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# STUDIES ON THE ANTIMICROBIAL POTENTIAL OF PLANT MATERIALS AGAINST BACTERIAL AND FUNGAL STRAINS

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#### ABSTRACT

Ayurveda is a science of health, medicine and treatment of diseases. According to ayurveda there is not a single plant on earth which is not having any therapeutic value. Considering this statement very limited research has been done in this field. Attempts have been made to detect the antimicrobial potential of some medicinal plants. For this 30 plants were selected randomly, different parts of which were crushed to make the aqueous and ethanolic extract. The extract was then used to detect the antimicrobial activity by using ditch plate method. Twelve bacteria, one yeast and one fungal isolate were used as test organisms. Standard antibiotics were also used for detecting its antimicrobial activity. The results obtained were compared with that of plant extract. Out of thirty, seven plants were showing broad spectrum activity.

KEYWORDS: Medicinal plants, Antimicrobial activity.

#### INTRODUCTION

From the ancient time "Dhanvantari" is considered as a god of health who formulated the Ayurveda in which the information of different disorders and use of medicinal plants in treatment of specific diseases is given. India is one of the largest treasure of plant wealth. The antibiotics produced by microorganisms are commonly used in the treatment of various diseases. It is known that many antibiotics have side effects, damaging different organs of our body. Ayurvedic medicines prepared from plants do not have detectable side effects on human body. They are comparatively slower in action than antibiotics. Many scientists have worked to detect the antimicrobial potential of different plant materials against different bacterial, fungal and protozoal strains.

### MATERIALS & METHODS

## Collection of plant material

The plant material was collected by using sterile knife and taken in sterile plastic bags to minimize contamination. It was then kept in refrigerator at  $4^{\circ}$ c until further use. Powder form of different parts of plants was obtained from Ayurvedic medicine shops.

#### **Identification of plants**

The plants from which sample was taken were identified on the basis of the methods given in Cock's flora vol. I, II and III.

#### **Preparation of plant extracts**

Fresh plant sample was rinsed with distilled water to remove the soil and dirt. It was washed with alcohol, rinsed with distilled water, again washed with 1:1000  $HgCl_2$  Solution and finally washed with sterile distilled water.

About 10g of plant material was chopped with sterile knife under aseptic condition into small pieces. The chopped pieces were crushed in the sterile mortar and pestle. 10ml sterile distilled water was added during crushing to

facilitate oozing of juice. The crushed extract was transferred to 100ml capacity conical flask. Additional 15ml sterile distilled water was added in the flask. The flasks were kept at room temperature for 6-8 hours on a rotary shaker for extraction. The liquid was then filtered using microfilter assembly under aseptic conditions. The filtrate was then used for detection of antimicrobial activity. The ethanol extract was prepared in the same manner as mentioned above by using ethanol instead of distilled water. The powder of different parts of plants, obtained from medicinal shop was added in 25 ml sterile distilled water in 100ml capacity conical flask .The flask was kept at room temperature for 6-8 hours on a rotary shaker. The liquid was then filtered using microfilter assembly under aseptic conditions. The filtrate was then used for detecting antimicrobial activity.

#### Detection of antimicrobial activity of plant extract

For detecting the antimicrobial activity of plant extract a ditch plate diffusion method was used. Test organisms used for detecting antimicrobial activity were Salmonella typhi, Salmonella paratyphi A, Salmonella paratyphi B, Escherichia coli, Shigella flexneri, Pseudomonas aerogenosa, Klebsiella pneumonae, Proteus mirabilis, Proteus vulgaris, Staphylococcus aureus, Bacillus cerus, Candida albicans, Mycobacterium tuberculosis and Aspergillus niger. The nutrient medium used during overall experiment was nutrient agar for all organisms except for Mycobacterium tuberculosis for which Lowenstin-Jensen medium was used and for Aspergillus niger potato dextrose agar was used. For performing the ditch plate method, a ditch of 2cm length and 0.5 cm breadth was prepared in the centre of the plate. The test organism from pure culture was streak inoculated on the surface of agar perpendicular to the ditch.0.5ml of plant extract was added in ditch with sterile pipette. The plates were kept in refrigerator  $(4^{\circ}C)$  for 1 hour for pre diffusion of plant extract. The plates were then incubated at 37°c for

two days for bacteria and yeast and at room temperature for five days for fungi. Precaution was taken to avoid flowing of plant extract over the agar surface. After incubation the plates were observed for the zone of inhibition.

While using ethanol based plant extract one control was kept by adding only ethanol in one ditch. The zone of inhibition was calculated by subtracting the value of control.

#### Antimicrobial activity of standard antibiotics

The nutrient medium used during overall experiment was nutrient agar for all organisms. The plates were initially spreaded with 0.1ml of pure culture of test organism. The combined microbial sensitivity disc (COMBI disc) was placed on the plates. The plates were kept in refrigerator  $(4^{\circ}C)$  for 1hour for pre diffusion of plant extract. The plates were then incubated at  $37^{\circ}c$  for two days.

#### **RESULTS & DISCUSSION**

The results of antimicrobial activity of crude extracts of thirty plants are shown in table 1 and 2. The direct crude aqueous and alcoholic extract of plant material was tested against Salmonella typhi, Salmonella paratyphi A, Salmonella paratyphi B, Escherichia coli, Shigella Klebsiella flexneri. Pseudomonas aerogenosa, pneumonae, Proteus mirabilis, Proteus vulgaris, Staphylococcus aureus, Bacillus cerus, Candida albicans, Mycobacterium tuberculosis and Aspergillus niger. The results indicates that out of 30 plants only nine plants namely Azadirachta indica, Nelumbium speciosum, Hibiscus rosa-sinensis, Ocimum sanctum, Mentha Terminalia bellerica, Curcuma longa, sulvestris. Bryophyllum pinnetum, Parthenium hysterophosous shows antimicrobial activity in aqueous extract.

TABLE 1: Antibacterial activity of water based plant extract

Sr. no.	Botanical and Vernacular names of plants	Parts of Plants Examined				Tes	st organ	isms, Z	one of	inhibiti	on (m	m)				
			Salmonella Typhimurium	Salmonella paratyphi A	Salmonella paratyphi B	Escherichia coli	Shigella flexineri	Pseudomonas aeruginosa	Kebsiella preumonae	Proteus mirabilis	Proteus vulgaris	Staphaylococcus aureus	Bacillus cerus	Mycobacterium	Candida albicans	Aspergillus nier
1	<i>Curcuma amada</i> (Ambehalad)	Powder	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	Saraca indica (Ashoka)	Leaves	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	Carum copticum (Ova)	Powder	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	Azadirachta indica (Neem)	leaves	3	-	-	3	5	6	3	-	3	-	-	-	-	-
5	Nelumbium speciosum	Flowers	-	-	-	-	-	6	-	-	-	3	-	-	-	-
	(Kamal-White)	Stem	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	. ,	Leaves	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	<i>Nymphoea lotu</i> s (Kamal-	Flowers	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Red)	Stem	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Leaves	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	Citrullus vulgaris	Gel	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	(Watermion)	Cover of	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Fruit														
7	Cucumis utilissimas	Fruit	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	(Kakdi)	Stem	-	-	-	-	-	-	-	-	-	-	-	-	-	-
_		Leaves	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	Momoradica charatia	Fruit	-	-	-	-	-	-	-	-	-	-	-	-	-	-
~	(Karale)	Leaves	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	Canna indica (Kardal)	Leaves Flowers	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10	Aloe vera (Korfad)	Leaf-gel	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10	Lantana camara	Leaves	-	-	-	-	-	-	-	-	-	-	-	-	-	-
••	(Kanheri)	Flowers	-		-		-				-	-	-		- 1	
12	Hibiscus rosasinensis	Leaves	5	7	-	5	9	7	-	_	7	-	-	_	_	_
	(Jaswand)	Flowers	-		-	-	-		-	-	2	-	-	-	-	
13	Ocimum sanctum (Tulsi)	Leaves	5	6	-	4	7	-	2	-	-	-	-	-	-	-
14	Cephalandra indica	Fruit	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	(Tondale)	Leaves	-	-	-	-	-	-	-	-	-	-	-	-	-	-
15	, Vitis vinifera (Draksh)	Leaves	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	. ,	Fruits	-	-	-	-	-	-	-	-	-	-	-	-	-	-
16	Luffa acutangulla	Fruit	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	(Dodaka)	Leaves	-	-	-	-	-	-	-	-	-	-	-	-	-	-
17	Menthe sulvestris	Leaves	-	6	-	-	-	-	-	-	-	-	-	-	-	-
	(Pudina)	Stem	-	-	-	-	-	-	-	-	-	-	-	-	-	-
18	<i>Acorus calamus</i> (Vekhand)	Powder	-	-	-	-	-	-	-	-	-	-	-	-	-	-
19	Acacia arabica (Babhul)	Leaves	-	-	-	-	-	-	-	-	-	-	-	-	-	-

20	Terminalia bellerica (Behada)	Powder	2	-	-	5	2	2	-	2	-	-	-	-	-	-
21	Capsium fruiteseans	Fruit	-	-	-	-	-	-	-	-	-	-	-	-	-	
	(Chilli)	Leaves	-	-	-	-	-	-	-	-	-	-	-	-	-	
22	Lesiosiphon ericephalus (Rametha)	Leaves	-	-	-	-	-	-	-	-	-	-	-	-	-	•
23	Ocimum basilicum (Sabja)	Fruit	-	-	-	-	-	-	-	-	-	-	-	-	-	•
24	Erigeron asteodes (Sonsali)	Leaves	-	-	-	-	-	-	-	-	-	-	-	-	-	
25	<i>Citrus aurantium</i> (Santra sal)	Powder	-	-	-	-	-	-	-	-	-	-	-	-	-	
26	Curcuma longa (Halad)	Stem	-	-	-	-	-	-	-	-	-	5	-	-	-	
		Leaves	-	-	-	-	-	-	-	-	-	-	-	-	-	
27	Bryophyllum pinnetum (Panfuti)	Leaves	-	-	-	-	-	-	-	2	2	1	-	-	-	
28	Parthenium	Leaves	5	5	3	4	6	3	2	-	2	1	-	-	2	
	hysterophorous	Flowers	-	-	-	-	-	-	-	-	-	-	-	-	-	
	(Congress Gavat)	Stem	-	-	-	-	-	-	-	-	-	-	-	-	-	
29	Jatropha curacus (Mogale Aerand)	Latex	-	-	-	-	-	-	-	-	-	-	-	-	-	
30	Solanum melongene	Leaves	-	-	-	-	-	-	-	-	-	-	-	-	-	
	(Brinjal)	Fruits	-	-	-	-	-	-	-	-	-	-	-	-	-	

- = No zone of inhibition

## **TABLE 2:** Antibacterial activity of ethanol based plant extract.

Sr. no.	Botanical and Vernacular names of plants	Parts of Plants Examined						rganisr	ms, Zon	e of in	hibitio	n (mm)				
			Salmonella Typhimurium	Salmonella paratyphi A	Salmonella paratyphi B	Escherichia coli	Shigela flexineri	Pseudomonas aeruginosa	Klebsiella pneumonae	Proteus mirabilis	Proteus vulgaris	Staphaylococcus aureus	Bacillus cerus	Mycabacterium	Candida albicans	Aspergillus nier
1	<i>Curcuma amada</i> (Ambehalad)	Powder	-	•	-	-	-	-	-	-	-	-	-	-	-	-
2	Saraca indica (Ashoka)	Leaves	-	-	-	-	5	-	2	2	2	-	-	-	-	-
3	Carum copticum (Ova)	Powder	-	2	2	2	3	6	-	2	2	-	-	-	-	-
4	Azadirachta indica (Neem)	Leaves	2	2	2	12	5	-	10	2	2	6	4	-	3	-
5	Nelumbium speciosum	Flowers	-	-	-	1	-	-	-	1	-	-	-	-	1	-
	(Kamal-White)	Stem	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Leaves	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Nymphoea lotus (Kamal-	Flowers	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Red)	Stem	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Leaves	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	Citrullus vulgaris	Gel	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	(Watermion)	Cover of Fruit	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	Cucumis utilissimas	Fruit	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	(Kakdi)	Stem	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Leaves	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	Momoradica charatia	Fruit	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	(Karale)	Leaves	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	Canna indica (Kardal)	Leaves	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Flowers	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10	Aloe vera (Korfad)	Leaf-gel	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11	Lantana camara	Leaves	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	(Kanheri)	Flowers	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12	Hibiscus rosasinensis	Leaves	5	9	2	4	1.2	-	1	1	6	5	-	-	2	-
	(Jaswand)	Flowers	-	-	-	-	2	-	2	2	-	-	-	-	-	-
13	Ocimum sanctum (Tulsi)	Leaves	2	4	-	1	5	-	-	-	-	-	-	-	-	-
14	Cephalandra indica (Tondale)	Fruit Leaves	-	-	-	-	-	-	-	-	-	-	2	-	-	-
15	Vitis vinifera (Draksh)	Leaves	-	-	-	-	-	-	-	-	-	-	-	-	-	-
40	1 - <del>6</del>	Fruits	-	-	-	-	-	-	-	-	-	-	-	-	-	-
16	Luffa acutangulla	Fruit	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4-	(Dodaka)	Leaves	-	-	-	-	-	-	-	-	-	-	-	-	-	-
17	Menthe sulvestris	Leaves	2	2	2	2	2	-	1	2	-	-	-	-	3	-
40	(Pudina)	Stem	-	-	-	-	-	-	-	-	-	-	-	-	-	-
18	Acorus calamus (Vekhand)	Powder	-	-	-	-	-	-	-	-	-	-	-	-	-	-

19	<i>Acacia arabica</i> (Babhul)	Leaves	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20	Terminalia bellerica (Behada)	Powder	-	-	-	-	-	-	-	-	-	-	-	-	-	-
21	Capsium fruiteseans	Fruit	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	(Chilli)	Leaves	-	-	-	-	-	-	-	-	-	-	-	-	-	-
22	Lesiosiphon ericephalus (Rametha)	Leaves	-	-	-	-	2	-	-	-	-	4	-	-	-	-
23	Ocimum basilicum (Sabja)	Fruit	-	-	-	-	-	-	-	-	-	-	-	-	-	-
24	Erigeron asteodes (Sonsali)	Leaves	-	-	-	-	-	-	-	-	-	-	-	-	-	-
25	<i>Citrus aurantium</i> (Santra sal)	Powder	-	-	-	9	2	-	6	-	-	2	2	-	-	-
26	Curcuma longa (Halad)	Stem	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Leaves	-	-	-	-	-	-	-	-	-	-	-	-	-	-
27	Bryophyllum pinnetum (Panfuti)	Leaves	-	-	-	-	4	-	-	5	-	-	-	-	-	-
28	Parthenium	Leaves	2	-	-	-	5	2	-	1	-	-	-	-	-	-
	hysterophorous	Flowers	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	(Congress Gavat)	Stem	-	-	-	-	-	-	-	-	-	-	-	-	-	-
29	Jatropha curacus (Mogale Aerand)	Latex	-	-	-	-	-	-	-	-	-	-	-	-	-	-
30	Solanum melongene	Leaves	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	(Brinjal)	Fruits	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Antimicrobial potential of plant materials against bacterial and fungal strains

Out of these nine plants only five plants were possessing broad spectrum antimicrobial activity. Antimicrobial activity of 30 ethanol based plant extracts was tested against above mentioned pathogens, it was observed that only eleven plants namely Parthenium hysterophorous, Bryophyllum pinnetum, Citrus aurantium, Lesiosiphon ericephalus, Menthe sulvestris, Ocimum sanctum, Hibiscus rosasinensis, Nelumbium speciosum, Azadirachta indica, Carum copticum, Saraca indica, were showing the antimicrobial activity. Out of these eleven only six plant extracts were possessing broad spectrum antimicrobial activity. Activity of plant extracts was further compared with standard antimicrobial discs, as shown in table 3. On the comparison of the zone of inhibition of plant extracts with that of standard antibiotics it was observed that standard antibiotics have better antibacterial activity than the plant extracts. This might be due to poor release of active principles from the plants in ethanolic or aqueous solvents or a higher concentration of the extracts may be required to have the equal effect.

Studies evaluating the *in vitro* antimicrobial activity of methanolic extracts of some medicinal plants against *Escherichia coli, Salmonella Typhimurium, Staphylococcus aureus* and *Enterococcus sp.* was done by Ushimaru P.I. *et al.* (2007)

The antibacterial activities of the alcohol, ethyl acetate, acetone and chloroform extracts of *Pimpinella anisum* (L.) (anise, aniseed) (seed), *Coriandrum sativum* (L.) (coriander, cilantro) (seed), *Glycyrrhiza glabra* (L.) (liquorice) (root), *Cinnamomum cassia Blume* (cassia bark, Chinese cinnamon) (bark), and *Juniperus oxycedrus* (L.) (juniper) (seed) were tested in vitro against 13 bacterial species and strains by the agar diffusion method

were studied. (Ates D.A. and O. T. Erdoúrul 2003). Eighteen plant species used in folklore medicine in west Nepal were tested for their antibacterial activity by the disk diffusion method. The bacteria employed were grampositive (Staphylococcus aureus) and gramnegative (Escherichia coli, Pseudomonas aeruginosa and Shigella bovdii) (Panthi M.P. and R.P.Chaudhary 2006) The methanol leaf extracts of Acacia nilotica, Sida cordifolia, Tinospora cordifolia, Withania somnifera and Ziziphus mauritiana showed significant antibacterial activity and antifungal activity. A. nilotica and S. cordifolia leaf extract showed highest antibacterial activity against B. subtilis. A. *nilotica* bark and leaf extract showed significant antifungal activity against A. flavus. (Mahesh B. and S. Satish 2008) .The antimicrobial activity of hot water and ethanolic extracts of six plant extracts, were studied against seven bacterial species and two fungal species by well-diffusion method and microdilution methods. The extract of Sphareranthu hirtus was the most active against multidrug resistant Pseudomonas aeruginosa and enterohemorrhagic E. coli 0157 EHEC. The ethanolic extract of S. hirtus exhibited a higher effect than the hot water extract. (Hassan M. et.al. 2009). Nine plants were screened for testing potential antibacterial activity against 6 bacterial strains. Both aqueous and organic solvents were used for preparation of abstract. The activity was studied by using Agar disk diffusion and Agar ditch diffusion (Nair R. et.al. 2005) Mehta B.K. et.al.(1993) observed antimicrobial efficacy of Triphala in vitro. Sharma V.D. et.al. (1977) studied antimicrobial property of Allium sativum lin in vitro and in vivo. Anticandidal activity of Azadirachta indica was studied by Lloyed et.al. (2005).

<b>TABLE 3:</b> Comparison of Antimicrobial activity of plant extract and antibiotic against various microorganisms (Zone of
inhibitions values given in parenthesis, in mm)

Sr.No.	Organisms	Plants*	Antibiotics*
1	Salmonella typhimurium	Ai (3)	CF (11)
•	Gamonala cyprimanam	Hr (5)	GI (11) GI (12)
		Os (5)	NA (15)
		Tb (2)	101 (10)
		Ph (5)	
2	Salmonella paratyphi A	Hr (7)	CI (15)
		Os (6)	CR (10)
		Ms (6)	CX (15)
		Ph (5)	
3	Salmonella paratyphi B	Ph (3)	CI (14)
4	Escherichia coli	Ai (3)	AK (20)
		Hr (5)	I (12)
		Os (4)	CR (5)
		Tb (5)	CP (13)
		Ph (4)	NF (12)
			PF (24)
			K (20)
			CI (23)
5	Shigella flexineri	Ai (3)	PF (17)
		Hr (9)	K (15)
		Os (7)	I (15)
		Tb (2)	NF (15)
		Ph (6)	
6	Pseudomonas aeruginosa	Ai (6)	AK (3)
		Hr (7)	K (12)
_		Ns (6)	
7	Klebsiella pneumonae	Ai (3)	AK (12)
		Os (2)	PF (15)
			CI (12)
			CR (15)
			CX (24)
•	Drata va minabilia	Th. (2)	NR (18)
8	Proteus mirabilis	Tb (2)	CP (8)
		Bp (2)	I (15) ∐(20)
			NF (10)
9	Proteus vulgaris	Ai (3)	AK (15)
5	Troleus vulgans	Hr (7)	NF (8)
		•• (7)	CX (15)
10	Staphylococcus aureus	Ns (3)	AM (22)
		a (5)	CP (15)
		Bp (1)	NF (24)
		Ph (1)	K (23)
		()	CX (20)
			I (15)
			CD (11)
			T (11)
11	Bacillus cerus	Nil	AM (15)
			CP (19)
			NF (15)
			K (18)
			CD (15)

\*Azadirachta indica – Ai, Hibiscus rosa-sinensis – Hr, Ocimum sanctum – Os, Terminalia belleria – Tb, Parthenium hysterophorous- Ph, Byophyllum pinnetum- Bp, Neturnbium speciosum- Ns, Ourcuma longa- O, Mantha sulvestris- Ms, Amikacin-AK, Ampicillin-AM, Tetracyclin – T, Imipenem – I, Kanamycin – K, Ciprofloxacin – Cl, Naliclixic acid –NA, Cefadxine-CX, Cefazolin-CF, Chloramphenicol- CP, Nafcillin – NF, Carbenicillin – CR, Clindamycin – CD, Profloxacin – PF, Norfloxacin – NR, Lincomycin – LI.

#### CONCLUSIONS

Antimicrobial activity of thirty plant extracts was compared with different standard antibiotics, so it is concluded that,

- Direct crude aqueous extract exhibited the highest degree of antimicrobial activity.
- The seven plants *Carum capticum, Citrus aurantium, Azadirachta indica, Hibiscus rosa-sinensis, Mentha sulvestris, Ocimum sanctum* and *Parthanium hysterophorus* shows broad spectrum of antimicrobial activity.
- The results could have been more promising if the active principles would have been isolated from the crude extracts and used against microorganisms.
- As the present results are clearly illustrating the potential of plant extracts as antibacterial/antifungal agents, the plants can be exploited for its secrete hidden quality by further research.

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