benzene, petroleum ether, chloroform, methanol and aqueous extracts of five plant species, traditionally used for treatment of ailments of infectious nature were screened for *in vitro* antibacterial activity against different species of bacteria, *Staphylococcus aureus*, *Streptococcus lactis*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Salmonella typhi*. Antibacterial activity was tested using the disc diffusion method. Most of the activity detected was against Gram positive bacteria.

Introduction

Medicinal plants are still major parts of traditional medicinal systems in developing countries. Many infectious diseases are known to be treated with herbal remedies throughout the history of mankind. Even today, plant materials continue to play a major roll in primary health care as therapeutic remedies in many developing countries. (Zakaria, 1991). Medicinal plants, which form the backbone of traditional medicine, have in the last few decades been the subject of very intense pharmacological studies. This has been brought about by the acknowledgement of the value of medicinal plants as potential source of new compounds of therapeutic value and as source of new compounds in drug development.

In many parts of the world medicinal plants are used for antibacterial, antifungal and antiviral activities. Plant derived drugs serve as a prototype to develop more effective and less toxic medicines. Tribal medicine has not been studied extensively. Hence, an attempt has been made to study the *in vitro* antibacterial activity of five ethnomedicinal plants used by tribals in Grizzled Giant Squirrel Wildlife Sanctuary, Tamil Nadu.

Materials and Methods

Ethnomedicinal information and plant collection

Information on usage presented in this paper was based on literature surveys presented in Table 1. Plant materials *Bauhinia purpurea* L., *Cardiospermum helicacabum* L., *Cissampelos pareira* L., *Rhinacanthus nastus* (L.) Kurz. var. *nastus* and *Swertia corymbosa* Wighti were collected from the well grown plants found in the natural forest of Grizzled Giant Squirrel Wildlife Sanctuary, Srivilliputhur, Western Ghats, Tamil Nadu. The collected plant materials were shade dried at room temperature for 10-15 days.

Extraction of plant material

Various organic solvents were used for the extraction of bioactive compound. The leaf/ stem powder (10g) was first extraction with petroleum ether for defatting in a Soxhlet apparatus. The defatted powdered residue was dried and successively extracted with benzene, petroleum ether, chloroform, methanol and Aqueous then water in a Soxhlet apparatus. The extracts obtained were completely evaporated by using vacuum rotary evaporator. The final weight of the various crude extracts were weighted and prepared the concentration.

Microorganisms

Bacterial strains of *Staphylococcus aureus*, *Streptococcus lactis*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Salmonella typhi* 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 Mueller 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 testing (Barry and Thornsberry, 1985). A loopful bacteria 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 Mueller 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 (30µg/disc) and tetracycline (30µg/disc) were used as reference or positive control. Plates were incubated at 37°C for 24 hours. 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 results of the antibacterial activity of the plant extracts were tabulated in Table 2. A total of 50 extracts from 5 different plant species were investigated. All the extracts have exhibited different degrees of antibacterial activity against the tested bacteria; among them leaf extract showed broad spectrum activity against *Bacillus subtilis* and *Pseudomonas aeruginosa*. Leaf extract of *Rhinacanthus nastus* var. *nastus* showed activity against all the test pathogenic bacteria. Antibacterial activity was found in the methonal extracts of *Swertia corymbosa* leaves, benzene extracts of *Swertia corymbosa* stem, both benzene and methanol extracts of leaves and stems of *Bauhinia purpurea*, *Rhinocanthus nastus* var *nastus*, stem of *Cardiospermum halicacabum*.

Most antibacterial activity was found against the Gram positive bacteria. Forty four extracts were found to be active against Gram positive bacterium *Bacillus subtilis*, 40 against *Streptococcus lactis* 39 against *Staphylococcus aureus*. The inhibition zone diameters were compared with antibiotics drugs Chloramphenicol and tetracycline in table 3 as a standard reference.

These observations suggested that the aqueous and organic extracts from the same plants showed different activities. There are no common rules for this, but in most cases, the organic extracts showed the same or greater activity than the aqueous extracts. In addition, the effectiveness of plant was not due to one main active constituent, but to the combined action of the chemical compound involved in it. This study has identified five plants with some antibacterial activity. This finding lends some support to traditional knowledge and can serve as a basis for selecting the most active medicinal plants to use in traditional medicine practices in the future.

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Table1. Ethnobotanical data of studied plants.

Botanical names, family and vernacular names	Part used	Ethnomedicinal uses	References
<i>Bauhinia purpurea</i> L. (Caesalpiniaceae) Sivappu Mandarai	leaf	Parts of leaf along with the latex of <i>Jatropha curcas</i> is taken to treat jaundice and liver disorders	Apparanantham and Chelladurai, 1986 Singh <i>et al.</i> , 1988.
	Stem bark	Paste of stem bark is used to treat smallpox	Srivastava <i>et al.</i> , 2003
		powder is used to arrest diarrhea and dysentery	Girach and Aminuddin, 1995 Manandhar, 2003
<i>Cardiospermum helicacabum</i> L. (Sapindaceae) Mudakkathan	Leaf	Paste of leaf is applied to cure skin diseases.	Singh <i>et al.</i> , 1997
		Decoction of fresh leaf along with seed of <i>Cuminum</i>	Muthukumara samy <i>et al.</i> , 2004

		<i>Cyminum</i> , leaves of <i>Solanum trilobatum</i> , <i>S. surattense</i> and <i>Ferula asafoedita</i> is taken internally to get relief from cold and cough	
<i>Cissampelos pareira</i> L. (Menispermaceae) Malaitaangivaer	Root	Paste of root is taken along with 100ml of hot water in empty stomach to cure stomachache	Muthukumarasamy <i>et al.</i> , 2003a
	Aerial part	Infusion of aerial part is taken for the treatment of fever and headache	Purkayastha <i>et al.</i> , 2005
	Leaf	Paste of leaf is applied on joints for rheumatism	Pawar and Patil 2006
<i>Rhinacanthus nastus</i> (L.) kurz. var. <i>nastus</i> (Acanthaceae) Nagamalli	leaf	Aqueous extract of fresh leaves is taken orally or one or two grams of shade dried leaf powder is consumed along with 100mlg cow's milk for snake bites	Muthukumarasamy <i>et al.</i> , 2003b
<i>Swertia corymbosa</i> Wight (Gentianaceae) Malaisiriyangai	Entire plant	Shade dried plants powder is taken along with water in empty stomach to get relief from stomachache.	Muthukumarasamy <i>et al.</i> , 2003a

Table 2. Antibacterial activity of various solvent extracts on some ethnomedicinal plants.

Baacterial Name	Parts tested	Extraction/Solvent used ^b	Zone of Inhibition (mm) Bacteria ^a					
			Sa	Sl	Bs	Pa	Ec	St
<i>Bauhinia purpurea</i>	leaf	I	-	2	3	3	-	-
		II	4	3	4	6	2	3
		III	-	2	3	-	3	2
		IV	2	-	6	3	4	2
		V	2	-	2	-	-	-
	Stem	I	-	2	-	2	3	-
		II	5	4	3	4	3	5
		III	2	-	6	4	-	-
		IV	6	3	5	6	5	4
		V	-	2	2	2	-	2
<i>Cardiospermum halicacabum</i>	Leaf	I	1	-	2	3	1	-
		II	3	2	4	1	2	-
		III	3	4	5	6	-	5
		IV	2	4	-	-	3	5
		V	3	-	2	1	1	-
	Stem	I	2	3	2	2	-	-
		II	4	2	3	3	5	3
		III	2	3	2	4	2	2
		IV	3	2	6	5	1	2
		V	1	2	-	-	2	-

<i>Cissampelos pareira</i>	Leaf	I	-	3	2	2	2	2
		II	2	5	6	3	-	-
		III	2	5	-	5	3	4
		IV	4	3	5	4	-	2
		V	-	1	3	-	2	-
	Stem	I	1	2	-	2	3	2
		II	2	1	4	-	2	2
		III	3	2	3	-	3	2
		IV	-	2	4	1	2	-
		V	-	2	2	3	1	-
<i>Rhinacanthus nastus var nastus</i>	Leaf	I	2	1	1	2	5	3
		II	4	2	6	5	3	4
		III	2	4	3	2	2	6
		IV	4	2	6	5	4	3
		V	4	2	6	5	4	3
	Stem	I	2	-	2	4	2	4
		II	3	4	4	4	2	3
		III	2	-	2	1	3	1
		IV	3	4	6	3	3	2
		V	3	4	1	2	3	2
<i>Swertia corymbosa</i>	Leaf	I	-	2	3	3	-	1
		II	1	2	-	2	1	-
		III	5	4	1	4	-	3
		IV	3	3	1	1	3	2
		V	1	-	2	-	1	-
	Stem	I	1	-	2	2	-	4
		II	2	2	2	3	1	1
		III	2	2	3	-	2	-
		IV	1	-	2	2	-	5
		V	-	-	1	-	1	2

^a Microorganisms: Sa, *Staphylococcus aureus*; Sl, *Streptococcus lactis*; Bs, *Bacillus subtilis*, Pa, *Pseudomonas aeruginosa*; Ec, *Escherichia coli*; St, *Salmonella typhi*;

^b Extraction/Solvent used: I, Petroleum ether; II, Benzene; III, Chloroform; IV, Methanol; V, Water.

Table 3. Antibiotic reference standards.

Bacteria	Antibiotics zone of inhibition (mm)	
	Chloramphenicol 30µg/disc	Tetracycline 30µg/disc
<i>Staphylococcus aureus</i>	10	9
<i>Streptococcus lactis</i>	11	10
<i>Bacillus subtilis</i>	9	8
<i>Pseudomonas aeruginosa</i>	10	9
<i>Escherichia coli</i>	9	10
<i>Salmonella typhi</i>	8	9

