



ANTIFUNGAL AND CYTOTOXIC ACTIVITIES OF FIVE TRADITIONALLY USED INDIAN MEDICINAL PLANTS

Adhikarimayum Haripyaree*¹, Kshetrimayum Guneshwor², Maibam Damayanti²

Address: Dr. Adhikarimayum Haripyaree,

¹ Manipur University, Department of Life Sciences, PIN-795003, Imphal, Manipur State,
India. Mob. No. +919862421978

² Manipur University, Department of Life Sciences, PIN -795003, Imphal, Manipur State,
India.

*Corresponding author: adhikarimayum_devi@yahoo.com

ABSTRACT

Hexane, Methanol and Distilled water extracts of five Indian Medicinal plants viz., *Mimosa pudica* L, *Vitex trifolia* Linn, *Leucas aspera* Spreng, *Centella asiatica* (L) Urban and *Plantago major* Linn belonging to different families were subjected to preliminary antimicrobial screening against six standard organisms viz., *Ceratocystis paradoxa*, *Aspergillus niger*, *Penicillium citrinum*, *Macrophomina phaseoli*, *Trichoderma viride* and *Rhizopus nigricans*. To evaluate antifungal activity agar well diffusion method was used. In addition LD₅₀ of the same plant extracts were determined by using Range test on *Mus musculus* for cytotoxic activity. Methanolic extract of *M. pudica* showed the highest and significant inhibitory effect against some fungal species. Again, methanolic extract of *M. pudica* displayed the greatest cytotoxic activity.

Keywords: Antimicrobial activity, minimum inhibitory concentration (MIC), agar well diffusion method, range test

INTRODUCTION

Five widely used medicinal plants viz., *Mimosa pudica* L. belongs to the family Mimosaceae, *Vitex trifolia* Linn, family Verbanaceae, *Centella asiatica* (L) Urban, family Apiaceae, *Leucas aspera* Spreng, family Lammiaceae and *Plantago major* Linn belongs to the family Plantaginaceae were selected for present study because of their popular medicinal values (**Prajapati et al., 2003**). The plant extracts of *Mimosa pudica* was used in treating haemorrhoids, urinary infections, dysentery, fever, syphilis and leprosy (**Sinha, 2000**). *Vitex trifolia* fruit is used in treating coryza, fever, headache, photopsia, vertigo and ophthalmagia (**Prajapati et al., 2003**). *Centella asiatica* (L) Urban possesses antibacterial, anti-inflammatory, anti-febrile, diuretic and galactogogic activity (**Prajapati et al., 2003**). The plant extract of *Leucas aspera* Spreng exhibited significant anti-inflammatory activity (**Savadane, 2000**). Alcoholic extract of leave shows antibacterial activities. *Plantago major* extract was used for complaints specifically of toothache, earache and enuresis. Traditionally it is used for bee sting, incised wounds and bleeding piles. Plant is semi boiled with litter water and the material is applied as bandage in boils, muscular sprains and gout (**Singh et al., 2003**). Flavonoids from *Plantago asiatica* have inhibitory effects on HIV-reverse transcriptase activity (**Nishibe et al., 1997**).

Many plants, fruits and vegetables were infected by various types of fungi. Due to infection by these fungal pathogens, these materials do not usually keep for longer period without preservatives. The use of plant extracts to reduce the incidence of various plant diseases was recorded (**Sarvamangala et al., 1993; Egawa et al., 1997**). On the other hand, several plants have therapeutic and pharmaceutical effect for antimicrobial, antiinfectious and antitumour activities (**Akroum et al., 2009**). The identification of plant extracts would be of immense practical use for safe and effective control of these fungal pathogens. Moreover from the economic point of view, it would be quite inexpensive.

The present study attempts to investigate plant extracts having maximum antifungal activity against six fungal pathogens *Ceratocystis paradoxa*, *Aspergillu niger*, *Macrophomina phaseoli*, *Rhizopus nigricans*, *Penicillium citrinum* and *Tricoderma viride*. The agar well diffusion method was employed in the screening of plant extracts against six fungi. In the cytotoxic assay Range test was used for determination of LD₅₀ (LD₅₀ is defined as the dose of the sample treated that can survived half of the experimental animal).

MATERIAL AND METHODS

Collection of plant materials and test organisms

Traditionally used Indian medicinal plants viz., *M. pudica*, *V. trifolia*, *C. asiatica*, *L. aspera* and *P. major* were collected from the local area of Imphal, India. They were made for all accessions and conserved at Herbarium of Manipur University. Different fungal pathogens viz., *C. paradoxa*, *A. niger*, *M. phaseoli*, *P. citrinum*, *T. viride* and *R. nigricans*, were collected from the Department of Plant Pathology, Life sciences, Manipur University, Manipur, India.

Preparation of plant extracts

Plant material used in the screening procedure was dried at 50 °C and extracted separately in distilled water (polar) and methanol (moderately polar) and hexane (non polar) solvents by using Soxhlet apparatus in a concentration of 1g.10 mL⁻¹. The different extracts were concentrated at reduced pressure to dryness and stored at - 10 °C until assayed.

Screening of antimicrobial activity

Antimicrobial activity was determined by the agar well diffusion method with a slide modification (**Rios et al., 1988**). MH agar (Biolab) plates were prepared using sterile 90 mm petridishes. Each plant extract was dissolved in 95% ethanol and tested at five different concentrations (5.00, 2.50, 1.25, 0.62 and 0.31 mg.mL⁻¹). They were evaluated in triplicate for each fungus. Ketoconazole at different concentrations are used as positive control for antimycotic tests. The solvents which are used to dissolve the respective extracts are considered as negative control. In this procedure the degree of micro-organism inhibition by each plant extract was assayed by measuring the diameter of the inhibition zone (mm). Then the minimum inhibitory concentration (MIC) was obtained. The plates were evaluated at 37 °C for 24 hrs. The end point (MIC) is the least concentration of the antimicrobial agent that completely inhibits the growth.

Cytotoxic activity assays

In vivo cytotoxic lethality test were carried out using Swiss albino, *Mus musculus* following the Range test for determination of LD₅₀ (**Guneshwor and Bhagirath, 2005**). The methanol and n-hexane extracts were dissolved in distilled water. Each plant extracts were treated with different concentrations to the experimental mice. First lethal doses (LD) of the plant extracts were determined; it was serially diluted with distilled water till LD₀ (LD₀ is defined as the dose of the sample treated that

can survived the entire experimental animal) was achieved. LD₀ was taken as MTD (maximum tolerated dose). Positive control animals received Ethyl methane sulphonate (EMS) at the dose of 240 mg.kg⁻¹ body weight dissolved in 1 ml distilled water. Each treatment and control groups consisted of five animals.

LD₅₀ in mg.mL⁻¹ for each plant extract was obtained by interpolation in the graph of mortality percentage versus the concentrations (mg.mL⁻¹) through a linear regression analysis.

RESULTS AND DISCUSSION

Results of the antimicrobial activities and determination of LD₅₀ of the crude plant extract in distilled water, methanol and hexane are shown in table. Among the five plants examined, *M. pudica* shows highest antifungal activities against one or more than one micro-organism. None of the plant extract evaluated in this research inhibited the growth of *P. citrinum*. Distilled water, methanol and hexane extract of *M. pudica* & *V. trifolia* shows moderate & strong activities against *C. paradoxa*. These results are in concordance with the antifungal activity of *M. pudica* that found to contain high amount of tannins (Savadane, 2003). *C. asiatica* extract shows its activity against *M. phaseoli*. Antibacterial activity of *C. asiatica* was reported (Srivastava, 1997). The positive effect of *C. asiatica* against the fungus *M. phaseoli* might be due to the presence of triterpenoid trisaccharide since triterpenoids show antifungal activity (Eloff, 1998). *Leucas aspera* exhibit antifungal activity against pineapple fruit rotting fungus *C. paradoxa* (Damayanti et al., 1996). In our experiment, this extract shows its effectiveness against two tested fungi *C. paradoxa* and *T. viride*. This may be due to the presence of terpenoids which possess antifungal, antibacterial and anti-insect activities. This is a common feature particularly in the plants belong to Labiatae (Cole, 1996; Verma et al., 1998). Extracts of *P. major* displayed no activity against all the fungi in the experiment.

The present study reveals that these plant extracts have significant effect on one or other tested fungi. So there is the possibility of using such plant as fungitoxic agent in controlling various fungal agents by reducing the severity of disease. This is the preliminary screening of antifungal activity found in the plant juices and extracts of *M. pudica*, *V. trifolia*, *C. asiatica*, *L. aspera* and *P. major*.

In the present experiment highest effect of cytotoxicity was exhibited by methanol and hexane extracts of *M. pudica* since the LD₅₀ dose (mg.mL⁻¹) is around the positive control EMS which exhibit 0.01 mg.mL⁻¹. LD₅₀ dose (mg.mL⁻¹) is lowest in *M. pudica*. The lower LD₅₀, the higher cytotoxic activity. Moreover the effects of these medicinal plants to the

experimental animals are dose dependent. The authors previously claimed the cytotoxic activity of the distilled water extract of *M. pudica* (Haripyaree et al., 2004). These activities may be due to the presence of certain bioactive phytochemicals. Therefore further detailed study is needed for the isolation and identification of active compounds responsible for antifungal activities particularly against the most resistant fungus and their cytotoxic activities.

Table 1 Minimum inhibitory concentration (MIC) (mg.mL^{-1}) and lethal dose fifty (LD_{50} mg.mL^{-1}) of crude extracts of five medicinal plants

Species (Voucher number & parts used)	Extractant	Fungi (MIC) (mg.mL^{-1})						<i>Mus musculus</i> LD_{50} mg.mL^{-1}
		<i>C.paradoxa</i>	<i>A.niger</i>	<i>M.phaseoli</i>	<i>P.citrinum</i>	<i>T.viride</i>	<i>R.nigricans</i>	
<i>M.pudica</i>	D.W	2.50	1.25	–	–	–	–	0.48
MU37	Met.	1.25	0.62	2.5	–	–	–	0.08
Whole plant	Hex.	0.62	1.25	–	–	–	1.25	0.12
<i>V.trifolia</i>	D.W	5.0	–	–	–	–	–	1.12
MU178	Met.	2.5	–	–	–	1.25	–	0.54
Leaves &fruits	Hex.	1.25	–	–	–	2.50	–	0.98
<i>C.asiatica</i>	D.W	–	–	–	–	–	–	1.20
MU750	Met.	–	–	2.5	–	–	–	0.95
Whole plant	Hex.	–	–	1.25	–	–	–	1.12
<i>L. aspera</i>	D.W	–	–	–	–	–	–	1.18
MU1252	Met.	1.25	–	–	–	2.50	–	0.69
Leaves & Flower	Hex.	2.50	–	–	–	5.00	–	0.87
<i>P. major</i>	D.W	–	–	–	–	–	–	1.14
MU1528	Met.	–	–	–	–	–	–	0.92
Whole plant	Hex.	–	–	–	–	–	–	1.00
Positive control	Ketoke- nazole	0.25	0.25	0.25	0.25	0.25	0.25	NE
	EMS	NE	NE	NE	NE	NE	NE	0.01

Legend: DW = distilled water; Met = Methanol; Hex = n-hexane; – = No growth inhibition at the concentration tested; EMS = Ethyl methane sulphonate; NE = Not evaluated.

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