



Search for antibacterial and antifungal agents from selected Indian medicinal plants

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Abstract

A series of 61 Indian medicinal plants belonging to 33 different families used in various infectious disorders, were screened for their antimicrobial properties. Screening was carried out at 1000 and 500 µg/ml concentrations by agar dilution method against *Bacillus cereus* var *mycoides*, *Bacillus pumilus*, *Bacillus subtilis*, *Bordetella bronchiseptica*, *Micrococcus luteus*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Streptococcus faecalis*, *Candida albicans*, *Aspergillus niger* and *Saccharomyces cerevisiae*. Twenty-eight plant extracts showed activity against at least one of the test organisms used in the screening. On the basis of the results obtained, we conclude that the crude extracts of *Dorema ammoniacum*, *Sphaeranthus indicus*, *Dracaena cinnabari*, *Mallotus philippinensis*, *Jatropha gossypifolia*, *Aristolochia indica*, *Lantana camara*, *Nardostachys jatamansi*, *Randia dumetorum* and *Cassia fistula* exhibited significant antimicrobial activity and properties that support folkloric use in the treatment of some diseases as broad-spectrum antimicrobial agents. This probably explains the use of these plants by the indigenous people against a number of infections.

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1. Introduction

India has a rich flora that is widely distributed throughout the country. Herbal medicines have been the basis of treatment and cure for various diseases and physiological conditions in traditional methods practiced such as Ayurveda, Unani and Siddha. Several plant species are used by many ethnic groups for the treatment of various ailments ranging from minor infections to dysentery, skin diseases, asthma, malaria and a horde of other indications (Dhar et al., 1968; Perumal Samy and Ignacimuthu, 1998, 2000; Dahanukar et al., 2000). The past three decades have seen a dramatic increase in microbial resistance to antimicrobial agents (Chopra et al., 1996; Baquero, 1997) that lead to repeated use of antibiotics and insufficient control of the disease (NCID, 2002). New prototype antimicrobial agents are needed to address this situation. This prompted us to evaluate plants as source of potential chemotherapeutic agents antimicrobial activity based on their ethnomedical use.

In this screening we have studied the antimicrobial activity of dichloromethane:methanol (1:1, v/v) extract of 61 plant species against a battery of microorganisms including Gram-positive and Gram-negative bacteria and fungi.

2. Materials and methods

2.1. Plant materials

Plant materials were collected from various localities of India. Their identity was confirmed and voucher specimens were deposited at the Department of Pharmacognosy and Phytochemistry of this institute. The parts of the different plants used in the experiment and their voucher numbers are given in Table 1.

2.2. Preparation of the extract

Different parts of the plants were air-dried at room temperature and powdered. About 10 g of powdered drug was extracted with a mixture of dichloromethane and methanol (1:1, v/v) (2 × 50 ml) under reflux for 30 min and filtered. The filtrates were combined and concentrated to dryness under reduced pres-

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Table 1 (Continued)

Voucher no.	Botanical name/family	Part tested	Ethnomedical use	Microorganisms														
				Bc	Bp	Bs	Bb	Ml	Sa	Se	Ec	Kp	Pa	Sf	Sc	Ca	An	
MB1016	<i>Abutilon indicum</i> G. Don (Malvaceae)	Fruit	Cough, gonorrhoea	a	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
				b	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
MB5045	<i>Abutilon indicum</i> G. Don (Malvaceae)	Root	Gonorrhoea, leprosy	a	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
				b	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
MB2187	<i>Abutilon indicum</i> G. Don (Malvaceae)	Leaf	Eye wash, mouth wash, catarrh, bilious diarrhoea	a	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
				b	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
AB5156	<i>Aristolochia indica</i> L. (Aristolochiaceae)	Root	Cholera, diarrhoea	a	–	–	–	–	–	–	–	+++	–	+++	–	–	–	++
				b	–	–	–	–	–	–	–	+++	–	+++	–	–	–	–
CB4156	<i>Artemisia annua</i> L. (Compositae)	Whole plant	Stomachic, skin diseases, jaundice	b	+++	+++	+++	+++	++	+++	+++	+++	+++	+++	+++	+++	–	+++
CB6178	<i>Doronicum hookeri</i> Hook.f. (Compositae)	Root	Aromatic, tonic	a	+++	+++	++	++	+++	+++	+++	+++	+++	+++	–	–	–	+++
				b	+++	+++	++	++	+++	+++	+++	+++	+++	+++	+	–	–	–
CB4324	<i>Achillea millefolium</i> L. (Compositae)	Aerial parts	Used in colic, styptic	a	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
				b	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
CB1459	<i>Sphaeranthus indicus</i> L. (Compositae)	Aerial parts	Blood purifier, skin diseases	a	–	–	–	–	–	–	–	+++	–	+++	–	–	–	–
				b	–	–	–	–	–	–	–	+++	–	+++	–	–	–	–
CB2156	<i>Eclipta alba</i> (L.) Hassk. (Compositae)	Root	Externally as antiseptic	a	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
				b	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
VB6145	<i>Vitex nigundo</i> L. (Verbenaceae)	Leaf	Anti-parasitic, vermifuge gonorrhoea, cartarrh, eczema	a	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
				b	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
VB3456	<i>Lantana camara</i> L. (Verbanaceae)	Leaf	Antiseptic for wounds, tetanus, rheumatism, malaria	a	–	–	–	–	–	–	–	+++	–	+++	–	–	–	–
				b	–	–	–	–	–	–	–	+++	–	+++	–	–	–	–
LB4157	<i>Gloriosa superba</i> L. (Liliaceae)	Root	Purgative, skin affections, anthelmintic	a	+++	+++	+++	+++	+	–	+++	+++	+++	+++	–	–	–	+++
				b	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	–	–	–	–
LI2169	<i>Dracaena cinnabari</i> (Balf.f) (Liliaceae)	Exudes of red resin	Astringent, stops haemorrhage	a	–	–	–	–	–	–	–	+++	–	–	–	+++	+++	–
				b	–	–	–	–	–	–	–	+++	–	–	–	+++	+++	–
RB1004	<i>Ruta graveolens</i> L. (Rutaceae)	Leaf	Worms, colic	a	+++	+++	+++	+++	+	–	+++	+++	+++	+++	–	–	–	+++
				b	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	–	–	–	–
RB6156	<i>Aegle marmelos</i> (L.) Correa (Rutaceae)	Fruit	Stomachic, diarrhoea	a	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
				b	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
RB4189	<i>Murraya exotica</i> L. (Rutaceae)	Leaf	Rheumatic fever, cough	a	+++	+++	+++	+++	–	+	+++	+++	+++	+++	+++	+++	+++	+++
				b	+++	+++	++	++	+++	++	+++	+++	+++	+++	+++	++	–	–
AS7163	<i>Calotropis gigantea</i> (L.) R.Br. (Asclepiadaceae)	Root bark	Diarrohea, dysentery, leprosy	a	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	–	–	–	+++
				b	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	–	–	–
AS6173	<i>Hemidesmus indicus</i> (L.) R.Br. (Asclepiadaceae)	Root	Blood purifier, skin diseases	a	+++	+++	+	–	–	+	+++	+++	+++	+++	–	+++	++	–
				b	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	–	+++	+++	+++

Table 1 (Continued)

Voucher no.	Botanical name/family	Part tested	Ethnomedical use	Microorganisms														
				Bc	Bp	Bs	Bb	Ml	Sa	Se	Ec	Kp	Pa	Sf	Sc	Ca	An	
PB7156	<i>Plantago lanceolata</i> L. (Plantaginaceae)	Seed	Wound healing	a	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
				b	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
PI1893	<i>Pinus gerardiana</i> Wall. (Pinaceae)	Nut	Wound healing, bronchitis	a	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
				b	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
PI7826	<i>Abies webbiana</i> Lindl. (Pinaceae)	Leaf	Cough, chronic bronchitis, catarrh	a	+++	+++	+++	+++	–	+	+++	+++	+++	+++	–	+++	+++	+++
				b	+++	+++	+++	+++	–	+	+++	+++	+++	+++	–	+++	+++	+++
ZB7826	<i>Fagonia arabica</i> Hook.f. (Zygophyllaceae)	Leaf	Sore mouth, smallpox	a	+++	+++	+++	+++	+	+++	+++	+++	+++	+++	+++	+++	+++	+++
				b	+++	+++	+++	+++	+	+++	+++	+++	+++	+++	+++	+++	+++	+++
SB7259	<i>Symplocos racemosa</i> Roxb. (Symplocaceae)	Stem bark	Diarrhoea, dysentery	a	+++	+++	+++	–	–	+++	+++	+++	+++	+++	+++	+++	+++	+++
				b	+++	+++	+++	+++	–	+++	+++	+++	+++	+++	–	+++	+++	+++
CR7269	<i>Raphanus sativus</i> L. (Cruciferae)	Seed	Gonorrhoea	a	+++	+++	+++	+++	++	+++	+++	+++	+++	+++	+++	+++	+++	+++
				b	+++	+++	+++	+++	++	+++	+++	+++	+++	+++	+++	+++	+++	+++
LA7826	<i>Leucaena glauca</i> (L.) Benth. (Labiatae)	Seed	Diarrhoea	a	+++	+++	+++	+	+	+	+++	+++	+++	+++	+++	+++	+++	+++
				b	+++	+++	+++	+++	+	+	+++	+++	+++	+++	+++	+++	+++	+++
AL1598	<i>Alangium salvifolium</i> (L.f.) Wang. (Alangiaceae)	Root	Purgative, anthelmintic, skin diseases	a	+++	+++	+++	+++	–	+++	+++	+++	+++	+++	+++	+++	+++	+++
				b	+++	+++	+++	+++	–	+++	+++	+++	+++	+++	+++	+++	+++	+++
GI7156	<i>Gisekia pharmaceoides</i> L. (Ficoideaceae)	Whole plant	Anthelmintic	a	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
				b	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
GU1893	<i>Mesua ferrea</i> L. (Guttiferae)	Flower	Dysentery, cough	a	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
				b	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++

Abbreviations: a, 1000 µg/ml; b, 500 µg/ml; N, negative control; P, positive control [ciprofloxacin (3 µg/ml) for bacteria and amphotericin-B (3 µg/ml) for fungi]; B, blank. Grading of results: +++, no inhibition; ++, moderate inhibition; +, partial inhibition; –, complete inhibition; Bc, *Bacillus cereus* var *mycoides* (ATCC 11778); Bp, *Bacillus pumilus* (ATCC 14884); Bs, *Bacillus subtilis* (ATCC 6633); Bb, *Bordetella bronchiseptica* (ATCC 4617); Ml, *Micrococcus luteus* (ATCC 9341); Sa, *Staphylococcus aureus* (ATCC 29737); Se, *Staphylococcus epidermidis* (ATCC 12228); Ec, *Escherichia coli* (ATCC 10536); Kp, *Klebsiella pneumoniae* (ATCC 10031); Pa, *Pseudomonas aeruginosa* (ATCC 9027); Sf, *Streptococcus faecalis* (MTCC 8043); Ca, *Candida albicans* (MTCC 10231); An, *Aspergillus niger* (MTCC 1344); Sc, *Saccharomyces cerevisiae* (ATCC 9763).

sure at 45 °C with a rotary evaporator and were stored at 4 °C until further use.

2.3. Microbial cultures and growth conditions

Bacillus cereus var *mycoides* (ATCC 11778), *Bacillus pumilus* (ATCC 14884), *Bacillus subtilis* (ATCC 6633), *Bordetella bronchiseptica* (ATCC 4617), *Micrococcus luteus* (ATCC 9341), *Staphylococcus aureus* (ATCC 29737), *Staphylococcus epidermidis* (ATCC 12228), *Escherichia coli* (ATCC 10536), *Klebsiella pneumoniae* (ATCC 10031), *Pseudomonas aeruginosa* (ATCC 9027), *Streptococcus faecalis* (MTCC 8043), *Saccharomyces cerevisiae* (ATCC 9763), *Candida albicans* (MTCC 10231) and *Aspergillus niger* (MTCC 1344) were used as test microorganisms. Cultures of bacteria were grown on nutrient broth (HiMedia) at 37 °C for 12–14 h and of fungus on Sabouraud dextrose broth (HiMedia) at 28 °C for 48 h and were maintained on respective agar slants at 4 °C.

2.4. Antimicrobial assay by agar dilution-streak method

The test organisms maintained on agar slants were recovered for testing by inoculating into nutrient broth (NB) and incubated at 37 °C in a shaker at 180 rpm till the concentration of the test organisms matched with the 0.5 McFarland standard. Fungal cultures were inoculated into Sabouraud dextrose broth (SDB) and incubated at 28 °C.

Antibacterial and antifungal activity was carried out by agar dilution-streak method (Mitscher et al., 1972). DCM extract of all plant materials were tested at two different concentrations, viz. 500 and 1000 µg/ml in nutrient agar (NA) medium or in Sabouraud dextrose agar (SDA) media for either antibacterial or antifungal testing. Test extracts were incorporated into the media and poured into the different petriplates and allowed to solidify. Bacteria or fungi inocula were then streaked at different areas on the respective agar plates. Plates were incubated at 37 °C (for bacteria) and 28 °C (for fungus) and observed after 24 h for bacteria and 48 h for *Candida albicans*. Growth of *Sac-*

charomyces cerevisiae and *Aspergillus niger* were observed after 4 days. Those extracts found to be active were retested for confirmation. Two blank plates each containing only NA and SDA, two negative controls containing DMSO only and two positive control plates containing ciprofloxacin (3 µg/ml) for bacteria and amphotericin-B (3 µg/ml) for fungi were also maintained. All the experiments were done in duplicate.

3. Results and discussion

Table 1 lists the plant extracts and their level of activity against the various organisms. Plants showing reproducible activity at 1000 µg/ml were considered for retesting. In the present investigation, extract of 61 plants belonging to 33 different families were screened, of which 28 plant extracts showed activity against at least one of the test organisms.

Bordetella bronchiseptica was completely inhibited by 10 plant extracts and weakly inhibited by 6 plant extracts. *Bacillus* species was the second most inhibited microorganism with eight plant extracts completely inhibiting its growth and three others weakly doing so. Ten plant extracts were most active in this series, of which extracts of *Dorema ammoniacum*, *Sphaeranthus indicus* and *Lantana camara* inhibited the growth of 12 microorganisms. *Aristolochia indica* and *Dracaena cinnabari* inhibited the growth of 11 microorganisms. *Jatropha gossypifolia*, *Mallotus philippinensis* and *Nardostachys jatamansi* showed activity against 8–10 microorganisms. *Cassia fistula* and *Randia dumetorum* showed activity only at 1000 µg/ml against 12 and 11 microorganisms, respectively. *Staphylococcus aureus* was inhibited by seven extracts completely and three extracts moderately. *Candida albicans* was inhibited by many plants tested. The growth of this microorganism was completely inhibited by 23 plants and partially by 2 plants.

In general, the plant antibiotic substances appear to be more inhibitory to Gram-positive organisms than to the Gram-negative type. It may be remembered that penicillin and some of the other prominent antibiotic agents of fungal origin are also rather selective in their inhibitory action, most of them being inhibitory to Gram-positive organisms. Unlike Gram-positive bacteria, the lipopolysaccharide layer along with proteins and phospholipids are the major components in the outer surface of Gram-negative bacteria (Burn, 1988). Access of most compounds to the peptidoglycan layer of the cell wall is hindered by the outer lipopolysaccharide layer. This explains the resistance of Gram-negative strains to the lytic action of most extracts exhibiting activity.

Eight plant extracts showed complete inhibition whereas five plant extracts showed moderate inhibition against the Gram-positive bacteria tested. The negative results obtained against Gram-negative bacteria were not unexpected since this class of bacteria is usually more resistant than Gram-positive bacteria (Tomas-Barberan et al., 1988). Antimicrobial extracts from tested plants can be assumed to be useful to the producing plant in warding off infectious diseases and there is therefore a compelling reason to suppose that anti-infective agents could be active against human pathogens as was suggested by folk-

loric and historical accounts (Kirtikar and Basu, 1968; Nadkarni, 1976).

Infections caused by *Pseudomonas aeruginosa* are among the most difficult to treat with conventional antibiotics (Levison and Jawetz, 1992). The growth of *Pseudomonas aeruginosa* was partially inhibited by two extracts and completely by the extract of *Dracaena cinnabari* (500 µg/ml). These plants may thus, be a source which could yield drugs that could improve the treatment of infections caused by this organism.

Bacillus species are common microbes found in most natural environments including soil, water, plant and animal tissues. While most *Bacillus* species are regarded as having little pathogenic potential, both *Bacillus cereus* and *Bacillus subtilis* have been known to act as primary invaders or secondary infectious agents in a number of diseases and have been implicated in some cases of food poisoning (Turnbull and Kramer, 1991). Many of the plants in the food and medicine of the indigenous people may have helped to combat these microbes.

4. Conclusion

Our results allow us to conclude that the crude extracts of *Dorema ammoniacum*, *Sphaeranthus indicus*, *Dracaena cinnabari*, *Mallotus philippinensis*, *Jatropha gossypifolia*, *Aristolochia indica*, *Lantana camara*, *Nardostachys jatamansi*, *Randia dumetorum* and *Cassia fistula* exhibited significant antimicrobial activity and properties that support folkloric use in the treatment of some diseases as broad-spectrum antimicrobial agents. This probably explains the use of these plants by the indigenous people against a number of infections since generations.

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