

SCREENING OF PAKISTAN PLANTS FOR ANTIBACTERIAL ACTIVITY- II

Baqir Shyum Naqvi, Dilnawaz Shaikh and Rafi Shaikh*

Department of Pharmaceutics, University of Karachi, Karachi-32

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The present study involves the screening of 63 plant materials obtained from 42 indigenous herbs of Pakistan using 50 % ethanol extract. The antibacterial activity was determined against 18 different species of bacteria, commonly associated with human infections. Out of 63 plant materials 18 exhibited broad spectrum antibacterial activity (28.6%) 19 plant extracts showed narrow spectrum activity against either Gram negative or Gram positive bacteria (30 %).

Key words: Antibacterial principles of plants.

INTRODUCTION

The use of plants for the treatment of human ailments dates back to prehistoric times. In the Indo-Pakistan sub-continent, medicinal properties of plants have been studied since very early times [1-2], but they were not based on any scientific ground. Since the middle of 19th century workers focused their attention on the screening of plant material for their possible biological activity [3-28]. Most of these studies were performed on flora of the region of the sub-continent which is to-day's India. This type of work has been conducted in Pakistan on a very limited scale.

The present work is an extension project on the screening of Pakistan plants for antibacterial activity. In a previous study the results of the screening of 100 plant material were presented [29]. In present report studies on antibacterial activity of 63 plant material from 42 plant species found in Pakistan have been presented.

EXPERIMENTAL

Plants were collected from different parts of the country. Each specimen was properly identified and voucher specimens were kept.

Preparation of extracts. Different parts of the plants like roots, stems, flowers and leaves were separated, washed and dried. The air dried plant material was powdered mechanically and extracted with 50 % ethyl alcohol in 500 g. lots by three cold percolations.

The combined extracts were concentrated below 40° under reduced pressure. The dried material thus obtained was dissolved in distilled water (5 mg/ml).

Antibacterial assay. The antibacterial activity was determined against 18 different species of Gram negative and Gram positive bacteria (Table 1)†.

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*

*Department of Microbiology, University of Karachi, Karachi-32, (Pakistan).

†National Culture Collection (Ottawa), Canada.

Seed culture was prepared in trypticase soy broth and seed broths were incubated at $37^{\circ}\text{C} \pm 1^{\circ}\text{C}$ for 24 hr.

For assay, petridishes (10 X 10 cm) were prepared with trypticase soy agar, Bhakuni [24]. 0.1 ml of the 1:100 diluted culture in saline was poured on each plate. The plates were dried and wells of 6 mm diameter were made with a sterile borer. They were filled with a 50% ethanolic extract. The control was run with 50% ethanol in water. The plates were observed for zones of inhibition after 24 hr incubation at 37° . Tests were run in triplicate.

RESULTS

The results of the screening of 63 plant materials are summarised in Table 2. As indicated by the results 18 plant material out of 63 showed broad spectrum antibac-

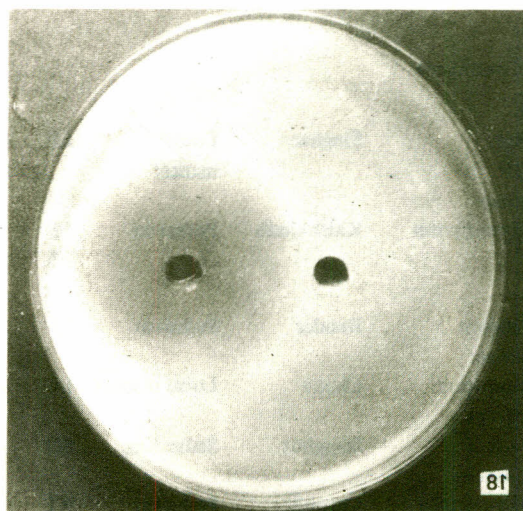


Plate 1. Inhibition caused by the crude extract of *Eugenia jambolana* (jamun) against *Salmonella typhi*

Table 2. Summary of results of antimicrobial screening*.

Family Botanical Name	Common name	Locality	Part used	Antibacterial activity against																	
				B. subtilis	B. megaterium	Sarcina lutea	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
Malvaceae:																					
<i>Althaea officinalis</i>	Khatmi	Local market**	FR	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Abutilon indicum</i>	Khanghi	Local market**	FR	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Abutilon muticum</i>	Gidawar	University**	FL	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Campus	LF	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Thespesia populnea</i>	Palas pipla	Local market**	LF	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
			FR	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Meliaceae:																					
<i>Cedrela toona</i>	Tuni	Herb dealer**	LF	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Melia azadirachta</i>	Bakain	Stadium**	FL	10	10	10	8	8	8	8	8	-	-	-	-	-	-	-	-	-	-
		Road	LF	10	10	15	8	8	8	8	8	-	-	-	-	-	-	-	-	-	-
<i>Melia azadirachta indica</i>	Neem	Stadium**	FR	10	10	14	15	15	15	15	15	15	-	-	-	-	-	-	-	-	-
		Road	LF	10	10	14	15	15	15	15	15	15	-	-	-	-	-	-	-	-	-
Menispermaceae:																					
<i>Tinospora cordifolia</i>	Zakhm-i-hayat	Peshawar	LF	5	5	8	8	6	6	5	-	5	5	5	5	6	4	-	-	-	-
			ST	5	5	6	6	6	6	6	-	5	5	5	4	4	4	-	-	-	-
Myricaceae:																					
<i>Eugenia jambolana</i>	Jamun	Nazimabad**	LF	10	10	10	22	15	15	15	8	-	24	24	24	15	15	15	10	-	-
			FR	12	12	12	20	15	15	15	10	-	30	25	26	15	12	15	10	-	-
<i>Eucalyptus globulus</i>	Eucalyptus	University**	LF	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Campus	LF	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Myrica comminis</i>	Vilayati menhdi	Local** market	LF	8	8	10	10	8	8	10	10	-	10	10	10	12	12	12	8	-	-

(Continued.)

(Table 2, Continued)

Oleaceae:																				
<i>Nyctanthes arbortristis</i>	Singhar	Local** market	LF	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Papaveraceae																				
<i>Papaver somniferum</i>	Khishkhash	Peshawar	LF	5	5	8	8	8	8	8	4	-	5	6	6	5	5	5	-	
			FR	6	6	8	10	8	6	6	4	-	8	6	5	5	5	3	-	
Pinaceae:																				
<i>Cedrus deodara</i>	Deodar	Peshawar	ST	4	4	6	6	5	5	5	-	-	-	-	-	-	-	-	-	
Polygonaceae:																				
<i>Rumex vesicarius</i>	Chuka	Local market**	LF	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Plantaginaceae:																				
<i>Plantago ovata</i>	Isapghot	Sadar Kar.	SD	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Plumbaginaceae																				
<i>Plumbago zeylanica</i>	Chitruk	Peshawar	RT	6	6	12	12	15	15	15	5	-	6	6	6	8	8	8	4	
Ranunculaceae:																				
<i>Aconitum heterophyllum</i>	Atis	Peshawar	RT	5	5	6	6	4	4	4	2	-	-	-	-	-	-	-	-	
Rutaceae:																				
<i>Aegle marmelos</i>	Beal fruit	Local** market	FR	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>Citrus aurantium</i>	Santra	Sadar Kar.	FR	5	5	6	6	10	10	10	-	-	-	-	-	-	-	-	-	
<i>Skimmia laureola</i>	Nera	Muree Hills	LF	6	6	8	8	12	12	12	5	4	6	6	6	5	5	5	4	
Rubiaceae:																				
<i>Anthocephalus cadamba</i>	Kadam	Peshawar	RT	6	6	10	10	8	8	8	4	-	-	-	-	-	-	-	-	
<i>Rubia cordifolia</i>	Margit	Local** market	LF	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Sapotaceae:																				
<i>Bassia latifolia</i>	Mahwa	Peshawar	SD	8	8	12	12	10	10	10	5	-	-	-	-	-	-	-	-	
<i>Mimusops elengi</i>	Bakul	Local** market	LF	5	5	8	8	10	10	12	4	-	-	-	-	-	-	-	-	
Solanaceae:																				
<i>Datura stramonium</i>	Datura	Stadium** Road	LF	25	20	25	30	15	25	25	15	-	6	6	-	-	-	-	-	
			FL	15	15	15	25	10	18	15	10	-	-	-	-	-	-	-	-	
<i>Hyoscyamus niger</i>	Khorasani a jawine	Local** market	SD	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>Solanum indicum</i>		Stadium** Road	LF	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
			FL	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>Solanum xanthocarpum</i>	Khantikiari	University** Campus	LF	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
			FL	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>Solanum nigrum</i>	Kakmacki	University** Campus	LF	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
			FL	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>Withania coagulans</i>	Ashvagandha	Malir**	LF	12	14	10	20	14	11	12	12	12	-	-	-	-	-	-	-	
			FL	10	10	10	20	12	11	12	12	12	-	10	10	10	10	10	10	
<i>Withania somnifera</i>	Aksen	University** Campus	LF	12	14	10	20	14	11	12	12	12	-	-	-	-	-	-	-	
			FL	10	10	10	20	12	11	12	12	12	-	10	10	10	10	10	10	
<i>Solanum indicum</i>	Byakura	University** Campus	LF	8	8	10	12	8	8	8	6	5	-	8	8	8	6	6	6	
			FL	8	8	10	12	8	8	8	6	5	-	8	8	8	6	6	6	
Scitaminaceae:																				
<i>Musa sapientum</i>	Kala	Stadium** Road	LF	14	14	15	12	14	16	16	15	10	8	16	16	16	16	16	16	
			SK	10	10	10	10	14	14	14	14	8	5	10	10	10	10	10	10	
			FR	12	12	10	12	14	15	15	15	4	4	15	15	15	15	16	16	
Umbelliferae																				
<i>Carum copticum</i>	Ajwain	Local** market	SD	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	

(Continued.)

(Table 2, Continued)

<i>Ferula foetida</i>	Hing	Local** market	Resin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
<i>Hydrocotyle asiatica</i>	Barhmi	Local** market	LF	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
			FR	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
Urticaceae																					
<i>Cannabis sativa</i>	Bhang	Islamabad	LF	15	15	15	22	15	16	16	16	-	-	12	12	12	12	12	12	10	8
<i>Ficus bengalensis</i>	Bargad	University	LF	-	12	8	8	4	4	4	-	-	-	-	-	-	-	-	-	-	-
		Campus	FR	-	12	5	6	4	6	6	4	4	-	-	-	-	-	-	-	-	-
<i>Ficus religiosa</i>	Pipal	University**	LF	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Campus	FR	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Nymphaeaceae:																					
<i>Nymphaea lotus</i>	Kanwal	University**	LF	8	8	10	10	8	8	8	8	-	-	-	-	-	-	-	-	-	-
		Campus	FL	-	-	5	5	6	6	6	6	-	-	-	-	-	-	-	-	-	-
Zingiberaceae:																					
<i>Curcuma amada</i>	Amba haldi	Local market**	ST	10	10	18	21	20	22	20	20	8	3	-	-	-	-	-	-	-	-
<i>Zingiber officinale</i>	Adrak	Local market	ST	8	8	8	8	10	10	10	5	-	10	10	10	12	12	12	-	-	-

FL, flower or inflorescence; Fr, fruit; LF, leaf, PL, whole plant; RT, root ST, stem; BK, bark; SD, seed; SK, skin of fruit. Zone of inhibition measure in mm. * Cup plate method.

** Karachi.

terial activity against most of the Gram negative and Gram positive bacteria used in the study (28.6 % of the extract), whereas 19 plant extracts exhibited narrow spectrum activity against either Gram negative or Gram positive bacteria (30 % of the extract).

DISCUSSION

Biological screening of the plant extracts is most frequently carried out as the determination of antibacterial activity. These evaluations are usually done by means of standard *in vitro* assays utilizing a broad selection of pathogenic as well as common non-pathogenic bacteria. In the present study eighteen bacterial strains were used (Table 1).

The results indicate that among the species studied, the fruits of *Eugenia jambolana* (jamun) exhibited most promising broad spectrum activity against all Gram positive bacteria and most of Gram negative bacteria used in the screening. Even the leaves of this species showed remarkable broad spectrum activity. The seeds and fruits of this plant find uses in both Unani and Ayurvedic Systems of medicine as antispasmodic, appetiser, tonic for the stomach and intestine, antiemetic, antidiarrhoeal and antidiabetic [30]. However, this seems to be the first report in literature in which antibacterial activity of the fruits and leaves have been determined by standard methods.

The second plant species which showed a remarkable broad spectrum activity is *Musa sapientum* (kela or banana). Extracts of the leaves, fruits and even the skin of the fruit were active against Gram negative and Gram positive bac-

teria. It has been reported that the fruit of the plant can combat the development of colon form of bacteria, i.e. Gram negative organisms which are commensals in G.I.T. or those which parasitize G.I.T. (*E.coli*, *Salmonella* spp. and *Shigella* spp.) [30]. The present study confirms the previous report of Nadkarni [30].

As indicated in Table 2 among 63 plant extracts screened during the study only a few were active against *Pseudomonas aeruginosa*, the most prominent were *Sphaeranthus indicus* [29] and *Cannabis sativa* (bhanga). *Pseudomonas aeruginosa* is a very difficult pathogen, since it is resistant to most of the known commercial antibiotics. Further studies on these plant species are needed for the effective treatment of *Pseudomonas* infections.

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