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RESEARCH ARTICLE

ANTI-CANCER POTENTIALS OF *MUKIA MADERASAPATANA* (LINN) M. ROEM LEAF EXTRACTS AGAINST COLO320 CELLS

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ABSTRACT

Understanding traditional medicines better has been the priority for scientists looking for a safer alternative for their synthetic counterparts. In the present study, an attempt was made to understand the anti-cancer potentials of *Mukia maderasaptana* (Linn) M. Roem. leaf extracts against human colon cancer cell lines Colo320 DM. The plant materials were subjected to sequential cold percolation extraction in Hexane, Chloroform and Methanol solvents. The phytoconstituents were determined using standard procedures. MTT based tetrazolium microculture assay was performed to understand the cytotoxicity of the extracts against the colon cancer cell lines, which showed significant activity. Further, the apoptosis induction potential of the extracts in the cancer cells was determined by CASPASEs quantification assay which showed the upregulation of these enzymes vital to apoptosis. The experiments revealed apoptotic induction in the cells by the extracts.

INTRODUCTION

Cancer is a growing public problem whose estimated worldwide new incidence is about 6 million cases per year and it is the second major cause of deaths after cardiovascular diseases and the disease is characterized by unregulated proliferation of cells (Srivastava *et al*., 2005). Colon, or colorectal cancer is a cancer that starts in the large intestine or the rectum. Colorectal cancer is one of the leading causes of cancer-related deaths in the world, owing to the modern lifestyle (Cunningham *et al*., 2010). Multidisciplinary scientific investigations are making best efforts to combat this disease, but the sure-shot, perfect cure is yet to be brought into world medicine (Ayyanar, 2012). Cancer-targeted phytotherapy falls into this category of approach. The use of natural products as anticancer agents has a long history that began with folk medicine and through the years has been incorporated into traditional and allopathic medicine (Costa-Lotufo *et al*., 2005).

Mukia maderasapatana (Linn.) M. Roem. Syn.*Melothria maderasapatana* (Linn.) Cogn; *Cucumis maderasapatana* (Linn.); *Mukia scabrella* (Linn. f) Arn.; *Bryonia scabrella* Linn. f., locally known as *Musumusukkai* (Tamil) (Family: Cucurbitaceae), is an annual monoecious, climbing vine or prostrate herb, densely covered with white hairs and found commonly in waste places and vacant fields, extending from the plains of the coast, ascending upto 1800m in the hills, almost throughout India and in China, Taiwan, Malaysia, Australia, New Zealand and in Africa (Chatterjee and Pakrashi, 1997; Khare 2007). The leaves of this plant find a prominent place in the Siddha and Ayurveda systems of medicine (Petrus *et al*., 2011). Folkloric traditional medicine claims that the leaves and tender shoots are useful as aperient, diuretic, stomachic, antipyretic, antiflatulent, antiasthmatic, antitussive, antihistaminic, antibronchitic and as an expectorant, in addition to its prescription against vertigo and biliousness (Muthu *et al*., 2006). The present study is an attempt at understanding the anti-cancer potentials of *M.maderasapatana* leaf extracts against human colorectal cancer cell lines.

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MATERIALS AND METHODS

Sample Collection

The leaf materials of the plant *Mukia maderaspatana* were collected from Vandaloor, Chennai, Tamil Nadu, India. It was taxonomically authenticated at the Department of Botany, Government Arts College (Men), Nandanam, Chennai, Tamil Nadu, India. A Voucher specimen was deposited in the department for future reference. The leaves were shade dried for a month and ground into powder.

Chemicals

Rosewell Park Memorial Institute 1640 (RPMI 1640) medium, Trypsin Phosphate Versene Glucose (TPVG) and Fetal Bovine Serum (FBS) were purchased from HiMedia, India. Dimethyl sulfoxide (DMSO), Penicillin and Streptomycin was bought from Sigma Chemical Co. (St. Louis, MO, USA). Cell culture plastic wares were from Nunc Co. (Denmark). All other chemicals and solvents were of analytical grade.

Cell lines

Human Colon carcinoma cell lines Colo320 DM were purchased from the National Centre for Cell Sciences (Pune, India). The cells were cultured in RPMI 1640 containing L-Glutamine and 25 mM 4-[2-hydroxyethyl]-1-piperazineethanesulfonic acid (HEPES), Penicillin (100 µg/mL), Streptomycin (50µg/mL) and 10% foetal bovine serum. Cells were maintained at 37°C in a humidified incubator with an atmosphere of 5% CO_2.

Extract Preparation

The leaves of *M.maderaspatana* were air-dried at room temperature (36°C) for 2 weeks, after which it was ground to a uniform powder.

The extracts of the leaf samples were prepared in a sequential procedure by soaking 100 g of dried powder in 900 ml of different solvents (Hexane, Chloroform and Methanol) for 48 h. At the end of each respective extraction, the extracts were filtered using Whatman filter paper. The filtrate was concentrated under reduced pressure in vacuum at 40°C for 25 min using a rotary evaporator.

Phytochemical Analysis

The leaf extracts were screened for the presence of various phytoconstituents like phenols, tannins, flavonoids, coumarins, cardiac glycosides, etc. (Harborne, 1973).

MTT Assay

The cytotoxicity of the plant extracts against Colo320 cells were analysed using the MTT assay (Mossman, 1983). Approximately 5x10³cells/well (cell line) were seeded into 96 well plate, 100µl of RPMI 1640 medium was added and incubated at 37ºC. After 24hours, the medium was discarded and fresh medium was added with different concentration of plant extracts (100µg/ml, 200µg/ml, 300µg/ml). The plates were incubated for 48h at 37ºC in a CO_2 incubator. After the incubation period, medium was discarded and 100µl fresh medium was added with 10µl of 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT 5mg/ml). After incubation at 37ºC in a CO_2 incubator for 4h, the medium was discarded and 200µl of DMSO was added to dissolve the formazon crystals. Then the absorbance was read in a microplate reader at 570nm and cell survival was calculated by the following formula.

Viability % = (Test OD/Control OD) X 100
Cytotoxocity % = 100 – Viability% The positive control used was Cyclo-90 (Cyclophosphamide) at a concentration of 90µg/ml.

CASPASEs Assay

CASPASEs 3 and 9 activities were determined by chromogenic assays using CASPASE-3 and 9 activation kits according to the manufacturer's protocol (Calbiochem, Merck).

After treating with plant extracts (100, 200 and 300 µg/ml), the cells were lysed using Lysis buffer (50mM HEPES, 100mM NaCl, 0.1% CHAPS, 1mM DTT, 100mM EDTA). Lysates were centrifuged at 10,000rpm for 1 min. The supernatants (cytosolic extract) were collected and protein concentration was determined by the Lowry's method (Lowry, 1951) using BSA as a standard. 100µg protein (cellular extracts) was diluted in 50 µl cell lysis buffer for each assay. Cellular extracts were then incubated in 96-well microtiter plates with 5 µl of the 4mM p-nitroanilide (pNA) substrates (CASPASE-9 activity), DEVD-pNA (CASPASE-3 activity) for 2 h at 37°C. CASPASEs activity was measured by cleavage of the above substrates to free pNA. Free pNA (cleaved substrates) was measured at 405nm in a microtiter plate reader. Relative CASPASE-3 and 9 activities were calculated as a ratio of the absorbance of treated cells to untreated cells. Camptothecin (1µM) was used as positive control.

RESULTS AND DISCUSSION

M. maderasapatana leaf extracts were prepared in Hexane, chloroform and methanol, by sequential cold percolation extraction method. The phytochemical analysis revealed the presence of flavonoids and carbohydrates in all the extracts, while tannins, glycosides and coumarins were found to be present in hexane and methanolic extracts. Phenols were detected in the chloroform and methanol extracts (Table 1). MTT assay is based on the ability of live but not dead cells to reduce a yellow tetrazolium dye to a purple formazan product (Mossman, 1983). Cytotoxicity analysis of leaf extracts of *B.acutangula* and *S.colais* against Colo320 cells suggested the anti-cancer potential of these medicinal plants (Florida *et al*., 2012). MTT assay was also used to analyse the cytotoxic potentials of root extracts of *M.citrifolia* against Colo 320 cells (Samuel *et al*., 2012). In the current study, Colo320 cells were exposed to various concentrations of *M.Maderasapatana* leaf extracts and their cytotoxicity was analysed by MTT assay. Results showed that the chloroform and methanolic extracts of the plant leaves were toxic to the cancer cells (Table 2, Figure 1).

Table 1. Phytochemical constituents present in different solvent extracts of *M.maderasapatana*

Phytochemical Tests	Extracts		
	Hexane	Chloroform	Methanol
Molisch's test (carbohydrates)	+	+	+
Ferric chloride test (tannins)	++	_	+
Foam test (saponins)	_	_	+
Alkaline test (flavonoids)	+	+	++
Mayer's test (alkaloids)	_	_	_
Quinones	+	_	++
Anthraquinone	_	_	_
Glycosides	_	_	_
Cardiac glycosides	+	_	+
Terpenoids test	_	_	_
Triterpenoids	+	+	_
Ferric chloride test (phenols)	_	+	+
Alkaline test (coumarins)	+	_	++
Ninhydrin test (protein)	_	_	_
Steroids and Phytosteroids	_	_	_
Phlobatannins	_	_	_

+ indicates presence; - indicates absence; ++ indicates strongly present

Table 2. MTT assay results after treatment of Colo320 cells with plant extracts

	Hexane			Chloroform			Methanol			PC	C
	100µg	200µg	300µg	100µg	200µg	300µg	100µg	200µg	300µg	90µg	
Viability%	68.452	63.987	55.321	42.762	36.872	31.986	44.652	38.873	32.557	24.745	100
Cytotoxicity%	31.548	36.013	44.679	57.238	63.128	68.014	55.348	61.127	67.443	75.255	0

PC= Positive control, C=Control

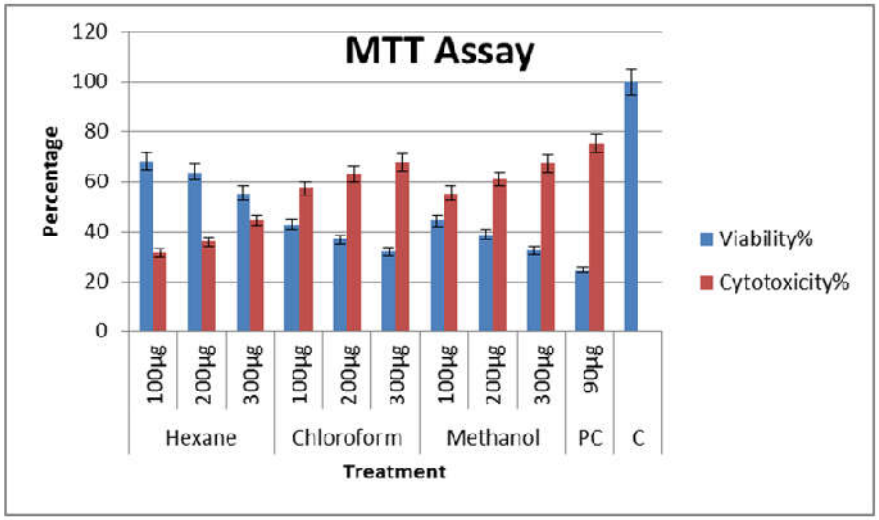

Figure 1. Graphical representation of MTT assay results

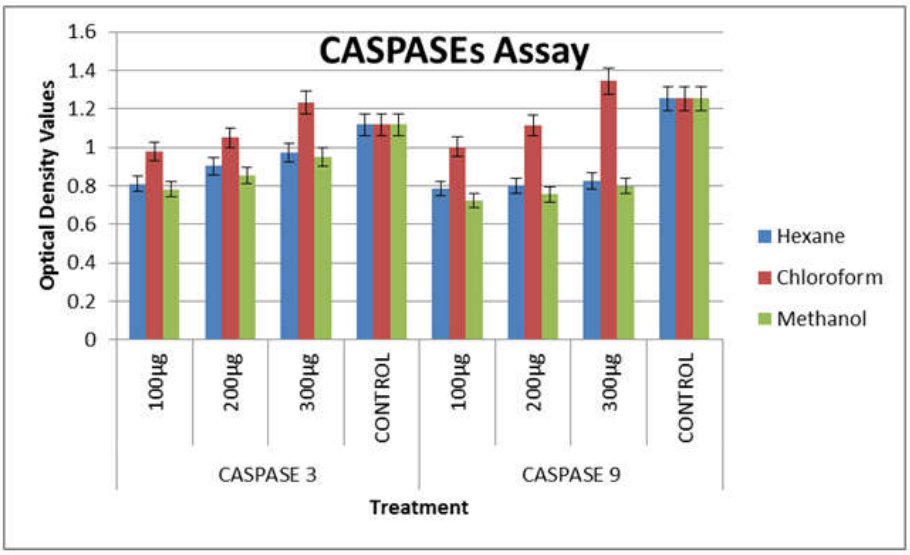

Figure 2. Graphical representation of CASPASEs quantification

The extracts were further analysed for their apoptosis inducing potentials using the CASPASEs quantification assay. The intrinsic and extrinsic apoptotic pathways converge to CASPASE-3, a protease which cleaves the inhibitor of the CASPASE-activated deoxyribonuclease, and the CASPASE activated deoxyribonuclease becomes active leading to nuclear apoptosis. The upstream caspases that converge to CASPASE-3 are CASPASE-9 and CASPASE-8 in the intrinsic and extrinsic pathways, respectively (Florida *et al*., 2012). The present study showed upregulation in the CASPASE-3 and 9 activities in Colo320 cells exposed to *M. Maderasapatana* leaf extracts. Cells treated with chloroform extracts showed considerable upregulation in CASPASEs level, compared to those treated with hexane and methanolic extracts (Table 3, Figure 2). This indicated the activation of apoptosis in human colon cancer cells treated with chloroform extracts of *M. maderasapatana* leaves, with possible involvement of mitochondrial proteins.

Conclusion

India is one of the twelve mega-biodiversity countries of the world having rich vegetation with a wide variety of medicinal plants and a tradition of plant-based knowledge distributed amongst a vast number of ethnic groups. The use of ethnobotanical information in medicinal plant research has gained considerable attention in segments of the scientific community. The importance of medicinal plants in traditional healthcare practices is providing clues to new areas of medical research (Ayyanar, 2012). The present communication is a preliminary report of a scientific approach in validating the existing knowledge about medicinal plants and extending the knowledge to various illnesses. Through the experiments performed, it is suggested that *Mukia maderasapatana* is a medicinal plant with possible anti-cancer potential, and this study needs to be validated with further research.

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