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In vitro Antimicrobial Activity of Certain Medicinal Plant Extracts Against Pathogens of Sorghum

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This study examines the antimicrobial activity of Chloroform, Methanolic and aqueous extracts from different plant parts of 15 species which were collected in and around the Visakhapatnam, A.P. The extracts were used to screen *in vitro* for antimicrobial activity against different phytopathogenic microorganisms. Out of these plants eleven species showed antifungal as well as antibacterial activity based on inhibition zones in well diffusion assays. Significant antimicrobial activity was observed in the methanolic extracts of *Terminalia chebula*, *Ocimum sanctum, Hyptis sueolences, Tephrosia villosa, Catheranthus roseus,* etc. on *Pseudomonas syringae*, *Xanthomonas campestris*, *Macrophomina phaseolina*, and *Curvularia lunata*. The susceptibility of different phytopathogenic micro organisms to these plant extracts varied. The antimicrobial activity of extracts of few plants was compared with that of synthetic pesticides of Bavistin, Streptomycin etc. This study indicates the potentiality of these plant extracts in curing the diseases caused by *Pseudomonas syringae*, *Xanthomonas campestris*, *Macrophomina phaseolina*, and *Curvularia lunata*.

Keywords: Phytopathogenic microbes; Plant extracts; Antimicrobial screening; Well diffusion assays; Traditional Indian medicine.

Phytopathogenic bacteria and fungi exploit the living plant cells as a main nutrient source. These bacteria and fungi are highly specialized to circumvent plant defense system and efficiently invade host plant tissues and cause diseases. Once invasion of host tissues takes place, secondary responses are initiated in the plant, which constitute of manifestations of the disease symptoms. The major *sorghum* diseases and their causal organisms are, bacterial leaf spot (*Pseudomonas syringae*), bacterial leaf streak (*Xanthomonas campestris*) fusarium head blight disease (*Fusarium moniliforme, Curvularia lunata),* rust (*Puccinea purpurea),* downy mildew (*Sclerospora sorghi),* ergot (*Sphacelia sorghi),* charcoal rot (*Macrophomina phaseolina*) and other leaf diseases (*Helminthosporium turcicum, Colletotrichum graminicola, Cercospora sorghi, Ascochyta sorghi, Ramulispora sorghi)* etc., causing grate damage/loss to the crop.

Indiscriminate application because of chemical pesticides has caused health hazards in

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animal and humans due to their residual toxicity. In recent years a large number of synthetic pesticides have been banned in the western world because their undesirable attributes such as high and acute toxicity, long degradation periods and accumulation in the food chain (Bernard *et al.* 1997). In developing countries such as India, they are still being used despite their harmful effects (Aron, 1996). Considering the deleterious effects of synthetic pesticides on life supporting system, there is an urgent need for alternative agents for the management of *Sorghum* pathogenic microorganisms. In this context, green plants represent a reservoir of effective chemotherapeutants and can provide valuable sources of natural bactericides and fungicides (Balandrin *et al*. 1985; Hostettmann and Wolfender, 1997). Reports are available on the use of active agents from higher plants (Plant by-products) in place of fungicides, which are non-phytotoxic, more systematic, easily biodegradable and eco-friendly.

In this context, it proposed to elucidate the microorganisms of the antibacterial and antifungal activities of certain medicinal plant extracts on *sorghum* pathogenic organisms of bacterial leaf spot (*Pseudomonas syringae*) bacterial leaf streak (*Xanthomonas campestris*) grain mold disease (*Curvularia lunata),* charcoal rot (*Macrophomina phaseolina*) etc. were chosen to study. In this attempt, *Terminalia chebula* (Combritaceae) fruit (Fig.1) extract, shown significant activity. Hence, this plant is intended to taken up for detailed study for isolation, characterization and identification of compounds responsible for claimed activity. The chemical profile work is in the midway.

MATERIAL AND METHODS

Plant material

The plant materials of 15 different plant species (Table. 1) were collected in and around the Visakhapatnam district and shade dried. After complete dryness they are chopped into small pieces and are coarsely powdered in a wily mill.

Preparation of plant extracts

The extraction method employed here is a known amount of coarsely powdered plant materials were successively extracted with organic solvents like chloroform, methanol and water basing on order of polarity using soxhlet apparatus. The different extracts obtained were subsequently concentrated under reduced pressure to get their corresponding residues. The extracts were screened for antimicrobial activity using the method described under the section.

Organisms and Media

The organisms used were purchased from Microbial Type Culture Collection & Gene Bank (MTCC), Chandigarh.

The strains are maintained and tested on Nutrient Agar (NA) for bacteria and Potato Dextrose Agar (PDA) for fungi.

Antimicrobial assays

The crude extracts of the different plant parts of different species were subjected to antimicrobial assay using the cup plate method of Murray *et al* (1995) modified by Olurinola (1996).

20 ml of nutrient agar was dispensed into sterile universal bottles these were then inoculated with 0.2 ml of cultures mixed gently and poured into sterile petri dishes. After setting a number 3-cup borer (6mm) diameter was properly sterilized by flaming and used to make five uniform cups/ wells in each petri dish. A drop of molten nutrient agar was used to seal the base of each cup.

The cups/wells were filled with 50µ–! of the different extracts of 100mg/ml, 200mg/ml 300mg/ml, 400mg/ml and 500mg/ml and allowed diffusing for 45 minutes. The solvents used for reconstituting the extracts were similarly analyzed. The plates were incubated at 37°c for bacteria and 25°c for fungal organisms.

The standard antibiotic (Streptomycin) and antifungal (Bavistin) drugs were used at different concentrations to get MIC (Minimum inhibitory concentrations). The zones of inhibition were measured with Antibiotic zone scale in mm and the experiment was carried out in duplicates.

RESULTS AND DISCUSSION

The data on the antimicrobial activities of the different plant parts of different species against different bacterial and fungal pathogens of important crop plant Sorghum are shown in table 2

In comparison with plant extracts *Terminalia chebula(Tc)* (Fig.1), *Hyptis*

Table 1. The plants and their parts used are

S.No.	Plant Name	Family	Common Name	Part Used
1.	*Adhatoda vasica*	Acanthaceae	Vasaka	Leaves
2.	*Andrographis paniculata*	Acanthaceae	Nelavamu	Whole plant
3.	*Catherantus roseus*	Apocyanaceae	Billaganneru	Whole plant
4.	*Cassia occidentalis*	caesalpinaceae	Kasinda	Whole plant
5.	*Centella asiatica*	Apiaceae	Saraswati aaku	Whole plant
6.	*Hyptis sueolences*	Lamiaceae	Sema tulasi	Leaves
7.	*Kyllinga nemoralis*	Cyperaceae	Kanda tunga	Leaves
8.	*Melia azadirach*	Meliaceae	Turaka vepa	Leaves
9.	*Ocimum sanctum*	Lamiaceae	Tulasi	Whole plant
10.	*Emblica officinales*	Euphorbiaceae	Usiri	Leaves
11.	*Phyllanthus niruri*	Euphorbiaceae	Nela Usuri	Whole plant
12.	*Pongamia pinnata*	Leguminaceae	Kanuga	Flowers
13.	*Terminalia chebula*	Combritaceae	Karakkaya	Galls
14.	*Tridox procumbens*	Asteraceae	Gaddi chamanti	Whole plant
15.	*Vitex negundo*	Verbenaceae	Tella vavili	Leaves

Table 2. The tested organisms used in this study

S.No.	Bacterial strains	Fungal strains	Imtech No
1.	*Pseudomonas syringae*		MTCC B1604
2.	*Xanthomonas campestris*		MTCC B2286
3.		*Macrophomina phaseolina*	MTCC F2168
4.		*Curvularia lunata*	MTCC F2030

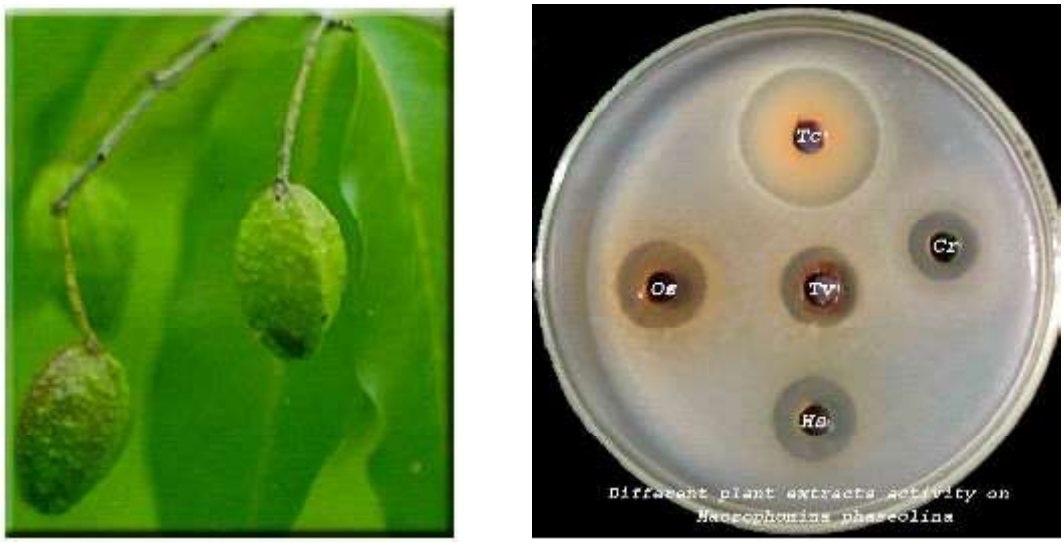

Fig. 1. *Terminalia chebula* small twig Plate.1 with fruits

Table 3. Different herbals antimicrobial properties on phytopathogens A=500mg/ml, B=400mg/ml, C=300mg/ml, D=200mg/ml, E=100mg/ml 6mm cup borer used, Antibiotic = Streptomycin (10mg/ml), Antifungal = Bavistin (10mg/ml)

Plant name	P.syringae					*X.campestris*					*C.lunata*					*M.phaseolina*				
	Zone of inhibition (mm)					Zone of inhibition (mm)					Zone of inhibition (mm)					Zone of inhibition (mm)				
	A	**B**	**C**	**D**	**E**	**A**	**B**	**C**	**D**	**E**	**A**	**B**	**C**	**D**	**E**	**A**	**B**	**C**	**D**	**E**
A.paniculata	15	12	13	11	10	15	12	13	13	12	19	18	17	16	17	16	15	16	15	14
A.vasica	12	11	10	10	9	11	8	7	7	-	19	16	17	16	14	17	16	15	14	14
C.asiatica	9	8	7	8	-	13	11	10	7	-	19	15	11	8	7	21	20	18	19	16
C.occidentalis	-	-	-	-	-	9	8	7	7	7	8	8	8	7	6	13	11	10	9	8
C.roseus	16	15	14	11	11	23	22	18	17	16	22	21	19	20	22	23	21	21	20	17
E.officinales	11	11	9	8	-	12	9	8	8	-	17	15	14	12	11	13	11	9	7	7
H.sueolences	18	16	15	13	14	11	10	10	9	7	15	13	9	8	-	25	23	23	21	20
K.nemoralis	9	7	8	-	-	10	9	9	8	-	8	-	8	7	-	14	12	13	10	11
M.azedarach	12	11	10	7	8	-	-	-	-	-	10	8	8	7	-	11	10	8	7	7
O.sanctum	17	17	16	15	14	28	27	27	25	22	16	15	14	14	13	28	26	24	23	21
P.niruri	11	9	8	8	7	19	18	18	16	16	21	18	17	16	15	21	21	19	18	14
P.pinnata	8	7	-	-	-	12	10	9	8	8	17	16	15	13	11	16	15	15	15	13
T.chebula	22	22	22	21	22	33	28	27	25	28	31	30	30	29	28	35	35	34	31	30
T.procumbens	16	15	13	12	9	15	14	12	13	10	21	19	18	16	14	23	21	20	19	18
V.negundo	9	7	8	8	-	24	18	16	16	15	16	13	12	13	11	20	18	17	15	13
Antibiotic	17	20	-	-																
Antifungal	-	-	18	22																
Chloroform	-	-	-	-																
Methanol	-	-	-	-																

sueolences(*Hs*), *Tridox procumbens(Tp)*, *Ocimum sanctum(Os)* and *Catheranthus roseus*(*Cr*) Showed the maximum inhibition (Plate.1) when compared with rest of the plant extracts against bacteria and fungal pathogens. Where the remaining plant extracts showed a noticeable activity on phytopathogenic microbes.

The solvents used for reconstitution of the extracts showed no activity. The chloroform extract and water extracts were showing less activity when compared with methanolic extracts. Methanolic extracts showed superior activity over fungal pathogens compared to bacterial organisms. Hence methanolic extracts data was reported here.

Since *Terminalia chebula* has showed good inhibitory activity it is to be taken up for isolation of individual compounds. Then screening is to be done with the pure compound/fraction responsible for the claimed activity.

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