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ANTIMICROBIAL ACTIVITY OF SOME MEDICINAL PLANT'S METHANOLIC EXTRACTS AGAINIST PATHOGENS OF COTTON

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The antimicrobial activity of crude methanolic extracts of 15 medicinal plants, used in traditional medicine, were tested against 13 species of microorganisms; *Erwinia herbicola, Agrobacterium tumefaciens, Xanthomonas campestris, Sclerotium rolfsii, Thielaviopsis basicola, Rhizoctonia solani, Verticillium dahliae, Alternaria alternata, Phoma exigua Desmaz, Cochliobolus spicifer, Tiarosporella phaseolina, Fusarium oxysporum, Aspergillus flavus* causing diseases in cotton. Of the fifteen plants tested, nine plants showed interesting activity against seven species of microorganisms. *Rubia cordifolia* proved to be most effective with maximum zone of inhibition against all microorganisms compared to other plants.

Keywords : Antimicrobial activity; Cotton; Methanol extracts; Zone of inhibition.

Introduction

Cotton is one of the most important crops amongst fiber and cash crops of India accounting for over 30% of the country's foreign exchange. *Gossypium hirsutum* is most widely grown and contributes 80% of the total production. In spite of having largest area (9.25 million ha) under cotton in the world, India's share is only 1/10th of the world production with a total production of 321 Kg/ha. The diseases have become the major limitation in the production of cotton. Quick and effective management of plant diseases is generally achieved by the regular use of synthetic fungicides and bacteriocides. But many pathogenic microorganisms developed resistance to these synthetic fungicides and bacteriocides. More over, large number of synthetic fungicides and bacteriocides have been banned in several countries as they cause harmful effects on soil biosphere, accumulation in food chain, creates health hazards in human and animals due to their residual toxicity. So, there is an urgent need to explore bioactive fungicides for the management of pathogenic microorganisms which are eco-friendly, safe and more effective.

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from plant sources and many based on their use in traditional medicine, the wide spread use of herbal remedies, health care preparations and bio-fungicides. Several higher plants are known to posses antifungal metabolites and the exploration of other plants continues in search of new sources of fungicidal activity of plant extracts in the control of foliar, soil borne and post harvest fungal diseases[1-9]. In particular, the search for components with antimicrobial activity gained increasing importance in recent years, due to growing world wide concern about alarming increase in the rate of infection by antibiotic resistance microorganisms[10]. As a result there is a need to explore alternative fungicides of plant origin with additional advantages of cost effective fungicides, nonphytotoxicity and biodegradable[11].

Because of this reason the present investigation is taken up and the report describes the results of screening for *in vitro* antimicrobial activity with the extracts of a set of 15 different medicinally important plant species against 13 species of phytopathogens causing burning diseases i.e. boll rot, lint degradation, seedling diseases, rust disease, leaf spot disease and wilt diseases in cotton.

Materials and Methods

Plant material and extract preparation- Fifteen taxa (Listed in Table 1) belonging to angiosperms were utilized in the study. These plants were collected in and around Visakhapatnam, A.P. India. Plant materials were identified with the help of Gamble volumes (Flora of the Presidency of Madras) and later confirmed by comparing with herbarium available in the Department of Botany, Andhra University.

The plant materials collected were dried on paper towel in laboratory at 37±2°C. After drying, the plant materials were ground in a grinding machine. Exposure to sunlight was avoided to prevent the loss of active

Table 1. List of plant materials used for the study.

S.No.	Plant name	Family	Trade Name	Parts collected
1.	*Abutilon indicum (L) sweet*	Malvaceae	Country Mallow	Twigs
2.	*Aegle marmelos(L.) correa*	Rutaceae	Holy fruit tree	Leaves
3.	*Bambusa arundinacea (Retz.) willd*	Poaceac	Thorny bamboo	Leaves
4.	*Boerhaavia diffusa L.*	Nyctaginaceae	Hog weed	Whole plant
5.	*Carica papayaL.*	Caricaceae	Papaw tree	Leaves
6.	*Cassia fistula L.*	Caesalpiniaceae	Indian Laburum	Leaves
7.	*Lawsonia Inermis L.*	Lythraceae	Henna	Leaves
8.	*Melia azedarach L.*	Meliaceae	Persian lilac	Leaves
9.	*Phyllanthus emblica L.*	Euphorbiaceae	Indian goose berry	Leaves
10.	*Piper longum L.*	Piperaceae	Long Pepper	Fruit
11.	*Ricinus communis L.*	Euphorbiacea	Castor oil plant	Leaves
12.	*Rubia cardifolia L.*	Rubiaceae	Indian madder	Roots
13.	*Sappindus emarginatus Vahl.*	Sapindaceae	Soap nut tree	Fruit
14.	*Solanum nigrum L.*	Solanaceae	Black night shade	Whole plant
15.	*Trigonella foenum graecum L.*	Fabaceae	Fenugree	Seed

components. 500 ml of a methanol extraction fluid was mixed with 100 g each of the powdered plant material and the mixtures were kept for 24 h in tightly sealed vessels at room temperature and mixed several times with a sterile glass rod. The mixture was then subjected for Soxhlet extraction for 5 - 6 hr and then filtered through Whatman No.1 filter paper and the extracted liquid thus obtained was subjected to rotary evaporation in order to remove the methanol. The semisolid extract produced was stored in an air tight container at 4°C in refrigerator for further use.

Microorganisms: All the thirteen microorganisms tested were received from microbial type culture collection (MTCC), Chandigarh, India. Bacteria include *Erwinia herbicola* (MTCCB 110), *Agrobacterium tumefaciens* (MTCCB 431), *Xanthomonas campestris* (MTCCB 2286) and fungi include *Sclerotium rolfsii* (MTCCF 288), *Thielaviopsis basicola* (MTCCF 1467), *Rhizoctonia solani* (MTCCF 4633), *Verticillium dahliae* (MTCCF 1351), *Alternaria alternata* (MTCCF 1362), *Phoma exigua Desmaz* (MTCCF 2315), *Cochliobolus spicifer* (MTCCF 2112), *Tiarosplorella phaseolina* (MTCCF 166), *Fusarium oxysporum* (MTCCF 156) and *Aspergillus flavus* (MTCCF 281).

Antimicrobial assay: The assay was conducted by agar-well diffusion method [12]. The organisms grown on slants were inoculated in broth and was used as inoculum after 24 hr of incubation period. The inoculum was used to inoculate 90mm diameter Petri dishes and wells (6 mm diameter) were punched in the agar and filled with 50 µl

Table 2. *In vitro* antimicrobial activity screening of 15 medicinal plant methanolic extracts.

S. No.	Plant extracts	*E.herbicola* a b c	*A.tumefaciens* a b c	*X.campestris* a b c	*S.rolfsii* a b c	*T.basicola* a b c	*R.solani* a b c	*V.dahliae* a b c	*A.alternata* a b c	*P.e.Desmaz* a b c	*C.spicifer* a b c	*T.phaseolina* a b c	*F.oxysporum* a b c	*A.flavus* a b c
1.	*A.indicum*	9 12 13	10 11 13	7 9 10	15 16 18	19 27 30	10 15 17	9 9 10	17 20 21	14 17 20	10 10 16	11 15 16	13 14 19	9 10 11
2.	*A.marmelos*	6 8 10	0 5 6	10 10 12	10 13 16	6 10 14	6 6 7	12 12 13	9 9 9	9 9 9	5 7 8	8 9 9	10 10 11	15 17 20
3.	*B.arundinacea*	10 12 15	0 0 0	12 14 15	12 14 14	12 14 15	9 10 10	0 6 7	9 9 10	11 13 13	0 0 6	6 8 10	7 9 9	9 10 11
4.	*B.diffusa*	7 7 8	0 0 0	0 0 0	14 20 22	10 9 9	10 10 10	11 12 14	10 12 12	15 19 19	16 18 19	12 14 15	10 10 10	9 10 10
5.	*C.papaya*	10 10 10	0 6 10	13 15 16	11 11 14	12 12 13	12 12 12	17 18 19	12 13 14	12 13 14	6 8 10	7 10 10	13 13 13	10 15 18
6.	*C.fistula*	0 0 0	14 16 25	12 14 16	17 18 19	18 19 20	0 0 0	20 20 20	12 13 13	15 17 17	19 20 20	0 0 0	0 0 0	10 12 12
7.	*Linemis*	12 14 15	12 13 11	8 11 12	12 15 17	19 20 21	9 9 9	15 17 17	13 15 16	16 18 20	12 14 14	8 11 13	8 8 8	10 12 14
8.	*M.azedarach*	9 9 10	6 7 9	0 0 0	19 21 24	11 11 13	9 10 10	10 11 12	10 12 13	15 17 19	14 17 18	13 15 16	9 9 9	10 12 13
9.	*P.emblica*	0 0 0	0 0 0	0 0 0	13 14 16	7 9 9	8 9 9	6 8 9	7 8 8	0 10 10	13 16 18	9 10 10	7 11 9	0 0 0
10.	*P.logum*	9 9 11	9 9 10	8 9 10	21 22 23	10 12 13	11 11 11	16 18 20	10 12 15	13 16 19	16 19 21	9 11 12	12 14 17	10 15 17
11.	*R.communis*	9 11 13	7 9 10	8 9 10	12 14 15	13 18 20	10 11 12	10 13 16	11 14 17	20 23 24	15 17 22	10 11 13	13 15 18	6 7 8
12.	*R.cordifolia*	20 22 23	18 20 21	12 16 17	23 24 25	23 27 30	19 22 24	27 28 29	21 23 24	30 32 33	22 25 26	20 21 22	25 26 27	20 24 26
13.	*S.marginatus*	6 7 9	10 11 12	11 18 21	9 10 11	10 12 15	11 15 17	12 14 16	13 15 16	11 14 18	8 10 11	10 11 13	12 13 15	0 0 0
14	*S.nigrum*	10 10 10	8 9 9	7 8 10	14 16 19	11 11 11	10 11 11	14 16 17	10 12 13	0 0 0	14 17 20	11 11 11	0 0 12	7 8 9
15.	*T.fgraecum*	8 9 9	7 8 8	8 10 11	13 15 17	9 9 10	8 8 8	10 10 10	9 10 10	7 7 7	12 13 15	10 10 10	12 12 13	10 13 14

Concentration ofextract in 50 µl (mg/ml) ; a =100mg/ml, b=300mg/ml, c=500mg/ml
Zone of inhibition in mm, includes diameter of the well 6mm,the mean of two replicates

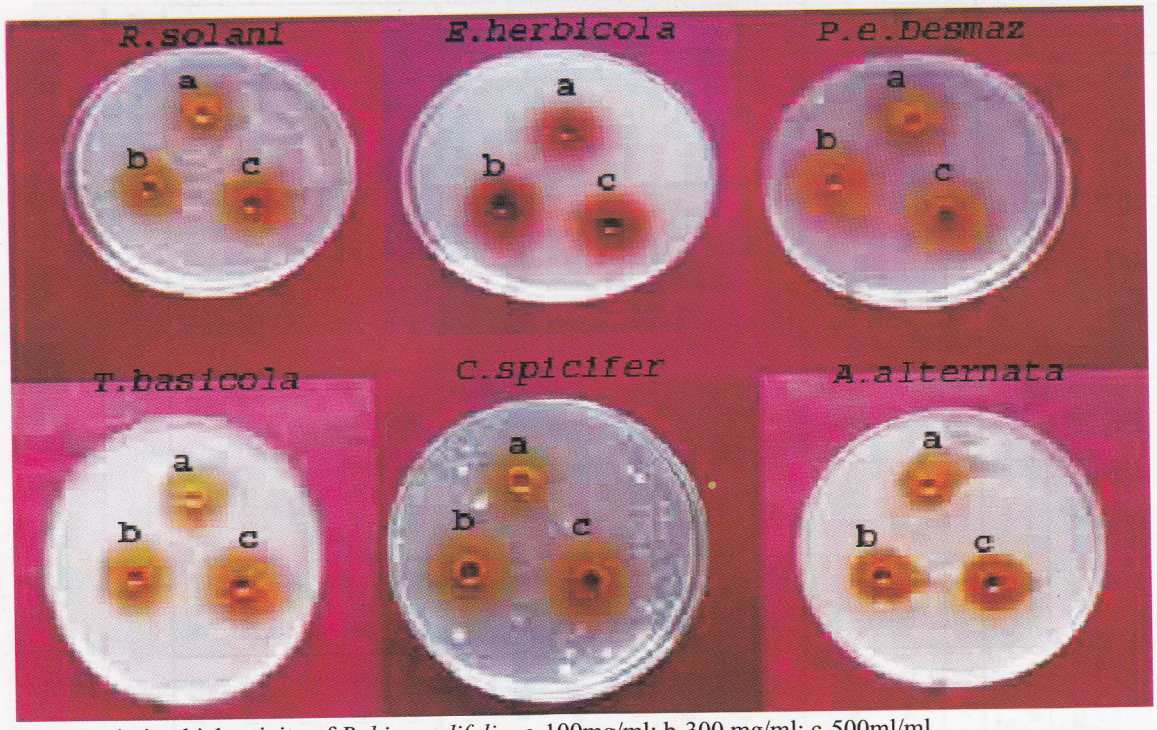

Fig.1. Antimicrobial activity of *Rubia cordifolia;* a-100mg/ml; b-300 mg/ml; c-500ml/ml.

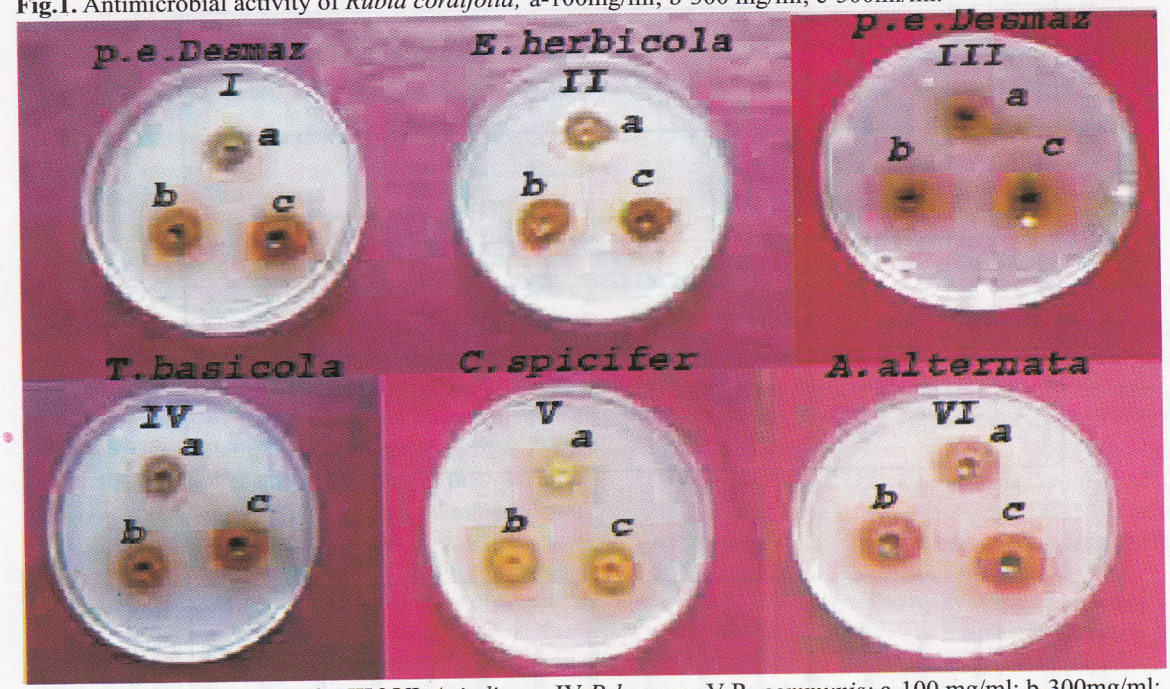

Fig.2. *L. inermis*; II-*C. fistula;* III&VI-*A. indicum;* IV-*P. longum;* V-R. *communis;* a-100 mg/ml; b-300mg/ml; c-500 mg/ml.

100 mg/ml 300 mg/ml and 500 mg/ml extracts, separately in their respective wells. The dissolution of the organic extracts (methanol) was aided by 1% (v/v) DMSO which did not effect the growth of microorganisms, in accordance with our control experiments. Plates were incubated in air at 37±°C for 24 hr. Antimicrobial activities were evaluated by measuring inhibition zone diameter. The experiments were conducted twice in aseptic conditions; the mean of two sets of data was taken for the sake of accuracy.

Results and Discussion

The preliminary screening tests of methanolic extracts of 15 plants against both bacteria and fungi causing diseases in cotton by using agar-well diffusion method are given in Table 2. It was considered that if the extract displayed an zone of inhibition more than 15mm the antimicrobial activity is good; from 10mm to 15mm the antimicrobial activity was moderate; from 7mm to 10mm the antimicrobial activity was weak; below 7mm the extract was considered inactive. *Rubia cordifolia* presented good activity with maximum zone of inhibition against all the tested microorganisms (Fig.1). Among the other plant extracts, 9 extracts were active against 7 organisms with considerable zone of inhibition (Table 2 and Fig. 2). This finding is in general agreement with the previous reports[6,7,13]. *Sclerotium rolfsii* is sensitive to plant extracts of *Abutilon indicum, Melia azedarach, Piper longum, Cassia fistula; Thielaviopsis basicola* to *Abutilon indicum, cassia fistula, Lawsonia inermis, Ricinus communis, Boerhaavia diffusa; Alternaria alternata* to *Abutilon indicum; Phoma exigua Desmaz* to *Melia azedarach, Cassia fistula, Lawsonia inermis, Ricinus communis* and *Boerhaavia diffusa; Verticillium dahliae to Piper longum, Cassia fistula, Lawsonia inermis and Carica papaya; Cochliobolus spicifer to Piper longum, Cassia fistula, Ricinus communis and Boerhaavia diffusa and Aspergillus flavus* is sensitive to *Aegle marmelos.*

Although the plant extracts differ significantly in their activities against the micro organisms tested, extract of *Rubia cordifolia* is most effective against all organisms compared to other plant extracts. These findings supports the earlier report that *R. cordifolia* was more specific even towards the gram positive strains and gram negative *P. aeruginosa* [14]. As *R. cordifolia* has shown significant activity against all the tested microorganisms in this study, it is further aimed to isolate the compounds responsible for this claimed activity through phytochemical analysis. The above 9 extracts were active against 7 organisms and remaining plant extracts exhibited minimum activity against all tested microorganisms. Extracts of *Cassia fistula* does not show any activity against *Erwinia herbicola Rhizoctonia solani, Tiarosporella phaseolina, Fusarium oxysporum; Solanum nigrum* does not show any activity against *Phoma exigua Desmaz ; Phyllanthus emblica* does not show any activity against *Erwinia herbicola, Agrobacterium tumefaciens, Xanthomonas campestris, Aspergillus flavus; Melia azedarach* is inactive against *Xanthomonas campestris; Boerhaavia diffusa* against *Agrobacterium tumefaciens, Xanthomonas campestris* and *Sapindus emarginatus* against *Aspergillus flavus* are inactive. However, the *Cassia* leaf extracts showed greater activity against *F. oxysporum*[7].

From this study it can be concluded that the plant extracts have given encouraging results, indicating their potentiality in pathogenic microorganisms management. The extract of *Rubia cordifolia* possessed the highest antimicrobial activity with maximum zones of inhibition against all the tested microorganisms. These results may provide a basis for the fruitful approach in the search of new bioactive fungicides of plant origin.

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