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SCREENING OF HIGHER PLANTS FOR ANTIBACTERIAL ACTIVITY

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The crude alcoholic extracts of 65 plants species belonging to 34 familes, which were selected on the basis of literature and medicinal folklore reports, were tested for antibacterial activity against 14 different pathogenic bacteria. A total number of 88 plant extracts were evaluated. 26% of the total extracts exhibited broad spectrum antibiosis, while 21% were totally inactive. The rest showed some level of bacteriostatic activity against few specific microorganisms.

Key words: Screening; Plant extracts; Antibacterial activity.

INTRODCUTION

The antibiotics are widely used for the treatment of infectious diseases in modern medicine throughout the world. During the last few decades an intensive effort has been made to discover new and clinically useful antibiotics. This is beacuse of the fact that certain diseases remain serious problems and some major antibiotics have considerable drawbacks in terms of limited antimicrobial spectrum or serious side effects. Today the need of developing safe and effective antibiotics is well recognized.

Previously the search for new antimicrobial agents was mainly focussed on microorganisms and as a results a number of antibiotics having microbial origin were obtained. However, with the development of resistant mutants, it was felt necessary to find out new compounds from the sources other than the traditional microorganisms. It has been revealed from the literature that higher plants could be another potential source of new antimicrobial agents. A number of surveys have been conducted by various research groups [1-11] for the screening of plants having antimicrobial activity and some are successful is isolating and characterizing the responsible compounds [12-20].

Considering the world-wide interest in searching new antimicrobial agents from higher plants, an attempt was made to screen out the flora of Pakistan for antimicrobial activity. From the previous screening reports, [21-24] it is evident that many of the plants growing wildly in this region have significant biological activities. Present communication is an addition to the previous reports.

MATERIALS AND METHODS

Plant material. The plants included in the present study were (a) rhizomes, fruits and seeds of edible plants (b) those having reputed medicinal value and (c) wild plants growing abundently in this region. Where possible plants, either wild or cultivated, were collected fresh from Karachi and its suburbs in their flowering and fruiting season, others were purchased from the local market in dried condition.

Preparation of crude extracts. Different parts of the fresh/dried plants were chopped separately and soaked in 90% Ethanol. The crude extracts were obtained by percolation method, first after 48 hours and then thrice after every 24 hours. The pooled extracts were concentrated under reduced pressure.

Test organisms. All the organisms used in the present study were clinical isolates and were obtained from the Department of Microbiology, University of Karachi, Karachi. They are listed in Table 1.

Antimicrobial testing. The hole-plate diffusion method was used for testing the crude plant extracts against the test organisms. The test organisms were maintained on nutrients agar slants. The 24 hours broth cultures were prepared by inoculating the organisms into 5 ml sterile nutrient broth. 25 ml of sterile liquid nutrient agar was poured into sterilized petri dishes of 8.5 cm diameter. The agar was left to solidify at room temperature. The agar surface was then swabbed with the 24 hours broth culture of the test organisms. Cavities were made in the centre of the petri plates with a sterile cork borer of 1.0 cm diameter and filled aseptically with 0.3 ml of 4% solution of the palnt extracts in 90% ethanol. In the control plates 0.3 ml of 90% ethanol was filled in the cavities. Neomycine sulphate obtained from Messrs SEARL Pakistan Ltd. in the form of tablets was used as standard. Each tablet contained 540 mg Neomycine. The tablets were ground to fine powder. As the antibiotic was insoluble in alcohol, an aquous solution (1 mg/ml) was prepared, and 0.3 ml of the standard was used for filling the cavities for compa-

Table 1. Organisms used in screening higher plants for antibacterial activity.

S. No.	Organisms	Classification
1.	*Salmonella typhy*	Gram negative
2.	*Salmonella typhy* para A	Gram negative
3.	*Salmonella typhy* para B	Gram negative
4.	*Shigella dysenteriae*	Gram negative
5.	*Shigella flexneri*	Gram negative
6.	*Escherichia coli*	Gram negative
7.	*Klebsiella pneumoniae*	Gram negative
8.	*Streptococcus faecalis*	Gram positive
9.	*Streptococcus pyogenes*	Gram positive
10.	*Vibrio Cholera Inaba*	Gram negative
11.	*Vibrio Cholera Eltor*	Gram negative
12.	*Staphylococcus aureous*	Gram positive
13.	*Diplococcus pneumoniae*	Gram positive
14.	*Corynebacterium diphtheriae*	Gram positive

rison. The plates were incubated at 37°C for 24 hours. Each set of experiment was repeated thrice for confirmation and the average zones of inhibition were noted.

RESULTS

The antibacterial activity of the crude palnt extracts have been summarized in Table 2. A total number of 88 extracts of 65 plant species belonging to 34 families were tested for the antibacterial activity in the present study. Out of 88 extracts 23 were found to have broad spectrum antibacterial activity (26.1 %). 19 extracts were found to be inactive against all of the test organisms (21.4 %), and the rest showed their activity against few specific pathogens.

DISCUSSION

The extracts of medicinal plants have been used in medicine for the treatment of various ailments. Many of them are used for infectious diseases in traditional medicine

Table 2. Results of the antibacterial screening of higher plants.

S. No.	Plants**	Family	Parts tested	Test organisms* 1	2	3	4	5	6	7	8	9	10	11	12	13	14
1.	*Adenanthera pavonia* L. (Barigumchi)	Mimosoideae	Leaves	–	–	–	–	–	–	–	–	–	-	–	–	–	–
			Seeds	–	–	–	–	–	–	–	–	–	–	–	–	–	–
			Bark	B	B	C	C	B	B	B	B	C	B	B	C	B	C
2.	*Agave americana* L. (Jungli Kanwar)	Amaryllidaceae	Leaves	C	–	–	B	–	C	C	–	–	C	–	C	C	C
3.	*Allium cepa* L. (Onion, piaz)	Lilliaceae	Bulb	C	–	–	C	–	–	C	–	C	–	–	C	–	–
4.	*Alysicarpus monilifer* L.	Papilionoideae	Shoot	–	–	–	–	–	–	–	–	–	–	–	–	–	–
5.	*Amberboa ramosa* Roxb.	Compositae	Shoot	B	B	B	B	B	B	–	B	B	C	B	B	B	C
6.	*Annona squamosa* L.	Annonaceae	Leaves	C	B	B	C	B	C	C	C	B	A	C	C	C	B
	(Sharifa)		Bark	A	C	B	C	C	B	–	–	B	A	B	C	B	B
			Root	B	B	C	B	B	C	C	C	B	B	B	C	B	C
7.	*Arachis hypogaea* L. (Ground Nut, Moongphali)	Papilionoideae	Shoot	–	C	C	B	–	–	–	–	–	–	–	–	–	C
8.	*Aristida hystricula* Edg.	Graminae	Shoot	–	–	–	–	–	–	–	–	C	–	–	A	–	–
9.	*Arnebia hispidissima* DC.	Boraginaceae	Shoot	–	–	A	–	–	–	C	–	–	–	A	–	B	B
10.	*Calendula officinalis* L.	Compositae	Shoot	B	B	B	C	B	C	B	C	C	C	B	–	B	–
	(Zergul, Marigold)		Roots	–	–	–	–	–	–	–	–	–	–	–	–	–	–
11.	*Canna indica* L. (Hakik, Sabbajaya)	Cannaceae	Shoot	B	B	B	B	–	C	–	B	–	–	–	C	–	B
12.	*Carica papaya* L.	Caricaceae	Leaves	B	B	B	–	–	B	–	–	–	–	–	B	–	–
13.	*Cicer arietinum* L. (Gram, Chana)	Papilionoideae	Seedling	–	–	–	–	–	–	–	–	–	–	–	–	–	–

(continued.....)

(Table 2 continued. . . . .)

14. *Cinnamomum zeylanticum* (Cinnamon, Darchini)	Lauraceae	Bark	B	B	B	B	B	B	B	B	C	B	A	B	B	B
15. *Citrus limon* Linn. (Lemon)	Rutaceae	Leaves	B	–	A	–	A	B	C	–	B	–	–	C	–	–
16. *Clerodendron odoratum* (Hiranpaddi)	Verbenaceae	Shoot	C	C	C	B	C	C	–	–	B	C	C	C	C	C
17. *Convolvulus arrensis* L. (Hiranpaddi)	Convolvulaceae	Shoot	B	B	B	B	B	B	B	A	B	B	B	C	–	A
18. *Coriandrum sativum* L. (Coriander, Dhania)	Umbelliferae	Leaves	C	–	–	B	–	C	C	C	–	–	–	–	–	C
19. *Curcuma longa* Linn. (Turmeric, haldi)	Zingiberaceae	Rhizome	–	–	B	–	C	–	–	B	–	–	–	–	–	–
20. *Curcuma zedoaria* Rosc. (Jadwer Khatta)	Zingiberaceae	Rhizome	C	C	C	B	–	B	–	C	C	B	–	C	–	C
21. *Daucus carota* Linn. (Carrot, gajar)	Umbelliferae	Leaves	–	–	–	C	–	–	–	–	–	–	–	–	–	–
22. *Dipteracanthus patulus*	Acanthaceae	Shoot	–	B	C	–	C	–	C	A	C	B	C	B	B	B
23. *Emblica officinalis* (Amla)	Euphorbiaceae	Fruit (dried)	B	–	B	B	–	B	B	A	–	–	–	–	–	B
24. *Euphorbia pulcherrima*	Euphorbiaceae	Shoot	–	–	–	C	C	C	C	C	C	C	–	C	C	C
25. *Ficus bengalensis* L. (Bargad, Ber)	Moraceae	Leaves	–	–	–	–	–	C	–	C	–	B	–	B	B	C
		Fruit (Fresh)	C	C	C	–	–	–	–	–	–	–	–	–	C	–
		Roots (Aerial)	C	C	B	C	C	B	–	C	B	C	C	C	C	–
26. *Ficus carica* L. (Fig, Anjir)	Moraceae	Leaves	–	–	–	–	–	–	–	–	–	–	–	–	–	–
27. *Glycyrrhiza glabra* L. (Liqourice, Mulhatti)	Papilionoideae	Root	A	B	A	B	A	B	A	B	B	B	A	A	B	B
28. *Hedychium spicatum* (Kachri)	Zinigiberaceae	Fruit (dried)	B	B	C	C	C	–	–	A	A	B	–	–	–	B
29. *Helianthus annus* L. (Surajmukhi, sunflower)	Compositae	Shoot	–	–	–	–	–	–	–	–	–	–	–	–	–	–
30. *Heliotropium brevifolium* (Tindu)	Boraginaceae	Root	B	B	C	B	B	C	C	B	C	B	B	B	B	B
		Shoot	–	–	–	C	B	B	–	B	–	B	A	B	C	B
31. *Hyoscymus niger* L. (Ajowan, Henbane)	Solanaceae	Seeds	C	–	–	C	–	C	C	–	–	–	–	C	–	C
32. *Lawsonia inermis* L. (Hena, Mehndi)	Lythraceae	Leaves	C	–	C	C	–	–	C	–	–	–	–	B	–	–
		Fruit (dried)	B	C	–	C	–	C	–	C	–	–	–	–	–	–
33. *Lycopersicon esculentum* (Tomato)	Solanaceae	Twigs	C	B	C	B	C	C	B	–	B	C	B	B	–	–
34. *Mangifera indica* L. (Mango, Aam)	Anacardiaceae	Bark	–	B	B	–	B	–	–	–	–	–	B	–	–	B
		Leaves	C	–	C	–	B	–	–	–	–	–	B	–	–	–
35. *Matricaria chamomilla* L. (Babuna, chamomile)	Composite	Shoot	C	C	C	C	C	C	C	C	C	C	C	–	C	C
36. *Morous acedosa Griff.* (Tut)	Moraceae	Leaves	–	–	–	–	–	–	–	–	–	–	–	–	–	–

(continued . . .)

(Table 2 continued. . . .)

		Brak	C	C	C	B	C	B	B	B	–	C	B	B	B	B
		Fruit	C	C	B	B	C	–	B	–	–	C	B	B	B	–
37. *Nerium indicum* Mill.	Apocynaceae	Twigs	C	–	–	–	–	C	–	–	B	B	–	B	–	C
		Root	C	–	–	–	–	–	–	–	–	–	–	B	–	–
		Leaves	–	B	–	–	–	–	–	–	–	B	–	–	C	–
38. *Nigella sativa* Linn. (kalongi)	Ranunculaceae	Seeds	C	–	–	B	–	–	–	–	–	–	–	B	–	–
39. *Peganum harmala* L.	Rutaceae	Seeds	–	B	B	A	B	B	B	C	B	B	B	B	B	–
(Isband)		Shoot	B	–	–	C	B	C	–	B	A	B	B	B	C	–
40. *Phoseolus tribobus* L. (Mugani, Mukuya)	Papilionoideae	Shoot	B	–	C	B	C	C	B	B	C	C	C	–	C	C
41. *Phyllanthus niruri* L. (Jar, Amla)	Euphorbiaceae	Shoot	B	C	B	B	B	C	B	C	C	B	B	–	C	C
42. *Pinus longifolia* Roxb.	Pinaceae	Bark	B	–	–	B	B	B	C	–	C	B	C	C	C	–
(Pine tree, Chir)		Fruit	–	–	–	B	C	B	–	C	–	B	–	C	–	–
		Flower	–	–	–	–	B	B	–	–	–	B	B	B	C	–
43. *Piper cubeba* L. (Kabab-chini)	Piperaceae	Fruit	–	B	B	–	B	–	B	B	B	B	B	B	B	–
44. *Piper longum* L. (Clove)	Piperaceae	Flower	B	–	B	B	–	–	C	C	–	–	–	–	–	B
45. *Piper nigrum* L. (Black pepper)	Piperaceae	Fruit	–	–	C	–	–	–	–	C	–	–	–	–	–	–
46. *Pithecellobium dulce*	Mimosoideae	Shoot	B	C	–	C	C	B	B	–	C	C	–	C	A	C
(Dakhnibabul)		Bark	–	A	B	B	B	B	B	B	B	B	B	B	B	B
47. *Prunus amygdalus* (Badim Talkh)	Rosaceae	Seedling	–	–	–	–	–	–	–	–	–	–	–	–	–	–
48. *Psoralea corylifolia* L. (Babchi)	Papilionoidae	Shoot	C	C	–	–	C	–	B	B	B	B	–	B	–	B
49. *Quisqualis indica* Linn. (Rangoon Creeper)	Combretaceae	Shoot	–	–	–	C	–	B	B	–	B	–	–	C	–	–
50. *Rhazya stricta* Dcne (Sewar)	Apocynaceae	Shoot	B	B	C	B	B	B	C	–	B	B	C	B	B	C
51. *Rhyncosia minima* DC. (Nahanikamalavel)	Papilionoideae	Shoot	B	–	C	C	C	–	–	C	–	C	–	–	–	–
52. *Ricinus communis* L.	Euphorbiaceae	Seedling	B	B	B	B	–	–	–	C	–	–	–	B	–	–
(Castor, arand)		Leaves	–	–	–	–	–	–	–	–	–	–	–	–	–	–
53. *Senra incana* Cav.	Malvaceae	Shoot	–	–	C	–	C	C	–	C	–	–	–	B	C	–
54. *Solanum incanum* Linn.	Solanaceae	Leaves	–	–	–	–	–	–	–	–	–	–	–	–	–	–
55. *Sonchus asper* L.	Compositae	Shoot	–	–	–	–	–	–	–	–	–	–	–	–	–	–
56. *Sonchus oleraceus* L.	Compositae	Shoot	–	–	–	–	–	–	–	–	–	–	–	–	–	–
(Dodak)		Root	–	–	–	–	–	–	–	–	–	–	–	–	–	–
57. *Spinacia oleracea* L. (Spinach, Palak)	Chenopodiaceae	Leaves	B	C	B	B	B	C	C	B	B	C	C	B	B	B
58. *Syzygium cumini* L.	Myrtaceae	Leaves	B	–	B	A	B	B	C	C	B	B	C	B	B	B
(Java Plum, Jamun)		Seedling	B	–	B	A	–	–	–	B	A	B	A	A	–	B
59. *Tamarix gallica* Linn. (Jhau)	Tamaricaceae	Shoot	B	C	B	B	B	C	B	B	B	C	B	B	B	B
60. *Tecoma undulata* (Rohira)	Bignoniaceae	Shoot	–	–	–	–	–	–	–	–	–	–	–	–	–	–

(Continued . . .)

(Table 2 continued. . . .)

61. *Tephrosia uniflora* Pers.	Papilionoideae	Shoot	–	C	C	B	–	C	C	C	C	–	C	C	C	–
62. *Thespesia populnea* L. (Paraspipal)	Malvaceae	Shoot	–	–	–	–	–	–	–	–	–	–	–	–	–	–
		Bark	–	–	–	–	–	–	–	–	–	–	–	–	–	–
		Fruit (dried)	B	C	C	B	B	B	B	B	B	C	B	C	B	B
63. *Verbena officinalis* L. (Faristariun)	Verbenaceae	Shoot	C	C	C	B	–	–	–	–	–	–	–	–	–	–
64. *Withania coagulans* Dunal. (Akri, Kaknaj)	Solanaceae	Shoot	–	–	–	–	–	–	–	–	–	–	–	–	–	–
65. *Ziziphus Jujiuba* Lamk. (Ber)	Rhamnaceae	Shoot	B	–	–	C	A	–	–	B	–	B	A	C	C	–

*No. 1-14, are the serial numbers of test organisms as represented in Table 1.

**The common name of the plant is indicated in parenthesis.

Zones of inhibition with average diameter 8.5-5.0, 4.9-3.0 and 2.9-1.5 cm are denoted by the letter A, B and C. Neomycine sulphate (1 mg/ml) produces B type of inhibition zones.

although their efficacy has not been scientifically proved. A number of such plants have been tested for their antibacterial activity in the present study. *Ficus bengalensis* is reported to be antidysentric, its therapeutic property was confirmed, during the present study, by the positive antibacterial activity of its aerial roots against intestinal pathogens. *Peganum harmala* which is used as lactagogue, protozoacidal, coronary dialator, and in the treatment of posterior encephalitis showed broad spectrum antibacterial properties. *Thespesia populnea* is commonly prescribed in Unani medicine for external use in cutaneous diseases such as scabies, psoriasis and as stringent. The dried furits of this plant showed bacteriostatic activity against all the test organisms. In *Adenanthera pavonia* and *Thespesia populnea* the whole broad spectrum antibiotic activity is concentrated in the bark and fruit respectively, while the leaves and seeds of the former and shoot and bark of the latter are completely inactive. The broad spectrum antibacterial activity was also observed in the leaves, bark and root of *Annona squamosa* but its seeds were reported [26] to have no appreciable antibacterial effect. The assessed antibacterial properties could be well utilized in case of those plants, which are already being used as therapeutic agents againt various diseases and have no toxic effects in the prescribed doses.

Allium cepa, Cinnamomum zeylanicum, Coriandrum sativum, Curcuma longa, Piper cubeba, Piper longum and *Piper nigrum* are used as spices and condiments throughout the world. Among the above mentioned plant species *Cinnamomum zeylanicum* was found to have broad spectrum antibacterial activity, as it formed mostly B and A catagory zones of inhibition against all the test organisms. *Piper cubeba* was also active against many gram +ve and –ve bacteria, while *Coriandrum sativum* leaves and *Piper longum* were mostly active against gram –ve organisms. *Spinacia oleraceae* which is commonly used as green vegetable also showed broad Spectrum antibacterial activity.

Embilica officinalis fruits used for diarrhoea, dysentary and various other purposes showed activity against intestinal pathogenic bacteria which confirm the results of Khorana *et. al.* [25]. *Tamarix gallica* recommended for sore throat, diarrhoea and dysentary in traditional medicine showed broad spectrum antibacterial activity which is also in accordance with the results of Naqvi *et. al.* [24]. There are, however, a few conflicting reports [9, 24] in some plant species. The leaves of *Agava americana,* which showed C type of activity against a number of test organisms in the present study, were reported to be completely inactive by Naqvi *et. al.* [24] but even *et. al.* [9] reported the same plant active against *E. coli, Pseudomonas aeroginosa* and *Proteus vulgaris. Carica papaya* leaves were active against *S. aureus, E. coli* and *Salmonella species* in our case. Its activity was only confirmed for *S. aureus,* while negative results were reported for *E. coli* and *Salmonella species* by other workers [24, 9]. We did not find any activity in the case of *Helianthus annus* L. but its leaves were reported to be active and the flowers inactive by Naqvi *et. al.* [24]. These discrepencies are expected, as chemical constituents of plants vary with environmental conditions, like temperature, altitude, soil conditions, time of collection etc.

It is interesting to note that many plants which are cultivated only for edible purpose showed therapeutic property in various parts, as for example, leaves, bark and root of *Annona squamosa,* leaves, bark and fruit of *Morus acedosa,* leaves of *Carica papaya, Citrus lemon, Syzygium cumini, Licopersicon esculentum, Ziziphus jujuba,* and

bark of *Magnifera indica.* All of the above mentioned plants showed clear zones of inhibition against most of the organisms tested.

The most promising plants are those which exhibited a broad spectrum activity. This suggest that the active principle is present either in good potency or in high concentration. From the present screening report a number of plants have been identified for isolation, purification and characterization of the responsible active agent, a few interesting ones are under study, the results of which would be communicated separately.

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