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# IN VITRO CYTOTOXIC ACTIVITY OF ADINA CORDIFOLIA PLANT EXTRACT

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## Abstract

In-vitro cytotoxicity assays are laboratory techniques used to evaluate the toxicity of substances, such as drugs or chemicals, on cultured cells. These assays help determine the potential harmful effects of these substances on cell viability, proliferation, and function before they are tested in vivo (in living organisms). Adina cordifolia is a plant known for its medicinal properties, particularly in traditional medicine. Its phytoconstituents have been the subject of various studies due to their potential therapeutic effects.

**Keywords:** Adina cordifolia, Rubiaceae, MTT assay, cytotoxic activity,

## Introduction

Adina cordifolia is a species of flowering plant in the family Rubiaceae. It's commonly known as the heart-leaf adina or by its local names in various regions. This plant is typically found in tropical forests and can be native to areas in Australia, Papua New Guinea, and parts of Southeast Asia. The plant is characterized by its distinctive heart-shaped leaves, which is where it gets its common name. It produces small, tubular flowers, and its fruit is usually a berry[1].

The MTT assay is a widely used laboratory technique for assessing cell viability and proliferation. Its name stands for "3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide," which is a yellow compound that gets converted into a purple formazan product by active cells. This color change can be quantified to determine the number of viable cells in a sample [2].

Researchers use the MTT assay to evaluate how cancer cells respond to different drugs or treatments. By measuring the reduction in formazan production, scientists can gauge the effectiveness of anti-cancer agents in killing or inhibiting cancer cell growth. This research work was aimed to determine in vitro cytotoxic activity of Adina cordifolia plant extract

## Materials and method

### Selection of the plant

The purpose of this research is to assess the anticancer properties of A. cordifolia by reviewing the available literature and consulting with traditional medical practitioners in the Indian cities of Ujjain, Indore, and Bhopal (M.P.).

### Collection and Authentification of the plant

The A. cordifolia leaves were gathered in June and July 2021 from the National Botanical Research Institute in Lucknow and the Sanjivini Botanical Garden in Bhopal, India. Dr.

 

Sayeeda Khatoon, a chemotaxonomist, verified the authenticity of the plants, and voucher specimens were archived at the department herbarium.

**Preparation of crude drug for extraction**

The extract was prepared using the chosen plant leaves. The leaves were harvested, dried in the shade, and then mechanically ground into a coarse powder. Powder was sieved through a No. 16 to provide the best extraction [3].

**In-vitro cytotoxicity study**

**Cell Line**

Standard cell culture procedures were used to cultivate the Elrish Acectic Carcinoma cell line that was generously provided by the Amala Cancer Research Centre in Thrissur and is now kept in the Pharmacology Department of the Oriental University of India in Indore at 37 degrees Celsius and 5% carbon dioxide [4].

**Maintenance of cell line**

There were a few steps involved in keeping the cell line alive and well

**Preparation of cell medium**

a) Ingredients

| | |
|---|---|
| DMEM | 10gm |
| Sodium bi carbonate | 2.2gm |
| HEPES | 10ml |
| Antibiotics | 10ml |
| FBS | 100ml |

Autoclaved water to make the volume up to 1 lit.

b) Method of Preparation

Dissolve DMEM and sodium bicarbonate in 850 ml of autoclaved water after adding HEPES to the water.

• Put in a total of 1 litre of autoclaved water,

100 millilitres of foetal bovine serum (FBS),

10 millilitres of antibiotics into the medium.

Keep it at 40 degrees and filter it twice.

**Passaging of cell line**

The process of cell passaging or splitting was developed so that cells could be maintained in culture for long periods of time. At 90%-100% confluence, cells should be passed on.

a) Reagents:

 

- Ethanol.
- Media with 10% serum and antibiotics.
- Trypsin.
- PBS.

b) Procedure:

• A 37°C water bath was used to gently heat the media and Trypsin.

• Verify if cells in a T flask are 90%-100% confluent using a microscope.

Ethanol was used to clean the hood.

Clean all bottles, containers, and other items that went into the hood.

Ethanol was used to clean the hands. The T flasks were taken out of the incubator and put under the hood.

• After removing the culture media, T-flasks were washed twice with PBS to get rid of the dead cells.

• To the T-75 flask, 4 cc of trypsin was added.

Under a microscope, we were able to verify that the cells had successfully shed their attachment to the surface.

After the cells were trypsinized, the culture media was added, and the mixture was incubated in a CO2 atmosphere.

Every 24 hours, the medium in the T flask was replaced until the mixture became confluent.

**Seeding of cells**

a) Reagents:

- Ethanol
- Media with 10% serum and antibiotics.
- Trypsin and PBS.

b) Procedure:

• A 37°C water bath was used to gently heat the media and Trypsin.

• Verify if cells in a T flask are 90%-100% confluent using a microscope.

Ethanol was used to clean the hood.

Trypsinization of the cells.

 

A hemocytometer was used to determine the total cell count.

• Each 100 l of diluted cell suspension contained between 2500 and 5000 cells, which was achieved by diluting the cell suspension with growth media.

• A 96-well plate was used to cultivate the cells, and 100 l of cell suspension was placed in each well.

These plates were kept in a CO 2 incubator for 24, 48, and 72 hours. The drugs were tested on these plates.

**Preparation of extract solution**

**Reagents**:

- Culture media.
- DMSO.

**Procedure:**

- • The stock solution with concentration 20mg/ml was prepared by dissolving 2000 mg of powdered extracts in 100 ml of DMSO.
- • The extract solution was diluted to a final concentration of 2mg/ml by dissolving 10 ml into 90 ml of culture media.

**Treatment with extract solution**

Reagents:

- Culture media.
- Extracts stock solution.

Procedure:

• Removed culture dishes containing seeded cells from incubator; discarded associated culture medium.

Extracts solution (100 l) was used instead of culture media (100 l).

• Eight wells were set aside as negative controls.

• After that, we put the plates in a CO2 incubator to let the drugs take effect for 24 hours.

• After 24 hours in the incubator, the plates were removed, and the drug's cytotoxic activity was measured using several assays [5].

**In-vitro Cytotoxic Assays**

These were the following cytotoxic assays which were used to evaluate the cytotoxicity of extracts to the cancer cells.

 

**MTT Assay**

The MTT assay is a common colorimetric assay used to evaluate the activity of enzymes responsible for the purple colour produced by the reduction of MTT to formazan. This is primarily a mitochondrial process, hence it can be used as a proxy for mitochondrial function in live organisms. It can also be used to test the toxicity of chemicals and drugs in development.

When adding a solubilization solution, such as dimethyl sulfoxide, acidified ethanol, or sodium dodecyl sulphate in diluted hydrochloric acid, the insoluble purple formazan product is dissolved into a coloured solution. This coloured solution's absorbance can be measured with a spectrophotometer by observing its colour at a specific wavelength, often between 500 and 600 nm. Maximum absorption varies with solvent type.

Because this conversion can only occur in cells with functional mitochondrial reductase enzymes, it is commonly used as a proxy for cell viability. A dose-response curve can be generated to determine the efficacy of an agent in causing cell death or altering cellular metabolism by comparing the amount of purple formazan produced by treated cells to the amount of formazan produced by untreated control cells [6].

Reagents

- MTT.
- PBS.

a) Procedure

The 10ml syringe and syringe filter were used to filter a solution containing 25mg of MTT powder dissolved in 5ml of PBS. The cell plates were removed from the incubator, and the culture media was thrown away. The culture media in the extract was used instead. After that, we left the plates in a CO2 incubator for 24 hours to see how the extracts fared. Add 20 l of MTT solution to each well containing cells 5 hours before the incubation is to terminate. Keep the plate at 37 degrees Celsius for 5 hours. To dissolve the crystals, empty the media from the wells and add 200 l of DMSO to each one, pipetting up and down to mix. To determine the optical density, move the sample to a plate ELISA reader and read the absorbance at 550 nm. Then, employ the formula to derive the percentage of inhibition.

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

**Results and discussion**

**In-vitro cytotoxic activity of extract of A. cordifolia by MTT assay**

At a concentration of 500 µg/mL, MEAC achieved a maximum percentage inhibition value of 65.54±1.05%, whereas the reference drug achieved a value of 83.03±1.0%.The findings of the MTT experiment are shown in Table 1.

 

**Table No. 1: In-vitro cytotoxic activity of extract of A. cordifolia by MTT assay**

| Concentration (µg/ml) | % growth Inhibition | | | | |
|---|---|---|---|---|---|
| | MEAC | CEML | PEAC | AEAC | 5-FU |
| 500 | 65.54±1.05 | 62.13±1.08 | 6.85±1.58 | 4.45±0.70 | 83.03±1.0 |
| 166.66 | 52.66±1.32 | 50.56±1.21 | 5.09±0.67 | 2.31±0.16 | 69.26±1.04 |
| 55.55 | 50.30±1.66 | 48.80±0.28 | 4.01±0.95 | 2.54±0.76 | 65.64±0.62 |
| 18.52 | 47.05±2.87 | 46.90±1.18 | 4.95±0.97 | 2.30±1.07 | 63.08±0.99 |
| 6.17 | 44.51±2.42 | 43.71±0.83 | 4.64±1.1 | 2.40±1.29 | 59.82±0.37 |
| 2.06 | 41.91±2.75 | 41.52±0.44 | 4.45±0.90 | 1.39±0.55 | 57.57±0.99 |
| 0.68 | 39.60±2.76 | 39.22±0.62 | 3.27±1.00 | 1.20±0.74 | 55.02±0.90 |
| 0.23 | 37.54±2.65 | 37.21±0.64 | 3.43±0.50 | 1.25±0.74 | 52.70±1.06 |
| 0.076 | 35.11±3.14 | 35.26±0.82 | 3.36±0.47 | 1.60±0.90 | 50.45±1.72 |
| 0.025 | 33.10±3.10 | 33.07±0.46 | 3.53±0.84 | 1.41±0.97 | 48.43±1.51 |

Values are expressed as mean ± SEM, n=3, P<0.01Vs Standard

## Conclusion

Since **A. cordifolia** contain these phytoconstituents, it's reasonable to assume that the anticancer activity seen in these plant is due, at least in part, to these phytoconstituents' pharmacologically dynamic properties.The cytotoxic effect of plant extracts was also tested in vitro using EAC cell lines. Extracts of **A. cordifolia** were shown to be effective in this investigation against the cell lines, and each was extracted using a different solvent. These findings informed the selection of these concentrates for their potential anticancer and other pharmacological effects. Methanolic extract shows 65.54 % inhibition as compared to 83.03% inhibition by 5-FU.

## References

1. Chaudhary P, Goel B, Ghosh AK. Antidiabetic activity of Adina cordifolia (Roxb) leaves in alloxan induced diabetic rats. Asian Pacific Journal of Tropical Biomedicine. 2012 Jan 1;2(3):S1630-2.
2. Grela E, Kozłowska J, Grabowiecka A. Current methodology of MTT assay in bacteria–A review. Acta histochemica. 2018 May 1;120(4):303-11.
3. Mukherjee PK. Quality control and evaluation of herbal drugs: Evaluating natural products and traditional medicine. Elsevier; 2019 May 30.
4. Richardson AD, Ireland CM. A profile of the in vitro antitumor activity of lissoclinolide. Toxicology and applied pharmacology. 2004 Feb 15;195(1):55-61.
5. Borenfreund E, Borrero O. In vitro cytotoxicity assays. Potential alternatives to the Draize ocular allergy test. Cell Biology and Toxicology. 1984 Oct;1:55-65.
6. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. Journal of immunological methods. 1983 Dec 16;65(1-2):55-63.