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In-vitro Cytotoxic activity of leaves of Adina Cordifolia against Ehrlich Ascites Carcinoma (EAC) cell lines

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ABSTRACT

Recent investigation was performed to explore the cytotoxic activity of various extracts from the leaves of *Adina Cordifolia* against Ehrlich Ascites Carcinoma (EAC) cell lines using different *In-vitro* cytotoxic assay at 200µg/ml. Results found that acetone and ethanol extract exhibited significant cytotoxic activity, but higher cytotoxic activity was found in acetone extract.

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Introduction

The use of medicinal plants to treat diseases is as old as human civilization. Human beings of all ages in both developing and undeveloped countries use plants in seek to treat numerous diseases and to get relief from physical ailments. Cancer is a class of diseases in which a cell or a group of cells display uncontrolled growth, invasion and sometimes metastasis. It is largest non-communicable disease and it has a sizable contribution in the total number of deaths. The World cancer report documents that cancer rates are set to increase at an alarming rate globally. Cancer rates could increase by 50% new cases for the year 2020[1]

India is a rich source of medicinal plants and a number of plant extracts are used against diseases in various systems of medicine such as Ayurveda, Unani, and Siddha. Only a few of them have been scientifically explored. There were good number of plant products such as flavonoids, terpenes, alkaloids [2, 3, 4] and these can are used as remedies to treat various diseases and disorders. Because of their distinct pharmacological qualities including cytotoxic and cancer chemopreventive effects, it is inspired many scientists to take up independent investigations on a number of medicinal plants.[5]

Adina cordifolia, (Rubiaceae) (commonly known as Haridru) is found throughout central and south India to Srilanka. The 7-hydroxycoumarin-1 and 7-β-D-glucosylcoumarin-2 were isolated from the root bark of *Adina cordifolia*, having antiamoebic activity.[6] *Adina cordifolia* was also reported for its antiulcer potential activity[7], antifertility activity[8], anti-inflammatory and anti-nociptive activity.[9]

Method and materials

Collection and Authentification of the Leaves

The leaves of *Adina Cordifolia* were collected from Sajeevini Botanical Garden, Bhopal, Madhya Pradesh 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.

Preparation of Crude Drug for Extract[10]

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 *Adina Cordifolia* [10]

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[11]

The EAC cell line was procured through the courtesy of Amala Cancer Research Center, Thrissur and maintained at Pharmacology Department, TIT- Pharmacy, Bhopal in Dulbecco's modified eagle medium (DMEM) 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.

In-vitro Cytotoxic Activity

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

MTT ASSAY[11]

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

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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 1.

Neutral Red Uptake Cytotoxic assay[11]

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. 1 ml of NR stock solution was dissolved in the 99 ml of culture media to get the final concentration 0.33%. 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 2 and in Fig no 1.

Result and discussion

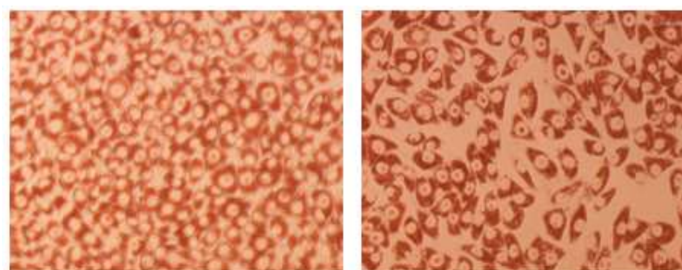
Effect of Adina cordifolia extracts by MTT assay

The results of cytotoxic activity by MTT Assay were showed in Table no. 1. The results showed that the acetone and ethanol extract of Adina cordifolia, showed the remarkable cytotoxicity against EAC. At 200 µg/ml concentration, 91.34 ± 4.56 % inhibition was found in acetone extract and 89.11 ± 2.97 % inhibition was found in ethanol extract 8 wells /group OD at 550 nm, Values are expressed as mean ± SEM

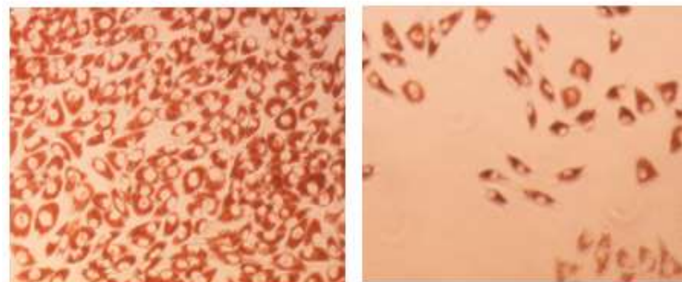
Effect of *Adina cordifolia* extracts by NR Uptake cytotoxic assay

The results of cytotoxic activity by NR Uptake cytotoxic assay were showed in Table no. 2 and fig no. 1. The results

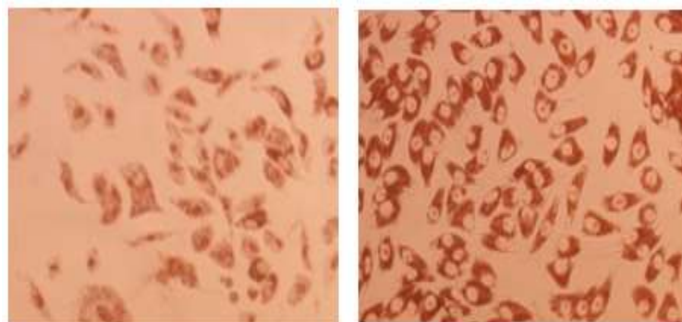
Fig No 2: Effect of *Adina cordifolia*'s Extracts on EAC by NR Cytotoxic Assay



Normal Control (No Treatment) **Treated with Pet. Ether Extract**



Treated with Chloroform Extract **Treated with Acetone Extract**



Treated with Ethanol Extract **Treated with Aqueous Extract**

These pictures showed the effect of *Adina cordifolia*'s extracts on EAC cells. In the normal control slide there is no vacant space as there is no cell death whereas in all other slides vacant space showed the cell death ie % inhibition caused by the extract treatment.

Conclusion

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

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Table No 1. *In-Vitro* Cytotoxic activity of *Adina cordifolia*'s extracts

S No	Sample	Concentration	Optical density	% inhibition
1	Control	No treatment	0.3660	0.00 ± 1.31 %
2	Pet. Ether Extract	200 µg/ml	0.2943	19.56±3.16 %
3	Chloroform Extract	200 µg/ml	0.2930	19.94±1.31 %
4	Acetone Extract	200 µg/ml	0.0317	91.34 ± 4.56 %
5	Ethanol Extract	200 µg/ml	0.0399	89.11 ± 2.97 %
6	Aqueous extraxt	200 µg/ml	0.3146	14.03.±2.74 %

Table No 2. *In-Vitro* Cytotoxic activity of *Adina cordifolia*'s extracts

S No	Sample	Concentration	Optical density	% inhibition
1	Control	No treatment	0.3800	0.00 ± 1.88 %
2	Pet. Ether Extract	200 µg/ml	0.3091	18.68 ± 4.13%
3	Chloroform Extract	200 µg/ml	0.3482	08.42 ± 3.79%
4	Acetone Extract	200 µg/ml	0.0252	93.36 ± 4.56%
5	Ethanol Extract	200 µg/ml	0.0380	90.00 ± 3.64%
6	Aqueous extraxt	200 µg/ml	0.2523	33.68 ± 1.96%

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