Pharmaceutical Biology

ISSN: 1388-0209 (Print) 1744-5116 (Online) Journal homepage: informahealthcare.com/journals/iphb20

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To cite this article: Yogesh Mahida & J.S.S. Mohan (2006) Screening of Indian Plant Extracts for Antibacterial Activity, Pharmaceutical Biology, 44:8, 627-631, DOI: 10.1080/13880200600897551

To link to this article: https://doi.org/10.1080/13880200600897551

Published online: 07 Oct 2008.

Pharmaceutical Biology
2006, Vol. 44, No. 8, pp. 627–631

informa
healthcare

Screening of Indian Plant Extracts for Antibacterial Activity

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Abstract

Methanol extracts of 22 Indian plants belonging to 12 families were studied for their antibacterial activity. All the examined plant extracts were effective against more than one organism, and the results are comparable with antibiotics. Out of the plants tested, *Blumea lacera* (Burm f.) DC (Asteraceae), *Canscora diffusa* (Vahl) R. Br. (Gentianaceae), *Cassia alata* L., *C. biflora* L., *C. fistula* L. (Ceasalpiniaceae), and *Putranjiva roxburghii* Wall (Euphorbiaceae) extracts were found to be more effective against both Gram-positive and Gram-negative bacteria. *Staphylococcus aureus* was found to be susceptible to 68% of the tested plant extracts, whereas *Pseudomonas aeruginosa* showed resistance to most of the plant extracts.

Keywords: Agar diffusion method, antibacterial activity, gram-negative bacteria, gram-positive bacteria, methanol extracts.

Introduction

Since their discovery, antibiotics have completely transformed humanity's approach to infectious diseases and have substantially reduced the threat posed by infectious diseases. However, the emergence of drug-resistant microorganisms is swiftly reversing the advances of the previous 50 years of research. These drug-resistant microorganisms have complicated the treatment of infectious diseases in immunocompromised AIDS and cancer patients (Davis, 1994). In this context, there is an urgent need to find new antimicrobial agents against resistant microorganisms from different sources, especially of plant origin. Scientists from divergent fields are now investigating plants with a new view of their antimicrobial usefulness and as an alternative source to existing drugs. Approximately 119 pure chemical substances extracted from higher plants are used in medicine throughout the world (Farnsworth & Soejarto, 1985). The World Health Organization estimates that 80% of the people in developing countries of the world rely on traditional medicine for their primary health care needs, and about 85% of traditional medicine involves the use of plant extracts. This means that about 3.5 million to 4 million people in the world rely on plants as sources of drugs (Farnsworth & Soejarto, 1985; Kamboj, 2000). Among 45,000 plant species in India, 3000 plants are officially documented with medicinal potential, whereas traditional practitioners use more than 6000 plants. India is the largest producer of medicinal herbs and is appropriately called the botanical garden of the world (Ahmedullah, 1999). In recent years, antimicrobial properties of medicinal plants are being increasingly reported from India (Perumal Samy, 1998, 2000; Ahmad & Beg, 2001; Srinivasan, 2001).

In the current investigation, methanol extracts of 22 plants belonging to 12 different families, used by herbalists for primary health care, were screened for their potential antibacterial activity against opportunistic bacteria, which can cause serious infections in hospitals and the community.

Materials and Methods

Plant material

Twenty-two plant samples were collected either in the form of leaves or bulbs (*Crinum pretense* Herb., Liliaceae, and *Urginea indica* L., Liliaceae) from different areas of Gujarat State (Table 1). All the plants were identified by Dr. A.S. Reddy, Department of Biosciences, Sardar Patel University. The collected plant material was air-dried under shade at room temperature and powdered with a grinder.

Accepted: June 21, 2006

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DOI: 10.1080/13880200600897551

Table 1. Antibacterial activity of plant extracts against Gram-positive and Gram-negative bacteria.

	Zone of inhibition[a] (in mm)											
	Gram positive							Gram negative				
Plant name	BC	BS	BM	SA	SE	ML	EN	EC	ST	SP	PA	KP
Alstonia scholaris Br. (Apocynaceae)	4	6	5	—	—	5	—	3	6	10	—	6
Blumea lacera (Burm.f.) DC (Asteraceae)	12	9	5	8	9	5	—	12	20	5	1	4
Canscora diffusa (Vahl) R. Br. (Gentianaceae)	6	5	7	7	—	4	6	10	9	2	3	5
Cassia alata L. (Ceasalpinaceae)	10	12	12	10	10	12	9	10	11	15	3	—
Cassia biflora L. (Ceasalpinaceae)	12	12	9	12	8	10	6	5	6	12	—	—
Cassia fistula L. (Ceasalpinaceae)	12	12	12	10	10	7	5	12	9	13	2	4
Combretum ovalifolium Roxb. (Combretaceae)	3	2	6	16	8	—	8	4	—	7	4	7
Crinum pratense Herb. (Liliaceae)	—	—	—	16	—	12	8	4	—	6	4	7
Dregia volubilis (L. f.) Bth. (Asclepidaceae)	8	5	6	6	—	5	6	10	—	7	5	5
Enicostema hyssopifolium (Willd.) Verdoon (Gentianaceae)	6	6	7	4	8	5	5	6	—	4	3	5
Exacum pedunculatum L. (Gentianaceae)	4	3	5	5	5	5	—	3	18	8	4	6
Hyptis suaveolens (L.) Poit (Lamiaceae)	9	3	1	6	—	—	7	12	6	5	3	4
Ipomoea fistulosa Mart. ex Choisy (Convolvulaceae)	7	3	5	6	—	—	6	—	10	—	3	5
Murraya exotica L. (Rubiaceae)	3	—	—	2	—	—	5	—	5	10	2	10
Nerium indicum Mill. (Apocynaceae)	1	2	3	—	3	—	—	4	—	—	1	5
Plumeria rubra L. (Apocynaceae)	—	4	2	3	—	13	—	—	—	3	—	—
Putranjiva roxburghii Wall. (Euphobiaceae)	10	10	8	18	—	10	5	8	30	12	2	6
Schweinfurthia papilionace A.Br. (Scrophulariaceae)	—	5	7	6	—	—	6	—	—	3	2	—
Streblus asper Lour. (Moraceae)	—	4	2	10	5	—	—	2	9	4	—	7
Tabebuia argentea (Bru. & Sch.) Britt. (Bignoneaceae)	6	5	4	2	—	2	—	5	—	—	1	7
Urginea indica L. (Liliaceae)	6	8	9	8	4	—	6	8	4	6	—	8
Wrightia tinctoria R. Br. (Apocynaceae)	6	4	—	6	—	—	5	3	8	3	3	5
Ciprofloxacin	11	11	9	10	8	11	10	10	22	9	11	24

BS, *Bacillus subtilis*; BC, *Bacillus cereus*; BM, *Bacillus megaterium*; EN, *Entertococcus faecalis*; SA, *Staphylococcus aureus*; SE, *Staphylococcus epidermidis*; ML, *Micrococcus luteus*; KP, *Klebsiella pneumoniae*; EC, *Escherichia coli*; ST, *Salmonella typhi*; SP, *Salmonella paratyphi A*; PA, *Pseudomonas aeruginosa*.

[a]Zone of inhibition (in mm) excluding the diameter of well.

Preparation of extract

Extracts were prepared by the cold extraction method, in which 100 g of dry powdered material was soaked in methanol (5×200 mL) for 12 h at room temperature and shaken occasionally. Each extract was filtered by Whatman filter paper no. 1. The methanol filtrates were dried by water vapor in a water bath at 50°C. Dried extracts were collected and stored in a refrigerator until further use.

Microorganisms

In the current study, seven Gram-positive and five Gram-negative bacteria (Table 1) were selected, which were obtained from V.P. & R.P.T.P Science College, Vallabh Vidyanagar, Gujarat, and Pramukh Swami Medical College, Karamsad, Gujarat. The bacterial cultures were grown in nutrient broth medium (Hi Media, Mumbai, India, pH 7.4) and maintained on nutrient agar slants.

Antimicrobial assay

Sensitivity tests were performed by the agar-well diffusion method (Perez et al., 1990). An inoculum size of 10^8 CFU/mL of bacteria, compared with 0.5 McFarland turbidity standards, was used. About 100 μL of plant extract (stock 100 mg/mL) was added carefully in a well of 10 mm diameter in a nutrient agar plate. Plates were kept in a refrigerator for prediffusion of extracts.

Minimum inhibitory concentration (MIC) was determined by twofold serial broth dilution method. The MIC was tested in the concentration range of 0.125–8.0 mg/mL. Plates and tubes were incubated at 37°C in an incubator. Zone of inhibition surrounding the well was measured to evaluate the antimicrobial activity. Tubes showing no turbidity was recorded as the MIC value. Antibiotics such as ciprofloxacin at 20 μg/mL and 100% DMSO, a dissolving solvent, were used as positive and negative controls respectively. Bioassay was carried out in duplicate, and experiments were repeated twice.

Results and Discussion

In the current study, the crude methanol extracts of 22 plants belonging to 12 different families were screened for antibacterial activity against seven Gram-positive and five Gram-negative opportunistic bacterial strains. Only methanol was used for the extraction of plant materials because it was found to be a better solvent for the extraction of phytochemicals having antibacterial properties, compared with other polar and nonpolar solvents (Cowan, 1999). The results of antibacterial activity of the extracts and their potency were comparatively assessed by the diameter of zone of inhibition in millimeters, excluding the well diameter (Table 1). Most plant extracts exhibited inhibitory activity against one or more tested bacterial strains, but plant extracts displaying zones of inhibition more than 5 mm were taken into consideration for the preparation of sensitivity sequence of organisms. Sensitivity of test strains was in decreasing order: SA > BC > ST > BM = EN = KP > EC > BS > PA toward different plant extracts (Fig. 1). In the current study, *Staphylococcus aureus* was found to be most susceptible, whereas *Pseudomonas aeruginosa* showed resistant to the different plant extracts (Table 1). The growth of *S. aureus* was inhibited by 68% plant extracts. Among these extracts, of *Cassia alata*, *Cassia biflora*, *Cassia fistula*, *Combretum ovalifolium*, *Crinum pretense*, *Putranjiva roxburghii*, and *Streblus asper* were found to be most effective.

The growth of *Bacillus cereus* was inhibited by approximately 59% of the plant extracts, among which *Blumea lacera*, *Cassia* spp., and *Putranjiva roxburghii* extracts were most effective (Table 1). *Salmonella typhi*, which causes enteric fever in children, was inhibited effectively by 54% of the plant extracts, mostly with *Blumea lacera*, *Cassia alata*, *Exacum pedunculatum*, *Ipomoea fistulosa*, and *Putrangiva roxburghii* extracts. However, extracts of *Alstonia scholaris*, *Cassia* spp., *Exacum pedunculatum*, *Murraya exotica*, and *Putrangiva roxburghii* were found to be most effective against *Salmonella pertyphi A* among 50% of the plant extracts. About 45% plant extracts inhibited the growth of *Bacillus megaterium*, *Enterococcus faecalis*, and *Klebsiella pneumoniae* more effectively. The extracts of *Cassia alata*, *C. fistula*, and *Urginea indica* showed inhibitory efficacy against *Bacillus megaterium*. *Enterococcus faecalis* was inhibited by *Combretum ovalifolium* and *Crinum pretense* including *Cassia alata*. However, *Murraya exotica* extract was least effective against most tested organisms, but inhibited the growth of *Klebsiella pneumoniae* effectively. *Blumea lacera*, *Canscora difusa*, *Cassia alata*, *Cassia fistula*, *Dregia volubilis*, and *Hyptis suaveolens* inhibited the growth of *Escherichia coli* out of 40% effective plant extracts. The growth of *Bacillus subtilis* was inhibited by 36% of the plant extracts, which include *Cassia alata*, *C. biflora*, *C. fistula*, and *Putranjiva roxburghii* extracts.

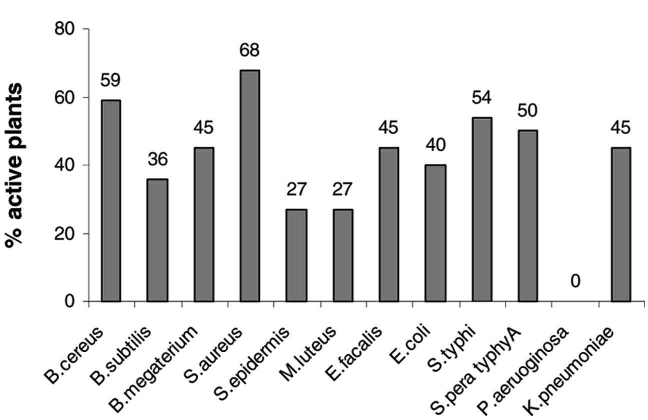

Figure 1. Number of plant extracts that showed antibacterial activity against each individual organism.

Staphylococcus epidermis, which can cause wound and urinary tract infection, was inhibited by *Cassia alata*, *C. biflora*, *C. fistula*, and *Enicostema hyssopifolium* extracts, which were found to be effective. The growth of *Micrococcus luteus* was inhibited by the extracts of *Cassia alata*, *C. biflora*, *C. fistula*, *Crinum pretense*, *Plumeria rubra*, and *Putranjiva roxburghii*. None of the plant extracts were found to effectively inhibit the growth of *Pseudomonas aeruginosa*. *Alstonia scholaris* (except *Salmonella peratyphi A)*, *Nerium indicum*, and *Plumeria rubra* extracts showed little or no inhibitory activity against one or more tested organisms. These observed results are comparable with the antibiotic ciprofloxacin for most of the bacteria.

The observed MIC values of the effective plant extracts varied in the range 0.5–8.0 mg/mL for most of the tested organisms (Table 2). Among different plant extracts, *Cassia alata*, *C. biflora*, *C. fistula*, and *Putranjiva roxburghii* extracts showed the MIC values at 2 mg/mL against most of the organisms tested (Table 2). Leaf extract of *Cassia alata* was found to be effective against most of the tested bacterial strains, except *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* (Table 1). Khan et al. (2001) reported methanol extracts of *Cassia alata* leaves, flowers, bark, and roots at 4 mg/mL concentration inhibited *E. coli* and *S. aureus*, but in this study, *C. alata* showed a MIC value at 0.5–4.0 mg/mL against most of the tested organisms. Other workers (Ogunti et al., 1991; Ali et al., 1999; Somechit et al., 2003) also reported antimicrobial activity of *Cassia alata*, but have used different plant materials, fractions, and test organisms. Perumal Samy et al. (1998) reported antibacterial activity of *Cassia fistula* against Gram-negative organisms (i.e., *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris*, and *Pseudomonas aeruginosa*). In the current investigation, *Cassia fistula* was also found to be effective at a MIC value of 1–4 mg/mL against both Gram-positive and Gram-negative bacteria, except *Microcouus luteus* (8 mg/mL). The methanol extract of *Putranjiva roxburghii* revealed significant inhibitory activity against most of the tested organisms except *Enterococcus faecalis* with a MIC value of 0.5–4.0 mg/mL (Tables 1 and 2). However, Saxena et al. (1983) reported that oil from seed kernel of *P. roxburghii*

Table 2. Minimum inhibitory concentration of effective plant extracts.

	MIC (mg/mL)											
	Gram positive							Gram negative				
Plant name	BC	BS	BM	SA	SE	ML	EN	EC	ST	SP	PA	KP
Blumea lacera (Burm.f.) DC (Asteraceae)	2	8	–	8	8	–	–	4	1	–	–	–
Canscora diffusa (Vahl) R. Br. (Gentianaceae)	4	–	4	–	–	–	–	4	4	–	–	–
Cassia alata L. (Ceasalpinaceae)	1	0.5	1	2	2	2	4	1	1	4	–	–
Cassia biflora L. (Ceasalpinaceae)	2	2	2	8	4	4	–	–	–	1	–	–
Cassia fistula L. (Ceasalpinaceae)	2	2	2	4	2	8	–	2	2	4	–	–
Combretum ovalifolium Roxb. (Combretaceae)	–	–	–	2	>8	–	–	–	–	–	–	–
Crinum pratense Herb. (Liliaceae)	–	–	–	2	–	8	>8	–	–	–	–	–
Exacum pedunculatum L. (Gentianaceae)	–	–	–	–	–	–	–	–	2	8	–	–
Hyptis suaveolens (L.) Poit (Lamiaceae)	2	–	–	–	–	–	–	2	–	–	–	–
Ipomoea fistulosa Mart. ex Choisy (Convolvulaceae)	–	–	–	–	–	–	–	–	2	–	–	–
Putranjiva roxburghii Wall. (Euphobiaceae)	2	2	2	1	–	1	–	4	0.5	2	–	–

BS, *Bacillus subtilis*; BC, *Bacillus cereus*; BM, *Bacillus megaterium*; EN, *Entertococcus faecalis*; SA, *Staphylococcus aureus*; SE, *Staphylococcus epidermidis*; ML, *Micrococcus luteus*; KP, *Klebsiella pneumoniae*; EC, *Escherichia coli*; ST, *Salmonella typhi*; SP, *Salmonella paratyphi A*; PA, *Pseudomonas aeruginosa*.

exhibited toxic activity for *Helminthosporium oryzae*. Extracts of *Alstonia scholaris* and *Ipomoea fistulosa* were found to be effective against *Salmonella peratyphi A* in the current study. However, it also showed significant inhibitory efficacy against *Bacillus* sp., *Micrococcus aureus*, *Escherichia coli*, *Shigella*, and *Mycobacterium tuberculosis* (Beloy et al., 1976; Reza et al., 1994). Wonkham et al. (2001) reported that the leaf extract of *Streblus asper* possesses a selective bactericidal activity toward *Streptococcus*. However, it is also effective against *Staphylococcus aureus* and *Salmonella typhi* in the current investigation.

Conclusions

The results obtained from the current study suggest that the extracts of *Combretum ovalifolium*, *Crinum pretense*, and *Putranjiva roxburghii* on *Staphylococcus aureus*, and *Blumea lacera, Exacum pedunculatum*, and *Putranjiva roxburghii* extracts on *Salmonella typhi* possess significant antibacterial properties. The extracts of *Cassia alata*, *C. biflora*, and *C. fistula* exhibitedrelatively more activity against most of the tested Gram-positive and Gram-negative organisms. Out of the 22 plant extracts tested, three plant extracts, *Nerium indicum*, *Plumeria rubra*, and *Tabebuia argentea*, showed the least or no activity against most of the organisms. Further study on the fractionation of active compounds and the mutual effect of active plant extracts may provide a better source for developing new therapeutic agents against infecting microbial species.

Acknowledgments

We are grateful to Head, Department of Microbiology, V.P. & R.P.T.P. Science College, and Dean, Pramukha Swami Medical College, for providing the microbial cultures. We also thank Dr. A.S. Reddy for identification of plants used in the current study. We that Dr. R.P. Vaidya, Dr. V. Rama Rao, and Mr. Kalpesh Ishnava for providing different plant samples.

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