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Screening of some angiospermic plants for antimicrobial activity

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Antibacterial and antifungal activities of 38 plants belonging to 17 families were tested. Solvent extracts of different morphological parts of these plants were tested against 14 bacterial and 18 fungal strains. Out of these, *Alpinia mutica, Cephalandra indica, Croton bonplandianum, Curcuma amada, Holarrhena antidysenterica, Moringa oleifera* and *Zingiber spectabile* were found to contain antimicrobial principles.

Key words: Plant extracts, antifungal activity, antibacterial activity

INTRODUCTION

Diseases of plants and animals caused by pathogenic bacteria and fungi are common in West Bengal and other parts of India. Plant extracts used to combat microbial infections are reported in our ancient Ayurvedic compendium - "Charaka Samhita" and "Sushruts Samhita" (Chopra, 1982; Chatterjee and Pakrashi, 1991). Screening of plant extracts for antimicrobial activity has been carried out by several authors (Osborn, 1943; Atkinson, 1946; Dhar *et al.*, 1968; Naqvi *et al.*, 1991; Tayor *et al.*, 1995). Detailed information regarding such screening has been reported in several reviews (Nickell, 1959; Sehgal, 1961; Stoessl, 1970; Cowan, 1999). In West Bengal, which is rich in medicinal plants, such screening work has also been done (Gupta and Banerjee, 1972; Ray and Majumdar, 1976). Present investigation was undertaken for screening of 38 angiospermic plants belonging to 17 families collected from different parts of West Bengal for their antibacterial and antifungal activities. Selection of plants was mostly random and partly on the basis of folklore.

MATERIALS AND METHODS

Collection and preservation

Plant materials from various localities were collected in plastic packets, identified, washed and stored at 4°C in separate packets. Later morphological parts were processed separately for extraction of active principles. Plant latex was collected directly from the field in sterile vials. The following plants were taken up for screening tests-*Alpinia mutica* Roxb; *Costus speciosus* (Koen ex Retz) Sm; *Curcuma amada* Roxb; *Zingiber spectabile* Griff (Zingiberaceae); *Arachis hypogaea* L., *Cicer arietinum* L., *Clitoria ternata* L., (Papilionaceae); *Acacia nilotica* (L) Willd. ex Del sub.sp.*indica* (Benth) Brenan. (Mimosaceae); *Acalypha hispida* Burnif ; *Croton bonplandianum* Baill; *Euphorbia antiquorum* L.; *E. hirta* L.; *E. ligularia* Roxb.; *E. nivulia* Buch-Ham.; *E.pulcherrima* Willd. ex Koltz.; *E.tirucalli* L, *Jatropha gossypifolia* L.; *Pedilanthus tithymaloides* (L) Poit (Euphorbeaceae); *Brassica nigra* (L) Koch. (Brassicaceae); *Barleria cristata* L. *Hygrophila auriculata* (Schum) Haine (Acanthaceae); *Cyperus rotundus* L (Cyperaceae); *Cocos nucifera* L.(Arecaceae); *Thevetia nereifolia* Juss. ex Steud; *Holarrhena antidysenterica* (L.) Wall. ex.ADC.; *Nerium indicum* Mill; *Tabernaemontana divaricata* (L) R.Br.ex Roem. &Schult;(Apocynaceae); *Calotropis procera* (Willd) Dry and ex Ait. subsp *hamiltonii* (W) Al.(Asclepiadaceae); *Cephalandra indica* Naud.; *Trichosanthes dioica* (Roxb) Swartz.(Cucurbitaceae); *Calendula officinalis* L.; *Eclipta prostrata* (L) L; *Enhydra fluctuans* Lour.;

(Asteraceae); Ficus *benghalensis* L. (Moraceae); *Moringa oleifera* Lamk. (Moringaceae); *Ocimum sanctum* L.*(Labiatae); Datura stramonium* L.(Solanaceae); and *Syzygium cuminii* (L); Skeels (Myrtaceae).

Extraction of active principles

For aqueous extract, 10 g of each plant part was crushed and homogenized in 10 ml of distilled water. The extract was sterilized by passing through a bacterial filter (G5) after adjusting the pH at pH 7. Plant latex was sterilized in a similar way. For solvent extraction plant parts were oven dried at 60°C, powdered and steeped in the respective solvent for 24 hrs at room temperature (1 g/10 ml of solvent). Solvents used included :dehydrated ethanol, 50% aqueous ethanol, methanol, chloroform, acetone, ethyl acetate, benzene, petroleum ether (60-80°C) and diethyl ether.

Assay of plant extracts for antimicrobial activity

Plant extracts and latex were tested for antimicrobial activity against a number of pathogenic and non-pathogenic bacteria and fungi. Bacterial strains were obtained from the Microbiology Laboratory, Department of Botany, Calcutta University and National Institute for Cholera and Enteric Diseases, Calcutta. Fungal strains were obtained from the Mycology Laboratory,Department of Botany and Microbiology Laboratory, Department of Biochemistry of Calcutta University. The test organisms were grown in their respective culture media- yeast extract mannitol (YEM) broth and YEM agar for *Rhizobium* and nutrient broth / nutrient agar for other bacteria, Sabouraud's agar for dermatophytes and potato dextrose agar for other fungi according to the recipes. Bacterial and fungal inocula were prepared from cell suspension and spore suspensions respectively in sterile distilled water.

Assay of plant extracts was done by agar cup - plate diffusion method. In each case inoculum was incorporated (0.1 ml for bacteria and 0.05 ml for fungi) in the respective agar media and cups were made on cooled agar bed by cork borer of 0.7 cm diameter. About 0.05 ml of sterile plant extract was added in each cup aseptically. Control cups received only the respective solvents. The plates were incubated at 30°C for fungi and 37°C for bacteria for 24-96 hrs. The plates were examined for any zone of inhibition around the agar cups and the diameter of the zone was measured.

RESULTS AND DISCUSSION

In the present screening of the 126 morphological parts of 38 plant species belonging to 17 families, extracts of 14 plant parts from 7 plant species were found to be antimicrobially active . Antibacterial activity was found an all of them (Table 1), while only four plants showed antifungal activity (Table 2).

Antibacterial activity

Extracts of dried rhizomes of *Alpina mutica, Curcuma amada* and *Zingiber spectabile* were active mostly against Gram-positive bacteria. Latex of *Croton bonplandianum* also revealed similar activity. Extracts of all morphological parts of *Moringa oleifera* were found positive against Gram-positive and some Gram-negative bacteria. Extracts of *Cephalandra indica* was found active only against *S. aureus* while the same of *Holarrhena antidysenterica* was found active only against *Bacillus subtilis* and *Bacillus cereus.*

Antifungal activity

Extracts of *M. oleifera, A.mutica* and *Z. spectabile* have shown antifungal activity mostly against *S.cerevissiae* and some dermatophytes. However *C.amada* was found active against all fungi tested.

The present study being a screening programme, employed only *in vitro* studies. Plants like *Moringa oleifera, Alpinia mutica, Curcuma amada, Zingiber spectabile* and *Croton bonplandianum* appear to be highly promising and further investigation regarding isolation and characterisation of active principles should be taken up.

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Table 1. Antibacterial activity of extracts of some selected plants

Name and Parts of the plant used	Solvent extracts	Bacterial strains													
		Bs	Bc	Sa	Ec	Sf	Pf	Pr	Ah	St	C	Pa	Kp	Sl	R
Alpinia mutica Roxb. (Zingiberaceae) rhizomes	E(A)	+++	++	++	-	-	-	-	-	-	-	-	-	-	-
	E(50%)	++	+	+	-	-	-	-	-	-	-	-	-	-	-
	M	++	++	++	-	-	-	-	-	-	-	-	-	-	-
	C	++	+	+	-	-	-	-	-	-	-	-	-	-	-
	A	+++	++	++	-	-	-	-	-	-	-	-	-	-	-
	EA	++	+	+	-	-	-	-	-	-	-	-	-	-	-
	B	+	+	+	-	-	-	-	-	-	-	-	-	-	-
Cephalandra indica Naud. (Cucurbitaceae) leaves	E(A)	-	-	+	-	-	-	-	-	-	-	-	-	-	-
	PE	-	-	++	-	-	-	-	-	-	-	-	-	-	-
Croton bonpland ianum Baill (Euphorbiaceae) latex	DW	+	+	+	-	-	-	-	-	-	-	-	-	-	-
	FL	++	++	++	-	-	-	+	+	-	-	-	-	-	-
	E(A)	+	+	+	-	-	-	-	-	-	-	-	-	-	-
	E(50%)	+	+	+	-	-	-	-	-	-	-	-	-	-	-
	M	+	+	+	-	-	-	-	-	-	-	-	-	-	-
	A	+++	+++	+++	-	-	-	+	+	-	-	-	-	-	-
Curcuma amada Roxb. (Zingiberaceca) rhizomes	E(A)	++	++	++	-	-	-	-	-	-	-	-	-	-	-
	E(50%)	+	+	+	-	-	-	-	-	-	-	-	-	-	-
	M	+	+	+	-	-	-	-	-	-	-	-	-	-	-
	A	+	+	+	-	-	-	-	-	-	-	-	-	-	-
	EA	+++	+++	+++	-	-	-	-	-	-	-	-	-	-	-
	B	++	+	+	-	-	-	-	-	-	-	-	-	-	-
Holarrhena antidysenterica (L.) Wall. ex DC. (Apocynaceae) stem and leaves	E(A)	+	+	-	-	-	-	-	-	-	-	-	-	-	-
Moringa oleifera L. (Moringaceae) Stem bark, leaves, inflorescence axis, flowers bud, fruit pulp, seed coat, cotyledons	DW	+++	++	+++	+	-	-	+	++	+	++	+	++	-	++
	E(A)	+	+	+	+	-	-	-	+	-	+	-	+	-	+
	E(50%)	+++	++	+++	++	-	-	+	++	+	++	+	++	-	++
	M	+++	+	+	+	-	-	+	++	+	++	+	++	-	++
	A	++	+	+	+	-	-	+	+	+	+	+	+	-	+
	EA	++	+	+	+	-	-	+	+	+	+	+	+	-	+
Zingiber spectabile Griff (Zingiberaceae) rhizomes	E(A)	+++	++	++	-	-	-	-	++	-	-	-	-	-	-
	E(50%)	++	++	++	-	-	-	-	++	-	-	-	-	-	-
	M	++	+	+	-	-	-	-	+	-	-	-	-	-	-
	EA	++	+	+	-	-	-	-	+	-	-	-	-	-	--
	B	+	+	+	-	-	-	-	+	-	-	-	-	-	-

"+" = Number of "+" indicate the degree of inhibition

"-" = No inhibition

Key : Bacterial strains : Bs = *Bacillus subtilis*, Bc = *B. cereus*, Sa = *Staphylococcus aureus*, Ec = *Escherichia coli*, Sf = *Shigella flegmeri*, Pf = *Pseudomonas fluorescence*, Pr = *Proteus retgeri*, Ah = *Aeromonas hydrophila*, St = *Salmonella typhi*, C = *Citrobacter* sp., Pa = *Providencia alcalifaciens*, Kp = *Klebsiella pneumoniae*, Sl = *Sarcina lutea*, R = *Rhizobium* sp.

Solvent extracts : DW = Distilled water; Fl = Fresh latex; E(A) = Ettyl alcohol (absolute); E(50%) = Ethhyl alcohol (50%); M = Melhanol, C = Chloroform, A= Acetone, EA = Ethyl acetate, B = Benzene, PE = Petroleum ether (60-80°C).

Table 2. Plant extracts exhibiting inhibitory activity against fungal strains *in vitro*

Name and parts of the plant tested	Solvent extracts	Fungal strains																	
		Rc	Pd	Sc	An	Af	Aa	Cl	Fs	Tm	Ca	Ef	C	Ba	Ho	Sr	Tr	Tt	Ti
Alpinia mutica Roxb. (Zingiberaceae) rhizome	E(A)	-	-	+++	++	++	-	-	-	+++	+++	+++	++	++	++	++	++	++	++
	E(50%)	-	-	++	+	+	-	-	-	++	++	++	+	+	+	+	+	+	+
	M	-	-	++	++	++	-	-	-	++	++	++	++	++	++	++	++	++	++
	A	-	-	++	+	+	-	-	-	+	+	+	+	+	+	+	+	+	+
	EA	-	-	++	+	+	-	-	-	+	+	+	+	+	+	+	+	+	+
Curcuma amada Roxb. (Zingiberaceae) rhizome	E(A)	-	-	+	+	+	-	-	-	+	+	+	+	+	+	+	+	+	+
	M	-	-	+	+	+	-	-	-	+	+	+	+	+	+	+	+	+	+
	A	-	-	++	++	++	+	+	+	++	++	++	+	+	+	+	++	++	++
	EA	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	++	+	+	+	+	++	++	++
	B	++	++	++	++	++	++	++	++	++	++	+	+	+	+	+	+	+	+
Moringa oleifera L. (Moringaceae) Stem bark, leaves, inflores -cence axis, flowers bud, fruit pulp,seed coat, cotyledons	DW	-	-	++	-	-	-	+	++	++	++	++	-	-	-	-	++	++	++
	E(A)	-	-	+	+	-	-	-	+	+	+	+	-	-	-	-	+	+	+
	E(50%)	-	-	+++	+	-	-	+	++	+++	++	++	-	-	-	-	++	++	++
	M	-	-	+	-	-	-	-	+	+	+	+	-	-	-	-	+	+	+
	A	-	-	++	+	-	-	+	+	+	+	+	-	-	-	-	+	+	+
	EA	-	-	+	-	-	-	+	+	+	+	+	-	-	-	-	+	+	+
Zingiber spectabile Griff (Zingiberaceae) rhizome	E(A)	-	++	++	-	-	-	++	++	++	++	++	+	+	-	-	-	++	++
	E(50%)	-	+	++	-	-	-	+	+	+	+	+	+	+	-	-	-	+	+
	M	-	++	++	-	-	-	++	++	++	++	++	+	+	-	-	-	++	++
	EA	-	+	++	-	-	-	+	+	+	+	+	+	+	-	-	-	+	+

"+" = Number of "+" indicate the degree of inhibition

"-" = No inhibition

Key : Fungal strains : Rc = *Rhizopus chinensis*, Pd = *Penicillium digitatum*, Sc = *Saccharomyces cerevisiae*, An = *Aspergillus niger*, Af = *Aspergillus fumigatus*, Aa = *Alternaria alternata*, Cl = *Curvularia lunata*, Fs = *Fusarium solani*, Tm = *Trichophyton mentagrophytes*, Ca = *Candida albicans*, Ef = *Epidermophyton floecosum*, C= *Colletotrichum* sp., Ba = *Botrytis alli*, Ho = *Helminthos - porium oryzae*, Sr = *Sclerotium rolfsii*, Tr = *Trichophyton rubrum*, Tt = *Trichophyton tonsuraus*, Ti = *Trichophyton interdigitalis*.

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