Screening of Aqueous and Alcoholic Extracts of Some Indian Medicinal Plants for Antibacterial Activity

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The antibacterial activities of 100 extracts of 50 Indian plant species were tested against six medically important bacterial strains, viz., *B. cereus, S. aureus, S. epidermidis, K. pneumoniae, A. fecalis* and *P. aeruginosa*. The antibacterial assay was done by both agar disc diffusion method and agar well diffusion method. The antibacterial activity exhibited by alcoholic extract was better than the aqueous extract. The results evaluated as the diameter of the inhibition zone of microbial growth showed that the extracts were more active against gram-positive bacteria than gram-negative bacteria. Amongst the investigated microorganisms, the most resistant bacteria were *P. aeruginosa* and *A. fecalis*. The most susceptible bacteria were gram-positive *B. cereus* and gram-negative *K. pneumoniae*, and the maximum activity was shown by *T. chebula, M. indica* and *E. citriodora*.

For centuries plants have been used throughout the world as drugs and remedies for various diseases since they have great potential for producing new drugs of great benefit to mankind. There are many approaches to search for new biologically active principles in higher plants[1]. One such approach is systematic screening, which may result in the discovery of novel effective compounds[2]. Despite the existence of potent antibiotics and antifungal agents, resistant or multi-resistant strains are continuously appearing, imposing the need for a permanent search and development of new drugs[3]. This revival of interest in plant-derived drugs is mainly due to the current widespread belief that "green medicine" is safe and more dependable than the costly synthetic drugs, many of which have adverse side effects.

The purpose of this study was to screen some Indian medicinal plant extracts that could be useful for the development of new tools as antimicrobial agents for the control of infectious diseases.

Fresh plant/plant parts were collected randomly from the semi-arid region of Rajkot, Gujarat, India. The taxonomic identities of these plants were confirmed at the Department of Biosciences, Saurashtra University, Rajkot. The voucher specimen numbers of the plants collected and their medicinal uses are reported in Table 1. Fresh plant material was washed thoroughly, air dried and then homogenized to fine powder.

Plant material (10 g) was extracted with 100 ml ethanol by subjecting it to agitation on rotary shaker (190-200 rpm) overnight, filtering it with muslin cloth and concentrating it to one-fifth of the volume[4]. Crude aqueous extract was prepared by subjecting plant material (10 g) to slow heat for 6 h and filtered through muslin cloth and concentrated to one-fifth of the total volume[4].

The microbial strains investigated (Table 2) were obtained from National Chemical Laboratory (NCL), Pune, India. Microorganisms were maintained at 4°C on nutrient agar slants (for bacteria) and MGYP slants (for yeast).

In vitro antimicrobial activity was examined for aqueous and alcoholic extracts from 50 different traditionally used medicinal plants. Antimicrobial activity of the aqueous extracts was evaluated by agar disc diffusion method[5] and that of alcoholic extracts was evaluated by agar well diffusion method[6].

Successful prediction of botanical compounds from plant material is largely dependent on the type of solvent used in the extraction procedure. The traditional healers use water primarily as the solvent, but in our studies we found that the plant extracts in organic solvent (methanol or ethanol) provided more consistent antimicrobial activity as compared to those extracted in water. These

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observations can be rationalized in terms of the polarity of the compounds being extracted by each solvent and, in addition to their intrinsic bioactivity, by their ability to dissolve or diffuse in the different media used in the assay. The growth media also seem to play an important role in the determination of the antibacterial activity.

Table 1 summarizes the data of the plant species selected for the study. Fifty aqueous and alcoholic extracts of the plants belonging to 30 families were tested against three gram-positive and three gram-negative bacteria. The results of antibacterial activity of the screened plants are presented in Table 2.

TABLE 1: SELECTED INDIAN MEDICINAL PLANTS TESTED FOR ANTIBACTERIAL ACTIVITY

Plant species (Common name)/ Voucher no.	Part used*	Medicinal uses[9,10]
Achyranthus aspera L. (Aghedo)/ PSN635	W	Cough, rheumatism, bronchitis, piles
Eranthemum spp. L. (Black Croton)/ SU/BIO/486/Thakrar	L	In boils and rashes
Calotropis gigantea (L.) R. Br. (Ankdo)/ PSN449	L	Purgative, antihelminthic
Eucalyptus citriodora Hook. (Nilgiri)/ PSN1058	L	Asthma, laryngitis, sore throat, colds, fevers
Fagonia cretica L. (Dhamasa)/ PSN98	W	Tonic, fevers, asthama, dysentry, bronchitis
Rauwolfia serpentina (L.) Benth ex Kurz. (Sarpgandha)/ SU/BIO/487/Thakrar	L	In bites of poisonous insects and reptiles
Musa paradisiaca L (Banana)/ PSN719	L	Scabies and inflammations
Ipomea pentphylla Jacq. (Gariya vel)/ PSN522	L	Ornamental
Solanum khasianum C. B. Clarke. (Ubhi ringani)/ SU/BIO/ 488/Thakrar	W	Antiinflammatory, antiarthritic
Croton bonaplanium Baill. (Croton)/ PSN673	W	Ornamental
Carissa congesta Wt. (Karamda)/ PSN440	L	Diarrhoea, anorexia, skin diseases,scabies
Tridax procumbens L. (Bhangro)/ PSN414	W	Emetic and purgative
Enicostema hyssopifolium (Willd.) I.C. Verd. (Mamejavo)/ PSN470	W	In fever, rheumatism, sciatica,
Vitex negundo L. (Nagod)/ PSN611	AP	In catarrhal dengue, rheumatism, dyspepsia, diarrhoea
Caesalpinia crista L. (Kakach)/ PSN244	AP	Useful in amenorrhoea, dysmenorrhoea, fevers, cough
Cymbopogon citratus (DC.) Stapf. (LeeliCha)/ PSN808	L	Chronic rheumatism, lumbago, myalgia
Ocimum canum Sims. (Shyam tulsi)/ SU/BIO/243A/Thakrar	W	In skin diseases
Sesamum indicum L. (Tal)/ PSN569	W	In gonorrhea, dysentry, piles, wounds, ulcers
Ocimum basilicum L. (Takmaria)/ PSN626	W	Cephalgia, colic worms, arthralgia, arthritis
Jasminum malabaricum L. (Jui)/ PSN432	A	Treatment of cataract, haematorrhoea
Thevetia peruviana (Pers.) Merr. (Pila Karen)/ PSN447	L	Cariotonic drug, insecticide
Launaea procumbens (Roxb.) Ram. and Raj. (Bhoy pathri)/ PSN397	W	Lactogogue, rheumatism
Thuja orientalis L. (Vidya)/ PSN1092	L	Ornamental
Cassia tora L. (Kuvadio)/ PSN258	W	Obstinate skin diseases, cardiac disorders
Quisqualis indica L. (Madhumalati)/ PSN289	A	Antihelminthic
Lantana camera L. (Abhagani)/ PSN605	W	antispasmodic, vulnerary, diaphoretic, carminative
Tephrosia purpurea (L.) Pers. (Sharpankho)/ PSN223	W	In obstruction of the liver, spleen and kidney, dropsy, boils
Andrographis paniculata (Burm. F.) Wall. (Lilu Karyatu)/ PSN574	L	Diarrhoea, dysentry, dyspepsia, constipation, tumor
Cassia angustifolia Vahl. (Mindhiaval)/ PSN248	L	Chronic constipation, abdominal disorders, leprosy
Embelica ribes Burm. F. (Vavding)/ SU/BIO/489/Thakrar	S	Brain tonic, skin diseases, neuro disorder, constipation
Dalbergia sisoo Roxb. (Moto Shisham)/ PSN179	L	Gonorrhoea, menorrhoea, excoriation, opthalmopathy
Aloe barbadensis Mill. (Kuamwarpanthu)/ PSN723	L	Eye infection, piles, cough, inflammation
Centella asiatica (L.) Urb. (Brahmi)/ PSN340	L	Skin diseases, insomnia, asthama, bronchitis
Mangifera indica L. (Mango)/ PSN1132	L	Hiccough, hyperdipsia, haemoptysis, haemorrhages,
Xanthium strumarium L. (Gadariyu)/ PSN419	W	Malarial fever, renal complaints, leucorrhoea, menorrhagia,
Ervatamia divaricata (L.) Burkill. (Chandani Tagar)/ PSN446	AP	Ornamental
Vitis vinifera L. (Grapes)/ PSN128	L	Bilious fever, anemia, heart disease, urinary disorder
Picrrohiza kurroa L. (Kadu)/ SU/BIO/493/Thakrar	Rh	To treat worms, constipation, low fever, scorpion sting
Cicer erientinum L. (Gingra)/ PSN159	W	Appetizer, tonic, skin diseases, dyspepsia, fever
Spinacia oleracea L. (Palak)/ SU/BIO/491/Thakrar	L	Carminative, laxative, hypoglycaemic, inflammation
Coleus aromaticus Benth. (Ajma pan)/ SU/BIO/492/Thakrar	L	In asthama, chronic cough, strangury, calculas
Allium sativum L. (Lasun)/ SU/BIO/490/Thakrar	W	Eye and heart diseases, inflammation, piles
Daucus carota L. (Carrot)/ PSN 343	AP	Anorexia, dyspepsia, diarrhoea, cough, asthama,ulcer
Lagenaria vulgaris Seringe (Dudhi)/ PSN 328	F	Antibiotic; Antidote; Diuretic; Emetic; Febrifuge
Mucuna pruriens (L.) DC. (Kaucha)/ PSN 209	W	Dyspepsia, neuro diseases, leucorrhoea, colic worms
Glycyrrhiza glabra L. (Jethimadh)/ SU/BIO/494/Thakrar	L	Sore throat, cold, cough, broncial infections, influenza
Bassella rubra L. (Velbondi, Poi)/ PSN654	L	Mild laxative, diuretic, used in urticaia, gonorrhoea
Solanum surattense Burm. F. (Bethi ringani)/ PSN543	W	Kidney disorder
Aristolochia bracteolata Lam. (Kidamari)/ PSN662	S	Constipation, inflammation, boils, syphilis, gonorrhoea
Terminalia chebula Retz. (Himej)/ PSN292	F	Asthama, urinary disorders, piles, ulceration, rheumatism

The ethnobotanical information like vernacular name, voucher no., part used and medicinal use of the selected Indian medicinal plants. *: W – Whole; L – Leaves; S – Stem; Rh – Rhizome; AP – Aerial parts

TABLE 2: ANTIBACTERIAL ACTIVITY OF AQUEOUS AND ALCOHOLIC EXTRACTS OF INDIAN MEDICINAL PLANTS

Sr. No.	*Inhibition zone (mm)											
	B. cereus		*S. aureus*		*S. epidermidis*		*K. pneumoniae*		*A fecalis*		*P. aeruginosa*	
	A	B	A	B	A	B	A	B	A	B	A	B
1	4	2	2	0	15	0	0	7	0	2	0	0
2	4	1	2	0	11	0	0	2	0	2	0	1
3	0	2	0	0	0	0	0	6	0	2	0	1
4	4	12	0	10	7	15	2	17	0	12	0	9
5	0	3	0	0	4	0	0	7	0	7	0	1
6	0	3	0	0	4	2	2	6	0	2	0	1
7	0	1	0	0	8	0	0	0	0	0	0	1
8	6	4	0	0	5	0	0	2	0	0	0	1
9	0	3	0	0	0	0	0	3	0	0	0	1
10	0	4	0	0	0	2	0	5	0	0	0	1
11	3	10	0	4	0	4	0	6	0	0	0	1
12	0	0	0	0	4	0	0	4	0	0	0	1
13	0	3	0	0	0	0	3	2	2	2	0	1
14	0	12	0	1	0	0	0	4	3	0	0	0
15	0	4	0	0	0	0	0	5	3	0	0	0
16	0	7	0	1	0	0	0	3	1	0	0	0
17	0	6	0	1	0	0	0	9	3	0	0	0
18	0	5	0	1	0	0	0	2	2	0	0	0
19	0	10	0	0	0	0	0	0	0	0	0	0
20	0	3	0	0	0	2	0	1	0	0	0	0
21	0	12	0	0	0	1	0	0	0	0	0	0
22	0	15	0	20	0	12	0	2	0	0	0	0
23	4	2	0	0	0	1	3	3	0	0	0	0
24	0	7	0	0	0	1	0	4	0	0	0	0
25	8	4	2	0	0	1	4	0	0	0	0	0
26	0	5	0	0	0	6	0	0	0	0	0	0
27	0	3	0	0	0	3	0	0	0	0	0	0
28	0	3	0	0	0	4	0	0	0	0	0	0
29	0	6	6	0	3	5	3	0	0	0	0	0
30	2	4	3	0	3	3	2	0	0	0	0	0
31	0	2	4	0	1	2	0	10	0	0	0	0
32	0	1	4	0	0	2	0	2	0	0	0	0
33	0	5	2	4	7	0	0	6	0	0	0	0
34	6	14	2	12	3	9	4	22	0	11	3	7
35	0	7	3	4	2	0	0	9	0	0	0	0
36	0	7	3	0	0	0	0	0	0	0	0	0
37	6	12	3	6	4	3	7	6	3	1	2	2
38	0	10	0	2	0	0	0	5	0	3	0	0
39	0	7	0	1	0	0	0	7	0	2	0	0
40	0	3	0	0	0	0	0	1	0	0	0	0
41	0	3	0	0	0	0	0	6	0	0	0	0
42	0	4	0	0	0	0	0	13	0	0	0	0
43	3	4	0	0	0	0	0	6	0	0	0	0
44	0	6	0	1	0	0	0	7	0	0	0	0
45	0	12	6	7	0	0	0	12	0	0	0	0
46	0	0	0	0	0	0	0	4	0	0	0	0
47	0	0	0	0	0	0	0	5	0	0	0	0
48	0	0	0	0	0	0	0	3	0	0	0	0
49	0	15	0	20	0	0	0	15	0	0	0	0
50	11	27	15	23	8	21	9	20	5	17	5	7

The evaluation of antibacterial activity of aqueous and alcoholic extracts of the selected medicinal plants. The results are mean values of diameter of zone of inhibition in millimetres. A: Aqua extract; B: Alcoholic extract (1-36: ethanol extract, 37-50: methanol extract); 1-50: plant species in order as shown in Table 1; *: mean values of zone diameter in millimetres (mm) are taken

Out of 100 extracts, 56 showed some degree of activity against one of the tested bacterial strains. The plants which exhibited significant antibacterial activity (defined as a perfectly clear zone with diameter >16 mm) were *Terminalia chebula, Aristolochia bracteolata, Mangifera indica, Launaea procumbens* and *Eucalyptus citriodora.* The gram-positive bacteria were more susceptible than gram-negative bacteria; the most susceptible gram-positive bacteria were *B. cereus*, while the most susceptible gram-negative bacteria were *K. pneumoniae*. The most resistant bacteria amongst those investigated were *P. aeruginosa* and *A. fecalis.*

The greatest zone of inhibition, with 27 mm diameter,

was displayed by *Terminalia chebula* seed extract against *B. cereus*. In fact, this plant extract (both aqueous and ethanolic) could inhibit all the bacterial strains investigated. Out of 50 aqueous extracts, 16 extracts showed activity against any one of gram-positive bacteria, while only 10 extracts showed activity against any one of gram-negative bacteria. The plant extracts inhibited the gram-positive microorganisms better than gram negative microorganisms. This is in agreement with previous reports that plant extracts are more active against gram-positive bacteria than gram-negative bacteria[7,8]. From our investigation for screening of different plant species, the results obtained confirm the therapeutic potency of some plants used in traditional medicine. In addition, these results form a good basis for selection of plant species candidates for further phytochemical and pharmacological investigation.

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