Scholars Journal of Applied Medical Sciences (SJAMS) **ISSN 2320-6691 (Online)**
Sch. J. App. Med. Sci., 2014; 2(4A):1235-1238 **ISSN 2347-954X (Print)**

(An International Publisher for Academic and Scientific Resources)
www.saspublishers.com **DOI:** 10.36347/sjams.2014.v02i04.013

Research Article

The Bioactive Compounds Obtained from the Fruit-Seeds of *Madhuca longifolia* (L) Act as Potential Anticancer Agents

Asish Bhaumik[1*], M. Upender Kumar[1]; Kaleem Ahmed Khan[2], Ch. Srinivas[3]
[1]Department of Pharmaceutical Chemistry[1], Teja College of Pharmacy, Kodad, Nalgonda-508206, India
[2]Department of Pharmacology[2], Teja College of Pharmacy, Kodad, Nalgonda-508206, India
[3]Department of Pharmaceutical Analysis[3],Teja College of Pharmacy, Kodad, Nalgonda-508206, India

***Corresponding author**
Asish Bhaumik
Email: bhaumik.asish@gmail.com

Abstract: *Madhuca longifolia* (Mahua)is a mediumto large sized deciduous tree belonging of amily Sapotaceae, commonly known as the Butter nut tree. *Madhuca longifolia*is reported to contain sapogenins, triterpenoids, saponins, steroids, flavonoids and glycosides. It is used as spasmogenic, uterotonic, oxytocic, anti-bacterial, anti-tumour, anti-implantation,anti-progestational,antiestrogenicagainstmenorrhagiaandanti-cancer. The extract was prepared by reflux condensation method. The objective of the present work is to search anticancer activity. Based on this,a new series of constituents have been planned to extract by Methanol (E1), Ethanol (E2), Acetone (E3), chloroform (E4)from fruit-seeds of *Madhuca longifolia*. The *in-vitro* anticancer studies were performed against human cancer cell line (HeLa) and MTT assay was used to analyze the cell growth inhibition. The results showed that the various extracts of fruit-seeds of *Madhuca longifolia* have a very good to moderate anticancer activity.
Keywords: *Madhuca longifolia*, Sapotaceae, Phytochemistry, HeLa, MTT assay

INTRODUCTION

Madhuca longifolia (Mahua)belongs to family Sapotaceae. *Madhuca* is also known as the Butter nuttree. It is a medium to large sized deciduous treeabout17mhigh with a large top, distributed in Nepal, India and Sri Lanka[1, 2].

Leaves: Clustered at end of the branches; coriaceous, elliptic, shortly acuminate, basecuneate.
Flower: numerous, near the ends of branches, drooping on pedicels.
Calyx: coriaceous, densely clothed rusty tomentum.
Corolla: yellowish-white, tubefleshy.
Stamens: 20-30,usually24or26,anthershispidat the back with stiff hairs.
Fruits: berries, ovoid, fleshy and green, seeds [1]

It is associated with diverse pharmacological activities. The flowers are traditionally used as cooling agent, aphrodisiac, tonic, astringent, demulcent and for the treatment of acute and chronic tonsillitis, helminthes, pharyngitis andbronchitis, inflammation, eczema[1, 3, 7, 8]. Flowers are used in the treatment of eye diseases, flowers mixed with milk are useful in impotency and general debility [4]. Juice of flower is use as treatment of skin disease [5]. The distilled juice of the flower is used as tonic, both nutritional and cooling [6, 7]

Leaves are used as expectorant for the treatment of chronic bronchitis and Cushing's disease [8]. The bark is a good remedy for itch, swelling, fractures and snake-bite poisoning, internally employed in diabetes mellitus [8].

Previous phytochemical studies on Mahua included characterization of sapogenins, triterpenoids, steroids, saponins, flavonoids and glycosides [9, 10].

MATERIALS AND METHODS

Weigh 50 g of fruit-seeds *Madhuca longifolia* (unriped can be mashed to prepare a paste) into a 500 ml round-bottomed flask. Add 200 ml of methanol. Heat the mixture under reflux for 5 min on stem-bath with frequent shaking. Filter the mixture under suction and transfer the filtrate to a separatory funnel. Wash this mixture containing bioactive compounds with three portions of 250 ml each with sodiumchloride solution. Dry the organic layer over anhydrous magnesium sulfate. Filter and evaporate most of the solvent in vacuum without heating**.**

Preliminary Phytochemical screening [11, 12]

Preliminary phytochemical tests of various extracts of fruit-seeds of *Madhuca longifolia* have shown the presence of following bioactive compounds.

Name of the Extracts	Bioactive compounds
E1 (+), E2(+), E3(+), E4(-)	Reducing sugar
E1 (-), E2(-), E3(-), E4(-)	Pentoses
E1 (+), E2(+), E3(+), E4(-)	Ketohexoses
E1 (+), E2(+), E3(+), E4(-)	Disaccharides
E1 (+), E2(+), E3(+), E4(-)	Aromatic aminoacids
E1 (+), E2(+), E3(-), E4(-)	Tyrosin
E1 (+), E2(+), E3(+), E4(-)	Tryptophan
E1 (+), E2(+), E3(+), E4(-)	Arginine
E1 (+), E2(+), E3(-), E4(-)	Alpha aminoacids and dipeptides
E1 (+), E2(+), E3(+), E4(-)	Phytosterol
E1 (+), E2(+), E3(+), E4(+)	Polyphenols (Flavanoids)
E1 (+), E2(+), E3(+), E4(-)	Alkaloids
E1 (+), E2(+), E3(+), E4(+)	Saponin glycosides
E1 (+), E2(+), E3(+), E4(-)	Mono and sesquiterpeniods
E1 (+), E2(+), E3(+), E4(-)	Long chain fatty acids -palmitic, stearic, arachidic, hexadecenoic, oleic, linoleic , and dihydroxy stearic acids

(+) = Presence, (-) = Absence

***In-vitro* evaluation of anticancer activity by MTT assay [13-15]**

Cell culture

The human cervical adenocarcinoma cell line (HeLa) was provided by National Centre for Cell Science (NCCS), Pune and was grown in Eagles Minimum Essential Medium (EMEM) which contained 10% fetal bovine serum (FBS). All cells were maintained at 37°C, 100% relative humidity, 5% CO2, 95% air and the culture medium was changed twice a week.

Cell treatment

The monolayer cells were detached and single cell suspensions were made using trypsin-ethylene diamine tetra acetic acid (EDTA). A hemocytometer was used to count the viable cells and the cell suspension was diluted with a medium containing 5% FBS in order to obtain final density of $1x10^5$ cells/ml. 96-well plates at plating density of 10,000 cells/well were seeded with one hundred microlitres per well of cell suspension and incubated for cell attachment at 37° C, 5% CO2, 95% air and 100% relative humidity. The cells were treated with serial concentrations of the test samples after 24 hr. Serial dilution method was used for preparing test samples of different concentrations. Cells were initially dissolved in dimethylsulfoxide (DMSO) and further diluted with serum free medium to obtain twice the desired final maximum test concentration. The required final drug concentrations of 1.25, 2.5, 5 and 10 µg/ml were obtained by adding aliquots of 100 µl of the different drug dilutions to the appropriate wells already containing 100 µl of medium.After addition of the drug the plates were incubated for an additional 48 hr at 37° C, 5% CO2, 95% air and 100% relative humidity. The medium without samples served as control and triplicate was maintained for all concentrations.

MTT assay

After 48h of incubation, to each well 15µl of MTT (5mg/ml) in phosphate buffered saline (PBS) was added and incubated at 37° C for 4h. The medium with MTT was flicked off and the formed formazan crystals were solubilized in 100µl of DMSO. Using micro plate reader the absorbance was measured at 570 nm. The % cell inhibition was determined using the following formula. % Cell Inhibition = [100- Abs (sample)/Abs (control)] x100. Same procedure was carried out for the extraction with different solvents.

Table 1: For Percentage (%) of Cell Growth Inhibition of Methanolic Extract (E1) of Fruit-Seeds of *M. longifolia* on He La Cells by MTT Assay

Sl.No.	Concentration of the Extracts (µg/mL)	Absorbance	Inhibition of Cell Growth (%)
1	10	1.519	62.61%
2	5	1.56	60.56%
3	2.5	1.62	57.54%
4	1.25	1.63	57.04%
5	Control	1.91	0

Table 2:For Percentage (%) of Cell Growth Inhibition of Ethanolic Extract (E2) of Fruit-Seeds of *M. longifolia* on He La Cells by MTT Assay

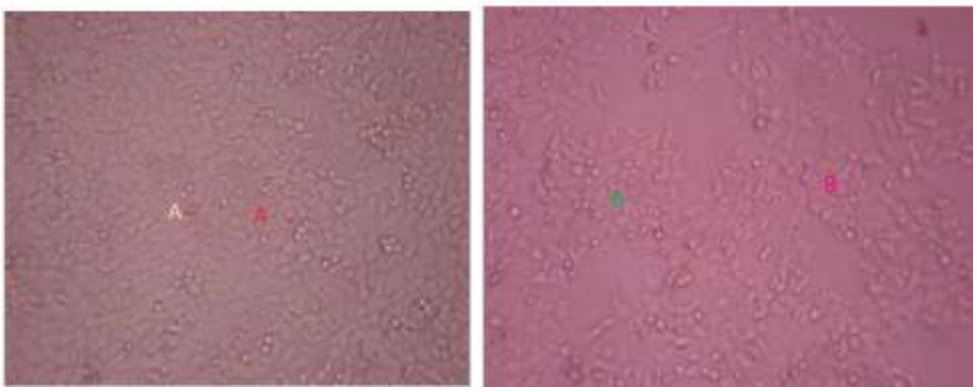
Sl.No.	Concentration of the Extracts (µg/mL)	Absorbance	Inhibition of Cell Growth (%)
1	10	1.518	64.61%
2	5	1.558	61.56%
3	2.5	1.59	59..94%
4	1.25	1.57	58.04%
5	Control	1.91	0

Table 3: For Percentage (%) of Cell Growth Inhibition of Acetone Extract (E3) of Fruit-Seeds of *M. longifolia* on He La Cells by MTT Assay

Sl.No.	Concentration of the Extracts (µg/mL)	Absorbance	Inhibition of Cell Growth (%)
1	10	1.658	55.62%
2	5	1.745	51.56%
3	2.5	1.77	49.94%
4	1.25	1.811	45.04%
5	Control	1.91	0

Table 4: For Percentage (%) of Cell Growth Inhibition of Chloroform Extract (E4) of Fruit-Seeds of *M. longifolia* on He La Cells by MTT Assay

Sl.No.	Concentration of the Extracts (µg/mL)	Absorbance	Inhibition of Cell Growth (%)
1	10	1.560	60.1%
2	5	1.657	56.56%
3	2.5	1.745	51.94%
4	1.25	1.751	49.04%
5	Control	1.91	0



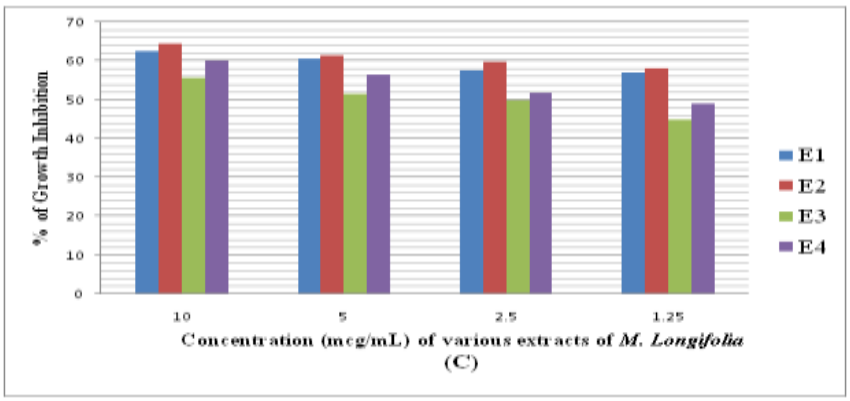


Fig. 3: Anticancer activity of various extracts (fruit-seeds) of *M. longifolia*against HeLa cells; A: control cells, B:10µg extract treated cells, C: Concentration Vs. % growth inhibition

RESULT AND DISCUSSION

In vitro anticancer activity

The results for cell growth inhibition by the extracts against HeLa cell lines for various concentrations are shown in table 1, 2, 3 and 4. As the concentration increases there is an increase in the cell growth inhibition and itwas found that with the highest 64.61 % growth inhibition at 10 µg of ethanolic Extract (E2) of Fruit-Seeds of
M. longifolia

CONCLUSION

The results obtained from the *in-vitro* studies performed using the He La cell lines reveals that the various extracts of fruit-seeds of *M. longifolia* have a very good to moderate anticancer activity. The levels of cytotoxicity of the extracts were effective. From the present studied it has been concluded that E2 and E1 extracts have good anticancer activity when compared with standard drug doxorubicin which has the 90% cell growth inhibition at concentration ranges between 1.2 to 2.5 µg/ml. The intencity of the cell growth inhibition of various extracts of *Madhuca longifolia* were given as below: E2 > E1 > E4 > E3.

REFERENCES

1. Yadav P; Review *Madhuca Lonigfolia*(Sapotaceae): A review of its traditional uses, Phytochemistry and pharmacology. International Journal of Biomedical Research, 2012; 3(7): 291-305.
2. Saluja MS, Sangameswaran B, Hura IS, Sharma A, Gupta SK, Chaturvedi M; In vitro cytotoxic activity of leaves of Madhuca longifolia against Ehrlich Ascites Carcinoma (EAC) cell lines. International Journal Of Drug Discovery and Herbal Research. 2011; 1(2): 55-57.
3. Chandra D; Analgesic effect of aqueous and alcoholic extracts of *Madhukalongifolia* (Koeing). Indian Journal of Pharmacology, 2001; 33: 108-111.
4. Acharya D, Shrivastava A; Indigenous herbal medicines: tribal formulation and traditional herbal practices. Avishkar Publishers Distributors, Jaipur, 2008.
5. Prashanth S, Kumarb AA, Madhub B, Kumar YP;Antihyperglycemic and antioxidant activity of ethanolic extract of *Madhuca longifolia* bark. International Journal of Pharmaceutical Sciences Review and Research, 2010; 5(3): 89-94.
6. Nadkarni KM; Indian MateriaMedica, 3rd edition, Bombay, India, Popular Books, 1954: 253-256.
7. Sharma P, Chaturvedi N, Upadhyay M, Varma S; Quantitative determination of total phenolic content in stem bark and leaves extracts of *Madhuca longifolia*. International Journal of PharmTech Research, 2013; 5(3): 1150-1154.
8. Rahman MA, Haque ME, Solaiman M, Saifuzzaman M; Anti nociceptive and antidiarrhoeal activities of *Madhuca indica* J. F. GMEL. Pharmacologyonline, 2011; 1: 473-480.
9. Yosiokal I, Inada A, Kitagawa I; Structures of genuine sapogenolprotobasic acid and a prosapogenol of seed kemel of *Madhucaindica*. Tetrahedron, 1974; 30: 707-714.
10. Yoshikawa K, Tanka M, Arihara S, Pal BS, Roy SK, Matsumura E, Katayama S; New oleanenetriterpenoidsaponins from *Madhucaindica*. J Nat Prod., 2000; 63(12):1679-1681.
11. Kokate CK, Purohit AP, Gokhale SB; Pharmacognosy. 42nd edition, A.1.
12. JaswantKaur, PV Chemistry of Natural Products, 2010 edition, PP-113-114, 116, 344-346, 381.
13. Sathish M, Tharani CB, Niraimathi V, Satheesh Kumar D; In-vitro cytotoxic activity on roots of Clerodendrumphlomidis against NIH 3T3 cell line and Hela cell line. Pharmacologyonline, 2011; 3: 1112-1118.
14. Dogra P; Study of antibacterial and anticancer activity of selected trifoliate plants. Biofrontiers, 2009; 1(2): 48.
15. Patel RM, Patel SK; Cytotoxic activity of methanolic extract of Artocarpusheterophyllus against A549, Hela and MCF-7 cell lines. Journal of Applied Pharmaceutical Science, 2011; 1(7): 167-171.