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An Alternative Approaches for the Control of Sorghum Pathogens Using Selected Medicinal Plants Extracts

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1. Introduction

Sorghum (*Sorghum vulgare* L.) belongs to the tribe Andropogonae of the grass family Poaceae. The genus Sorghum is characterized by spikelet's borne in pairs. Sorghum is treated as an annual, although it is a perennial grass and in the tropics it can be harvested many times. Sorghum crop production has considerably increased in several countries during the past few years. Sorghum is the fifth important cereals after wheat, rice and maize and are significant dietary food for one-third of the world population, these crops are the principal sources of energy, protein, vitamins and minerals for millions of the poorest people in these regions and sustain the lives of the poorest rural people and will continue to do so in the foreseeable future. India is the world's second largest producer of Sorghum. Like all crops, grain Sorghum is subject to infectious diseases which can sometimes limit production. Sorghum is susceptible to fungal and bacterial micro flora under certain environmental conditions. These mycoflora not only threaten plant growth but also affect food quality, causing huge economic losses. Every year, seed and seedling diseases of grain Sorghum are common in India. Grain Sorghum root rot can be a considerable problem in Sorghum production.

Synthetic pesticides are nowadays widely used for the control of plant diseases throughout the world because of their higher effectiveness in controlling disease causing organisms. However, excessive and unsystematic application of these chemicals has created several environmental and health hazards and also some phytopathogens have been developed resistance (Rhouma et al., 2009**)**. Therefore, there is an urgent need to search for effective, safe and biodegradable alternative pesticides. Diseases of cultivated crops remain the major limitation to increased agricultural production. Therefore, protection of plants from pathogens remains a primary concern of agricultural scientists. Despite serious environmental implications associated with the increased use, chemical fungicides remain the first line of defense against bacterial and fungal pathogens.

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Natural plant products and their analogues are an important source of new agricultural chemicals (Cardellina, 1988, Gulter, 1988). Medicinal plants as a group comprise approximately 8000 species and account for around 50% of all the higher flowering plant species of India. Over one and a half million practitioners of the Indian System of Medicine use medicinal plants in preventive, promotive and curative applications. In recent years, secondary plant metabolites (Phytochemicals), previously with unknown pharmacological activities, have been extensively investigated as a source of medicinal agents (Krisharaju et al, 2005). Plants have been formed the basis of natural pesticides, that make excellent leads for new pesticide development (Newman et al., 2000). The potential of higher plants as a source of new drugs is still largely unexplored. Hence, last decade witnessed an increase in the investigation on plants as a source of new biomolecules for human disease management (Grierson and Afolayan, 1999). Green plants are found to be an effective reservoir for the bioactive molecules and can provide valuable sources for the discovery of natural pesticides (Akhtar *et al.,* 1997). Therefore in recent years medicinal plant extracts are intensively analyzed with an aim of isolating novel bioactive compounds.

2. Materials and methods

2.1 Plant materials

Fifty medicinal plants (Table-1) were selected in this study based on the information collected from literature (Warrier *et al.,* 1994-1996 and Pullaiah, 2002). All the plant materials were collected in and around Visakhapatnam over the course of the respective growth season during February to April in the year 2005 because of the extracts were generally rich in antibacterial agents after the flowering (sexual) stage and plants from stressful environments (Mitscher *et al.,* 1972). Plant materials were identified with the help of Gamble, "Flora of the Presidency of Madras" and later verified by comparison with the authentic specimens available in the herbariums of NBRI, Lucknow and the Department of Botany, Andhra University, Visakhapatnam. Voucher specimens were deposited in the herbarium of the Botany Department, Andhra University, Visakhapatnam.

2.2 Solvents and chemicals used

All chemicals were purchased from Qualigens fine Chemicals, Mumbai and SD fine chemicals, Mumbai. All culture media components and antibiotics used in this study were procured from Hi Media, Mumbai, India.

2.3 Tested organisms

Based on the disease index of Sorghum (Horne and Frederiksen., 1993) crops in which five phytopathogenic microorganisms were selected to screen the antimicrobial inhibition of the selected plant extracts listed in Table-2. The organisms used were procured from Microbial Type Culture Collection & Gene Bank (MTCC), Chandigarh. The lyophilized form of pure strain is reconstituted in sterile water and produced a suspension of the microbial cells. Inoculation was done with sterile inoculating loop to liquid broth medium. Liquid cultures are then incubated to allow cell replication and adequate growth of the culture, for use in bioassays. Following incubation, liquid cultures are refrigerated to store for further use. Typically, 24 hours will provide sufficient growth to allow visibly thick spread of the

microbes as required for bioassay. The bacterial strains are maintained and tested on Nutrient Agar (NA) and Potato Dextrose Agar (PDA) for fungi.

2.4 Preparation of plant extracts

The collected plant materials were chopped into small pieces shade dried and coarsely powdered in Willy mill. The coarsely powdered material weighed and extracted with hexane, chloroform, methanol and water in sequential order of polarity using a soxhlet extractor for five to six hours at temperature not exceeding the boiling point of the solvent. For each gram of dry material 2ml of solvent was used. The extracted solvents were filtered through Whatman no-1 filter paper and subsequently concentrated under reduced pressure (in vacuo at 40°c) using a rotary evaporator. The residue obtained were designated as crude extracts and stored in a freezer at -20°c until assayed.

The dried plant extract residues obtained were redissolved in 0.1% Dimethyl Sulfoxide (DMSO) to get different concentrations (100mg/ml, 300mg/ml and 500mg/ml) of crude extracts and filtration through a 0.45µm membrane filter and stored in sterile brown bottles in a freezer at 20°c until bioassay.

The prepared hexane, chloroform, methanol and water extracts samples were tested for antimicrobial activity against the test organism's the plant pathogens using the agar cup plate method. Streptomycin (5µg) was placed as a positive control in all plates and inoculated with bacteria and for the bacterial cultures used that was incubated at 37°C for 18-24 hours. Bavistin (5µg) was placed as a positive control in all plates inoculated with fungi and for the fungal cultures that were incubated at 26°C for 36-48 h. The microbes were plated in triplicates and average zone diameter was noted.

2.5 Antibacterial activity

The antimicrobial activity of the chloroform, methanol and water extracts of each sample was evaluated by using well diffusion method or cup plate method of Murray *et al.*, (1995) modified by Olurinola, (1996). Which is the most widely used type for identifying the antimicrobial activity, which exploit diffusion of antimicrobial compounds through agar media to demonstrate inhibition of bacteria and fungi.

2.5.1 Composition of nutrient agar medium

Peptone	:	5grams
Meat extract	:	10 grams
Sodium chloride	:	5grams
Agar agar	:	15grams
Distilled water to make	:	1000ml
pH adjusted to	:	7.2 to 7.4

2.5.2 Procedure

This assay performed by two methods agar disc diffusion and agar well diffusion. In these two methods the agar well diffusion essay was used to screen for antimicrobial activity of the hexane, chloroform, methanol and water extracts of different plant species. In agar well

diffusion method peptone (0.5 grams), meat extract (1.0 grams), sodium chloride (0.5 grams) and agar (1.5 grams) were dissolved in small quantity of distilled water with the aid of heat on water bath and the volume was made up to 100 ml with purified water. The pH of the nutrient broth was adjusted to 7.2 using 5M sodium hydroxide, and then sterilized in an autoclave maintained at 121°C (15lbs/sq. in.) for 20 minutes.

After sterilization, the medium was inoculated with 3µl aliquots of culture containing approximately 105 CFU/ml of each organism of 24hours slant culture in aseptic condition and transferred into sterile 6 inch diameter petridishes and allowed to set at room temperature for about 10 minutes and then kept in a refrigerator for 30 minutes. After setting a number 3 cup borer (6mm) diameter was properly sterilized by flaming and used to make three to five uniform cups/wells in each petridish. A drop of molten nutrient agar was used to seal the base of each cup. The cups/wells were filled with 50µl of the different extracts of 100mg/ml, 300mg/ml, and 500mg/ml so final drug concentration will be 5mg/well, 15mg/well, and 25mg/well respectively and allow diffusing of plant extract into the medium for about 45 minutes.

Standard drugs Streptomycin (5µg/ml), control (0.1% DMSO) were transferred to the cups of each agar plate by means of sterile pipettes under a laminar flow unit. The solvents used for reconstituting the extracts were similarly analyzed. The plates thus prepared were left for 2 hours in a refrigerator for diffusion and then kept in an incubator at 37°C. After 24 hours, the agar plates were examined for inhibition zones, and the zones were measured in millimeters. The zones of inhibition were measured with antibiotic zone scale in mm and the experiment was carried out in triplicates.

2.6 Antifungal activity

2.6.1 Composition of PDA medium

Potatoes (peeled)	:	200grams
Dextrose	:	20grams
Agar-Agar	:	15grams
Distilled water to make up to	:	1000ml

2.6.2 Procedure

Peeled potatoes (20grams) were cut into small pieces and boiled with 100ml of water for 30 minutes. The pieces are crushed during boiling and the pulp was removed after cooling by filtration through muslin cloth. Dextrose (2grams) and agar (1.5grams) were added and the volume is made up to 100ml. the medium is then distributed in 20ml quantities in two 250ml conical flasks and were sterilized in an autoclave at 121°C (15lbs/sq. in.) for 30min. the medium was inoculated using 4 days cultures of the test organisms in aseptic condition and transferred to sterile 6 inch diameter petri dishes and allowed to set at room temperature for about 10 minutes. Four cups of 6mm diameter bore in medium at equal distance were made in each agar plate by using sterile borer.

Hexane, chloroform, methanol and water extracts in different concentrations (100mg/ml, 300mg/ml, and 500mg/ml) to get the final drug concentration 5mg/well, 15mg/well, and 25mg/well respectively, control (DMSO) and standard (Bavistin 5µg/ml), were transferred

to the cups of each agar plate, incubated at room temperature (28°C) and examined for inhibition zones of 36 hours of incubation. The results of different studies provide evidence that some medicinal plants might indeed be potential sources of new antibacterial agents even against some antibiotic-resistant strains (Kone *et al.,* 2004).

2.7 Minimum inhibitory concentration (MIC) assays

Based on the preliminary reports all the medicinal plants were identified to have potent antimicrobial activity and Minimum Inhibitory Concentrations (MIC) of the extracts was determined according to Elizabeth, (2001). A final concentration of 0.5% (v/v) Tween-20 (Sigma) was used to enhance crude extract solubility. A series of two fold dilution of each extract, ranging from 0.2 to 100 mg/ml, was prepared. After sterilization, the medium was inoculated with 3µl aliquots of culture containing approximately 10^5 CFU/ml of each organism of 24 hours slant culture in aseptic condition and transferred into sterile 6 inch diameter petridish and allowed to set at room temperature for about 10 minutes and then kept in a refrigerator for 30 minutes. After the media solidified a number 3-cup borer (6mm) diameter was properly sterilized by flaming and used to make three to five uniform cups/wells in each petridish. A drop of molten nutrient agar was used to seal the base of each cup. Different plant crude extracts ranging from 0.2 to 100mg/ml were added to the cups/wells of each petridish and the control plates without plant extract. Inhibition of organism growth in the plates containing test crude extracts was judged by comparison with growth in blank control plates. The MICs were determined as the lowest concentration of extracts inhibiting visible growth of each organism on the agar plate. Similarly the MICs of methanol extracts were determined against all other microorganisms.

3. Results

Among the 50 plant methanol extracts screened thirteen plant extracts showed antibacterial and antifungal activity by zone of inhibition. These results indicated that the plant extracts showed antibacterial as well as antifungal activity. Hexane, chloroform and aqueous extracts were showed very less activity against all the phytopathogens hence only the methanol extracts reports was analyzed. The methanol extracts activities were increased with increasing concentrations. However, the activity produced by the extract was low when compared with that of the standard. The methanol extracts of fifty medicinal plants (Table-1) showed broad spectrum of antimicrobial activity against the test organisms (Table-2) using agar cup plate method. The plant species were *Adenocalymna allicia*, *Acacia farnaciana*, *Avicenia officinales*, *Bridilia Montana*, *Coleus forskohlii*, *Phyllanthus niruri*, *Grewia arborea*, *Melia azadirach, Ocimum sanctum*, *Peltophorum pterocarpum*, *Scoparia dulcis, Terminalia chebula* and *Withania somnifera*, showed a significant activity against *Macrophomina phaseolina*, *Rhizoctonia solani* at less than 50mg/ml concentration.

Of all *Terminalia chebula* and *Melia azadirach* showed remarkable largest zones of inhibition against all the phytopathogens tested. Antimicrobial activities are different medicinal plants were represented in Table 3 and 4. Fruit extract of *Terminalia chebula* showed less than 2mg/ml and *Melia azadirach* below 15mg/ml concentrations showed significant activity on all the pathogens tested in this study.

Botanical Name	**Parts used**	**Uses / Ailments treated**
Acacia farnesiana (L.) Willd	Bark, roots	Astringent, Demulcent, Poultice, Stomachic.
Acalypha indica Linn.	Aerial parts	Skin diseases, Ulcers Bronchitis, Head ache, Snake bite
Acanthus ilicifolius Linn.	Leaf extract	Relieve rheumatism
Adenocalymma alliaceum (Lam.)	Leaves	Astringent,
Adhatoda vasica Nees.	Leaves, whole plant	Cough and chronic bronchitis, rheumatism and asthma.
Andrographis paniculata Nees.	Whole plant, leaves	Anti-biotic, anti-viral, anti-parasitic and immune system stimulant.
Avicennia officinalis L.	Seed	Relieving ulcers
Boerhaavia diffusa Linn.	Whole plant	Scabies, myalgia, aphrodisiac
Bridelia montana (Roxb.) Willd	Bark, Root Leaf	Stomach pains, sore eyes and headaches.
Cassia occidentalis Linn.	Whole plant	Boils, Spasm. Hysteria, Whooping cough
Catharanthus roseus Linn.	Leaves and roots	Anti-mitotic and Anti-microtubule agents
Centella asiatica Linn.	Whole Plant	Diuretic, treatment of leprosy, use as brain tonic and stimulates hair growth.
Cleome viscosa Linn.	*Leaves and seeds*	*Anthelmintic, carminative, diaphoretic and rubefacient.*
Coleus forskohlii (Willd.).	Roots	Treat heart and lung diseases, intestinal spasms, insomnia and convulsions. Antispasmodic.
Coriandrum sativum Linn.	Fruits	Colic, Laxative, Blood purifier, Indigestion, sore throat
Derris scandens (Roxb.) Benth	Stem	Arthritis, Anti-inflammatory
Eichhornia crassipes (C.Mart.)	Whole plant	Biomass, soil reclamation
Emblica officinalis Gaertn.	Fruit	Aperient, Carminative, Diuretic, Aphrodasiac, Laxative, Astringent and Refrigerant.
Gmelina arborea Linn.	leaves and roots	Gonorrhea, catarrh of bladder, cough, cleaning the ulcers, insanity, epilepsy, fevers, indigestion, nerve tonic.
Gynandropsis gynandra (L.)	Leaf	Anti-irritant
Hildegardia populifolia (Roxb.)	Stem bark	Dog bite, Malaria.
Holarrhena antidysenterica Foxh.	Bark and seeds	Dysentery, piles, leprosy, colic, dyspepsia, chronic chest complaints, , spleen diseases, jaundice, bilious, calculi
Hiptage benghalensis (L.) Kurz.	Leaves and bark	Insecticidal, cough, inflammation; skin diseases and leprosy
Hyptis suaveolens (L.) Poit.	Leaves	Antispasmodic, antirheumatic and antisoporific
Kyllinga nemaralis Rottb.	Whole Plant	Promotes action of liver, and relief prunitus
Lantana camara Linn.	Whole Plant	Antidote to snake venom, Malaria, wounds cuts ulcers, Eczema, Tumors

Botanical Name	**Parts used**	**Uses / Ailments treated**
Melia azedarach L.	Leaves, Seed Flower, Oil,	Vermifuge, Insecticide, Astringent, Antiseptic, antidiabitic, anti bacterial and anti viral
Mimosa pudica Linn.	Whole Plant	Menorrhagia, piles, Skin wounds Diarrhoea, Hydrocele, Whooping caugh and Filiriasis
Moringa heterophylla L.	Roots, Seeds,	Antibiotic Anti-inflammatory and Diabetes
Muntinga calabria Linn.	Leaves	Antiseptic
Marraya Koenigii (L.) Spreng.	Leaves	Skin diseases, Heminthiasis, Hyperdipsia, Pruritus, etc.
Ocimum sanctum Linn.	Leaves, Seeds	Malaria, bronchitis, colds, fevers, absorption, arthritis.
Peltophorum pterocarpum (DC.)	Whole plant	Reclamation
Phyllanthus niruri L.	Leaves or herb	Jaundice, Diabetes
Plumeria rubra Linn.	Leaves	Ulcers, leprosy, inflammations, rubefacient.
Pongamia pinnata (L.) Pierre.	Bark, seeds	Antimalaria, skin disease, rheumatic and leprous sores
Ricinus communis Linn.	Leaves	Jaundice, sores,
Salvadora persic, Linn.	Twigs, roots	Antimicrobial and dental diseases
Scoparia dulcis Linn.	Leaves, bark, roots	Used for upper respiratory problems, congestion, menstrual disorders, fever, wounds and hemorrhoids
Sesbania grandiflora (L.) Pers.	Flowers	Gonorrhoea
Strychnos nux vomica Linn.	Seeds	Cholera, chronic wounds, Ulcers, paralysis, Diabetes
Suaeda maritima (L.) Dumort.	Whole plant	Bioremediation
Tephrosia pumila (Lamk.) *Persoon.*	Root	Rheumatism, fevers, pulmonary problems, bladder disorders, Coughing, hair loss, and reproductive disorders
Tephrosia tinctoria Pers.	Root	Antisyphilitic
Tephrosia villosa (L.) Pers.	Root, Leaves, Bark	Anthelmintic, alexiteric, leprosy, ulcers, antipyretic, cures diseases of liver, spleen, heart, blood, asthma etc.
Terminalia chebula Retz.	Fruit	Antimicrobial, cures digestive problems, mouthwash/gargle and astringent,
Tinospora cordifolia (Willd.)	Stem	Analgesic and anti-inflammatory.
Tridax procumbens Linn.	Whole plant	Antimicrobial, Anti-oxidant and Anti-inflammatory,
Vitex pentaphyllal Linn.	Aerial parts	Foetid discharges, Febrifuge Rheumatism affections, catarrhal
Withania somnifera (L.) Dunal	Leaves	Sore eyes, Febrifuge, ulcers Cure sterility of women sedative

Table 1. List of Medicinal plants

Pathogen	MTCC	Disease
Pseudomonas syringae van Hall	B1604	Bacterial spot
Xanthomonas campestris (Pammel) Dowson	B2286	Bacterial leaf streak
Agrobacterium tumifaciens	B7405	Gall disease
Pantoea agglomerans	B2959	Unnamed disease
Erwinia carotovora	B3609	Stem rot
Aspergillus spp	F4633	Seed rot
Colletotrichum graminicola (Ces.) G.W. Wils.	F2232	Seedling blight and seed rot
Fusarium moniliforme J. Sheld	F156	Fusarium head blight, root and stalk rot
Macrophomina phaseolina	F2165	Charcoal rot
Rhizoctonia solani Kuhn.	F 4633	Rhizoctonia root rot, Sheath blight, stalk rot

Table 2. Pathogen index on *Sorghum vulgare* crop

Most of the methanol plant extracts were active towards pathogens. The plant extracts active against fungi are *T. chebula, Melia azadirach, R. communis, Acanthus ilcifolius, Andrographis paniculata, C. roseus, Derris scandens* and *Tephrosia pumila*. Of the five phytopathogenic fungi tested *Rhizoctonia solani* and *Macrophomina phaseolina* were found sensitive strains and evidenced by most of the methanol extracts showed good zone of inhibition on the agar well diffusion assays and *Colletotrichum graminicola* was found resistant when compared with all the fungi tested.

PLANT NAME	*A. tumefaciens*			*E. caratovara*			*P. agglomerans*			*P. syringae*			*X. campestris*		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
Acacia farnesiana	9	14	18	30	35	36	15	15	20	24	26	28	8	9	12
Acalypha indica	9	9	10	9	11	15	9	11	15	9	11	14	7	8	8
Acanthus ilcifolius	10	11	13	12	14	15	-	-	-	11	13	15	7	10	11
Adenocelima allicia	9	12	14	7	8	12	-	9	10	17	19	21	6	7	9
Adhatoda vasica	10	13	15	-	10	15	7	8	12	9	10	12	-	7	11
Andrographis paniculata	12	10	14	7	10	13	-	-	7	10	13	15	12	13	15
Avicenia officinalis	8	13	14	-	7	9	-	-	8	10	14	15	9	11	13
Boerhavia diffusa	8	7	11	10	12	15	7	8	12	-	-	-	-	-	8
Bridilia montana	16	19	25	24	28	29	11	15	18	21	25	24	23	25	26
Cassia occidentalis	8	11	13	-	7	9	-	-	7	-	-	-	7	7	9
Catharanthus roseus	11	10	10	-	8	9	7	11	15	11	14	16	16	18	23
Centella asiatica	9	10	10	-	-	9	-	7	9	-	7	9	-	10	13
Cleome viscosa	9	10	13	12	11	15	9	10	12	11	10	13	9	10	9
Coleus forskohlii	14	15	18	7	8	10	9	11	14	7	8	11	8	9	11
Coriandrum sativum	-	-	-	9	12	10	-	-	-	12	14	15	11	13	14
Derris scandens	10	12	11	-	8	12	-	-	-	16	17	20	7	7	9
Eichhornia crassipes	9	14	13	10	11	14	7	8	10	7	7	11	12	15	14
Emblica officinales	9	10	11	-	-	7	15	14	18	-	9	11	-	8	12
Grewia arborea	15	17	20	20	21	25	19	21	22	8	9	14	-	8	13
Gyanandropsis gyanandra	10	9	14	8	9	9	9	11	11	-	7	7	7	8	12
Heldigordia populipolia	13	15	15	11	14	15	13	15	16	8	9	9	-	7	9

PLANT NAME	*A. tumefaciens*			*E. caratovara*			*P. agglomerans*			*P. syringae*			*X. campestris*		
	A	**B**	**C**	**A**	**B**	**C**	**A**	**B**	**C**	**A**	**B**	**C**	**A**	**B**	**C**
Hoelarrhena antidysenterica	13	14	17	7	8	10	9	10	14	6	7	10	-	-	-
Hyptage bengalenses	-	-	9	-	7	8	-	-	-	9	11	12	7	8	11
Hyptis sueolences	12	11	13	12	13	14	9	10	13	14	15	18	7	10	11
Kyllinga nemoralis	-	-	-	-	7	10	-	-	7	-	8	9	-	9	10
Lantana camara	15	13	13	8	11	13	12	14	15	-	-	7	-	-	7
Melia azedarach	10	15	17	-	-	-	-	9	13	8	10	12	-	-	-
Mimosa pudica	11	12	15	14	13	13	-	-	8	-	-	-	-	-	-
Moringa heterophylla	9	10	12	10	9	12	10	11	14	-	7	8	8	9	11
Muntinga calebria	12	11	10	13	16	21	13	11	15	9	11	15	7	8	8
Murraya koenigii	9	9	11	9	11	12	7	9	9	11	10	15	7	8	10
Ocimum sanctum	9	10	12	-	-	8	13	14	15	14	16	17	22	27	28
Peltophorum pterophorus	21	24	24	19	24	25	20	21	24	9	13	15	18	21	22
Phyllanthus niruri	12	13	15	-	9	12	-	7	10	7	8	11	16	18	19
Plumaria rubrum	-	-	7	-	7	8	-	-	-	15	16	18	-	9	11
Pongamia pinnata	14	13	15	-	-	9	11	13	12	-	-	8	8	9	12
Recinus communis	-	-	-	-	9	9	-	-	-	-	-	9	-	-	9
Salvedara persia	-	-	7	7	8	11	-	-	-	7	10	12	-	7	7
Scoparia dulcis	16	21	20	15	18	17	9	13	14	7	9	11	13	17	19
Sesbanian grandiflora	11	13	16	8	7	10	-	-	-	11	13	14	10	11	15
Strynos nuxvomica	9	10	13	8	11	14	-	-	8	6	10	12	8	9	11
Suaeda maritima	10	9	11	9	12	13	9	10	11	14	15	18	9	8	13
Tephrosia pumila	7	7	9	7	8	10	9	11	13	-	-	-	7	8	8
Tephrosia tinctoria	8	10	11	-	7	7	-	7	10	-	7	8	7	9	10
Tephrosia villosa	14	15	16	-	7	11	-	-	7	-	8	9	6	7	12
Terminalia chebula	19	23	24	26	28	28	11	15	18	22	22	22	28	27	33
Tinospora cordifolia	9	13	14	9	9	11	7	8	10	7	7	9	-	9	10
Tridax procumbens	10	14	12	8	11	14	-	-	9	9	13	16	10	12	15
Vitex negundo	9	11	10	12	10	15	-	9	11	8	9	11	-	7	10
Withania somnifera	17	21	25	18	21	25	-	-	9	13	15	16	9	11	17
Streptomycin (5µg/well)	31			20			25			20			15		

Volume per well: 50µl, A: 100mg/ml=5mg/well, B: 300mg/ml=15mg/well,
C: 500mg/ml= 25mg/well, Borer size used: 6mm
-: no activity, Borer size used: 6mm, Extract /Drug concentration in mg/ml,

Table 3. Antibacterial activity of Medicinal plant crude extracts

PLANT NAME	*A. niger*			*C. graminicola*			*F. moniliformi*			*M. phaseolina*			*R. solani*		
	A	**B**	**C**	**A**	**B**	**C**	**A**	**B**	**C**	**A**	**B**	**C**	**A**	**B**	**C**
Acacia farnesiana	-	-	7	-	10	13	18	20	21	17	16	18	-	7	13
Acalypha indica	17	19	25	-	-	-	9	10	14	8	8	10	26	27	29
Acanthus ilcifolius	9	11	13	-	-	9	8	11	14	12	14	15	9	13	15
Adenocelima allicia	10	16	17	9	13	14	16	21	25	15	19	22	11	14	16
Adhatoda vasica	7	9	12	7	8	10	9	11	14	14	15	17	13	14	15
Andrographis paniculata	9	13	15	-	8	12	-	8	8	14	16	16	10	14	15

PLANT NAME	*A. niger*			*C. graminicola*			*F. moniliformi*			*M. phaseolina*			*R. solani*		
	A	**B**	**C**	**A**	**B**	**C**	**A**	**B**	**C**	**A**	**B**	**C**	**A**	**B**	**C**
Avicenia officinalis	10	15	17	-	7	7	11	12	15	20	24	27	10	11	14
Boerhavia diffusa	-	7	10	9	10	13	7	9	9	8	10	10	-	-	-
Bridilia montana	7	9	11	8	12	18	8	12	15	15	18	20	7	10	12
Cassia occidentalis	-	7	9	7	10	13	9	-	-	8	10	13			
Catharanthus roseus	-	-	8	7	9	16	12	14	15	17	21	23	9	11	12
Centella asiatica	-	-	7	-	7	10	-	8	13	16	18	21	7	11	14
Cleome viscosa	18	21	24	9	11	15	13	17	23	20	21	25	19	22	26
Coleus forskohlii	15	17	21	8	9	10	12	16	19	16	18	21	15	17	19
Coriandrum sativum	10	14	17	-	9	9	-	-	-	7	9	13	-	-	-
Derris scandens	19	21	24	7	7	9	11	13	17	16	15	19	13	14	17
Eichhornia crassipes	13	13	17	-	-	-	9	10	12	12	14	17	10	11	15
Emblica officinales	-	-	-	-	7	9	-	-	-	7	9	13	-	-	-
Grewia arborea	21	24	28	9	11	11	12	15	20	20	21	25	18	19	24
Gyanandropsis gyanandra	6	7	8	-	-	-	10	10	12	8	8	0	8	9	11
Heldigordia populipolia	-	-	-	8	10	12	7	9	11	8	9	11	7	7	9
Hoelarrhena antidysenterica	7	9	12	7	9	13	14	13	18	6	8	8	8	10	11
Hyptage bengalenses	12	13	16	-	7	8	10	13	16	9	10	12	9	10	11
Hyptis sueolences	-	-	-	9	11	14	7	8	11	20	23	25	12	13	14
Kyllinga nemoralis	7	8	11	-	-	8	-	8	14	11	13	14	7	8	8
Lantana camara	-	-	8	8	7	11	-	-	-	10	11	16	9	12	16
Melia azedarach	21	19	30	12	15	18	20	20	22	7	8	11	35	38	45
Mimosa pudica	9	10	13	-	7	10	8	9	12	10	12	14	-	-	7
Moringa heterophylla	-	-	-	7	9	15	8	8	12	8	10	14	12	15	17
Muntinga calebria	14	17	19	-	8	12	9	12	21	10	9	14	10	10	14
Murraya koenigii	10	13	17	9	11	18	12	15	19	13	15	18	15	16	18
Ocimum sanctum	9	11	12	8	10	14	13	15	16	21	24	28	10	13	14
Peltophorum pterophorus	21	22	29	10	14	19	11	13	17	22	24	27	33	35	40
Phyllanthus niruri	-	10	15	-	-	7	10	7	13	14	19	21	11	13	15
Plumaria rubrum	9	9	13	-	8	8	-	-	9	10	13	13	18	21	25
Pongamia pinnata	-	-	8	-	-	7	8	9	12	13	15	17	7	9	11
Recinus communis	10	14	21	7	10	11	-	-	-	15	18	21	-	-	-
Salvedara persia	12	15	19	-	8	11	-	-	-	19	21	21	12	15	16
Scoparia dulcis	17	21	24	9	11	16	12	16	22	11	14	18	14	19	22
Sesbanian grandiflora	8	8	11	7	8	8	16	19	22	21	25	29	12	15	17
Strynos nuxvomica	-	-	-	-	7	8	-	-	10	17	21	23	-	8	9
Suaeda maritima	12	15	19	-	-	-	10	13	14	13	16	17	14	17	21
Tephrosia pumila	7	7	9	-	-	-	7	9	13	-	-	-	-	-	-
Tephrosia tinctoria	7	7	10	7	10	12	-	-	-	10	12	14	-	-	7
Tephrosia villosa	-	-	8	7	9	16	-	-	7	20	25	26	10	11	15
Terminalia chebula	19	21	25	11	16	20	18	25	29	30	34	35	8	11	14
Tinospora cordifolia	7	9	13				11	15	18	17	18	24	9	8	10
Tridax procumbens	-	-	8	-	8	9	9	10	14	18	20	23	-	7	9
Vitex negundo	-	-	-	-	7	9	7	7	9	13	17	20	8	12	13
Withania somnifera	9	10	14	9	12	14	13	15	17	20	25	26	13	16	21
Bavistin (5µg/well)	32			25			28			20			35		

Volume per well: 50µl, A: 100 mg/ml = 5 mg/well, B: 300 mg/ml =15 mg/well,
C: 500 mg/ml= 25 mg/well, Borer size used: 6mm
na: no activity, Borer size used: 6mm, Extract /Drug concentration in mg/ml,

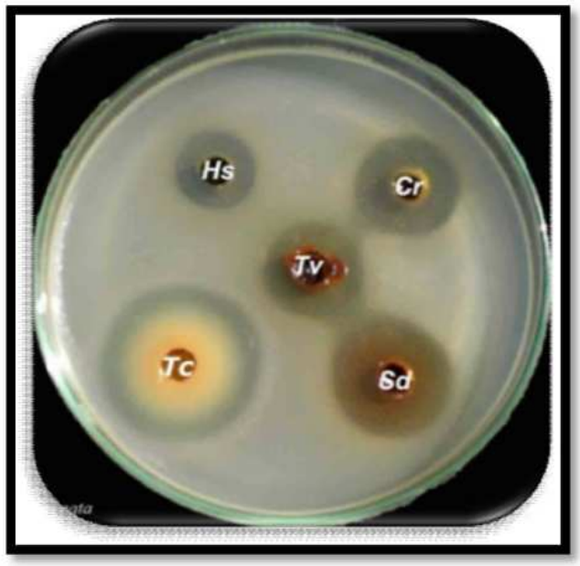
Table 4. Antifungal activity of Medicinal plant crude extracts

PLANT NAME	*A.tumifaciens*	*E.caratovora*	*P.agglomerans*	*P.syringae*	*X.campestris*	*A.niger*	*C.graminicola*	*F.moniliforme*	*M.phaseolina*	*R.solani*
Acacia farnaciana	75	2	50	100	85	na	100	50	50	300
Acalypha indica	90	90	100	100	100	75	na	100	100	50
Acanthus ilcifolius	na	85	na	75	100	90	na	100	85	100
Adenocelima allicia	90	90	300	90	150	90	75	75	75	90
Adhatoda vasica	90	300	85	100	100	100	100	100	85	85
Andrographis paniculata	85	90	na	100	85	90	na	300	85	90
Avicenia officinales	na	300	na	85	90	90	na	90	75	90
Boerhavia diffusa	100	100	100	100	na	300	28	26	22	18
Bridilia montana	50	25	90	25	25	100	100	100	100	na
Cassia occidentalis	100	100	100	100	100	300	100	100	75	100
Catheranthus roseus	85	100	100	100	75	na	150	100	100	150
Centella asiatica	90	90	85	100	300	na	150	90	75	90
Cleome viscosa	85	85	100	85	90	75	na	300	75	100
Coleus forskohlii	75	90	25	90	100	75	100	90	75	75
Coriandrum sativum	na	90	na	85	85	90	100	85	75	75
Derris scandens	100	300	na	75	100	75	150	na	100	na
Eichhornia crassipes	90	100	100	90	90	85	100	90	75	85
Emblica officinales	85	100	50	100	100	na	na	100	85	75
Grewia arborea	75	25	75	100	300	50	200	na	100	na
Gyanandropsis gyanandra	85	75	85	90	100	300	100	90	2.5	50
Heldigordia populipolia	75	100	75	100	300	na	na	90	100	100
Hoelarrhena antidysenterica	75	85	75	85	100	100	90	100	100	100
Hyptage bengalenses	300	300	na	90	100	85	100	75	300	100
Hyptis sueolences	75	85	75	100	100	na	150	90	100	90
Kyllinga nemoralis	100	300	na	90	300	100	100	100	50	85
Lantana camara	75	100	85	100	na	na	Na	300	85	100
Melia azedarach	75	na	100	25	na	50	100	na	90	75
Mimosa pudica	85	85	85	85	na	100	75	50	100	75
Moringa heterophylla	90	90	100	100	100	na	150	100	90	na
Muntinga calebria	85	85	85	85	100	75	100	100	100	85
Murraya koenigii	90	100	100	90	100	90	150	100	90	75
Ocimum sanctum	85	85	90	90	75	100	90	85	75	75
Peltophorum pterophorus	50	75	5	75	75	50	100	85	75	90
Phyllanthus niruri	85	100	85	90	90	300	90	90	50	10
Plumaria rubrum	na	300	200	75	300	100	na	90	85	90
Pongamia pinnata	75	na	85	100	100	na	na	na	85	50
Recinus communis	na	300	150	na	na	90	na	100	90	100
Salvedara persia	100	100	na	100	300	85	100	na	75	na
Scoparia dulcis	15	22	12	30	75	45	150	na	75	75
Sesbanian grandiflora	85	100	na	90	85	100	25	20	15	25
Strynos nuxvomica	90	85	85	100	100	na	150	na	75	300
Suaeda maritima	85	90	90	75	90	85	na	90	85	85
Tephrosia pumila	na	100	100	na	100	100	na	100	na	100
Tephrosia tinctoria	100	100	100	na	100	100	100	na	100	na
Tephrosia villosa	85	100	75	90	100	na	100	na	75	90
Terminalia chebula	25	75	2.5	75	50	50	75	50	5	85
Tinospora cordifolia	90	100	100	100	300	100	na	90	75	100
Tridax procumbens	85	100	100	na	100	na	150	100	75	300
Vitex negundo	90	85	100	90	300	na	150	100	85	100
Withania somnifera	50	75	25	85	100	100	90	85	75	75

Volume per well: 50µl, Borer size used: 6mm, na: no activity,
Borer size used: 6mm, Extract /Drug concentration in mg/ml,

Table 5. Antimicrobial activity (MIC) of different plant crude extracts

The methanol extracts of *Terminalia chebula* fruit had potent antimicrobial activity at less than 25mg/ml concentrations. The solvent control of hexane, chloroform, methanol, and DMSO had no effect on microbial growth. And the standard synthetic fungicide Bavistin and antibacterial drugs of Streptomycin and Penicillin had a variety of activity against all the pathogens tested.

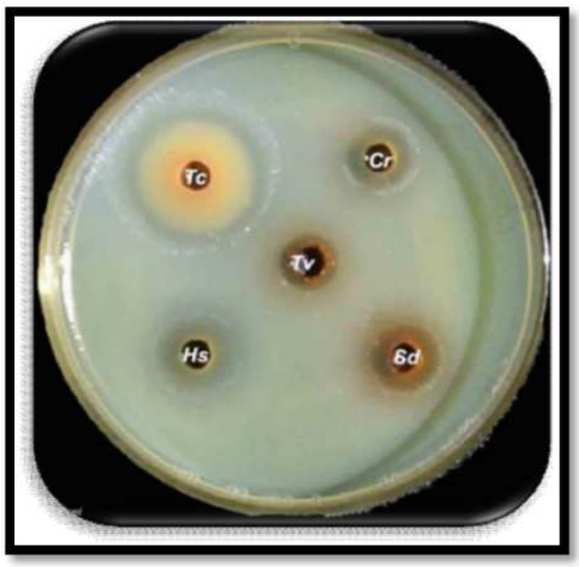


Tc - *T. chebula*, Sd - *S. dulcis*, Hs - *H. sueolences*, Cr - *C. roseus*, Tv - *T. villosa*, Bm - *B. montana*, Eo - *E. officinales*, Pn - *P. niruri*, Av - *A. vasica*, Ap - *A. paniculata*, Aa - *A. allicia*.

Fig. 1. Different plant extracts activity on *M. phaseolina*

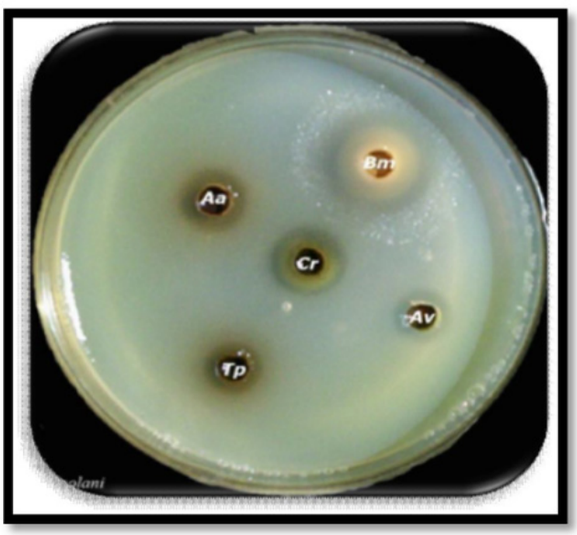
Tc - *T. chebula*, Sd - *S. dulcis*, Hs - *H. sueolences*, Cr - *C. roseus*, Tv - *T. villosa*, Bm - *B. montana*, Eo - *E. officinales*, Pn - *P. niruri*, Av - *A. vasica*, Ap - *A. paniculata*, Aa - *A. allicia*.

Fig. 2. Different plant extracts activity on *M. phaseolina*



Tc - *T. chebula*, Sd - *S. dulcis*, Hs - *H. sueolences*, Cr - *C. roseus*, Tv - *T. villosa*, Bm - *B. montana*, Eo - *E. officinales*, Pn - *P. niruri*, Av - *A. vasica*, Ap - *A. paniculata*, Aa - *A. allicia*.

Fig. 3. Different plant extracts activity on *R. solani*



Tc - *T. chebula*, Sd - *S. dulcis*, Hs - *H. sueolences*, Cr - *C. roseus*, Tv - *T. villosa*, Bm - *B. montana*, Eo - *E. officinales*, Pn - *P. niruri*, Av - *A. vasica*, Ap - *A. paniculata*, Aa - *A. allicia*.

Fig. 4. Different plant extracts activity on *R. solani*

4. Discussion

Natural products isolated from higher plants have been providing novel, antimicrobial drugs. Historically, many plant oils and extracts, such as tea tree, clove, Etc. have been used

as topical antiseptics, or have been reported to have antimicrobial properties (Hoffman 1987 and Lawless 1995). It is important to investigate scientifically those plants which have been used in traditional medicines as potential sources of novel antimicrobial compounds (Mitscher *et al.,* 1987). Also, the resurgence of interest in natural therapies and increasing consumer demand for effective, safe, natural products means that quantitative data on plant oils and extracts are required.

Majority of studies conducted the search of compounds with antimicrobial properties have targeted plants with a history of ethno botanical uses (Janovska *et al.,* 2003), most of the medicinal plant species screened in this study were previously been surveyed for antimicrobial activities on human pathogens. And very few citations were reported on phytopathogens (Kaushik and Arora, 2003; Jaspal singh and Tripathi, 1993; Krishna kishore and Suresh pande, 2005; Meena and Goplakrishnan, 2005). The observed antimicrobial activity of these plant extracts, and isolated compounds were of highly remarkable.

The present study was designed to obtain information on the antimicrobial effect of 50 Indian medicinal plants on certain plant pathogenic microorganisms. The well diffusion/cup plate method was used in this study since it was found to be better than the disc diffusion method. All the medicinal plant extracts and isolated compounds showed antimicrobial activity against selected pathogens of Sorghum.

Hexane extracts never showed antimicrobial activity. The chloroform and water extracts showed very less antimicrobial activity compared with methanol extracts. This may be due to little diffusion properties of these extracts in the agar or because fresh plants contain active substances which may be affected or disappeared by the steps of extraction methods.

The methanol extracts of all the medicinal plant screened (Table-1) exhibited grater antimicrobial activity. According to Darout *et al.,*(2000) the antimicrobial action of methanol extracts is due to the compounds such as thiocynate, nitrate, chloride and sulphates beside other high polarity soluble compounds which are naturally occurring in most plant materials.

Methanolic extracts of *T. chebula, B. Montana, M. azadirach, W. somnifera, O santum* and *P. pterocarpum* showed greater antimicrobial activity. *Terminalia chebula* possessed 32-40% of tannin content and the antibacterial activity may be indicative of the presence of some metabolic toxins or broad-spectrum antibiotic compounds (Fundter *et al.,* 1992). *M. azadirach* was exhibited good antimicrobial activity against most of the tested pathogens in this study. According to Jacobson, (1995) this activity is due to Nimbidin, extracted from *M. azadirach* demonstrated several biological activities. From this crude principle some tetranortriterpenes, including nimbin, nimbinin, nimbidinin, nimbolide and nimbidic acid have also been showing antimicrobial activities.

The observations reveal that tested medicinal plant methanol extracts activity against all phytopathogenic species. As evidenced, the fungal strains that were sensitive are *M. phaseolina*, *R. solani* species, *C. graminicola* and *F. moniliforme* found to be resistant strains. Among the tested medicinal plants methanol extracts against the phytopathogenic species, *Terminalia chebula* extracts showed greater antimicrobial activity on all plant pathogens.

In view of the changing agricultural policies throughout the world complete disease control is no longer a target of plant pathologist's reducing the threshold level using cost-effective and eco-friendly management option is the focus of the day. In this context identification of aqueous leaf extract of *T. chebula* and *M. azadirach* methanol extracts as bactericides and fungicides against the pathogens tested are highly significant recommendable. The result of these studies maybe helpful in developing/synthesizing the plant based natural fungicides and insecticides that may be for preventing and curing the common destructive diseases of *Sorghum* crop and other cereal crops. In this context the studied plant extracts is more appropriate and helpful in synthesizing the plant based biofungicides to reduce the pathogen population to lower economic threshold level using cost effective and eco friendly management. This will also offer a great help in facing the emergence spread of antimicrobial resistance.

5. References

Akhtar M.A., M.H. Rahber-Bhatti, M. Aslam, *International Journal of Pest Management*, 1997, 43, *2*, 149-153.

Cardellina J. H. (1988): Biologically active Natural Products: Potential Use in Agriculture, 305-311.

Darout, I., Cristy, A., Skaug, N., and P. Egeberg, 2000. Identification and quantification of some potentially antimicrobial anionic components in Miswak extract. *Ind J Pharm*, 32:11-4.

Elizabeth, M., Adrien Szekely, Johnson and David W. Warnock., 2001. *Journal of Clinical Microbiology*, 37(5):1480-1483.

Fundter, J.M., *et al.*, 1992. *Terminalia chebula* Retz. In Lemmens, R.H.M.J. & Wulijarni-Soetjipto, N. (Eds.): Plant Resources of South-East Asia. No. 3: *Dye and tannin-producing plants*, pp 122-125.

Grierson DS, Afolayan AJ (1999). An ethnobotanical study of plants used for the treatment of wounds in the Eastern Cape, South Africa. Ethnopharmacol 67: 327-332.

Gulter H.G. (1988): Natural products and their potential in agriculture. A personal over review., 1-22.

Hoffman, D. L. 1987. *The Herb User's Guide*. Wellingborough, UK: Thorsons Publishing Group.

Horne, C. W., and R. A. Frederiksen, 1993. (http://www.apsnet.org/online/common/names/sorghum.asp).

Jacobson, M., 1995. In the Neem Tree: Source of Unique Natural Products for Integrated Pest Management, Medicine, Industry and other Purposes (ed. Schmutterer, H.), pp. 484-495.

Janovska, D., Kubikova, K., Kokoska, L. 2003. Screening for antimicrobial activity of some medicinal plant species of traditional Chinese medicine. *Czech. J. Food Sci*. 21: 107-111.

Jaspal Singh., and N. N. Tripathi., 1993. *Journal of the Indian Botanical Society*, 72 (1-2) :51-53.

Kaushik, R. D. and Charu Arora, 2002. Fungitoxic activity of methanol extracts of some plants of kamaun, garhwal and tarai regions against fungal pathogens of rice. *Journal of Indian botanical sciences*, 81:327-331.

Kone, W. M., Atindehou, K. K., Terreaux, C., Hosetettman, K., Traore, D., and M. Dosso, 2004. Screening of 50 medicinal plants for antibacterial activity. *Ethnopharmacol Bul*, 93(1):43-49.

Krisharaju A.V. and Rao T. V. N. Sundararaju (2005): Assessment of bioactivity of Indian medicinal plants using Brine shrimp (Alternaria solania) lethality assay. Int. J. Appl. Sci. Engg.,2, 125- 134.

Krishna Kishore, G., and Suresh Pande, 2005. Integrated management of the late leaf spot and rust disease of groundnut with *Prosopis* leaf extract and chlorothalonil. *International journal of pest management*, 51(4):327-334.

Lawless, J., 1995. *The Illustrated Encyclopedia of Essential Oils*. Shaftesbury, UK: Element Books Ltd.

Meena, C., and J. Gopalakrishnan, 2005. Efficacy of plant extracts against bacterial blight. *Annals of plant protection sciences*, 12(2):344-346.

Mitscher, L. A., Drake, S., Gollapudi, S.R. and S. K. Okwute, 1987. A modern look at folkloric use of anti-infective agents. *Journal of Natural Products,* 50:1025–1040.

Mitscher, L. A., R. P. Leu., M. S. Bathala., W. N. Wu., and J. L. Beal, 1972. Antimicrobial agents from higher plants. Introduction, rationale and methodology. Lloydia, 35:157-166.

Murray, S. S., Chappell, J. H., Kenter, A. T., Kraft, R. P., Meehan, G. R., and M. V. Zombeck, 1995. *Proc, SPIE* 3356:974.

Newman D. J., Cragg G. M. and Snader K. M. (2000): The influence of natural products upon drug discovery. Natural Product Research, 17, 215- 234.

Olurinola, P. F., and Ibrahim, Y. K., 1991. Comparative Microbial Contamination Levels in Wet Granulation and Direct Compression Methods of Tablet Production, *Pharm. Acta. Helv*, 66:298-301.

Pullaiah, T., 2002. Medicinal Plants in India, Regency Publications. New Delhi.

Rhouma A, Ben Daoud H, Ghanmi S, Ben Salah H, Romdhane M, Demak M, *Journal of Plant Pathology*, 2009, 91, *2*, 339-345.

Warrier P. K., (1994-1996) Indian Medicinal Plants- A compendium of 500 species Vol. 5, p 396.

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