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IN VITRO CYTOTOXICITY OF *MADHUCA INDICA* AGAINST DIFFERENT HUMAN CANCER CELL LINES

Asima Shaban [1], Satish K. Verma*[1], Rajesh Nautiyal [1] Santosh Kumar Singh [2], Reena Purohit [3] and Madhvi Lata Chimata [4]

K.L.P.G College [1], Naini, Allahabad, Uttar Pradesh, India
Department of Chemistry, JNPG College [2], Lucknow, Uttar Pradesh India
Department of Biotechnology SIPAS Dehradun [3], Uttarakhand, India
Department of Biotechnology& Biochemistry, DPMC [4], Dehradun, Uttarakhand, India

ABSTRACT

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Correspondence to Author:

Dr. Satish Kumar Verma

Lecturer, Department of Zoology, K.L.P.G College, Naini, Allahabad, Uttar Pradesh, India

Cancer is a public health problem all over the world. Large number of plants and their isolated constituents has been shown to potential anticancer activity. Ethanolic whole plant extract of *Madhuca indica* showed *in vitro* cytotoxicity against different human cancer cell lines such as lung, neuroblastima, and colon. There was no growth of inhibition recorded against liver cancer cell line. Sulforhodamine B dye (SRB) assay was done for *in vitro* cytotoxicity test assay. The *in vitro* cytotoxicity was performed against five human cancer cell lines namely of lung (A-549), liver (Hep-2) colon (502713 HT-29) and neuroblastima (IMR-32). The activity was done using 100µg/ml of the extract. Against lung (A-549) cell line plant extract showed 83% growth of inhibition. In case of liver (Hep-2) showed no activity reported, where as in case of colon 502713 cell line plant extract showed maximum activity. In case of HT-29 liver human cancer line and IMR-32 neuroblastima cell line plant extract showed 99% and 98% activity respectively.

INTRODUCTION: *Madhuca indica* commonly known as mahua belongs to the family Sapotaceae. Mahua is a large, shady, deciduous tree doting much of the central Indian landscape, both wild and cultivated. Mahua seeds are of economic importance as they are good source of edible fats [1]. The distilled juice of the flower is considered a tonic, both nutritional and cooling and also in treatment of helminthes, acute and chronic tonsillitis, as well as bronchitis [2]. The leaves are applied as a poultice to relieve eczema. The aerial parts are used for treatment of inflammation [3]. The bark is a good remedy for itch, swelling, fractures and snake-bite poisoning, internally employed in diabetes mellitus.

Previous phytochemical studies on *Madhuca indica* included characterization of sapogenins, triterpenoids, steroids, saponins, flavonoids and glycosides [4, 5].

In the present communication, we report its *in vitro* cytotoxicity activity against different human cancer cell lines.

Experimental:

Plant material: *Madhuca indica* was collected in February to March 2009 from Allahabad. The whole plant ethanolic extract was used for *in vitro* cytotoxicity properties.

Preparation of Plant Extracts: Plant material was dried at 37°C, powdered and extracted in ethanol. Extract was fine-filtered and freeze dried. For the preparation of the extracts, dried ground plant material was percolated with 95% ethanol and concentrated to dryness under reduced pressure. Extract was redissolved in Dimethylsulphoxide (DMSO) to form stock solutions, which were filter sterilized (0.2µm) before testing on cell lines.

Human Cell Lines: Human cancer cell lines namely of lung (A-549), liver (Hep-2) colon (502713 HT-29) and neuroblastima (IMR-32) were grown in RPMI-1640 with 2 mM L-glutamine medium pH 7.2. Penicillin was dissolved in PBS and sterilized by filtering through 0.2µ filter in laminar air flow hood. The media was stored in refrigerator (2-8°C). Complete growth medium contains 10 % FCS. The medium for cryopreservation contains 20 % FCS and 10 % DMSO in growth medium. The cell lines were maintained at 37°C in a 5% CO_2 atmosphere with 95% humidity.

***In vitro* assay for Cytotoxic Activity:** The anticancer activity was determined by the cytotoxic potential of the test material using human cancer cell lines which were allowed to grow on tissue culture plates in the presence of test material. The cell growth was measured using ELISA reader after staining with Sulforhodamine B dye (SRB) which binds to basic amino acid residues in the trichloroacetic acid (TCA) fixed cells.

Preparation of Cell Suspension for Assay: Human cancer cell lines were grown in multiple tri conical flasks (TCFs) at 37°C in an atmosphere of 5% CO_2 and 90% relative humidity in complete growth medium to obtain enough number of cells. The flasks with cells at subconfluent stage were selected. Cells were harvested by treatment with Trypsin-EDTA.

Cells were separated to single cell suspension by gentle pipetting action and the viable cells were counted in a hemocytometer using trypan blue. Cell viability at this stage should be >97%. Viable cell density was adjusted to 5,000 - 40,000 cells/100µl depending upon the cell line Monks [6]. 100µl of cell suspension together with 100µl of complete growth medium was added into each well. The plates were incubated at 37°C for 24 hours in an atmosphere of 5% CO_2 and 90% relative humidity in a CO_2 incubator. After 24 hours, the test material, DMSO (vehicle control) and positive control were added.

Sulforhodamine B (SRB) Assay: The antiproliferative SRB assay was performed to assess growth inhibition. This is a colorimetric assay which estimates cell number indirectly by staining total cellular protein with the SRB dye [7]. The microtiter plates were taken out after 48 hours incubation of the cells with test materials and gently layered with chilled 50% TCA in all the wells to produce a final concentration of 10%. The tissue culture plates were incubated at 4°C for one hour to fix the cells attached to the bottom of the wells. The supernatant was then discarded.

The plates were washed five times with distilled water to remove TCA, growth medium, low molecular weight metabolites, serum proteins etc. Plates were air dried and stored until further use. SRB solution was added to each well of the plates and incubated at room temperature for 30 minutes. The unbound SRB was removed quickly by washing the wells five times with 1 % acetic acid and then air dried. 100µl of Tris buffer (0.01 M, pH 10.4) was added and shaken gently for 5 minutes on a mechanical shaker. Optical density was recorded on ELISA reader at 515 nm.

RESULTS & DISCUSSION: Plants are storehouse of good variety of compounds. Latest and previous studies have concluded the beneficial aspects of plant derived drugs as good source of Anticancer activity agents [8, 9]. The test sample showing growth inhibition more than 70% at 100µg/ml is considered to be active. The *in vitro* cytotoxicity was performed against five human cancer cell lines namely of lung (A-549), liver (Hep-2) colon (502713 HT-29) and neuroblastima (IMR-32). Against lung (A-549) cell line plant extract showed 83% growth of inhibition. In case of liver (Hep-2) showed no activity. Where as in case of colon 502713 cell line plant extract showed maximum activity. In case of HT-29 liver human cancer line and IMR-32 neuroblastima cell line plant extract showed 99% and 98% activity respectively.

In the present study, we conclude that the plant extracts showed selective *in vitro* cytotoxity, active against some human cancer cell lines and other not showed activity.

Activity depends upon the morphology and mechanisim of action plant extract. Many plant extract kill the cancer cell lines through activating apoptosis and some through effecting growth regulators. Other than anticancer activity this plant extract also show the other activity. It is not possible at this juncture to single out the most effective *in vitro* cytotoxicity constituent of plant. However, based on the published studies alkaloids seem to be most likely candidates eliciting *in vitro* cytotoxicity effect. Its reported *in vitro* cytotoxicity effects warrant further investigation for its use in the cases of clinical anticancer activity.

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