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Ecofriendly approach to microbial control

S. SEN GUPTA, S. N. GHOSH, S. B. GHOSH* AND A. K. DAS
*Mycology & Plant Pathology Research Laboratory, P. G. Department of Botany, Presidency College, Kolkata 700 073, W. Bengal, India. * Department of Botany, S. C. College, Habra 743268, W. Bengal, India.*

In the present investigation three lichens, two pteridophytes and seven angiospermic plants were screened for their antimicrobial activities against four bacterial and eight fungal strains. The various solvent extracts of all these plants were found to be antimicrobially active.

INTRODUCTION

Varieties of diseases of both plants and animals are caused by several pathogenic bacteria and fungi. Insect pests of important cultivars, pets and farm animals together is a major cause of concern of our time. In most of these clinical or pathological cases, a huge varieties of synthetic bactericides, fungicides, antibiotics and pesticides are used to control the diseases or are used as chemotherapeutic agents. The majority of these antimicrobial compounds and pesticides are toxic, in one form or other, to different biological systems and as such are the sources of constant threats to our environment. The progress in biotechnology coupled with the global environmental awareness, have prompted us to search for safe, eco-friendly, non-toxic and effective antimicrobial agents from the safest possible sources as plants. Screening and reviewing of plant extracts for their antimicrobial activities have extensively been carried out by several workers since middle of the twentieth century, till date (Osborn, 1943 ; Dhar *et al.*, 1968 ; Ray and Majumder, 1976 ; Ghosh *et al.*, 1980 ; Naqvi *et al.*, 991 ; Cowan, 1999 ; Bhat and shukla, 2001 ; Sengupta *et al.*, 2002 ; Banerjee and Mukherjee, 2003 ; Daswani and Bohra, 2003 ; Ghosh and Das, 2003).

In this investigation, an attempt has been made to test different groups of plants from West Bengal, India, for their antibacterial and antifungal activities.

MATERIALS AND METHODS

The collected plant materials were thoroughly cleaned, dried (at 40°C), powdered and steeped separately in different pure solvents (Ethanol, Methanol and water only in the present sets of experiments) at different proportions (1g/10ml for *Holarrhena antidysenterica* and *Ramalina* sp. ; and 1g/16ml for other plants). The aqueous extraction was prepared by maceration of the plant materials with additional water (1ml/g plant material), followed by filtration. The extracts were concentrated (to ½ of its volume) by evaporating the solvents in a rotary evaporator. The extrcts from *Usnea barbata, Peltigera* sp., & *Ramalina* sp. (the lichens), *Spheromeris chinensis, & Christiella* sp. (the pteridophytes), and *Holarrhena antidysenterica, Coriandrum sativum, Mentha piperita, Murraya exotica, Centella asiatica, Vitex negundo & Tephrosia candida* (the angiosperms), were tested for their antimicrobial activities against *Bacillus cereus, Bacillus subtilis, Staphylococcus aureus* and *Escherichia coli* (the bacteria), and *Aspergillus niger, Acremonium kiliense, Alternaria brassicicola, Alternaria triticina, Curvularia lunata, Fusarium udum, Macrophomina phaseolina* and *Rhizoctonia solani* (the fungi).

Antimicrobial activities of these extracts were assayed by disc diffusion technique. The bacterial

inocula, in the form of cell suspensions (10^6/ml), were prepared from 20-22 hr old nutrient broth cultures. The fungal inocula, as spore or mycelial suspensions (10^{5-6}/ml), were prepared by scrapping the surfaces of 90-95 hr old cultures on PDA and subsequently by suspending the scrappings in sterile water. In all the experimental sets, the plating medium for bacteria was Nutrient Agar (NA) and for fungi it was Potato Dextrose Agar (PDA), with 1.8% (w/v) agar in both, to which the bacterial or fungal inocula (as the case might have been) were incorported (1ml suspension/plate of 20 ml medium). Sterilized discs (diameter 6mm : made from Whatman No. 1 filter paper) soaked in the extract, were allowed to stand for 5 minutes and were then carefully placed in the freshly prepared agar plates seeded with the test organisms. The control plates, also seeded with the test organisms, received only the solvents in discs. The bacterial plates were incubated at 37°C for 24 hr and the fungal plates were incubated at 28°C for 96 hr. The diameter of the zone-of-inhibition thus developed around the paper discs, were measured and compared with the control sets.

RESULTS AND DISCUSSION

It was revealed from the zone-of-inhibition resulting from disc diffusion assay that, almost all the plant extracts tested so far have antibacterial activities against all the test bacteria. Moreover, a good number of plants, tested in this screening programme, showed antifungal activities as well against a number of test fungi (Table 1). The plants as *Christiella* sp. and *M. piperita,* although are fairly active against the test bacteria, did not have any antifungal property. The extracts from *C. sativum* and *M. exotica* showed feeble antifungal

Table 1 : *In vitro* activities of the plant extracts against some bacteria and fungi

The plants (& parts used)	Extraction Solvents	Diameter of inhibition zone against test organisms											
		Bacteria				Fungi							
		Bc	Bs	Sa	Ec	An	Ak	Ab	At	Cl	Fu	Mp	Rs
U. barbata (thallus+ascocarp)	Ethanol	NP	x	33	25	—	NP	12	15	x	NP	NP	NP
Peltigera sp. (thallus+ascocarp)	Ethanol	NP	x	9	14	—	NP	7	—	—	NP	NP	NP
Ramalina sp. (thallus+ascocarp)	Ethanol	21	16	x	20	—	8	x	x	x	—	—	—
S. chinensis Bory. (leaves)	Ethanol	NP	x	—	9	—	NP	15	—	7	NP	NP	NP
Christiella sp. (leaves)	Aqueous	16	x	8	8	—	—	x	—	x	—	x	x
H. antidysenterica (leaves) (L). wall	Ethanol	13	16	7	15	—	15	—	x	x	—	—	7
C. sativum L. (leaves)	Methanol	9	x	8	7	—	—	x	x	—	—	x	7
M. piperita L. (leaves)	Aqueous	8	7	x	7	—	x	—	x	x	—	x	—
M. exotica L. (leaves)	Aqueous	8	8	7	7	—	—	x	—	x	—	x	7
C. asiatica (L). Urban (leaves)	Ethanol	9	x	—	8	—	—	x	—	10	—	x	x
V. negundo L. (leaves)	Ethanol	x	16	17	—	x	—	14	x	x	x	x	—
T. candida dc. (leaves)	Aqueous	—	x	9	8	—	18	x	19	15	14	7	15

Legends/key words : '—' =no inhibition ; "x" = very poor inhibition ; "NP" = *Not Performed* ; BC = *Bacillus cereus* ; Sa = *Staphylococcus aureus* ; Ec = *Escherichia coli* ; An = *Aspergillus niger* ;Ak = *Acremonium kiliense* ; Ab = *Alternaria brassicicola* ; At = *Alternaria triticina* ; Cl = *Curvularia lunata* ; Fu = *Fusarium udum* ; Mp = *Macrophomina phaseolina* ; Rs = *Rhizoctonia solani.*

activities only against *R. solani,* whereas the *Peltigera* sp. was only active against the fungus *A. brassicicola.* However, none of the test plants could show any activity against the test fungus *A. niger.* The extracts of *T. candida, H. antidysenterica* and *Ramalina* sp. showed commendable activities against *A. kiliense,* while the fungus *A. brassicicola* was found to be very sensitive against *A. kiliense,* while the fungus *A. brassicicola* was found to be very sensitive against *S. chinensis, V. negundo* and *U. barbata. The fungus A. triticina* was well inhibited by *T. candida* and *U. barbata.* Similarly *C. lunata, F. udum* and *R. solani* were inhibited by *T. candida,* and *C. lunata* by *C. asiatica.* On the other hand, among bacteria, *B. cereus* showed very high sensitivity against *Ramalina* sp., *H. antidysenterica, Christiella* sp., *C. sativum* and *C. asiatica ; B. subtilis* against *Ramalina* sp., *H. antidysenterica* and *V. negundo.*

Similarly, *Staph. aureus* was sensitive against *U. barbata, V. negundo, T. candida* and *Peltigera sp.* and *E. coil* was very sensitive towards *U. barbata, Ramalina* sp. *H. antidysenterica, Peltigera* sp. and *S. chinensis.* The extract of *Usnea barbata, Peltigera* sp. and *Sphaeromeris chinensis* have already been tested against the microorganisms, *B. cereus, A. kiliense, F. udum, M. phaseolina* and *R. solani* previously from this laboratory (Sengupta *et al.* 2002), so the experiment on this plant materials does not performed.

It is now evident from the results thus obtained that, the tested plant extracts have more antibacterial activities than antifungal, under the present experimental conditions, against the microorganisms tested so far. Nevertheless, the antimicrobial activities of any plant extract may be attributed to a single compound or there may be a synergistic effect of the active principles present in the extract. Of all, the extracts from *U. barbata, Ramalina* sp., *H. antidysenterica* and *T. candida* are found to be very promising because of their effectivencess against a greater number of microorganisms under this test condition.

Taking a cue from the present screening programme, further investigations, regarding the isolation, purification and characterization, are in progress.

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