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Antimicrobial and phytochemical studies on 45 Indian medicinal plants against multi-drug resistant human pathogens

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Abstract

Ethanolic extracts of 45 Indian medicinal plants traditionally used in medicine were studied for their antimicrobial activity against certain drug-resistant bacteria and a yeast *Candida albicans* of clinical origin. Of these, 40 plant extracts showed varied levels of antimicrobial activity against one or more test bacteria. Anticandidal activity was detected in 24 plant extracts. Overall, broad-spectrum antimicrobial activity was observed in 12 plants (*L. inermis*, *Eucalyptus* sp., *H. antidysentrica*, *H. indicus*, *C. equistifolia*. *T. belerica*, *T. chebula*, *E. officinalis*, *C. sinensis*, *S. aromaticum* and *P. granatum*). No correlation was observed between susceptibility of test strains with plant extracts and antibiotic resistance behaviour of the microbial strains (*Staphylococcus aureus*, *Salmonella paratyphi*, *Shigella dysenteriae*, *Escherichia coli*, *Bacillus subtilis*, *Candida albicans*). Qualitative phytochemical tests, thin layer chromatography and TLC-bioautography of certain active extracts demonstrated the presence of common phytocompounds in the plant extracts including phenols, tannins and flavonoids as major active constituents. © 2001 Published by Elsevier Science Ireland Ltd.

Keywords: Medicinal plants; Antimicrobial activity; Multidrug resistance; TLC-bioautography

1. Introduction

Infectious diseases are the world's leading cause of premature deaths, killing almost 50 000 people every day. In recent years, drug resistance to human pathogenic bacteria has been commonly reported from all over the world (Piddock and Wise, 1989; Singh et al., 1992; Mulligen et al., 1993; Davis, 1994; Robin et al., 1998). However, the situation is alarming in developing as well as developed countries due to indiscriminate use of antibiotics. The drug-resistant bacteria and fungal pathogens have further complicated the treatment of infectious diseases in immunocompromised, AIDS and cancer patients (Rinaldi, 1991; Diamond, 1993). In the present scenario of emergence of multiple drug resistance to human pathogenic organisms, this has necessitated a search for new

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antimicrobial substances from other sources including plants.

Traditionally used medicinal plants produce a variety of compounds of known therapeutic properties (Iyengar, 1985; Chopra et al., 1992; Harborne and Baxter, 1995). The substances that can either inhibit the growth of pathogens or kill them and have no or least toxicity to host cells are considered candidates for developing new antimicrobial drugs. In recent years, antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world (Grosvenor et al., 1995; Ratnakar and Murthy, 1995; Silva et al., 1996; David, 1997; Saxena, 1997; Nimri et al., 1999; Saxena and Sharma, 1999). It is expected that plant extracts showing target sites other than those used by antibiotics will be active against drug-resistant microbial pathogens. However, very little information is available on such activity of medicinal plants (Hasegawa et al., 1995; Lee et al., 1998). In the present study, we have selected 45 Indian medicinal plants to be screened against multi-drug resistant bacteria including *Staphylococcus aureus*, *Salmonella paratyphi*, *Escherichia coli*, *Shigella dysenteriae* and *Candida albicans*. The selection of medicinal plants is based on their traditional uses (32 plants) in India and our reported antimicrobial activity of 13 plants (Chopra et al., 1992; Ahmad et al., 1998; Mehmood et al., 1999). However most of these plants were not previously screened against multi-drug resistant, pathogenic organisms. Phytochemical analysis of active plant extracts for their major group of phytoconstituents and the active group of certain extracts is also reported here.

2. Materials and methods

2.1. Plant material

Thirty-five authenticated plant samples were collected locally and 10 plant samples were kindly provided by the Himalaya Drug Co. (New Delhi, India). All the plant materials were further identified in the Department of Botany, AMU, Aligarh, India by a senior plant taxonomist, Professor Wazahat Hussain. Voucher specimens have been deposited in the Department of Agricultural Microbiology, RAK Institute of Agricultural Sciences, AMU, Aligarh, India. The details of medicinal plants along with their acquisition code numbers are listed in Table 1.

2.2. Preparation of plant extracts

Plant extracts were prepared by the method of Alade and Irobi (1993) with minor modification. Briefly 100 g of each powdered plant material were soaked in 100 ml of 70% alcohol for 72 h. Each mixture was stirred every 24 h using a sterile glass rod. At the end of extraction each extract was passed through Whatman filter paper No. 1 (Whatman, UK). The alcoholic filtrates obtained was concentrated in vacuo at 30°C and stored at 4°C until further use.

2.3. Microorganisms used

The test organisms used included *E. coli* UP 2566, resistant to M, Cx, Do, Nv, (Central Drug Research Institute, Lucknow, India), *Bacillus subtilis* MTCC-121 (Microbial Institute of Technology, Chandigarh, India) and drug-resistant clinical isolates of *S. aureus* IOA-106 (Am, Cu, Cx, Cj, Nal, Co), *S. paratyphi* IOA-107 (M, Nv, Cx, Do), *S. dysenteriae* IOA-108 (M, Tc, Do) and *C. albicans* IOA-109 (Fu, Ns) , kindly provided by the Department of Medical Microbiology, JN Medical College, AMU, Aligarh, India. The clinical isolates were biochemically and serologically characterized by standard methods.

2.4. Culture media and inoculum

Sabouraud Dextrose (SD) and Soyabean Casien Digest (SCD) media (Hi-Media Pvt. Ltd., Bombay, India) were used for *C. albicans* and test bacteria, respectively. Microbial cultures, freshly grown at 37°C were appropriately diluted in sterile normal saline solution to obtain the cell suspension at 10^5 CFU/ml.

Table 1
Ethnobotanical and phytochemical data of 45 Indian medicinal plants

Specimen no.	Botanical name; family, voucher specimen	Common name	Part used	Known phytoconstituents of part used	Traditional uses (Chopra et al., 1992)
1.	*Acorus calamus* L.; Araceae, HDCO-166/297	Bach/Vaj	Rhizome	Glucoside acorin, alkaloid, 1.5–3.5% essential oil, methyl isoeugenol (Chopra et al., 1992; Harborne and Baxter, 1995)	Emetic, stomach-ache, nerve dyspepsia, colic, tonic in bronchitis, dysentery in children
2.	*Allium cepa* L.; Liliaceae, IOA-01/99	Piyaz	Leaves	Methylallyl, diallyl dimethyl, propanethiol, phloroglucinol, propanethiol (Harborne and Baxter, 1995)	Expectorant
3.	*Allium sativum* L.; Liliaceae, IOA-02/99	Lasan	Bulb	Allicin 0.06–0.1%, diallyl catechols, protocatechuic acid Allistin I & II, ajoene, allyl propyl sulfide (Harborne and Baxter, 1995; Ratnakar and Murthy, 1995; Nagenawa et al., 1996)	Carminative, coughs in fevers, juice used in skin diseases, ear aches, atonic dysepsia, colic
4.	*A. sativum* L.; IOA-03/99	Lasan	Leaves	–	–
5.	*Azadirachta indica* A. Juss.; Meliaceae, IOA-04/99	Neem	Bark	Azadirachtin, margosic acid 0.04% nimbin, 0.001% nimbinin 0.4% nimbidin (Chopra et al., 1992)	Tonic, astrigent, anti periodic snake periodic bite
6	*Beta vulgaris* L.; Chenpodiaceae, IOA-05/99	Chokundar	Root	Betamic acid, Prebetanin, Vulgaxanthin I & II (Harborne and Baxter, 1995)	Cooling, diaphoretic
7.	*Calotropis procera* L.; Asclepediaceae, IOA-35/99	Madar	Leaves	Calotropin, Calotropagenin, latex-uscharin, Calotoxin, Calactin	Fever
8.	*Camelia sinensis* L.; Theaceae, Assam Tea Chowk Ltd.	Chai	Leaves	Caffeine, theaine, Theobromine, Chlorgenic acid, Myricetin, Epi-gallotannins, 3 galactosides of flavones and flavonols (Harborne and Baxter, 1995)	Astrigent, diuretic stimulant
9.	*Casuarina equistifolia* L.; Casuarinaceae, IOA-07/99	Jangli-saru	Bark	Casuarin, 6–18% Tannins (Chopra et al., 1992)	Astrigent, useful in diarrhoea and dysentery
10.	*C. equistifolia* L.; IOA-08/99	Jangli-saru	Leaves	–	Decoction used in colic
11.	*Citrus sinensis* L.; Rutaceae, IOA-09/99	Musambi	Rind	Tangetin, Sinensetin, Limonene decyclicaldehyde, Linalool diterpineol (Chopra et al., 1992; Harborne and Baxter, 1995)	Carminative, tonic used for acne
12.	*Cordia dichotama* L.; Boraginaceae, IOA-10/99	Lasora	Leaves	–	Used for headaches and ulcers; decoction used for sore throat

Table 1 (*Continued*)

Specimen no.	Botanical name; family, voucher specimen	Common name	Part used	Known phytoconstituents of part used	Traditional uses (Chopra et al., 1992)
13.	*Emblica officinalis* Gaerth.; Euphorbiaceae, IOA-11/99	Amla	Fruits	Vitamin C, phosphatides, seeds contain tannins, Chebulinic Acids (Chopra et al., 1992; Harborne and Baxter, 1995)	Acrid, cooling, refrigerant diuretic, used in diarrhoea, dysentery, anaemia, jaundice and cough
14	*Eucalyptus* sp.; Myrtaceae, IOA-12/99	Eucalyptus	Leaves	0.9–1.2% oil: Cineole, Pinene Sesquiterpene alcohols, Astrgin eudesmal, a-phellandrene (Harborne and Baxter, 1995)	Antiseptic, infections of upper respiratory tract, skin diseases, burns, rheumatism
15.	*Ficus carica* L.; Moraceae, IOA-13/99	Anjir	Leaves	Ficusin and bergaptene 0.06% (Chopra et al., 1992)	Acidant
16.	*Ficus religiosa* L.; Moraceae, IOA-14/99	Pipal	Leaves	Bergaptol and bergapten (Swami and Bisht, 1996)	Purgative
17.	*Hemidesmus indicus* R. Br.; Asclepiadaceae, HDCO-204/76	Anatamul	Roots	0.18% 2-OH, 4 Methyl benzaldehyde, sterols, glucosides, resinic acid, tannins (Chopra et al., 1992)	Demulcant, tonic, diaphoretic, skin diseases, syphilis, and blood purifier
18.	*Holarrhena antidysenterica* R.; Apocyanaceae, HDCO-120/235	Kurachi	Bark	Alkaloids — Conessine, Kurchine, Kurchicine, Holarhimine (Chopra et al., 1992)	Dropsy, diarrhoea
19.	*Lantana camara* L.; Verbenaceae, IOA-19/99	Ghaneri	Leaves	Essential oil carmene, isocamerene, micramene (Chopra et al., 1992)	Decoction used in malaria, atoxy, rheumatism
20.	*Lawsonia inermis* L.; Lythraceae, IOA-15/99	Hena/Mehdi	Leaves	Glucoside, hennotannic acid, Lawsone (Harborne and Baxter, 1995)	Headache, burning of skin, decoction used for sore throat
21.	*Morus alba* L.; Moraceae, IOA-16/99	Shahtut	Leaves	p-Cresol, phenol, morin (Harborne and Baxter, 1995)	Decoction used for sore throat
22.	*Musa paradisiaca* L.; Musaceae, IOA-17/99	Kala	Stem	–	Epilepsy, diarrhoea, dysentery
23.	*Nelumbo nucifera* Gaerth.; Nymphaceae, HDCO-01/99	Kamal	Flowers	Alk. Nelumbine, ammonaine pronuciferine (Harborne and Baxter, 1995)	Astrigent, diarrhoea cholera, fever
24.	*Nerium indicum* Mill.; Apocyanaceae, IOA-18/99	Kaner	Leaves	Glucoside (Chopra et al., 1992)	Swelling and skin diseases
25.	*Nigella sativa* L.; Ranunculaceae, HDCO-111/194	Kalongi	Seeds	Glucoside, melanthin, saponin (Chopra et al., 1992)	Eruptions of skin
26.	*Nyctanthes arbortristis* L.; Oleaceae, IOA-20/99	Harsingar	Leaves	Alkaloid, resins, glucoside (Chopra et al., 1992)	Fever, rheumatism, obstrinate sciata
27.	*Ocimum sanctum* L.; Labiatae, HDCO-23/28	Tulsi	Whole plant	71.3% Eugenol, 3.7% carvacrol plant 20.4% Methyl eugenol 1.7% Caryophyllene (Chopra et al., 1992)	Gastric disorders bronchitis, ear ache antiseptic, diaphoretic, hepatic affections

Table 1 (*Continued*)

Specimen no.	Botanical name; family, voucher specimen	Common name	Part used	Known phytoconstituents of part used	Traditional uses (Chopra et al., 1992)
28.	*Plumbago zeylanica* L.; Plumbaginaceae, HDCO-43/64	Chita	Root	Plumbagin (Bruneton, 1995)	Used in skin diseases, dyspepsy, piles, leprosy and skin diseases
29.	*Portulaca quadrifolia* L.; Portulacaceae, IOA-21/99	Chota-lunya	Plant	–	Application in erysipelas, infusion used as diuretic and in dysuria
30.	*Psidium guajava* L.; Myrtaceae, IOA-22/99	Amrud	Leaves	Eugenol (Chopra et al., 1992)	Astringent, for bowels, used for diarrhoea, ulcers, piles, cholera, vomiting
31.	*Punica granatum* L.; Punicaceae, IOA-23/99	Anar-ke-per	Rind	Water soluble yellow pigment (Chulasuri, 1997)	Diarrhoea and dysentery
32.	*Raphanus sativus* L.; Brassicaceae, IOA-24/99	Mouli	Root	Methyl mercaptan (Harborne and Baxter, 1995)	Urinary complaints, piles, gastrodynamic pains
33.	*Sapindus* sp.; Sapindaceae, IOA-25/99	Ritha	Fruit	–	Expectorant, emetic, epilepsy, asthma purgative
34.	*Saussurea lappa* C.B. Clarke; Compositae, HDCO-102/175	Kuth	Root	Alk. Saussurine, bicyclic lactone, kushtin (Chopra et al., 1992; Harborne and Baxter, 1995)	Tonic, spasmodic in cough, cholera, chronic skin diseases
35.	*Syzgium aromaticum* L.; Myrtaceae, IOA-26/99	Laung	Bud	Eugenol, eugeniin, Casuarji-citin (Chopra et al., 1992)	Stimulant, carminative used in dyspepsy
36.	*S. aromaticum* L.; Myrtaceae, Dabur India Ltd.	Laung	Oil	Eugenol, vanillin (Harborne and Baxter, 1995)	Toothache and constipation
37.	*Syzgium cumini* L.; Myrtaceae, IOA-27/99	Jamun	Bark	Jambosine (Chopra et al., 1992)	Astrigent, used for sore throat, diarrhoea
38.	*S. cumini* L.; IOA-28/99		Leaves	–	Dysentery
39.	*Terminalia arjuna* W.&A.; Combretaceae, IOA-29/99	Arjun	Bark	Arjunine, Lactone, Arjunetin tannin (Chopra et al., 1992)	Astrigent, bilious affections, heart diseases
40.	*Terminalia belerica* Roxb.; Combretaceae, HDCO-24/29	Bahera	Fruit	17% tannins, triterpenoid (Chopra et al., 1992)	Antipyretic, leprosy, diarrhoea, dropsy
41.	*Terminalia chebula* Retz.; Combretaceae, HDCO-70/116	Harir	Fruit	Chebulinic acid, tannic acid 20–40%, Anthroquinone chebulagic acid, Corilagin (Harborne and Baxter, 1995)	Laxative, ulcers, used in carious teeth, piles
42.	*Vitis vinifera* L.; Vitaceae, IOA-30/99	Angur	Leaves	p-OH benzoic acid (Harborne and Baxter, 1995)	Diarrhoea
43.	*Wattaka volubilis* L.; Asclepiadace, IOA-31/99	Madumalati	Leaves	Glucoside, dregein (Chopra et al., 1992)	Fevers, boils, abscesses
44.	*Zizyphus jujuba* L.; Rhamnaceae, IOA-32/99	Ber	Leaves	–	Plaster for strangury, eye sores
45.	*Z. jujuba* L.; IOA-33/99	Ber	Bark	–	Diarrhoea

2.5. Antibiotic resistance of test strains

Antibiotic sensitivity of test strains was determined by the standard Disc diffusion method of Baur et al. (1966) against a number of antibiotics including two antifungal drugs. The potency of antibiotics per disc are as follows.

Amoxycillin (Am), Cefuroxime (Cu), Cefaclor, Chloramphenicol, Doxycycline (Do), Nalidixic acid (Nal), Novobiocin (Nv), Tetracycline, (Tc) (30 μg/disc each); Cloxacillin (Cx), Methicillin (M), Fluconazole, (Fu) (10 μg/disc each); Nystatin (Ns), (100 μg/disc) and Nitofurantoin (300 μg/disc). *E. coli* B (a sensitive strain) was used to check the potency of the discs. All antibiotic discs were purchased from the Hi-Media Pvt. Ltd. (Bombay, India).

2.6. Antimicrobial assay

The agar well diffusion method (Perez et al., 1990) as adopted earlier (Ahmad et al., 1998) was used; 0.1 ml of diluted inoculum (10^5 CFU/ml) of test organism was spread on SDA/SCD agar plates. Wells of 8 mm diameter were punched into the agar medium and filled with 100 μl (150 mg/ml) of plant extract, solvent blanks and antibiotic (chloramphenicol, 100 μg/ml conc.) to which the test bacteria were sensitive. The plates were incubated for 18 h at 37°C. Antimicrobial activity was evaluated by measuring the zone of inhibition against the test organism.

2.7. Phytochemical analysis

Phytochemical analysis for major phytoconstituents of the plant extracts was undertaken using standard qualitative methods as described by various authors (Vogel, 1958; Kapoor et al., 1969; Rizk and Bashir, 1980; Fadeyi et al., 1989; Odebiyi and Sofowora, 1990). The plant extracts were screened for the presence of biologically active compounds like glycosides, phenolics, alkaloids, tannins, flavonoids, saponins and steroids.

2.8. Thin layer chromatography (TLC) of plant extracts

TLC of the 11 plant extracts with strong antimicrobial activity was carried out. Different solvent systems were used for different classes of compounds based on the polarity of the organic solvents. TLC microslides were prepared as described by Harborne (1984) with silica gel G. About 20 μl of plant extract was applied to the TLC chromatogram. Tannic acid, resorcinol, and anthrone were used as control. Solvent systems used were (1) petroleum ether and benzene, 1:1, (2) benzene and chloroform 1:1, (3) benzene and ethyl acetate 2:1, (4) acetone and alcohol, 1:1, and (5) methanol and water 1:1. Individual R_f for each spot was measured. TLC spots were visualized under UV light and adequate TLC reagents were used to detect the phytoconstituent.

2.9. TLC-bioautography

For direct bioautographic assay, agar overlay assay as described by Slusarenko et al. (1998) was used with minor modification. Drug-resistant *S. aureus* was used as test strain. About 10 μl of plant extract was spotted on preparative Merck 3×8 cm chromatographic silica gel-60 plates. Only one solvent system (acetone and ethanol, 1:1) was used. One milliliter of (10^5 CFU/ml) broth culture was used for every 10 ml of nutrient agar. The developed chromatogram was placed in sterilized Petri plates. Culture was added to 42°C nutrient agar, mixed and poured over the chromatograms as a thin layer. Plates were incubated at 37°C for 24 h. The zone of inhibition of bacterial growth could be seen around the active chromatogram spot.

3. Results and discussion

Emergence of multi-drug resistance in human and animal pathogenic bacteria as well as undesirable side effects of certain antibiotics has triggered immense interest in the search for new antimicrobial drugs of plant origin. In the

present study alcoholic extracts of 45 traditionally used Indian medicinal plants have been tested against drug-resistant bacteria and a pathogenic yeast, *C. albicans*. Ethnobotanical and phytochemical data, plant parts used along with their acquisition code number are given in Table 1. The antimicrobial activity of the extracts and their potency was quantitatively assessed by the presence or absence of inhibition zone and zone diameter, respectively as given in Table 2. Only the alcoholic extract was tested, as alcohol was found to be a better solvent for extraction of antimicrobially active substances compared to water and hexane (Ahmad et al., 1998). The results of screening are encouraging as out of the 45 plants, 40 extracts showed antibacterial activity against one or more test bacteria while 24 extracts showed anticandidal activity. Twelve plants namely *L. inermis*, *Eucalyptus* sp., *H. antidysenterica*, *H. indicus*, *C. equistifolia*, *T. belerica*, *E. officinalis*, *C. sinensis*, *S. aromaticum* and *P. granatum* demonstrated broad spectrum antibacterial activity. Similar reports on antibacterial and anticandidal activities of certain Indian medicinal plants such as *Nigella sativa*, *Plumbago zeylanica*, *P. granatum*, *Citrus sisensis*, *Allium sativum*, *Azadirachta indica*, *Lantana camara*, *Psidium guajava*, *Camelia sisensis*, *T. belerica*, *Zizyphus jujuba*, *Acorus calamus* and *Ocimum sanctum* were also reported by other workers (Anesini and Perez, 1993; Belachew Desta, 1993; Youraj et al., 1995; David, 1997; Saxena, 1997; Ahmad et al., 1998; Nimri et al., 1999). However antimicrobial activity of some Indian plants namely *Beta vulgaris*, *Sapindus* sp., *Casuarina equistifolia*, *Nelumbo nucifera*, *Portulaca quadrifolia*, *Vitis vinifera*, *Cordia dichotoma* and *Nyctanthes arbortristis* is reported here for the very first time. Overall percent inhibition by active plant extracts against test bacteria is shown in Fig. 1. Sensitivity of test strains was, in decreasing order: *S. aureus* > *S. dysenteriae* > *E. coli* > *S. paratyphi* > *C. albicans* > *B. subtilis*. In the case of test bacteria, the basis for their differences in susceptibility might be due to the differences in the cell wall composition of Gram + ve and Gram − ve bacteria. (Grosvenor et al., 1995). *B. subtilis* was least sensitive compared to other test bacteria, which may be due to their ability to form highly resistant resting stages called endospores. Drug-resistant strains of bacteria and *C. albicans* were found to be sensitive to the tested plant extracts. This has clearly indicated that antibiotic resistance does not interfere with the antimicrobial action of plant extracts and these extracts might have different modes of action on test organisms. Thirteen plants in this study were also screened previously against other test strains (Ahmad et al., 1998; Mehmood et al., 1999) and showed similar results to this study with varying degrees of potency. The difference in potency may be due to the stage of collection of the plant sample, different sensitivity of the test strains and method of extraction (Nimri et al., 1999).

Phytochemical analysis of 40 active extracts demonstrated the presence of common phytoconstituents like phenols (79.5%), epi/gallotannins or condensed salt tannins (77%), glycosides (49%), saponins (38%), flavonoids (28%) and alkaloids (25%). The presence of these compounds was also detected by thin layer chromatography (TLC). TLC analysis also differentiated between monomeric and dimeric forms of flavonoids. Our phytochemical analyses are in agreement with the reports of other workers (Iyengar, 1985; Chopra et al., 1992; Bruneton, 1995; Harborne and Baxter, 1995; Cai and Wu, 1996). To locate the major active constituents responsible for antimicrobial activity against the most sensitive test strains (*S. aureus*), TLC-bioautography was performed against six highly active plant extracts (Amla, Anar, Behera, Eucalyptus, Harir and Hena). In

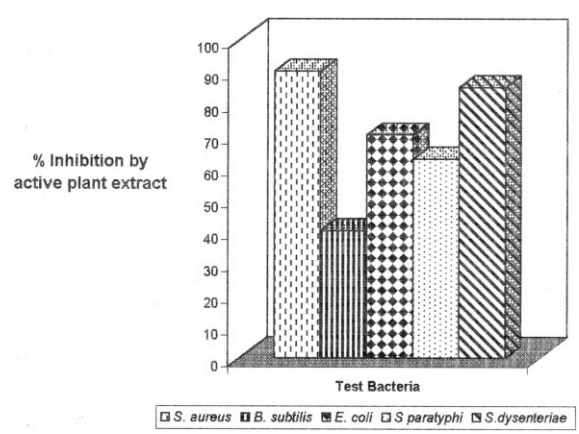

Fig. 1. Antibacterial spectrum of plant extracts.

Table 2
Antimicrobial and phytochemical properties of alcoholic extract of medicinal plants[a]

Plant name; family	Common name	Part used	Phytocompounds						Antimicrobial activity					
			A	F	G	P	T	S	SA	BS	EC	SP	SD	CA
1. *Acorus calamus* L.; Araceae	Bach/Vaj	Rhizome	−	+	−	−	−	+	1+	−	1+	−	3+	2+
2. *Allium cepa* L.; Liliaceae	Piyaz	Leaves	−	−	−	+	+	−	−	−	3+	−	−	−
3. *Allium sativum* L.; Liliaceae	Lasan	Leaves	−	−	−	+	+	−	−	−	−	2+	2+	2+
4. *A. sativum* L.; Liliaceae	Lasan	Bulb	+	+	−	−	−	−	2+	−	2+	−	2+	2+
5. *Azadirachta indica* A. Juss.; Meliaceae	Neem	Bark	−	−	−	+	+	+	2+	−	2+	−	−	−
6. *Beta vulgaris* L.; Chenopodiaceae	Chokundar	Root	−	−	+	−	−	−	2+	−	2+	−	−	−
7. *Calotropis procera* L.; Asclepiadaceae	Madar	Leaves	NT	NT	NT	NT	NT	NT	−	−	−	−	−	−
8. *Camelia sinensis* L.; Theaceae	Chai	Leaves	−	−	+	+	+	+	3+	2+	3+	2+	2+	−
9. *Casuarina equistifolia* L.; Casuarinaceae	Jangli saru	Leaves	+	−	+	+	+	−	3+	2+	3+	3+	2+	2+
10. *C. equistifolia* L.; Casuarinaceae	Jangli saru	Bark	+	−	+	+	+	+	2+	2+	2+	3+	3+	3+
11. *Citrus sinensis* L.; Rutaceae	Musambi	Rind	−	−	−	+	+	−	2+	−	2+	3+	2+	3+
12. *Cordia dichotama* L.; Boraginaceae	Lasora	Leaves	−	−	+	+	+	−	3+	−	−	−	2+	−
13. *Emblica officinalis* Gaerth.; Euphorbiaceae	Amla	Fruits	−	+	+	+	+	+	4+	2+	−	4+	2+	2+
14. *Eucalyptus* sp.; Myrtaceae	Eucalyptus	Leaves	+	−	−	+	+	−	3+	2+	2+	3+	3+	2+
15. *Ficus carica* L.; Moraceae	Anjir	Leaves	−	−	+	−	−	+	−	−	−	−	2+	−
16. *Ficus religiosa* L.; Moraceae	Pipal	Leaves	−	−	+	+	+	−	2+	−	2+	2+	2+	2+
17. *Hemidesmus indicus* R. Br.; Asclepiadaceae	Anantamul	Root	+	−	−	+	+	−	2+	2+	2+	3+	4+	5+
18. *Holarrhena antidysenterica* R.; Apocyanaceae	Kurachi	Bark	+	+	−	+	+	−	2+	2+	2+	3+	2+	2+
19. *Lantana camara* L.; Verbenaceae	Ghaneri	Leaves	−	−	−	+	+	+	2+	2+	−	3+	2+	−
20. *Lawsonia inermis* L.; Lyrthaceae	Mehdi	Leaves	+	−	+	+	+	+	4+	2+	2+	4+	2+	4+
21. *Morus albus* L.; Moraceae	Shahtut	Leaves	−	−	+	−	−	−	2+	−	−	2+	3+	−
22. *Musa paradisiaca* L.; Musaceae	Kala	Stem	NT	NT	NT	NT	NT	NT	−	−	−	−	−	−
23. *Nelumbo nucifera* Gaerth.; Nymphaceae	Kamal	Flowers	−	+	−	+	+	−	3+	2+	1+	−	2+	2+
24. *Nerium indicum* Mill.; Apocyanaceae	Kaner	Leaves	NT	NT	NT	NT	NT	NT	−	−	−	−	−	−
25. *Nigella sativa* L.; Ranunculaceae	Kalongi	Seeds	−	−	−	+	+	+	3+	−	−	2+	2+	−
26. *Nyctanthes arbortristis* L.; Oleaceae	Harsingar	Leaves	−	−	−	+	+	−	2+	−	−	2+	−	−
27. *Ocimum sanctum* L.; Labiatae	Tulsi	Whole plant	−	−	+	+	+	−	2+	−	1+	−	4+	3+
28. *Plumbago zeylanica* L.; Plumbaginaceae	Chita	Root	+	+	−	−	−	−	2+	−	2+	3+	2+	3+
29. *Portulaca quadrifolia* L.; Portulacaceae	Chota luniya	Stem/leaves	−	−	−	−	−	−	−	−	−	−	2+	−
30. *Psidium guajava* L.; Myrtaceae	Amrud	Leaves	−	−	+	+	+	+	2+	−	−	−	−	−
31. *Punica granatum* L.; Punicaceae	Anar	Rind	+	+	+	+	+	−	4+	2+	2+	4+	4+	3+
32. *Raphanus sativus* L.; Brassicaceae	Mouli	Roots	NT	NT	NT	NT	NT	NT	−	−	−	−	−	−
33. *Sapindus* sp.; Sapindaceae	Ritha	Fruits	−	+	−	−	−	+	2+	2+	−	−	−	2+
34. *Saussurea lappa* C.B. Clarke; Compositae	Kuth	Roots	+	+	+	+	+	−	2+	−	3+	3+	4+	3+
35. *Syzgium aromaticum* L.; Myrtaceae	Laung	Bud	−	+	+	+	+	−	3+	2+	3+	3+	4+	5+
36. *S. aromaticum* L.; Myrtaceae	Laung	Oil	−	+	−	+	−	−	2+	3+	3+	5+	3+	5+

Table 2 (*Continued*)

Plant name; family	Common name	Part used	Phytocompounds						Antimicrobial activity					
			A	F	G	P	T	S	SA	BS	EC	SP	SD	CA
37. *Syzgium cumini* L.; Myrtaceae	Jamun	Bark	−	−	−	+	+	−	1+	−	1+	1+	3+	−
38. *S. cumini* L.; Myrtaceae	Jamun	Leaves	−	−	−	+	+	+	2+	−	1+	1+	4+	−
39. *Terminalia arjuna* W. & A.; Combretaceae	Arjun	Bark	−	−	−	+	+	−	3+	−	2+	3+	2+	−
40. *Terminalia belerica* Roxb.; Combretaceae	Bahera	Fruits	−	+	+	+	+	+	3+	2+	2+	3+	2+	2+
41. *Terminalia chebula* Retz.; Combretaceae	Harir	Fruits	−	−	+	+	+	+	3+	2+	2+	3+	2+	2+
42. *Vitis vinifera* L.; Vitaceae	Angur	Leaves	−	−	+	+	+	−	3+	−	2+	−	2+	−
43. *Wattaka volubilis* L.; Aslepiadaceae	Madu malati	Leaves	NT	NT	NT	NT	NT	NT	−	−	−	−	−	−
44. *Zizyphus jujuba* L.; Rhamnaceae	Ber	Leaves	−	−	−	+	+	−	2+	−	2+	2+	3+	2+
45. *Z. jujuba* L.; Rhamnaceae	Ber	Bark	−	−	+	+	+	−	2+	2+	−	3+	−	2+
Total number of active plants			10	12	19	32	31	14	36	17	29	27	33	24

[a] Phytocompound key: A, alkaloid; F, flavonoid; G, glycoside; P, phenol; T, tannin; S, saponin; NT, not tested. Activity key: −, no inhibition; 1+, <10 mm zone of inhibition; 2+, 10–20 mm; 3+, 21–30 mm; 4+, 31–40 mm; 5+, 41–65 mm. Organism key: SA, *S. aureus*; BS, *B. subtilis*; EC, *E. coli*; SP, *S. paratyphi*; SD, *S. dysenteriae*; CA, *C. albicans*.

the majority of the plants tested by TLC and TLC bioautography, phenols and tannins were observed as the most common active constituents. These findings correlate with the observations of Tanaka et al. (1991) and Silva et al. (1996). Monomeric flavonoids were detected as the active constituent in *E. officinalis*. It is expected that more active compounds might have been detected by TLC bioautography if different solvent systems, microbial strains and more plant extracts were used. Thus our antimicrobial screening results also justify the traditional uses of these plants in various ailments including infectious diseases. Further, the active phytocompounds of these plants against multi drug-resistant bacteria and *C. albicans* has to be characterized and the efficacy of non-toxic extracts/preparations has to be evaluated in vivo. Study of the synergistic interaction of active phytocompounds with antibiotics is required to exploit these potential plant extracts in the combination therapy of infectious diseases caused by multi drug-resistant organisms.

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