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

Varaprasad Bobbarala¹,* and Chandrasekhar K. Naidu²

¹Translational Research Institute of Molecular Sciences (TRIMS), Visakhapatnam, AP, India
²Department of Botany, College of Science and Technology, Andhra University, Visakhapatnam, India

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.

* Corresponding Author
Natural plant products and their analogues are an important source of new agricultural chemicals (Cardellina, 1988, Guler, 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

| Component                  | Amount  |
|----------------------------|---------|
| 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 121ºC (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 37ºC. 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

| Ingredient       | Quantity |
|------------------|----------|
| Potatoes (peeled)| 200 grams|
| Dextrose         | 20 grams |
| Agar-Agar        | 15 grams |
| Distilled water  | 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 121ºC (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
An Alternative Approaches for the Control of Sorghum Pathogens Using Selected Medicinal Plants Extracts

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, Pentophorum 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, diarrhetic 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                                 |
| Echthornia crassipes (C.Mart.) | Whole plant       | Biomass, soil reclamation                                    |
| Emblica officinalis Gaertn. | Fruit               | Aperient, Carminative, Diuretic, Aphrodisiac, Laxative, Astringent and Refrigerant. |
| Gmelina arborea Linn.       | leaves and roots    | Gonorrhea, catarrh of bladder, cough, cleaning the ulcers, insanity, epilepsy, fevers, indigestion, nerve tonic. |
| Gymnandropsis 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 nemoralis 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, anti diabetics, anti bacterial and anti viral |
| *Mimosa pudica* Linn.          | Whole Plant      | Menorrhagia, piles, Skin wounds Diarrhoea, Hydrocele, Whooping cough and Filariasis     |
| *Moringa heterophylla* L.      | Roots, Seeds,    | Antibiotic Anti-inflammatory and Diabetes                                               |
| *Muntinga calabra* 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 punila* (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
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 ilicifolius, 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.

| 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                  |
| *Clotetotrichum 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, Sheath blight, stalk rot |

| PLANT NAME                 | *A. tumefaciens* A B C | *E. carotovara* A B C | *P. agglomerans* A B C | *P. syringae* A B C | *X. campestris* 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 ilicifolius      | 10 11 13               | 12 14 15              | - - -                 | 11 13 15            | 7 10 11               |
| Adenocelina allicia       | 9 12 14                | 7 8 12                | - 9 10                | 17 19 21            | 6 7 9                 |
| Adhatoda vasicia          | 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              |
| Aevicia officinalis       | 8 13 14                | - 7 9                 | - - 8                 | 10 14 15            | 9 11 13               |
| Boerhavia diffusa        | 8 7 11                 | 10 12 15              | 7 8 12                | - - -               | - - -                 |
| Bridalia 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                 |
| Catharanthus roseus       | 11 10 10               | - 8 9                 | 7 11 15               | 11 14 16            | 16 18 23              |
| Centella asiatica         | 9 10 10                | - 9                   | 7 9 11                | 7 9 12              | - 10 13               |
| Cleome viscosa            | 9 10 13                | 12 11 15              | 9 10 12               | 11 10 13            | 9 10 9                |
| Coleus forskohii          | 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 officinale        | 9 10 11                | - 7 15 14             | - 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                 |
### Table 3. Antibacterial activity of Medicinal plant crude extracts

| PLANT NAME                      | A. tumefaciens | E. caratovara | P. agglomerans | P. syringae | X. campestris |
|---------------------------------|---------------|---------------|----------------|-------------|--------------|
| Hoellarrhena 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            | 9            | 11           |
| Muntinga caerulea              | 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           | 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 pinata                | 14            | 13            | 15             | -            | 9            | 11           | 13           | 12           | -            | 8            | 8            | 9            | 12           |              |
| Recinus communis               | -             | -             | -              | 9            | 9             | -            | -            | -            | -            | -            | 9            | -            | -            | 9            |
| Salvadora persis               | -             | 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           |
| Sesbania 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 puniula              | 7             | 7             | 9              | 7            | 8             | 10           | 9            | 11           | 13           | -            | -            | 7            | 8            | 8            |               |
| Tephrosia tinctora             | 8             | 10            | 11             | -            | 7            | 7            | -            | 7            | 10           | -            | 7            | 8            | 7            | 9            | 10           |
| Tephrosia villosa              | 14            | 15            | 16             | -            | 7             | 11           | -            | 7            | -            | 8            | 9            | 6            | 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            | 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           | 20           |              |               |               |               |

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 4. Antifungal activity of Medicinal plant crude extracts

| PLANT NAME                  | A. niger | C. graminicola | F. moniliformi | M. phaseolina | R. solani |
|-----------------------------|----------|----------------|----------------|--------------|-----------|
| A                           | 10       | 15             | 7              | 12           | 15        |
| B                           | -        | 10             | 9              | 13           | -         |
| C                           | 7        | 11             | 8              | 12           | 8         |
| A                           | 9        | 11             | 8              | 12           | 8         |
| B                           | 10       | 13             | 9              | -            | 8         |
| C                           | -        | 10             | 13             | -            | 10        |
| A                           | 7        | 10             | 9              | -            | 7         |
| B                           | -        | 7              | 10             | 8            | 16        |
| C                           | -        | 8              | 12             | 14           | 15        |
| A                           | -        | 7              | 9              | 16           | 14        |
| B                           | 10       | 13             | 15             | 17           | 23        |
| C                           | -        | 17             | 21             | 23           | 9         |
| A                           | 10       | 14             | 17             | 9            | -         |
| B                           | 10       | 14             | 17             | 15           | 19        |
| C                           | -        | 20             | 21             | 25           | 7         |
| A                           | 7        | 9              | 13             | 17           | -         |
| B                           | 9        | 11             | 15             | 17           | 17        |
| C                           | 10       | 12             | 16             | 19           | 21        |
| A                           | 15       | 21             | 27             | 10           | 11        |
| B                           | 11       | 14             | 19             | -            | -         |
| C                           | 10       | 12             | 13             | -            | -         |
| A                           | 10       | 13             | 15             | 20           | 21        |
| B                           | 11       | 14             | 19             | 25           | 7         |
| C                           | 10       | 11             | 13             | 18           | 14        |
| A                           | 12       | 21             | 27             | 10           | 11        |
| B                           | 11       | 14             | 19             | 25           | 7         |
| C                           | 10       | 11             | 13             | 18           | 14        |
| A                           | 12       | 15             | 19             | 19           | 21        |
| B                           | 11       | 13             | 17             | 15           | 16        |
| C                           | 10       | 12             | 14             | 16           | 16        |
| A                           | 10       | 14             | 17             | 13           | 15        |
| B                           | 10       | 15             | 19             | 17           | 11        |
| C                           | 10       | 12             | 14             | 16           | 14        |
| A                           | 12       | 15             | 20             | 16           | 18        |
| B                           | 11       | 14             | 19             | 25           | 7         |
| C                           | 10       | 13             | 17             | 16           | 14        |
| A                           | 12       | 15             | 20             | 16           | 18        |
| B                           | 11       | 14             | 19             | 25           | 7         |
| C                           | 10       | 13             | 17             | 16           | 14        |
| A                           | 12       | 15             | 20             | 16           | 18        |
| B                           | 11       | 14             | 19             | 25           | 7         |
| C                           | 10       | 13             | 17             | 16           | 14        |

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,
| PLANT NAME | A. tumifaciens | C. carotovora | P. agglomerans | P. syringae | X. campestris | A. niger | C. graminicola | F. moniliforme | M. phaseolina | R. solani |
|------------|--------------|--------------|--------------|------------|------------|--------|--------------|--------------|--------------|---------|
| Acacia farneciana | 75 | 2 | 50 | 100 | 85 | na | 100 | 50 | 50 | 300 |
| Acalypha indica | 90 | 90 | 100 | 100 | 100 | 75 | na | 100 | 100 | 50 |
| Adenocaulon allicia | na | 85 | na | 75 | 100 | 90 | na | 100 | 85 | 100 |
| Adenocaulon alificia | 90 | 90 | 300 | 90 | 150 | 90 | 75 | 75 | 75 | 90 |
| Andrographis paniculata | 85 | 90 | na | 100 | 85 | 90 | na | 300 | 85 | 90 |
| Artenema officinatales | na | 300 | na | 85 | 90 | na | na | 90 | 75 | 90 |
| Benthavia diffusa | 100 | 100 | 100 | 100 | na | 300 | 28 | 26 | 22 | 18 |
| Brelia montana | 50 | 25 | 90 | 25 | 25 | 100 | 100 | 100 | na | 100 |
| Cassia occidentalis | 100 | 100 | 100 | 100 | 100 | 300 | 100 | 100 | 75 | 100 |
| Cattenarius rooseus | 85 | 100 | 100 | 100 | 75 | na | 150 | 90 | 75 | 90 |
| Cloroxicaciens | 85 | 85 | 100 | 85 | 90 | 75 | na | 300 | 75 | 100 |
| Coleus forskohlii | 75 | 90 | 25 | 90 | 100 | 75 | 100 | 90 | 75 | 90 |
| Coriandrum sativum | na | 90 | na | 85 | 85 | 90 | 100 | 85 | 75 | 75 |
| Derris scandens | 75 | 300 | na | 75 | 100 | 75 | 150 | na | 100 | na |
| Eichhornia crassipes | 90 | 100 | 100 | 90 | 90 | 85 | 100 | 90 | 75 | 85 |
| Emblica officinalis | 85 | 100 | 50 | 100 | 100 | na | na | 100 | 85 | 75 |
| Grewia arborea | 25 | 75 | 25 | 100 | 300 | 50 | 200 | na | 100 | na |
| Gyanandropsis ganewus | 85 | 75 | 85 | 90 | 100 | 300 | 100 | 90 | 2.5 | 50 |
| Heliodorina populifolia | 75 | 100 | 75 | 100 | 300 | na | na | 90 | 100 | 100 |
| Helianthus annuus | 85 | 75 | 75 | 85 | 100 | 100 | na | 90 | 100 | 100 |
| Hyptage bruguieres | 300 | 300 | na | 90 | 100 | 85 | 100 | 75 | 300 | 100 |
| Hyptis sueolences | 75 | 85 | 75 | 85 | 100 | 85 | 85 | na | 90 | 75 |
| Kalliena nemoralis | 100 | 300 | na | 90 | 300 | 100 | 100 | 100 | 50 | 85 |
| Laminae cannabla | 75 | 100 | 85 | 100 | na | na | Na | 300 | 85 | 100 |
| Melia azedarach | 85 | na | 85 | na | 100 | 75 | 100 | 50 | 100 | 75 |
| Morinda heterophylla | 90 | 90 | 100 | 100 | 100 | na | 150 | 90 | 100 | 90 |
| Mustanga calebria | 85 | 85 | 85 | 85 | 100 | 75 | 100 | 100 | 100 | 85 |
| Murraya koeingi | 90 | 100 | 100 | 90 | 100 | 90 | 150 | 100 | 100 | 85 |
| Ocimum saccharum | 85 | 85 | 90 | 90 | 75 | 100 | 90 | 85 | 75 | 75 |
| Peltophorum pterophorus | 85 | 100 | 85 | 90 | 90 | 300 | 90 | 90 | 50 | 10 |
| Plectranthus pterophorus | 50 | 75 | 5 | 75 | 75 | 50 | 100 | 85 | 75 | 90 |
| Plectranthus munari | na | 300 | 200 | 75 | 300 | 100 | na | 90 | 85 | 90 |
| Pongamia pinnata | 75 | na | 85 | 100 | na | na | na | 85 | 50 | 10 |
| Recinun communis | na | 300 | 150 | na | na | na | na | 90 | 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 |
| Scoparia grandiflora | 85 | na | 90 | 85 | 100 | 25 | 20 | 15 | 25 | 10 |
| Sesbania nucumnea | 90 | 85 | 85 | 100 | 100 | na | 150 | 75 | 300 | 100 |
| Sida marina | 85 | 90 | 90 | 75 | 90 | 85 | na | 90 | 85 | 85 |
| Tephrosia pumila | na | 100 | 100 | na | 100 | 100 | na | 100 | na | 100 |
| Tephrosia torroides | 100 | 100 | 100 | na | 100 | 100 | na | 100 | na | 100 |
| Tephrosia vellosa | 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 | 100 | na | na | 90 | 75 | 100 |
| Tridax procumbens | 85 | 100 | 100 | na | 100 | 100 | na | 150 | 100 | 75 |
| 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.

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

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*

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 greater 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 Nimbinid, 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., Hoasetetttman, 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.