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Research Article

In Vitro cytotoxic activity of leaves of *Madhuca longifolia* against Ehrlich Ascites Carcinoma (EAC) cell lines

M. S. Saluja[1*], B. Sangameswaran[2*], I. S. Hura[3], Ajay Sharma[1], S.K.Gupta[1] and M. Chaturvedi[4]

1, Research Scholars, Department of Pharmacy, Suresh Gyan Vihar University, Jaipur, Rajasthan, India
2, Head and Prof., Department of Pharmacognosy, TIT- Pharmacy, Bhopal, M.P., India
3, Technical Officer, DCGI, New Delhi, India
4, Dept. of Pharmaceutical Chemistry, Central India Institute of Pharmacy, Indore, M.P., India

Abstract

Present study was performed to explore the cytotoxic activity of acetone and ethanol extracts from the leaves of *Madhuca longifolia* against Ehrlich Ascites Carcinoma (EAC) cell lines using different *Invitro* cytotoxic assay at 200µg/ml. Results found that both extracts exhibited significant cytotoxic activity, but higher cytotoxic activity was found in ethanol extract.

Keywords: Cytotoxic Activity, Ehrlich Ascites Carcinoma (EAC) and *Madhuca Longifolia*

Introduction

It is well established that plants have been a useful source of clinically relevant antitumor 69 compounds.[1] Indeed there have been worldwide efforts to discover new anticancer agents from plants. There are different approaches for the selection of plants that may contain new biologically active compounds.[2] One of the approaches used is the ethnomedical data approach, in which the selection of a plant is based on the prior information on the folk medicinal use of the plant. It is generally known that ethnomedical data provides substantially increased chance of finding active plants relative to random approach.[3] However, as for cancer, the disease is complicated and heterogeneous, which makes it difficult to be well diagnosed, especially by traditional healers. The ethnomedical information obtained for a plant extract that is used to treat cancer might therefore not be reliable.[1] Traditional Indian and Chinese medicinal herbs have been used in the treatment of different diseases in the country for centuries. There have been claims that some traditional healers can successfully treat cancer using herbal drugs. Indeed, some traditional healers who were interviewed recently in the country stressed that they have successfully treated patients presented with cancer or cancer related diseases.

Madhuca commonly known as the Butter nut tree is a medium to large sized deciduous tree distributed in Nepal, India and Sri Lanka. The flowers are used as tonic, analgesic and diuretic. The bark is used for rheumatism, chronic bronchitis and diabetes mellitus. *Madhuca longifolia* leaves are expectorant and also used for chronic bronchitis and Cushing's disease.[4,5]

Material and Methods

Collection and Authentification of the Leaves

The leaves of *Madhuca longifolia* was collected from Garden of National Botanical Research Institute, Lucknow, India in month of July 2009. The leaves were authenticated by Dr. Sayeeda Khatoon, chemotaxonomist and the voucher specimens were deposited in the departmental herbarium for future reference.

*Corresponding Author
E-mail: manidewsoft@gmail.com
Mob. +91 9303155885

Preparation of Crude Drug for Extract[6]

The authenticated leaves were used for the preparation of the extract. The leaves were collected and dried under shade and then coarsely powdered with the help of mechanical grinder. The powdered was passed through sieve no. 40 and stored in an airtight container for extraction.

Preparation of extracts of *Madhuca longifolia*[6]

The powdered leaves (500g) were sequentially extracted using petroleum ether, chloroform, ethanol, acetone and aqueous solution in Soxhlet apparatus. After about forty siphons of each solvent extraction step, the materials were concentrated by evaporation.

Cell Cultures[7]

The EAC cell line was procured through the courtesy of Amala Cancer Research Center, Thrissur and maintained at Pharmacology Department, TIT- Pharmacy, Bhopal in Minimum essential medium (MEM) at 37°C and 5% CO_2 using standard cell culture methods. At confluence, cells were trypsinised and equally distributed in two standard cell culture flasks and were allowed to adhere for 24hr. In order to evaluate the effect of extracts on cancer cells, cells were transferred in 96 well cell culture plate and incubated for 24hr. After confluence, MTT assay and Neutral red uptake cytotoxic assay have been conducted to evaluate the cell death caused by the extracts, whereas trypan blue assay was carried out by direct method.

In Vitro Cytotoxic Activity

In Vitro cytotoxic activity was found using trypan blue cytotoxic assay, MTT assay and Neutral red uptake cytotoxic assay.

Trypan blue method[8]

- Given sample (leaves extract) i.e., acetone extract or aqueous extract dissolved in suitable solvent. (Water/ethanol).
- Sample solution pipetted out having concentration 200µg/ml.
- The volume in all the tubes is made up to 800µl with PBS. (Phosphate buffer saline).
- 100 µl EAC with concentration, 1 million cells/ml were added to all the test tube.
- A control having solvent alone is also prepared.
- Incubated at 37oC for three hours and add 100 µl of trypan blue to all test tubes.
- Count the no. of cells using the Haemocytometer with the help of compound microscope.
- No .of dead cells/total 100 cells counted give the percentage cytotoxicity.

The result was shown in Table no 1.

MTT ASSAY[7]

Preparation of MTT solution

25mg of MTT powder was dissolved in 5ml PBS then filtered it with the help of 10ml syringe and syringe filter.

Procedure

- Incubated cell plates were taken out from the incubator, and discard the culture media from the plates.
- Culture media was replaced by the extract containing culture media.
- Then the plates were incubated in CO_2 incubator for 24 hrs for the action of extracts.
- 5 hours before the end of the incubation, add 20µl of MTT solution to each well containing cells.
- Incubate the plate at 37ºC for 5 hours.
- Remove media and add 200µl of DMSO to each well and pipette up and down to dissolve crystals.
- Transfer to plate ELISA reader and measure absorbance at 550nm to get optical density.
- Then calculate the % inhibition using the formula

% inhibition = [(OD of untreated)-(OD of drug Treated) /(OD of untreated)] ×100

The result was shown in Table no 2.

Neutral Red Uptake Cytotoxic assay[7]

Preparation of NR stock solution

NR dye (3.3gm) was dissolved in 100 ml of double distilled water and then this stock solution was filtered by using syringe filter. It was stored at room temperature and used within 6 months.

Preparation of working solution

1 ml of NR stock solution was dissolved in the 99 ml of culture media to get the final concentration 0.33%.

Procedure

- Incubated cell plates were taken out from the incubator, and discard the culture media from the plates.
- Culture media was replaced by the extract containing culture media.
- Then the plates were incubated in CO_2 incubator for 24 hrs for the action of extracts.
- The extract containing culture media was then replaced with NR-containing medium.
- Plates were again placed to incubator for 4-8 hours depending on cell type and maximum cell density.
- At the end of the incubation period, the medium was carefully removed and the cells were quickly washed with PBS.

The washed solution was removed and the incorporated dye was then solubilized in a volume of Neutral Red Assay Solubilization Solution (ethanolic acetic acid) equal to the original volume of culture medium. The plates were allowed to stand for 10 minutes at room temperature. Gentle stirring in a gyratory shaker or pipetting up and down (trituration) enhanced mixing of the solubilized dye.

- The background absorbance was measured at 540 nm using ELISA reader to get optical density and pictures were captured using microscope.
- Then calculate the % inhibition using the formula

% inhibition = [(OD of untreated)-(OD of drug Treated) /(OD of untreated)] ×100

The result was shown in Table no 3 and in Fig no 1.

Results and discussion

The results of *In Vitro* Cytotoxic test were shown in Table 1, 2 and 3. The ethanol extract shows remarkable cytotoxic activity against the tested cells Ehrlich Ascites Carcinoma (EAC).The ethanol extract shows more activity compared to other extracts, against Ehrlich ascitic carcinoma.

At 200 µg/ml concentration, 84% of activity was found for ethanol Extract, and 78% of activity for acetone extract by trypan blue assay, whereas 82 ± 3.12 % inhibition was found in ethanol extract and 80 ± 2.28 % inhibition was found in acetone extract by MTT assay and 81.7 ± 1.53 % inhibition was found in ethanol extract and 78.2 ± 2.29 % inhibition was found in acetone extract by NR uptake cytotoxic assay which was showed in fig no 1.

Conclusion

The whole study concluded that both the extracts shows remarkable cytotoxic activity against the tested cells *i.e,* Ehrlich Ascetic Carcinoma (EAC). The ethanol extracts show more activity against Ehrlich ascitic carcinoma when compared with acetone extract.

Aknowledgement

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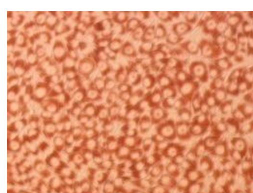
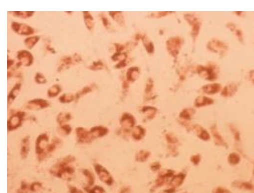
Fig. 1: *In-Vitro* Cytotoxicity activity by NR Uptake cytotoxic assay



Untreated group



Treated with Ethanol Extract

Treated with Acetone Extract

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Table 1: *In-Vitro* Cytotoxic activity by Trypan blue Assay

Sample	Concentration µg/ml	% Death
Ethanol Extract	200	84%
	Control	-
Acetone Extract	200	78%
	Control	-

Table 2: *In-Vitro* Cytotoxic activity by MTT assay

S No	Sample	Concentration	Optical density	% inhibition
1	**Untreated**	-	0.3660	0.00 ± 1.31 %
2	**Acetone extract**	200 µg/ml	0.0732	80 ± 2.28 %
3	**Ethanol extract**	200 µg/ml	0.0658	82 ± 3.12 %

Table 3: *In-Vitro* Cytotoxic activity by NR Uptake cytotoxic assay

S No	Sample	Concentration	Optical density	% inhibition
1	**Untreated**	-	0.380	0.00 ± 1.88 %
2	**Acetone extract**	200 µg/ml	0.106	78.2 ± 2.29 %
3	**Ethanol extract**	200 µg/ml	0.094	81.7 ± 1.53 %