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PRELIMINARY PHYTOCHEMICAL SCREENING AND ANTICANCER POTENTIAL OF ETHANOLIC EXTRACT OF *Madhuca neriifolia* (Moon) H.J. Lam

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ABSTRACT

The present study involves the preliminary phytochemical screening and evaluation of the anticancer potential of ethanolic extract of stem of *Madhuca neriifolia (Moon) H.J. Lam.* Anicancer potential of plant stems were investigated by standard MTT assay and the IC50 value was calculated from linear regression analysis. The invitro cytotoxic effect of the stem extract of *Madhuca neriifolia (Moon) H.J. Lam* was recognized and the percentage of growth inhibition was increased with increasing concentration of test compounds. IC50 value of this assay was 79.72 µg/ml. The present findings conclude that, the stem of *Madhuca neriifolia (Moon) H.J. Lam* acquired anticancer effect.

KEYWORDS: *Madhuca neriifolia (Moon) H.J. Lam,* Soxhlet extraction cytotoxic, MTT assay, linear regression analysis.

INTRODUCTION

India is one of the 12 important bio-diversity centers with the presence of over 45,000 different plants. But out of this strong resource only about 7,000 plants are used in Ayurveda, 600 in Siddha, 700 in Unani and 30 in modern medicine. Certainly, the plant kingdom still holds many species of plants containing substances of medicinal value, which have yet to be exposed.[1-4] Natural products are vital part of human health care system. Several mechanisms of action for herbal medicines and their bioactive components which may reduce cancer risk through their anti-oxidant and anti-tumorigenic properties were discovered through many

research activities. Literature survey revealed that, various medicinal plants are used for the treatment and management of cancer by various tribal communities.[5, 6]

Madhuca neriifolia (Moon) H.J. Lam belong to the family Sapotaceae, medium sized tree; bark dark brown. Leaves simple, crowded at the tips of branchlets,linear-oblong or oblanceolate, acute or obtuse, 7.5-25x 2.5-6 cm. Flowers yellowish white, in clusters of 4-10,axillary or from the scars of fallen leaves. Fruits ellipsoid, about 2.5 cm long. Flowers are used in the treatment of kidney complaints. Fruits are recommended in cases of rheumatism, biliousness, asthma and worm trouble. Oil from seeds are used to treat rheumatism and for improved growth of hair.[7]

Tumor is a mass of tissues which proliferate rapidly, spread throughout the body and may cause death of the host. The Chemotherapeutic agents are effective against various types of tumor are not totally free from side effects. Hence an attempt has been made to assess some plant products against cancer that are having lesser side effects. Many Indian Plants are used in different types of cancer. A vast literature collection did not generate a scientific evidence to prove the anti tumor activity of *Madhuca neriifolia* (Moon) H.J. Lam. But the plant is used as classical anticancer drug in certain regions of Kerala. Hence this study was planned.

The MTT assay was first described by Mosmann [8] and then improved by others.[9] It is a sensitive, quantitative and reliable colorimetric assay that measures the viability, proliferation and activation of cells. The assay is based on the capacity of the cellular mitochondrial dehydrogenase enzyme in living cells to reduce the yellow water-soluble substrate 3-(4, 5-dimethylthiazol-2yl)-2, 5-diphenyl tetrazolium bromide (MTT) into a dark blue/purple formazan product which is insoluble in water. The amount of formazan produced is directly proportional to the cell number in a range of cells lines.[8, 10] The results are consistent with those obtained from 3H-thymidine uptake assays.[11] The MTT assay has greater applicability in the detection of cells which are not dividing but are still metabolically active. Therefore it can be used to distinguish proliferation and cell activation.[10] An additional advantage to this assay is that it can be used in suspended or monolayer cell preparations.[12,13] Unfortunately, during conduct of the test, cells die making it impossible to conduct follow-up cell culture assessments.[14] Moreover, while MTT reduction only occurs in metabolically active cells, marked changes in metabolic activity can result in significant changes in results despite the fact that the number of viable cells remains constant.

IC 50 value is used to determine the concentration of an anti-cancer drug that kills half of the cells in a cancer cell line and the value was calculated by non-linear regression analysis.[15]

MATERIALS AND METHODS

Plant material and preparation of extracts

Fresh stem of *Madhuca neriifolia (Moon) H.J. Lam* was collected from the western guards of Kerala, authenticated and identified by Dr.St. Tessy Joseph,H.O.D ,Dept of Botany,Nirmala College of pharmacy, kerala. Shadow dried and powdered. Powdered material was passed through sieve No.60. Then extracted separately using hexane, petroleum ether, chloroform, ethyl acetate, ethanol by Soxhlet extraction method. Hot percolation method was employed for water for 48 hrs. The extracts were concentrated using rotary vacuum evaporator. Dried extracts were stored in an airtight container and placed in refrigerator.

Phytochemical analysis of *Madhuca neriifolia* (Moon) H.J. Lam stem extracts

Various qualitative tests were performed on the various stem extracts of *Madhuca neriifolia* (Moon) H.J. Lam for the identification of phytoconstituents [16-23] and the results were presented in Table 1.

Table 1: Phytochemical screening of stem extracts of *Madhuca neriifolia* (Moon) H.J. Lam

SI/NO	Test	H	P	C	E.A	E	W
1	Carbohydrates	-	-	-	-	-	+
2	Glycosides	-	-	-	-	+	+
3	Saponins	-	-	+	+	+	-
4	Alkaloids						
	Dragendorff's test	-	-	-	-	-	-
	Wagner's test	-	-	-	+	+	-
5	Flavonoids	-	-	-	-	+	-
6	Anthocyanosides	-	-	-	-	+	-
7	Phenolic and Tannins	-	-	-	-	+	+
8	Phytosterols and Triterpenoids						
	Salkowaski test	+	+	+	+	-	-
	Leiberman-Burcharat test	+	+	-	-	-	-
9	Fixed oils and fats	+	+	+	-	+	-

– : Absence ; + : Presence

Based on the presence of more phytoconstituents ethanolic extract was preferred for the investigation of cytotoxic activity.

Cell culture

SKMEL skin cancer cells were purchased from National Centre for Cell Sciences, Pune. It was maintained in Dulbecco's modified eagles media supplemented with 10% FBS and grown to confluency at 37°c in 5 % carbondioxide in a humidified atmosphere in a carbondioxide incubator.

Invitro cytotoxic activity of *Madhuca neriifolia* (Moon) H.J. Lam *s*tem extract

The cells were trypsinized for 2 minutes and passaged to T flasks in complete aseptic conditions. Extract was added to grown cells at the concentrations of 10 - 100µg from a stock of 10mg/ml in 0.1% DMSO and incubated for 24 hours. Dilution of stock solutions was prepared in culture medium yielding final extract concentrations with a final DMSO concentration of 0.1%. This concentration of DMSO did not affect the cell viability. Control cells were incubated in culture medium only. Every concentrations of the plant extract were in triplicates on the same cell batch.

MTT Assay

The % difference in viability was determined by standard MTT assay after 24 hours of incubation. The cell culture suspension was washed with 1x PBS and then 30 µl of MTT solution was added to the culture. Then incubated at 37^0C for 3 hours. MTT was removed by washing with 1x PBS and 200µlof DMSO was added to the culture. Incubation was done at room temperature for 30 minutes until the cell got lyses and colour was obtained. The solution was transferred to centrifuge tubes and centrifuged at 4000 rpm for 2minutes to precipitate cell debris. Optical density was examined at 540 nm using DMSO as blank in an ELISA reader.

% viability = (OD of Test/ OD of Control) X 100.

Statistical Analysis

Experimental results were expressed as mean ± SD. All measurements were replicated three times. The data were analyzed by an analysis of variance ($P < 0.05$). The IC50 values were calculated from linear regression analysis.[24]

RESULTS & DISCUSSION

Preliminary phytochemical screening of ethanolic extract of the stem of *Madhuca neriifolia (Moon) H.J. Lam* exhibited the presence of ,saponins,flavonoids,anthocyanosides,alkaloids,

glycosides,phytosterols and triterpenoids,fixed oils and fats and the results were offered in Table.no:1

Determination of Invitro cytotoxic activity of ethanolic extract of stem of *Madhuca neriifolia* (Moon) H.J. Lam .

The in vitro cytotoxic activity by MTT assay on SKMEL skin cancer cells was conducted. Control and the stem extract of *Madhuca neriifolia* (Moon) H.J. Lam (Test) were used. The test results were presented in Table 2. Cytotoxicity activity of plant extract was carried out against SKMEL skin cancer cell line at different concentrations to determine the IC50 (50% growth inhibition) by MTT assay. Results of different concentrations of ethanolic extract of *Madhuka neriifolia* from 10 – 100 µg/ml were tabulated in Table 2, and graphically represented in Figure 3. MTT assay of ethanolic extract of *Madhuca neriifolia* (Moon) H.J. Lam exhibited significant effect on SKMEL skin cancer cells at microgram levels. The highest cytotoxicity of this extract against SKMEL skin cancer cells was found to be 100µg/ml concentration with 51.68% of cell growth inhibition. It was found that, the percentage of growth inhibition is increasing with increasing concentration of test compounds. The MTT assay results revealed that, ethanolic extract of stem of *Madhuca neriifolia* (Moon) H.J. Lam exhibiting good anticancer activity and satisfactiory IC 50 value of 79.72 µg/ml. Hence the ethanolic extract of stem of *Madhuca neriifolia* (Moon) H.J. Lam can be considered as a potential source for anticancerous activity but further studies are required for isolation and identification of biologically active substances.

Table 2: Cytotoxicity activity of *Madhuca neriifolia* (Moon) H.J. Lam extract against SKMEL skin cancer cells at different concentrations by MTT Assay

Concentrations of *M.neriifolia*., µg/ml	Absorbance, Mean ± SD	Inhibition, %	IC50, µg/ml
10	0.2745±0.03	44.80	
20	0.2725±0.02	45.20	
30	0.2685±0.04	46.01	
40	0.2643±0.06	46.85	
50	0.2600±0.03	47.77	
60	0.2565±0.07	48.42	
70	0.2524±0.05	49.43	
80	0.2475±0.06	50.24	79.72
90	0.2444±0.08	50.85	
100	0.2406±0.02	51.62	
Control	0.4973±0.01		

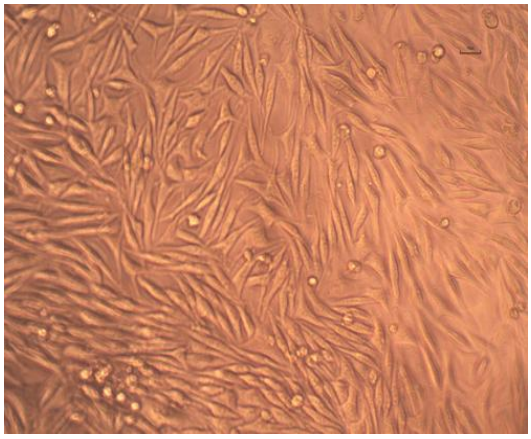

Fig. 1: Effect of control on SKMEL skin cancer cells.

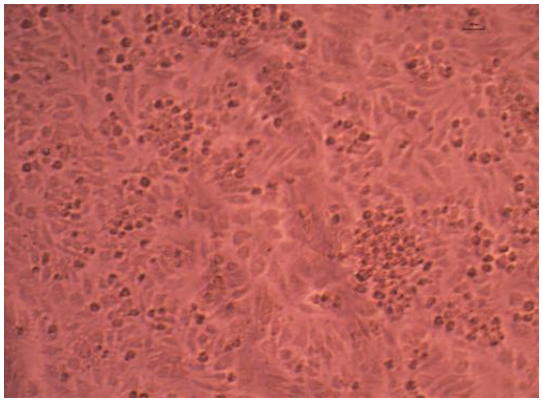

Fig. 2: Effect of ethanolic extract of *Madhuca neriifolia* (Moon) H.J. Lam on SKMEL skin cancer cells.

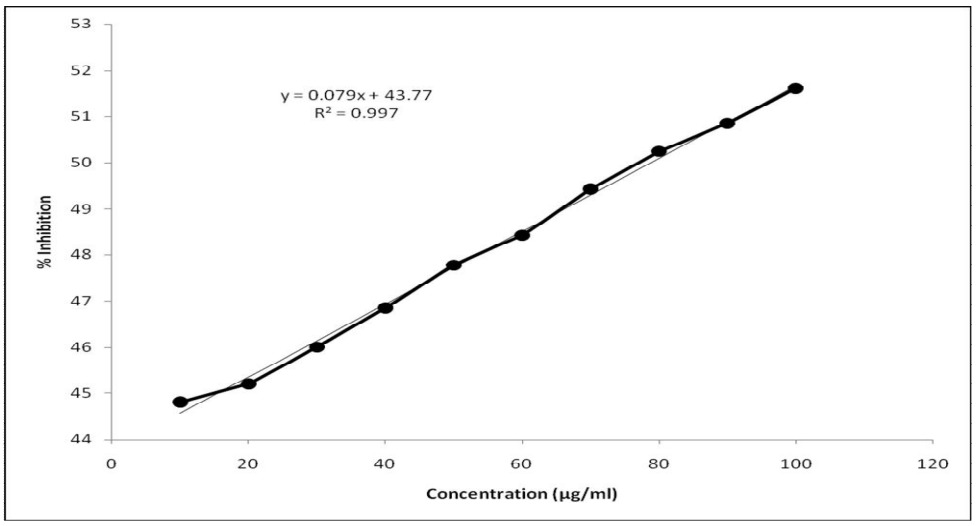

Fig. 3: Growth inhibition of *Madhuca neriifolia* (Moon) H.J. Lam extract against SKEMIL skin cancer cells by MTT

Abbreviations

MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide [24]

IC50: 50% inhibitory concentration [26]

FBS: Fetal bovine serum [25]

DMSO: Dimethyl sulfoxide [27]

PBS: Phosphate buffer saline [25]

ELISA: Enzyme-linked immunosorbent assay [29]

OD: Optical density [28]

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