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Antimycobacterial, antiviral and cytotoxic studies of *Indigofera aspalathoides Vahl*

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ABSTRACT - Alcohol extract of *Indigofera aspalathoides* was tested for antimycobacterial activity against *Mycobacterium tuberculosis* ($H_{37}Rv$), antiviral activity against HEL Cell culture (herpes simplex virus-1 KOS, herpes simplex virus-2 G, vaccinia virus, vesicular stomatitis virus and herpes simplex virus-TK KOS ACV), HeLa cell culture (vesicular stomatitis virus, coxsackie virus B4 and respiratory syncytial virus) and cytotoxic activity against human epithelial larynx cancer cells (HEp2), human breast cancer (HBL-100) and cervical carcinoma cells (HeLa) cell lines. The results showed that the extract produced 48% inhibition for antimycobacterial and >80 μg/ml of minimum inhibitory concentration against HEL and HeLa cell cultures of antiviral studies. In cytotoxic studies IC_{50} value of extract were found 117, >300 and 210 μg/ml against HEp2, HBL-100 and HeLa cells respectively. These studies clearly indicate that extract exhibited antimycobacterial, antiviral and cytotoxic activity.
KEYWORDS: Antimycobacterial; Antiviral; Cytotoxicity; Indigofera *aspalathoides*

INTRODUCTION

Indigofera aspalathoides Vahl (Family: Papilionaceae) is a low under shrub with wide distribution, found mostly in South India and Ceylon. Stem is traditionally used for various skin disorders and tumours (1). It also has anti-inflammatory and antitumour activity against transplantable tumours (2-5). Phytochemical studies revealed that presence of pterocarpan (6). The present study is aimed at evaluating the effect of the alcohol extract of *Indigofera aspalathoides* against *Mycobacterium tuberculosis*, antiviral and cytotoxic effect on human cancer cell lines.

MATERIALS AND METHODS

Plant materials and extraction

Stems of *Indigofera aspalathoides* were collected in and around Salem district in the month of December 2002 and authenticated at Botanical Survey of India, Coimbatore, Tamilnadu, India. A voucher specimen (PIA-02) has been kept in our laboratory for future reference. The stem samples were shade dried and pulverized. The powder was treated with petroleum ether for defatting and removal of chlorophyll. Then, it was extracted (250 g) in a Soxhlet apparatus for 8 hours using 450 ml of ethanol (95% v/v) as solvent. The extract was concentrated under vacuum and dried in a desiccator (yield = 11.25 g, 4.5% w/w). Later, the extract was dissolved in water and partitioned in to different fractions with benzene, diethyl ether, ethyl acetate and n-butanol.

Antimycobacterial assay

Antimycobacterial assay was performed using microplate Alamar blue assay (7) (MABA). Suspension of *Mycobacterium tuberculosis* $H_{37}Rv$ strain was prepared at a concentration of 10^5 cells/ml. Samples were dissolved in dimethyl sulphoxide (DMSO) and subsequent dilutions were performed in 0.1 ml of 7H9 medium in the microplate together with the plant

extract and its fractions (concentration 0.78-100μg/ml). The plates were incubated at 37° C for 7 days. At day 7 of incubation, 20 μl of Alamar blue solution were added to the control well. If the dye turned pink, indicating bacterial growth, the dye was then added to all remaining wells in the plate. The results were read on the following day and Minimum Inhibitory Concentration (MIC) values of the extract and fractions were calculated. Rifampicin was used as positive control. The MIC of rifampicin was 0.0047 - 0.0095 μg/ml.

Antiviral studies

Antiviral activity (8) of the extract was tested in HEL Cell culture (herpes simplex virus-1 KOS, herpes simplex virus-2 G, vaccinia virus, vesicular stomatitis virus and herpes simplex virus-TK KOS ACV) and HeLa cell culture (Vesicular stomatitis virus, coxsackie virus B4 and respiratory syncytial virus). Ribavirin and ganciclovir were used as standards. The Minimum Cytotoxic Concentration (MCC) to cause a microscopically detectable alteration of normal cell morphology and Minimum Inhibitory Concentration (MIC) to reduce virus-induced cytopathogenecity by 50% were recorded.

Cytotoxic studies

Human epithelial larynx cancer cells (HEp2), human breast cancer (HBL-100) and Cervical carcinoma cells (HeLa) cells were obtained from National Centre for Cell Science, Pune, India. Stock cells of these cell lines were cultured in RPMI-1640 or DMEM supplemented with 10% inactivated newborn calf serum, penicillin (100 IU/ml), streptomycin (100 μg/ml) and amphotericin-B (5 μg/ml) in a humidified atmosphere of 5% CO_2 at 37° C until confluent. The cells were dissociated in 0.2% trypsin, 0.02% EDTA in phosphate buffer saline solution. The stock culture was grown in 25cm^2 tissue culture flasks and cytotoxicity experiments were carried out in 96 well microtitre plates (Tarsons India Pvt. Ltd., Kolkata, India).

Cell lines in the exponential growth phase were washed, trypsinised and resuspended in complete culture media. Cells were plated at 10000 cells/well in 96 well microtitre plates and incubated for 24 h during which a partial monolayer forms. The cells were then exposed to various concentrations of the extract (25-300 μg/ml). Control wells received only maintenance medium. The plates were incubated at 37°C in a humidified incubator with 5% CO_2 for a period of 72 h. At the end of 72 h, cellular viability was determined by using MTT assay (9).

RESULTS AND DISCUSSION

The alcohol extract and fractions were tested for evaluation of the MIC to mycobacterium tuberculosis $H_{37}Rv$ strain with microplate technique using alamar

Table 1. Minimum inhibitory concentration of I. aspalathoides against Mycobacterium tuberculosis

Test Samples	MIC (μg/ml)	% Inhibition
Alcohol extract	>100	48 ± 1.46
Butanol fractions	>100	6 ± 0.14
Diethyl ether Fractions	>100	9 ± 0.16
Benzene Fractions	>100	61 ± 2.07
Ethyl acetate Fractions	>100	27 ± 0.94

Average of 3 determinations, 3 replication

Table 2. Antiviral activity of I. aspalathoides in HEL cell cultures

Test Samples	MCC[a] (μg/ml)	MIC[b] (μg/ml)				
		Herpes simplex Virus-1(KOS)	Herpes simplex Virus-2 (G)	Vaccinia virus	Vesicular stomatitis Virus	Herpes simplex virus-TK KOS ACV
I. aspalathoides	≥400	>80	>80	>80	>80	>80
Ribavirin	>400	16	>400	>400	>400	>400
Ganciclovir	>100	0.032	0.0064	>100	>100	0.8

a) Minimum cytotoxic concentration; b) Minimum inhibitory concentration.

Table 3. Antiviral activity of I. aspalathoides in HeLa cell cultures

Test Samples	MCC[a] (μg/ml)	MIC[b] (μg/ml)		
		Vesicular stomatitis virus	Respiratory syncytial virus	Coxsackie virus B4
I. aspalathoides	400	>80	>80	>80
Ribavirin	>400	48	48	48

a) Minimum cytotoxic concentration; b) Minimum inhibitory concentration.

Table 4. Cytotoxic action of I. aspalathoides on HEp2, HBL-100 and HeLa cell lines

Cancer cell lines	Cytotoxic concentration IC_{50} (μg/ml)
HEp2,	157 ± 2.04
HBL-100	>300 ± 4.61
HeLa	210 ± 3.72

Average of 3 determination, 3 replicate; IC50 , drug concentration inhibiting 50% cellular growth following 72 h drug exposure

blue. The results are shown in table 1. The antimycobacterial activity of alcohol extract and fractions were compared with rifampicin. The benzene, ethyl acetate fractions and extract showed inhibition 61%, 27% and 48% respectively at 100 μg/ml of MIC. The n-butanol and diethyl ether fractions were found to be less active□6 and 9 μg/ml□.

Data on antiviral activity are shown in table 2 and 3. The minimum inhibitory concentration (MIC) was 80 μg/ml against HEL and HeLa cell culture. The extract showed moderate antiviral activity against all viruses and produced significant cytoprotection to the HEL and HeLa cell culture when challenged with viruses.

The cytotoxic effects of the extract against different cell lines are shown in table 4. Among the three cell lines studies, the extract was found to most potent against HEp2 cell line. The IC_{50} values were found to be 157 μg/ml for HEp2 cell, >300 and 210 μg/ml for HBL-100 and HeLa cells respectively. Hence, HEp2 cells are more sensitive to the alcohol extract of *I. aspalathoides*. Results of our studies confirm the antimycobacterial, antiviral and cytotoxic nature of the alcohol extract of *I. aspalathoides*. Preliminary phytochemical studies have indicated the presence of alkaloid and flavonoid in the extract. Some alkaloids and flavonoids are known to possess antimycobacterial and antiproliferative activity (10-11). Hence, these compounds may mediate antimycobacterial, antiviral and cytotoxic properties of the extract. Further studies to characterise the active principles and to elucidate the mechanism of action are in progress.

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