Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 3, Suppl 5, 2011

Research Article

CYTOTOXICITY AND ANTIMICROBIAL STUDIES OF STEM BARK OF *MILLINGTONIA HORTENSIS* LINN

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Received: 9 July 2011, Revised and Accepted: 5 Nov 2011

ABSTRACT

The present study aims at exploring the cytotoxic potential and antimicrobial capacity of the aqueous extract of stem bark of *Millingtonia hortensis* Linn. Aqueous extract of stem bark of *Millingtonia hortensis* Linn is tested against the Human cervical cancer cell line by MTT Assay. Antimicrobial capacity of the extract is evaluated by means of agar diffusion method using 12 strains of microbes. The extract is found to be a poor cytotoxic and antibacterial agent, however it is found to be an effective antifungal agent. The isolation of phytoconstituent from the stem bark of *Millingtonia hortensis* Linn may lead to a novel antifungal agent rather than an antibacterial or cytotoxic agent.

Keywords: *Millingtonia hortensis*, Cytotoxicity, MTTAssay and antimicrobials.

INTRODUCTION

Medicinal plants serves as an important source of treatment for various ailments. Various medicinal plants are identified and studied using scientific and modern approaches. In recent years, attention has been focused on the anticancer properties of these medicinal plants. Many plant extracts and phytoconstituents had been tried for their cytotoxic and anticancer potential and most of these plants tend to exert their anticancer properties through antioxidant mechanisms[1]. Plant based antimicrobials represent a vast untapped source for medicines and further exploration of plant antimicrobials needs to occur[2].

Millingtonia hortensis Linn -is a medium sized tree belonging to the family of Bignoniaceae. It is found through out southern Asia and well known for its fragrant flowers. This tree is grown in gardens and parks as an avenue tree for its beautiful and fragrant flowers. This plant used as antipyretic, sinusitis, cholagogue and tonic in Thai folklore medicine[3] and it is a rich source of essential oil, flavonoids, tannin and alkaloids[4]. In the current study, a positive approach had been made to explore the cytotoxic and antimicrobial potential of the aqueous extract of stem bark of *Millingtonia hortensis* Linn.

MATERIALS AND METHODS

Plant Material

Millingtonia hortensis Linn trees were identified and herbarium specimen was deposited in, Centre for Advanced Research in Indian System of Medicine, SASTRA University, Thanjavur. The barks were collected during the month of September – October and shade dried.

The extract

Shade dried barks of *Millingtonia hortensis* Linn was coarsely powdered and aqueous extract was prepared with distilled water using 0.3% of chloroform as preservative. The extract prepared was filtered and condensed by vacuo evaporator.

Cytotoxic Studies

Cell lines

Human cervical cancer cell line (**HeLa**) was procured from National Centre for Cell Sciences (NCCS), Pune, India. Stock cells were cultured in DMEM supplemented with 10% inactivated Fetal Bovine Serum (FBS), penicillin (100 IU/ml), streptomycin (100 µg/ml) and amphotericin B(5 µg/ml) in an humidified atmosphere of 5% CO_2 at 37°C until confluent. The cells were dissociated with TPVG solution (0.2% trypsin, 0.02% EDTA, 0.05% glucose in PBS). The stock cultures were grown in 25 cm^2 culture flasks and all experiments were carried out in 96 microtitre plates (Tarsons India Pvt. Ltd., Kolkata, India).

Preparation of Test Solutions

For cytotoxicity studies, weighed test drug was dissolved in distilled DMSO and volume was made up with DMEM supplemented with 2% inactivated FBS to obtain a stock solution of 1 mg/ml concentration and sterilized by filtration. Serial two fold dilutions were prepared from this for carrying out cytotoxic studies.

Determination of cell viability by MTT Assay

The monolayer cell culture was trypsinized and the cell count was adjusted to 1.0 x 10^5 cells/ml using DMEM medium containing 10% FBS. To each well of the 96 well microtitre plate, 0.1 ml of the diluted cell suspension (approximately 10,000 cells) was added. After 24 h, when a partial monolayer was formed, the supernatant was flicked off, washed the monolayer once with medium and 100 µl of different test concentrations of test drugs were added on to the partial monolayer in microtitre plates. The plates were then incubated at 37° C for 3 days in 5% CO_2 atmosphere, and microscopic examination was carried out and observations were noted every 24 h interval. After 72 h, the drug solutions in the wells were discarded and 50 µl of MTT in PBS was added to each well. The plates were gently shaken and incubated for 3 h at 37° C in 5% CO_2 atmosphere. The supernatant was removed and 100 µl of propanol was added and the plates were gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader at a wavelength of 540nm. The percentage growth inhibition was calculated using the following formula and concentration of test drug needed to inhibit cell growth by 50% (CTC_{50}) values is generated from the dose-response curves for each cell line[5-7].

$$\text{Percentage growth inhibition} = 100 - \left(\frac{\text{Mean OD of individual group X 100}}{\text{Mean OD of control group}} \right)$$

Antimicrobial studies

Test Microorganisms

The following bacterial strains and fungi strains were used for screening the antimicrobial activity on a broad spectrum basis. All microbial human pathogens used were procured from IMT, Chandigarh. Bacteria such as *Staphylococcus aureus* (NCIM 2079), Bacillus *subtilis* (NCIM 2063), *Salmonella paratypi* (NCIM 2501), *Salmonella paratypi* (NCIM 2501), *Klebsiella pneumoniae* (NCIM 2707), *Micrococcus luteus* (NCIM 2169), *Staphylococcus albus* (NCIM 2178), *Vibrio cholerae* (NCIM 1738), *Corynebacterium diphtheriae* (NCIM 2640), *Pseudomonas aureginosa* (NCIM 2200) and fungi such as *Monococcus purpurea* (MTCC1090, *Aspergillus fumigatus* (MTCC 1811) were utilized for the study.

Media used

Nutrient agar (NA) and potato Dextrose agar (PDA) were used respectively for testing the antibacterial and antifungal activity and their compositions were given below.

Composition of Nutrient Agar: (gm/ litre)

Peptone : 5.0

Beef extract : 3.0

Yeast extract : 3.0

Sodium chloride : 5.0

Agar : 20.0

Distilled water : 1 litre

pH : 7.2 ± 0.2

Composition of potato Dextrose Agar (PDA) (gm/l)

Infusion from potatoes : 200 g

Dextrose : 20 g

Agar : 15 g

Distilled water : 1 litre

pH : 5.6 ± 0.2

Inoculation

Inoculation of each bacterial and fungi strain were suspended in nutrient broth and incubated for 8 hrs at 37^{0}C.

Determination of anti-microbial activity

Agar well diffusion method[8-12] was followed to determine the anti-microbial activity. Nutrient Agar (NA) and potato dextrose agar (PDA) were inoculated with the organisms and allowed to solidify. Four wells of 10mm diameter were made in each of this plate using sterile cork borer. About 0.3 ml of different concentration of plant extract were added using sterilized dropping pipette into the wells and allowed to diffuse at room temperature for 2 hrs. The plates were incubated at 37^{0}C for 18 – 24 hrs for bacteria pathogens and 3 days for fungal pathogens. Diameter of inhibition zones were recorded for determining the anti-microbial activity.

RESULTS AND DISCUSSIONS

The results of cytotoxic studies of aqueous extract of *Millingtonia hortensis* Linn against human cervical cancer cell line were given in the Table.01.

Table 1: Cytotoxic properties of *Millingtonia hortensis* Linn in human cervical cancer cell line by MTT assay

S. No	Cell lines	Test Concn. In µg/ml	% Cytotoxicity	CTC_{50} in µg/ml
1	Human cervical cancer cell line	1000	45.98	> 1000
		500	39.54	
		250	38.32	
		125	31.63	
		62.5	23.72	
		31.25	0.00	

The results of antimicrobial activity of aqueous extract of *Millingtonia hortensis* Linn against various human pathogens were given in the Table.02.

Table 2: *In vitro* antimicrobial activity of *Millingtonia hortensis* Linn

S. No	Microorganism	Inhibition zones in mm			
		Std (Ciprofloxacin) 5µg/ml	50 µg /ml	100 µg/ml	250 µg /ml
Bacteria					
1.	*Staphylococcus aureus* (NCIM 2079)	25	-	-	10
2.	*Bacillus subtilis* (NCIM 2063)	35	-	-	13
3.	*Salmonella paratypi* (NCIM 2501)	37	-	-	09
4.	*Salmonella paratypi* (NCIM 2501)	18	-	-	09
5.	*Klebsiella pneumoniae*(NCIM 2707)	13	-		12
6.	*Micrococcus luteus* (NCIM 2169)	30	-	-	09
7.	*Staphylococcus albus* (NCIM 2178)	40	-	-	10
8.	*Vibrio cholerae*(NCIM 1738)	19	-	-	8
9.	*Corynebacterium diphtheriae* (NCIM 2640)	37	-	-	10
10.	*Pseudomonas aureginosa*(NCIM 2200)	13	-	-	10
Fungi					
		Std(Clotrimazole) 20µg	**50 µg /ml**	**100 µg/ml**	**250 µg /ml**
1.	*Monococcus purpurea* (MTCC1090	7	-	-	12
2.	*Aspergillus fumigatus* (MTCC 1811)	7	-	-	12

In the present study, the effect of aqueous extract of *Millingtonia hortensis* Linn against human cervical cancer cell line (**HeLa**) and various microbes were qualitatively and quantitatively assessed. The results for the MTT assay and inhibition of zones were tabulated in table 01 and 02 respectively.

Cytotoxicity studies were widely used in *in vitro* toxicology studies. In the present study, cytotoxic effect of aqueous extract of *Millingtonia hortensis* Linn against Human cervical cancer (HeLa) cell lines were determined by MTT assay. The results indicate that *Millingtonia hortensis* stem bark is poor cytotoxic agent against human cervical cancer line. This was clearly depicted by the occurrence of CTC_{50} value more than 1000mµg/ml.

Millingtonia hortensis Linn against various microbial pathogens also indicate poor antibacterial property. Their zone of inhibition against most of the bacterial microbes at various concentrations was found to be very poor at about their highest concentration itself. However, at about 250µg/ml they had shown good antimicrobial activity against few bacterial strains such as *Pseudomonas aureginosa* (NCIM 2200), *Klebsiella pneumoniae* (NCIM 2707) and fungi *Monococcus purpurea* (MTCC1090, *Aspergillus fumigatus* (MTCC 1811).

The isolation and purification of phytoconstituents from stem bark of *Millingtonia hortensis* Linn may yield a novel antifungal agent rather than an antibacterial or cytotoxic agent.

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