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# Bioefficacy of some plant extracts against microorganisms

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Antimicrobial properties of plants from different groups have been tested against a number of microorganisms. Extracts of different solvent systems of *Rhizoclonium hieroglyphicum (alga), Peltigera sp. and Usnea barbata* (lichen), *Nephrolepis tuberosa* and *Spheromeris chinensis* (pteridophytes). *Mimosa pudica, Nerium odoratum* and *Ocimum sanctum* (angiosperms) were found to contain antimicrobial principles. The antimimirobial activities or these plants are due to one or more active principles present in them. Four major groups of active principles (phenols and phenolic acids, natural lipids, amino acids and alkaloids etc.) have been separated by TLC and antimicrobial activities of different fractions of three plant extracts that have been tested against test organisms as they showed maximum inhibitory effects.

**Key words :** Plant extract, microorganism, biocontrol

## INTRODUCTION

Diseases of plants and animals caused by several pathogenic bacterial and fungi are common throughout the world. Growing concern about the harmful effects of chemical pesticides on the environment and human health together with great progress in biotechnology, have prompted a search for safer environment friendly alternatives. Although biological control is now of immense importance in modern agriculture, the performance of biological control is now of immense importance in modern agriculture, the performance of biopesticides are not yet upto the mark of chemical pesticides. With the objective finding alternative for chemical synthetic fungicides for plant disease control, different plant extracts have been tested against several plant pathogenic bacteria and fungi. Screening and reviewing of plant extracts for antimicrobial activities have been carreied out by several authors (Osborn 1943 ; Dhar *et al.* 1968 ; Naqvi *et al.* 1999 ; Bhat and Shukla, 2001; Dubey *et al.* 2001). In India, medicinal plants have extensively been studied for their antimicrobial activities against a number of plant and animal pathogenic and non pathogenic organisms. (Ray and Majumdar, 1976 ; Ghosh *et al.,* 1980 ; Cowan 1991, Jain and Singh 1998 ; Mathre *et al.,* 1999).

Present investigation was undertaken to screen different groups of plants from West Bengal, India, for their antimicrobial activities. The active principles (phenols and phenolic acids, natural lipids, amino acids alkaloids etc.) present in these plant extracts have also been separated by TLC (Stahl *et al.*, 1969) and antimicrobial activities of the different fractions have been tested against the test organisms (*Achremonium killense and Bacillus cereus.)*

## MATERIALS AND METHODS

Collected plant materials were properly cleaned, dried (over dry at 40°C), dusted and stored at 4°C. Different plant parts were processed separately for extraction of active principles. The following plants—*Rhizoclonium hieroglyphicum* (alga), *Peltigera* sp. and *Usnea barbata* (lichens) ; *Microsorum punctatum, Nephrolepis tuberosa* and *Spheromeris chinensis* (pteridophytes), and *Mimosa pudica, Nerium odoratum* and *Ocimum sanctum* (angiosperms) have been tested so for the present purpose.

The aqueous extract was prepared with fresh plant materials (approximately 16% W/V) in aseptic condition. Different solvent extracts of dusted plant materials were prepared separetely in soxhlet

apparatus (10g/160 ml). Each extract was concentrated in a rotaevaporator.

The antimicrobial activities of the plant extracts were tested against bacteria and fungi pure culture of *Bacillus cereus, Collectotrichum gloeosporioides, Fusarium oxysporum* f. sp. *udum, Macrophomina phoseolina, Sclerotium rolfsii, Phytophthora parasitica, Achremonium kiliense* and *Rhizoclonium hieroglyphicum* (all the strains except alga obtained from the Microbiology and the Mycology and Plant Pathology Laboratory, P. G. Deptt. of Botany, Presidency College, Kolkata-73) were used. The bacterial test organism was grown and maintained on nutrient agar slants and fungal test organisms were grown and maintained on P.D.A. media. Bacterial and fungal inocula were prepared from cell suspension and spore suspension respectively.

Antimicrobial activities of the extracts were assayed by filter paper disc diffusion method. In each case, inoculum ($10^6$/ml.) was incorporated (0.1 ml. for bacteria and 1 ml. for fungi) in respective agar media. Sterilized discs (Brand-Whatman and diam. of the paper disc) were soaked in different plant extracts, allowed to stand for 5 minutes and were then carefully placed at the centre of the freshly prepared petriplates seeded with test organisms. Control plates received only in respective solvents. After inoculation the plates were incubated at 37°C for bacteria and 28°C for fungi for 24-96 hrs. The plates were then examined for any inhibition zone around the discs and then the diameter of the zone was measured (Table 1).

The different fractions of the active principles in these plant/extracts have been separated by Thin Layer Chromatography (TLC). Plant extracts were applied as spot on TLC plate with silica gel G. Visualisation of spots made under iodine chamber and spraying with ninhydin reagent (probable solvent used for amino acids). Different solvent systems were used (Dhingra and Sinclair, 1995 ; Horborne, 1976) to separate these probable active principles which includes Acetic acid : Chloroform, 1 : 9 for phenolic acids; Pet. ether : Diethyl ether : Acetone, 90 : 10 : 1 for natural lipids; 96% Ethanol : Water, 90 : 30 for amino acids and Chloroform : Methanol : $NH_3$, 85 : 14 : 1 for alkaloids (Table 2). Among all the plant extracts, the antimicrobial activities of the different fractions of three plant extracts i.e. *Peltigera sp., Usnea barbata* and *Nephrolepis tuberosa* have been tested against the test organisms i.e. *B. cereus* and *A. kiliense* as they showed maximum inhibitory capacity against these organisms (Table 3). The rest plant extracts (except *O. sanctum*) will be done in due course, because they showed poor activities than the former three.

## RESULTS AND DISCUSSION

In the present screening programme, only nine

**Table 1 :** in *vitro* activities of plant extracts against some bacteria and fungi.

| Name and parts of the plant extracts tested | Solvent extracts | Bacterial strain (Bc) (inhibition zone including diameter of paper discs)* | Fungal strains (inhibition zone's diameter including diameter of the paper discs).* | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Cg | Fo f.sp. udum | Mp | Pp | AK | Sr. |
| *Rhizoclonium hireroglyphicum* (Whole plant body) | Ethanol | 10 mm | - | 16 | - | 25 | - | - |
| *Peltigera sp.* (thallus + ascocarp) | Ethanol | 21 mm | - | - | - | 10 mm | 20 | - |
| *Usnea barbata* (thallus + ascocarp) | Ethanol | 28 mm | 32 mm | - | - | 18 mm | 14 mm | - |
| *Nephorolepis tuberosa* (leaf) | Aqueous | 14 mm | 12 mm | - | 8 mm | - | 30 mm | - |
| *Spheromeris chinensis* (leaf) | Ethanol | 8 mm | - | - | - | - | 20 mm | - |
| *Mimosa pudica* (leaf) | Alcohol | 18 mm | - | - | - | - | 17 mm | - |
| *Nerium odoratum* (leaf) | Alcohol | 8 mm | - | - | - | - | - | - |
| *Ocium sanctum* (leaf) | Alcohol | 14 mm | - | 17 mm | - | 8 mm | 15 mm | - |

- : No inhibition zone. * Diam . 07 paper disc - 6mm.

Key : -

Bacillus Strain : Bc = Bacillus cereus

Fungal Strains :

Cg = *Collectorichum gloeosporioides* ; Fo = *Fusarium oxysporum* f sp. *udum* Mp = *Macrophomina phaseolina* ;
Pp = *Phytophthora parasitica* ; AK = *Achremonium kiliense* ; Sr = *Sclerotium rolfsii*

**Table 2 :** Determination ofsome probable active principles of the tested plant extracts by TLC with their Rf values.

| Name and parts of the plant extracts tested | Solvent extracts | Fractions of probable active principles with their Rf values | | | |
|---|---|---|---|---|---|
| | | Phenols etc. (Solvent system : Aa: Chl= 1: 9 | Natural Lipids (solvent system: Pe:Dee:Ac=90:10:1) | Amino acids (Solvent system: Eth : Dw=90=30) | Alkaloids (Solvent system: Chl: Meth: $NH_3$=85 : 14 :1 |
| *R. hireroglyphicum* (Whole plant body) | Ethanol | - | - | - | 0.544 |
| *Peltigera sp.* (thallus+ascocarp) | Ethanol | 0.90<br>1.78 | 0.09<br>0.216<br>0.41 | 0.61 | 0.22<br>0.86<br>1.50<br>1.69 |
| *Usnea barbata* (thallus + Ascocarp) | Ethanol | 0.77<br>1.25<br>1.76 | 0.108<br>0.41 | - | 0.10<br>0.64<br>0.85<br>1.10<br>1.78 |
| *Nephorolepis tuberosa* leaf) | Aqueous | 0.20<br>0.41<br>1.86 | - | - | - |
| *Spheromeris chinensis* (leaf) | Ethanol | 0.34 | 0.04<br>0.16 | - | 0.13 |
| *Mimosa pudica* (leaf) | Alcohol | 0.07<br>0.13<br>0.3<br>0.4<br>0.58 | - | - | |
| *Nerium odoratum* (leaf) | Alcohol | 0.18<br>0.316 | - | 0.63 | 0.33<br>0.57 |
| *Ocium sanctum (leaf)* | Alcohol | 0.34<br>1.56 | - | -<br>0.57 | 0.18<br>0.42 |

Key :
Aa : Acetic acid Chl : Chloroform Pe : Petroleum ether Dee : Diethyl ether Ac : Acetone Eth : Ethanol Dw : Distilled Water Meth : Methanol.

**Table 3 :** Antimicrobial properties of different active fractions.

| Name & parts of the plant extracts tested | Solvent Extracts | Probable active principles (as revealed from solvent system used in TLC) with their Rf values and activities (zone of inhibition in mm.) * | | | | | |
|---|---|---|---|---|---|---|---|
| | | Phenols etc. (Solvent system : Aa : Chl= 1 : 9 | | | Alkaloids (Solvent system : Chl:Meth : $NH_3$ 85 : 14 : 1 | | |
| | | Rf Values | Bc | Ak | Rf Values | Bc | Ak |
| *Peltigera sp.* (thallus+ascocarp) | Ethanol | 0 | 14 | - | 0 | - | 16 |
| | | 0.90 | 16 | 14 | 0.22 | 14 | 16 |
| | | 1.78 | 24 | 18 | 0.86 | - | 18 |
| | | | | | 1.50 | 24 | 22 |
| | | | | | 1.69 | 16 | 20 |
| *Usnea barbata* (thallus + Ascocarp) | Ethanol | 0.77 | - | 14 | 0.10 | - | 14 |
| | | 1.25 | 16 | - | 0.64 | - | 14 |
| | | 1.76 | 15 | 14 | 0.85 | - | - |
| | | | | | 1.10 | - | 14 |
| | | | | | 1.78 | - | - |
| *Nephorolepis tuberosa* (Leaves) | Aqueous | 0 | 14 | - | | | |
| | | 0.20 | - | - | | | |
| | | 0.41 | 16 | - | | | |
| | | 1.86 | 16 | 14 | | | |

* Diam. of paper disc - 6 mm.
Key : Aa = Acetic acid ; Chl = Chloroform ; Meth = Methanol.
Bc : *Bacillus cereus ;* Ak : *Achremonium kiliense*

plants belonging to different groups have been tested so far, of which eight plants have been found to be antimicrobially active. However, *M. punctatum* did not showed any antimicrobial activity.

The assay result indicated that the extracts except *N. odoratum* showed the antifungal activity. Among all the effective plant extracts, *Usnea barbata* active against *C. gloeosporioides. P. parasitica and A. kiliense* ; *N. tuberosa* against *A. kiliense, C. gloeosporioides* and *M. phaseolina* ; *O. sanctum* against *F. oxysporum f. sp. udum, P. parasitica ; S. chinensis* and *M. pudica* against *A. kiliense.* Out of the six fungal strains tested the mycelial growth of *A. kiliense* might be inhibited by most of the plant extracts.

The result of disc diffusion assay technique indicated the synergistic effect to active fractions of the plant extracts.

The probable active principles, as have been revealed from activities of different fractions separated by Thin Layer Chromatographic method, may be one or more varieties or different fractions of phenols and phenolic acids, natural lipids, amino acids, alkaloids etc.

After TLC, it has been revealed that the compounds of the plant extracts may be partially purified when these extracts (*Peltigera sp. Usnea barbata and Neohrolepis tuberosa)* have been tested against test organisms *(B. cereus and A. kiliense)* they showed more inhibitiry effect than unpurified form. So it can be concluded that more amount of chemical constituents of purified plant extracts have more activities than the extracts having mixtures of compounds with some impurities.

Further experiments are being carried out in the laboratory to determine and ascertain the extract's chemical nature of the active compounds and to find out subsequently their modes of actions against specific micoorganisms or target microoganisms.

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