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SCREENING OF PAKISTANI PLANTS FOR ANTIBACTERIAL ACTIVITY

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The present study involves the screening of 100 plant materials obtained from 64 important indigenous herbs of Pakistan using 50% ethanolic extracts. Eighteen different species of bacteria mostly involved in common infections of humans were used for the determination of antibacterial activity. Out of 100 plants material 29 showed broad spectrum antibacterial activity against Gram-negative and Gram-positive bacteria. 31 plants extract exhibited short spectrum activity against either Gram-negative and Gram-positive bacteria.

INTRODUCTION

The importance of plant material in the present-day therapy cannot be understimated since such useful compounds as digitonins, rutine, papain, morphine, codine, centonine and many other exhibit a broad range antibacterial and pharmacological activity. Studies on phytochemical, pharmacological and antibacterial activity of plant extracts present a unique challenge to strike new sources of medication. The object of present project is to screen out important indigenous medicinal plants of Pakistan which are commonly used in eastern medicine (Unani, Ayurvedic and folk meicines) to determine their antibacterial activity. This type of work has not been reported before from this part of the world.

Numerous surveys [1-16] had been conducted to determine the antibacterial activity of different species belonging to various plant families and genera against various pathogenic and non-pathogenic organisms.

EXPERIMENTAL

Important medicinal plants were collected from different parts of the country. Each specimen was properly identified. Voucher specimens were kept. Different parts of the plants were separated, washed and dried. The dried material was powdered mechanically and was extracted in 500 g. lots by three cold percolations with 50% ethyl alcohol. The extracts were combined and concentrated under reduced pressure below 40°. The concentrated extract was dissolved in distilled water (5 mg/ml) and aliquots were used to test antibacterial activity.

Antibacterial activity was tested against eighteen different species of Gram-negative and Gram-positive bacteria (Table 1). Seed cultures were prepared in trypticase soy broth which was incubated at $37^{\circ} \pm 1^{\circ}$ for 24 hr. A dilution

Table 1. Gram Positive and Gram Negative bacteria used in the study

Gram Positive		
A.	Aerobic spore formers	
1.	*Bacillus*	*subtilis*
2.	*Bacillus*	*megaterium*
B.	Micrococci	
1.	*Staphylococcus*	*aureus*
2.	*Staphylococcus*	*citreus*
3.	*Staphylococcus*	*albus*
4.	*Micrococcus*	*lysodeikticus*
5.	*Sarcina*	*lutea*
C.	Streptococci	
1.	*Streptococcus*	*faecalis*
2.	*Streptococcus*	*pyogenes*
Gram Negative		
1.	*Escherichia*	*coli*
2.	*Proteus*	*vulgaris*
3.	*Pseudomonas*	*aeruginosa*
4.	*Salmonella*	*typhi*
5.	*Salmonella*	*typhi para A*
6.	*Salmonella*	*typhi para B*
7.	*Shigella*	*shigi*
8.	*Shigella*	*flexneri*
9.	*Shigella*	*sonnei*

of 1:100 in saline was used in all tests.

ASSAY

The tests were run in triplicate. Petri plates (10 x 10 cm) were prepared with trypticase soy agar. 0.1 ml of dried culture was used as inoculum. Wells of 6 mm dia. were filled with a 50% ethanolic extract. The control was run with 50% ethanol in water. The results were checked after 24 hr. at 37° and inhibition zones were measured to nearest milli meter. (Plate 1 and 2).

RESULTS

The results of the antibacterial activity of crude ethanolic extracts of different species have been summarized in Table 2. The plants have been listed in alphabetical order according to families. Twenty-nine plants material out of hundred showed broad spectrum antibacterial activity against Gram-negative and Gram-positive bacteria. Thirty-one exhibited short spectrum activity either against Gram-negative or Gram-positive bacteria (plates 1 and 2).

DISCUSSION

Herbs have been a source of medicinal compounds since times immemorial. The history of the use of herbal medicine in treatment of diseases can be identified with the history of medicine. Plant extracts are used in the Ayurvedic, Tibbi, and allophathic systems of medicines for the treatment of number of human ailments such as wound infections, typhoid, dysentery, leprosy, erysipelas, foul ulcers, cough, cold, syphilis, pustular eruptions (specially those due small pox), jaundice, prurigo, boils, tetanus, urinary tract infections, piles and a number of skin diseases. They are also used for the treatment of viral and fungal diseases. However, studies with reference to their specific antibacterial activity had been done to a negligible extent only.

The results obtained with *Allium sativum* (garlic) extract confirm the results of Lehman [17] against *E. coli* and *Staph aureus* and Chester and John, [18], who isolated a substance allicin which shows antibacterial activity against Gram-negative and Gram-positive species. The present studies also confirm the results of Datta *et al.* [19], Lucas and Lawis [20] and Fletcher *et al.* [21].

Arnebia nobilis (ratanjot) is reported to possess strong antibacterial component in alcoholic extract. The results of Patel and Patel [22] are also confirmed by the present work. The present studies are in confirmity with the findings of Dhar *et al* [10] in the case of *Cassia auriculata, C. fistula,* and *C. occidantalis.* In the case of *Adhatoda vasica* Dhar *et al.* have reported activity against *B. subtilis* and *Staph. aureus,* whereas the present study indicates that the crude extract of the plant does not have any such activity. Furthermore in the case of *Cassia alata, C. obvata, C. reticulata, C. surattensis* and *C. augustifolia,* activity has

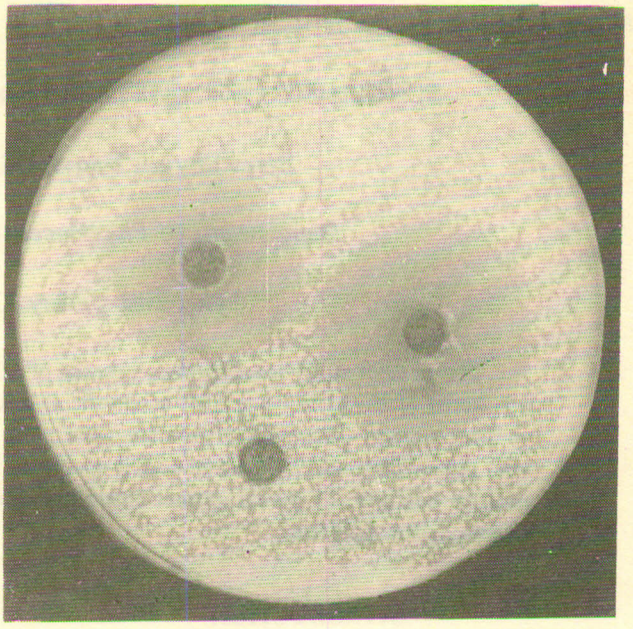

Plate 1. Inhibition caused by, the crude extract of *S. indicus* against *Staphylococcus aureus.*

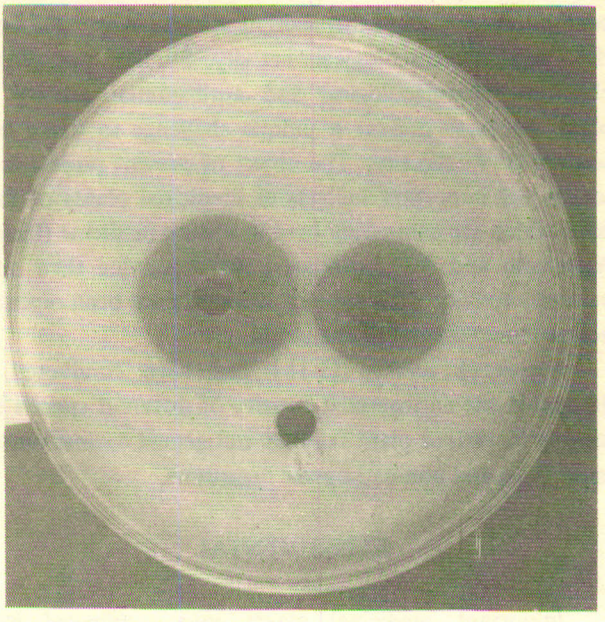

Plate 2. Inhibition caused by the crude estract of *S. indicus* against *Escherichia coli.*

been found against gram-positive bacteria, whereas Dhar *et al.* [10] could not find any such activity in these species of plants. These discrepencies of results are not unexpected as phytoconstituents are known to vary with ecological factors like the time of collection, climate and habitat [23, 10].

Table 2. Summary of results of antimicrobial screening

				Antibacterial activity against																		
Family	Common name	Locality	Part used	*B. subtilis*	*B. megaterium*	*Sarcina ultea*	*Micro. lysodeikticus*	*Staph. citreus*	*Staph. aureus*	*Staph. albus*	*Strept. pyogenes*	*Strept. faecalis*	*Sal. typhi*	*Sal. typhi P.A.*	*Sal. typhi P.B.*	*Shigella shigi*	*Shigella sonnei*	*Shigella flex*	*E. coli*	*Proteus vulgaris*	*Pseudo. aeruginosa*
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
Acanthacease *Adhatoda vasica*	*Adosa*	K.D.A.** Extension	LF	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Amaranthaceae *Celosia argenta*	*Sarpanka*	Peshawar	F1	–	–	–	–	–	–	–	–	–	5	5	5	8	8	8	5	4	–
			SD	4	4	5	5	4	4	4	4	4	6	6	6	5	6	5	5	2	–
Amaryllieaceae *Agave americana*	*Banskeora*	Peshawar	LF	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
			RT	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Anacardiaceae *Anacardium occidentable*	*Badam*	Local* market	FR	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Apocynaceae Alstatia *scholaris*	*Karabht*	Peshawar	LF	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
			BK	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Holarrhena antidysenterica	*Kurchi*	Peshawar	BK	5	4	6	6	5	5	5	-	-	5	5	5	8	8	8	5	4	-
Rauwolfia serpentina	*Chota chanda*	Local** market	LF	4	4	5	5	5	4	4	-	-	5	5	5	6	6	6	5	4	-
Aracaceae *Acorus calamus*	*Batch*	Peshawar	ST	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
			LF	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Asclepiadaceae *Calotropis gigantea*	*Madar*	University** campus	LF	–	–	–	–	–	–	–	–	–	–	–	–	–	–		–	–	–
			FL	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Bixaceae *hydnocarpus wightiona*	*Chal-moogra*	Local** market	LF	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
			FR	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Boraginaceae *Cordria myxa*	*Sipistan*	Local** market	FR	4	4	4	4	5	5	5	4	2	-	-	-	-	-	-	-	-	-
Heliotropium indicum	*Hatisura*	Local** market	LF	5	5	8	8	6	6	6	5	4	–	–	–	–	–	–	–	–	–

Contd.

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
Onosma echioides	Mahara-nges	Local** market	FR	4	4	5	5	6	6	6	4	2	–	–	–	–	–	–	–	–	–
Bursearaceae																					
Balsamo-dendron mukul	*Guggul*	Local** market	LF	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
			FR	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Cactaceae																					
Opuntia dillenii	*Phanior-monsa*	University** campus	ST	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Carbicaceae																					
Carica Papaya	*Papita*	Stadium** Road	LF	10	10	22	30	20	20	20	20	10	–	–	–	–	–	–	–	–	–
			FR	10	10	22	30	20	20	20	20	10	–	–	–	–	–	–	–	–	–
			FR	10	10	23	30	20	20	20	20	10	–	–	–	–	–	–	–	–	–
Capparidaceae																					
Capparis aphylla	*Kerin*	Local** market	LF	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
			FL	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Compositeae																					
Artemisia maritima	*Afsanthin*	Herb** dealer	LF	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
			FL	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Eclipta alba	*Bhangra*	University** campus	FR	4	4	6	6	6	6	6	5	2	–	–	–	–	–	–	–	–	–
Eclipta indica	*Keshwari*	Herb dealer**	FR	5	5	6	6	8	8	8	5	2	–	–	–	–	–	–	–	–	–
Helianthus annus	*Sunflower*	Nazimabad	LF	10	10	15	15	10	10	10	10	10	–	–	–	–	–	–	–	–	–
			FL	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Sphaeranthus indicus	*Mundi*	Local** market	FL	12	12	22	24	15	15	15	15	12	20	20	18	16	15	15	14	14	12
Enhydra fluctuans	*Hingcha*	Local** market	FR	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Combretaceae																					
Terminalia belerica	*Bahera*	Local** market	LF	5	5	6	6	5	5	5	5	4	5	5	6	6	5	5	5	4	–
Terminalia chebula	*Haritaki*	Local** market	FR	5	5	6	6	8	8	8	5	4	5	6	5	6	6	6	5	5	–
Quisqualis indica	*Rangoon*	Bunder Road Karachi	ST	4	4	5	5	5	5	5	5	2	4	4	3	4	4	3	3	3	–
Convolulaceae																					
Cuscuta reflexa	*Akas bail*	Jail Road**	ST	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Ipomoea batatos	Sweet potato	Empress** market	FR	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Cucurbitaceae *citrulus colocynthis*	*Indrayan*	New Karachi	LF	8	8	10	10	8	8	8	4	-	10	8	8	6	6	6	6	5	-
			FL	8	8	10	10	8	8	8	4	–	10	8	8	6	6	6	6	5	–
Citrulus vulgaris	*Tarbuza*	Dumbloti	FR	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Momordica charantia	*Karela*	Sadar Karachi	FR	5	5	6	6	5	5	6	5	2	5	5	6	6	5	5	5	5	–
Lagenaria vulgaris	*Kaddu*	Sadar Karachi	FR	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
			LF	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

Contd.

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
Euphorbiaceae *Euphorbia pilulifera*	*Budhi*	University** campus	FL	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Euphorbia rosea	*Dudiya*	University** campus	LF	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Filicineae *Adianaum capillus verneris*	*Hansraj*	Local** market	LF	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
			FR	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Geraniackeae *Arnebia nobilis*	*Ratanjot*	Local** market	BK	8	8	10	10	10	8	8	8	5	–	6	8	7	5	6	4	8	2
Gentianaceae *Swertia chirata*	*Chirata*	Local** market	LF	5	5	8	8	8	8	8	6	–	–	–	–	–	–	–	–	–	–
Graminae *Andropogon citrulus*	*Lemon grass; izkhar Makki*	Peshawar	LF	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Guttiferae *Mesua ferrea*	*Nagesar*	Peshawar	FL	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Labiatae *Hyssopus officinalis*	*Zupha*	Local market	LF	10	12	12	15	10	10	10	8	6	–	8	8	8	8	10	–	–	–
Mentha piperita	*Jungli pudina*	Sadar* Karachi	LF	–	–	–	–	–	–	–	–	5	5	5	8	8	8	5	–	–	–
Mentha viridis	*Pundina*	Sadar* Karachi	LF	–	–	–	–	–	–	–	–	5	5	5	10	10	10	6	–	–	–
Leucas aspera	*Chotahal kusa*	Local** market	LF	4	4	6	6	5	5	5	–	4	4	4	5	5	5	5	5	–	–
Ocimum dasilicum	*Niyazboo*	University** campus	LF	5	5	6	6	8	8	8	4	–	5	5	5	4	4	4	4	–	–
			FL	5	5	6	6	8	8	8	4	–	5	5	5	4	4	4	2	–	–
Ociumum sanctum	*Tulsi*	University** *campus*	LF	4	4	5	5	5	5	5	4	–	4	4	4	4	4	4	2	–	
Nepeta ruderalis	*Billilotan*	Local** market	FR	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Lauraceae *Cinnomonum camphora*	*Karpur*	Local** market	FR	5	5	8	8	10	10	10	5	–	5	5	5	6	6	6	5	2	–
Litsaea sebifera	*Kurkuchita*	Local** market	BK	5	5	6	6	8	8	8	5	–	5	5	5	8	8	8	5	2	–
Lamarieaceae *Tamarix gallica*	*Jhab Jhar*	Local** market	BK	5	5	6	6	8	8	8	4	–	16	16	16	18	18	18	5	4	–
Leguminosae *Acacia arabica*	*Babul*	Stadium** Road, Karachi	FL	8	8	10	10	8	8	8	5	5	3	4	8	8	8	7	7	7	–
			LF	8	8	10	10	8	8	8	5	5	3	4	8	8	8	7	7	7	–
Acacia catechu	*Katha*	Local** market	Resin		4	4	6	6	5	5	5	–	–	–	–	–	–	–	–	–	–

Contd.

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
Abrus precatoruis	*Rati*	Local market	LF	8	8	10	10	10	12	12	6	–	5	5	5	7	5	5	5	4	–
			Fl	8	8	10	10	12	12	12	6	4	5	6	6	4	6	5	5	4	–
Cassia alata	*Dadmari*	Local** market	LF	10	10	10	10	10	10	10	10	10	–	–	–	–	–	–	–	–	–
			Fl	8	8	8	8	10	9	10	10	10	–	–	–	–	–	–	–	–	–
Cassia fistula	*Amaltas*	Jail Road**	FR	12	14	10	20	12	12	12	12	–	–	–	–	–	–	–	–	–	–
		Road	LF	10	10	10	20	14	12	12	12	–	–	–	–	–	–	–	–	–	–
Cassia occidentalis	*Kalhashunda*	University** campus	LF	8	8	5	8	5	5	5	5	5	–	–	–	–	–	–	–	–	–
			FL	8	8	5	8	8	5	5	5	5	–	–	–	–	–	–	–	–	–
Cassia tora	*Chahunda*	*Malir*, Karachi	FL	10	10	10	8	8	6	–	–	–	–	–	–	–	–	–	–	–	–
			LF	10	10	10	8	8	6	–	–	–	–	–	–	–	–	–	–	–	–
Cassia obvata		University** Road	FL	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
			LF	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Cassia auriculata		University** campus	FL	8	8	8	10	8	8	8	8	–	–	–	–	–	–	–	–	–	–
			LF	10	10	8	10	8	8	8	8	–	–	–	–	–	–	–	–	–	–
Cassia reticulata		University** campus	LF	10	10	15	8	8	8	8	–	–	–	–	–	–	–	–	–	–	–
			FL	10	10	10	8	8	8	8	–	–	–	–	–	–	–	–	–	–	–
Cassia surattenis		*University*** campus	LF	8	8	20	20	20	10	10	10	–	–	–	–	–	–	–	–	–	–
			FL	8	8	20	20	20	10	10	10	–	–	–	–	–	–	–	–	–	–
Cassia angustifolia	*Sana-i Makki*	Local** market	LF	10	10	15	22	15	14	12	12	12	20	20	20	20	15	15	15	10	–
			FL	12	10	15	22	15	12	12	12	20	15	15	15	15	15	15	15	8	–
Caesalpina pulchamin		University** Road	LF	10	10	8	12	8	8	8	8	–	–	–	–	–	–	–	–	–	–
			FL	10	10	8	12	8	8	8	8	–	–	–	–	–	–	–	–	–	–
Glycyrrhiza glabra	*Mulhaiti*	Local** market	FR	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Peltophorum sonburgh		K.D.A.** Extension	LF	3	4	5	4	3	3	4	4	–	–	–	–	–	–	–	–	–	–
			FL	3	3	5	5	3	4	4	4	–	–	–	–	–	–	–	–	–	–
Liliaceae *Alliumcepa*	Onion	Local** market	ST	10	10	20	22	22	22	20	–	–	12	12	12	13	14	15	10	–	–
Allium sativum	Garlic	Local** market	ST	10	10	20	20	15	15	15	15	–	–	14	14	14	12	13	14	10	–
Aloe perfoliata	*Ghikanvar*	Local** market	ST	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Urginea indica	*Jangli*	Local** market	ST	5	5	8	8	8	8	8	8	5	–	6	6	6	5	5	4	–	
Punica granatum	*Anar*	Sadar, Karachi	FR	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Malvaceae *Althaea officinalis*	*Khatmi*	Local** market	FR	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

FL, flower or inflorescence; FR, fruit; LF, leaf; PL, whole plant; RT, root, ST, stem; BK, bark; SD, seed; SK, skin of fruit.

* Cup plat method. Zone of inhibition measured in mm.

** Karachi.

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