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Antibacterial Activity of Some Medicinal Plants

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Aqueous, Chloroform and methanol extracts of 10 medicinal plants were tested against 10 pathogenic bacteria for their antibacterial activity using disk diffusion method. Methanol extracts of *Withania somnifera, Lawsonia inermis, Datura metel, Datura stramonium* and *Bauhinia racemosa* showed significant antibacterial activity against certain bacteria. Methanol extracts of *Withania somnifera* revealed highest zone of inhibition against *Staphylococcus aureus, Proteus vulgaris* and *Leuconostoc mesenteroides.* Among all tested extracts, methanol extract of *Lawsnia inermis* exhibited antibacterial activity against almost all bacteria except *Leuconostoc mesenteroides*. Gram-positive bacteria were more susceptible to plant extracts than gram-negative bacteria.

Key words: Antibacterial activity, *Lawsonia inermis,* methanol extract and *Withania somnifera.*

Infectious diseases have been threatened the life of millions of people in the world. This situation has been arises due to the increase in the development of antibiotics resistance by various pathogens, high cost of antibiotics and side effect on the host.[1] A major part of the total population in developing countries still uses tradition folk medicine obtained from plant resources. More than 50 % of all modern clinical drugs are of natural product origin. Natural products play an important role in drug development program in the pharmaceutical industries.[2] The antimicrobials from plant origin may act on different site of action than those presently used antimicrobials, which has significant clinical values in treatment of resistant microbial strains.[3]

By keeping these things in mind, 39 extracts of 10 medicinal plants were tested for their antibacterial activity against eight human and two plant pathogenic bacteria.

MATERIAL AND METHODS

The healthy, infection free, mature parts (i.e. root, stem bark, leaves, flower, seeds and bulbs) of 10 plants were collected from Government Institute of Science campus, Aurangabad. Identities of plant species were authenticated by referring standard literature.

Preparation of extracts

Preparation of aqueous extracts

The plants parts were washed with fresh water followed by distilled water and finally

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grounded with sterile distilled water @ 1 g of tissues/ml in mortar with pestle. The macerate was succeeded through cotton wool followed by two layers of muslin cloth and finally through Whatman no.1 filter paper to get the extracts.

Preparation of solvent extracts

Collected plant parts were shade dried and grounded to a fine powder using grinder mixer. Extracts were prepared in chloroform and methanol at room temperature by simple extraction method.[4] Dried powder of plant parts (10 g) were mixed with 100 ml solvent in 250 ml conical flasks. The flasks were plugged tightly with cotton and wrapped with paper. All conical flasks were kept on shaker for 24 h then it was allowed to stand for five hours to settle the plant material. Thereafter, it was filtered and centrifuged at 5000 rpm for 15 min. The supernatant was collected and the solvent was evaporated at 45 ^{o}C in vacuum evaporator to make the final volume 1/5th of the original volume. It was stored at 4 ^{o}C in airtight bottles for further studies.

Bacterial cultures

Pure cultures of *Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumoniae, Proteus vulgaris, Escherichia coli, Bacillus megaterium, Anthrobacter simplex* and *Leuconostoc mesenteroides* were obtained from Department of Microbiology, Government Institute of Science Aurangabad. Whereas *Xanthomonas axonopodis* pv. *citri* and *Xanthomonas axonopodis* pv. *malvacearum* were isolated from citrus (*Citrus aurentifolia* L.) and cotton (*Gossypium herbacium* L.) respectively.

Preparation of standard culture inoculums of bacteria

A loop full of target bacteria (24 h old cultures) was inoculated in 5 ml nutrient broth and incubated at 37 ^{o}C for 5-8 h until moderate turbidity was developed to 0.5 OD McFarland standards (WHO Drug information, 1993).

Antibacterial assay

Antibacterial activity was determined using disk diffusing method.[5] Standard inoculums of each bacterium were spread on the surface of solidified nutrient agar plates using sterile cotton swab. The surface was allowed to dry for 10 min after which sterile paper disk (5 mm) was soaked in extracts allowed to dry and then placed on the inoculated surface. For comparison, Streptomycin (100 ppm) was used. Plates were incubated at 37 ^{o}C for 24 h. Each sample was tested in triplicates. Zone of inhibition was measure after 24 h of incubation.

RESULT AND DISCUSSION

Out of 39 extracts examined, 69.2 % (27) exhibited antibacterial activity [Table 1]. Those extracts, which showed 15 mm zone of inhibition, were considered as active extract. Among methanol extract, leaf extracts of *W. somnifera* showed highest zone of inhibition against *S. aureus, P. vulgaris* and *L. mesenteroides* (28 mm). In aqueous extracts, leaf extracts of *W. somnifera* and *C. roseus* revealed highest zone of inhibition against *P. vulgaris* and *E. coli* (24 mm) respectively. Among chloroform extracts, leaf extract of *W. somnifera* showed strongest antibacterial activity against *S. aureus, P. vulgaris* and *L. mesenteroides* (22 mm). Overall methanol extracts showed greater antibacterial activity as compared to other type of extracts. Similar finding was observed[6,7].

In this screening, only methanol leaf extract of *L. inermis* exhibited zone of inhibition against almost all bacteria except *L. mesenteroides*. Among 10 bacteria, *Xanthomonas axonopodis* pv. *citri* and *P. aeruginosa* were found to be resistant to the plant extracts; whereas *S. aureus, B. magaterium* and *P. vulgaris* were susceptible to plant extracts. Gram-positive bacteria were more sensitive than gram-negative bacteria to plant extracts. The sensitivity of gram-positive bacteria to plant extracts was also reported[8,9]. The difference in susceptibility between gram-positive and gram-negative bacteria may be due to the difference in chemical composition of cell wall and permeability of cell membrane. Among tested plants *W. somnifera, D. metel, D. stramonium, L. inermis, B. racemosa* and *C. roseus* were potent which posses antibacterial properties. The present finding agreed with previous reports elsewhere using the same plants[10-12].

Some plant extracts inhibited the growth of *P. aeruginosa*, which is the most prevalent burn patient's pathogen capable of causing life threatening illnesses. Some strains causing septicemia and pneumonia in cystic fibrosis and immuno-compromised patients are becoming difficult to treat with currently available antimicrobials agents. Therefore, present works

Table 1. Antibacterial activity of various plant extracts

Plant species	Part(s) used	Extract	Zone of inhibition (mm)*									
			Gram-positive bacteria					Gram-negative bacteria				
			S.a.	B.m.	L. m.	A.s.	P.a.	K.p.	P.v.	X.a.m.	E.c.	X. a. c
Withania somnifera	Lv	H_2O	-	-	-	-	18 ± 0.1	23 + 0.3	24 ± 0.2	8 ± 0.3	-	-
		$CHCl_3$	22 ± 0.1	16 ± 0.1	22 ± 0.3	-	20 ± 0.4	18 + 0.2	22 ± 0.3	20 ± 0.1	-	-
		MeOH	28 ± 0.1	16 ± 0.3	28 ± 0.3	-	-	24 ± 0.3	28 ± 0.1	22 ± 0.2	-	-
	Rt	H_2O	-	-	-	-	-	9 ± 0.2	-	-	-	-
Datura metel	Lv	H_2O	9 ± 0.2	-	-	9 ± 0.1	-	-	-	-	10 ± 0.2	-
		$CHCl_3$	14 ± 0.2	14 ± 0.2	12 ± 0.1	-	-	12 ± 0.3	12 ± 0.1	-	-	-
		MeOH	18 ± 0.1	18 ± 0.2	16 ± 0.3	28 ± 0.1	-	16 ± 0.2	16 ± 0.2	-	-	18 ± 0.1
Datura stramonium	Lv	H_2O	-	12 ± 0.2	-	-	-	-	14 ± 0.2	-	-	-
		$CHCl_3$	16 ± 0.1	14 ± 0.2	-	-	-	18 ± 0.3	12 ± 0.1	14 ± 0.2	-	-
		MeOH	18 ± 0.2	16 ± 0.1	16 ± 0.2	-	-	18 ± 0.2	18 ± 0.3	-	-	-
	Sd	H_2O	-	-	-	-	-	-	-	-	-	-
		$CHCl_3$	-	-	-	-	-	-	-	-	-	-
		MeOH	-	-	-	-	-	8 ± 0.2	-	-	-	12 ± 0.1
Lycopersicon esculentum	Lv	H_2O	-	-	-	-	-	-	-	-	-	-
		$CHCl_3$	-	-	-	-	-	-	-	-	-	-
		MeOH	-	12 ± 0.1	14 ± 0.2	-	-	-	-	-	-	8 ± 0.2
Catharanthus roseus		H_2O	-	-	-	-	-	10 ± 0.1	13 ± 0.1	14 ± 0.2	24 ± 0.1	-
		$CHCl_3$	-	-	-	-	-		-	-	-	-
		MeOH	16 + 0.1	16 + 0.1	-	-	-	16 + 0.1	18 + 0.2	-	-	-
	Fl	H_2O	-	-	-	10 + 0.2	-	-	-	-	-	-
Thevetia peruviana	Lv	H2O	-	-	-	-	-	-	-	11 + 0.1	-	-
		$CHCl_3$	16 + 0.1	-	-	-	-	-	-	-	-	-
		MeOH	-	-	-	-	-	-	-	-	-	-
	Sd	H_2O	-	-	-		-	-	16 + 0.2	-	-	-
		$CHCl_3$	-	-	-	-	-	-	-	-	-	-
		MeOH	-	-	-	-	-	-	-	-	-	-
Casuarina equisetifolia	Lv	H_2O	-	-	-	12 + 0.3	-	-	-	-	-	-
		$CHCl_3$	-	-	-	-	-	-	-	-	-	-
		MeOH	12 + 0.1	8 + 0.2	16 + 0.2	-	14 + 0.1	-	10 + 0.1	-	12 + 0.4	-

Table 1. Cont...

Lawsonia inermis	Lv	H_2O	-	-	-	-	-	-	-	-	8 + 0.1	-
		$CHCl_3$	10 + 0.2	-	-	-	-	-	-	-	-	-
		MeOH	15 + 0.1	16 + 0.2	-	11 + 0.1	10 + 0.1	11 + 0.2	18 + 0.2	10 + 0.2	12 + 0.2	12 + 0.1
Allium cepa	Bb	H_2O	-	-	-	-	-	-	-	-	-	7 + 0.4
Bauhinia racemosa	Lv	H_2O	-	8 + 0.2	16 + 0.4	-	18 + 0.2	-	-	-	-	-
		$CHCl_3$	-	-	-	-	-	-	-	-	-	-
		MeOH	8 + 0.1	8 + 0.2	-	11 + 0.3	-	7 + 0.1	8 + 0.2	10 + 0.2	12 + 0.1	-
	Sb	H_2O	-	-	-	8 + 0.2	-	-	-	8 + 0.3	10 + 0.1	-
		$CHCl_3$	-	-	-	-	-	-	-	-	-	-
		MeOH	9 + 0.1	15 + 0.1	-	-	-	-	-	-	-	-
Streptomycin (100ppm)			-	14 + 0.1	20 + 0.2	21 + 0.1	9 + 0.1	-	12 + 0.2	-	9 + 0.2	11 + 0.2

* Values expressed in mean + S.D. of triplicate , Lv = Leaves, Rt = Roots, Sd = Seeds, Fl = Flower, Bb = bulb, Sb = Stem bark. A.s. = *Anthrobacter simplex*, S.a = *Staphylococcus aureus*, X. a. m. = *Xanthaomonas axonopodis*, Pv. *malvacearum*, E. c. = *Escherichia coli*, P. v. = *Proteus vulgaris*, X. a. c. = *Xanthomonas axonopodis* Pv. citri, P. a. = *Pseudomonas aeruginosa*, K.p.= *Klebsiella pneumoniae*, B.m.= *Bacillus megaterium* and L.m.= *Leuconostok megenteroids*.

is useful for the development of new antimicrobial agents against these types of pathogens and rationalize the medicinal values of plants in traditional medicinal systems.

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