Evaluation Of Anticancer Activity of Tamarindus Indica by Using MTT Assay

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Abstract

Background
It is to investigate the anticancer potential of *Tamarindus indica* leaf extract using the cell line method, with the aim of establishing the plant as a source for treating chronic diseases like cancer.

Material and Method
The ethanolic extract from the selected plant was tested for anticancer activity using the MTT assay method. The A431 cell line, representing human epithelial cancer, was utilized in the study. Measurements were taken at a wavelength of 570 nm, and the percentage of cell viability was calculated.

Results
The concentration of the test compound required to inhibit cell growth by 50% (IC_{50}) 23.65 was determined from the dose-response curve for the cell line. The initial phytochemical screening of ethanolic extracts from *Tamarindus indica* indicated the presence of various bioactive compounds, including sugars, proteins, alkaloids, glycosides, and tannins.

Conclusion
IC_{50} value of extract and isolated compound 23.65 % was detected. The pharmacological activity was carried out using the MTT Assay Method; based on the viability of the cells, anticancer activity was evaluated. The leaf extract of *Tamarindus indica* showing potent activity for the treatment of various cancer diseases and that could be used to develop the herbal formulation. Hence this extract can used in various formulation to treat various life treating cancer disease naturally.

Key words: *Tamarindus indica*, phytochemical, MTT Assay Method, cell viability, IC_{50}

Introduction

Cancer is a complex disease that can affect approximately 200 different types of cells within the body. A key characteristic of cancer is the loss of control over cellular processes, including growth, differentiation, and programmed cell death. This loss of regulation leads to the uncontrolled proliferation of cells, allowing them to invade surrounding tissues and organs. Treating cancer poses significant challenges due to various factors. One of the major hurdles is the development of resistance to therapies, where cancer cells adapt to and evade the effects of treatment. Additionally, many treatments are associated with high toxicity, which can harm healthy cells and lead to severe side effects. Furthermore, achieving specificity in targeting cancer cells without damaging normal cells remains a critical issue, complicating the development of effective and safe therapies [1].

The use of natural compounds in cancer treatment has deep historical roots, originating in traditional and folk medicine before being integrated into allopathic and modern medical practices. Many of the chemotherapy drugs utilized today have either been directly sourced from plants or were developed based on plant-derived compounds. For instance, the vinca alkaloids, such as vinblastine and vincristine, are sourced from the plant *Catharanthus roseus*. These alkaloids play a significant role in cancer treatment due to their ability to disrupt cell division. Similarly, *Podophyllum* species have given rise to epipodophyllotoxins, from which the semisynthetic drugs etoposide and teniposide are derived. These drugs are crucial in the treatment of various cancers. Taxanes, another important class of chemotherapy agents, are derived from the yew tree species (*Taxus*). Paclitaxel, a prominent member of this class, is widely used in cancer therapy. Camptothecin, extracted from *Camptotheca acuminata*, has also been modified to produce semisynthetic derivatives like irinotecan and topotecan, both of which are used in chemotherapy. These examples underscore the critical role that natural products and their derivatives play in the development of effective cancer therapies, with numerous other compounds also contributing to the arsenal of treatments available today [2].

According to research by Cragg and Newman, over 50% of the anti-cancer drugs currently undergoing clinical trials are either derived directly from natural sources or have connections to naturally occurring compounds. This highlights the significant role that natural substances continue to play in the development of new cancer therapies, as many of these compounds provide the foundational structures or inspiration for the synthesis of novel drugs. The ongoing reliance on natural products in cancer research underscores their value as a rich source of bioactive molecules with therapeutic potential [3]. It is now essential to raise awareness and promote the use of Ayurvedic therapies in the fight against cancer, advocating for an integrated approach to tumor management and treatment. By combining Ayurvedic practices with conventional methods, we can create a more holistic strategy for cancer care [4].

Tamarindus indica belongs to the Leguminosae family. *Tamarindus* is a genus in the Fabaceae family, consisting of a single species, *Tamarindus indica* (tamarind). Native to tropical Africa, it is now cultivated globally in tropical and subtropical regions. The tamarind tree produces pod-like fruits with a tangy pulp used in cooking, traditional medicine, and industry.

Tamarindus indica (tamarind) is widely used in traditional medicine across various cultures for its numerous health benefits the plant is recognized for its diverse pharmacological effects, including antidiabetic and hypolipidemic activity, antioxidant activity, hepatoprotective activity, anti-inflammatory, analgesic properties and more [5]. The present work aims to investigate the phytochemical screening of leaf extract and also investigate the in vitro cytotoxic potential of the alcoholic extract of *Tamarindus indica* plant using MTT assay.

Methods & Materials

Collection, Authentication, and Drying of Plant Material:

Tamarindus indica will be gathered from the local area of Koradi, Nagpur. A herbarium sheet for the selected plant will be prepared and authenticated.

Extraction

The leaves will be separated from the plant, dried in the shade, and then ground into a coarse powder using mechanical methods. A measured amount of the coarse powder (1 kg) will be extracted with petroleum ether at a temperature of 50-60°C for 72 hours using a Soxhlet apparatus. After the petroleum ether extraction, the remaining marc will be dried and subsequently extracted with 95% ethanol at 60-70°C for up to 72 hours using the Soxhlet apparatus. The resulting alcoholic extract will be concentrated, yielding a brown residue, which will be stored in a desiccator.

Preliminary Phytochemical screening of ethanolic extracts of *Tamarindus indica*

Plants can be regarded as natural biosynthetic laboratories, producing a wide array of compounds such as alkaloids, glycosides, volatile oils, tannins, saponins, flavonoids, and sugars, which have physiological effects. These compounds are known as secondary metabolites. To determine the presence or absence of primary and secondary metabolites, all extracts were subjected to various chemical tests.

Test for Sugars

a) Molisch’s Test

Molisch's reagent was made by dissolving 10 g of alpha-naphthol in 100 ml of 95% alcohol. A small amount of the test residue was mixed with 0.5 ml of water in a test tube, followed by the addition of 2 drops of Molisch's reagent. Then, 1 ml of concentrated sulfuric acid was carefully added along the side of the test tube to form a separate layer beneath the aqueous solution. The appearance of a red-brown ring at the interface indicated the presence of sugars.

b) Fehling’s Test

Equal volumes of Fehling's A and Fehling's B solutions were mixed, and 2 ml of the test extract was added. The mixture was then warmed, resulting in a red precipitate of cuprous oxide, indicating the presence of reducing sugars.

Test for proteins

a) Millon’s Test

The aqueous extract was treated with 2 to 3 ml of Millon's reagent. The formation of a white precipitate that turned pink indicated the presence of proteins.

b) Xanthoproteic Test

A small amount of the extract was mixed with 2 ml of water and 0.5 ml of concentrated nitric acid. The appearance of a yellow color indicated the presence of proteins.

Test for amino acids

a) Ninhydrin Test

A 0.1% w/v solution of Ninhydrin in n-butanol was prepared, and a small amount was added to the test extract. The development of a violet or purple color indicated the presence of amino acids.

Test for alkaloids

A few milligrams of each extract residue were dissolved in 5 ml of 1.5% hydrochloric acid and filtered. The resulting filtrates were then tested for the presence of alkaloids.

a) Dragendorff's reagent

Dragendorff's reagent was prepared by mixing Solution A (17 g of bismuth subnitrate, 200 g of tartaric acid, and 800 ml of distilled water) with Solution B (160 g of potassium iodide and 400 ml of distilled water) in a 1:1 ratio. A working standard was then made by diluting 50 ml of this mixture with 100 g of tartaric acid and distilled water to 500 ml. Dragendorff's reagent was sprayed on Whatman No. 1 filter paper and dried. The test filtrate, after being basified with dilute ammonia and extracted with chloroform, was applied to the treated paper using a capillary tube. The development of an orange-red color indicated the presence of alkaloids.

b) Mayer's Reagent (Potassium mercuric iodide reagent)

1.36 g of mercuric chloride was dissolved in 60 ml of distilled water, and 5 g of potassium iodide was dissolved in 10 ml of distilled water. The two solutions were mixed and diluted to a final volume of 100 ml. A few drops of this reagent were added to a small amount of each extract in dilute hydrochloric acid on a watch glass. The formation of a cream-colored precipitate indicated the presence of alkaloids.

c) Wagner's Reagent (Iodine-potassium iodide)

1.27 g of iodine and 2 g of potassium iodide were dissolved in 5 ml of water and diluted to 100 ml. Adding a few drops of this reagent to the test filtrate produced a brown precipitate, indicating the presence of alkaloids.

Test for Glycosides

A small quantity of the extract was hydrolyzed with hydrochloric acid on a water bath for two hours. The hydrolysate was then tested with Legal's and Borntrager's tests to detect cardiac and anthraquinone glycosides, respectively.

a) Legal's Test

Adding 1 ml of pyridine and a few drops of sodium nitroprusside to the hydrolysate, then making it alkaline with sodium hydroxide, resulted in a pink to yellow color, indicating the presence of glycosides.

b) Borntrager's Test

The hydrolysate extract was treated with chloroform, and the chloroform layer was separated. Adding an equal quantity of dilute ammonia solution to this layer resulted in a pink color, indicating the presence of glycosides.

``Test for tannins

Each test residue was dissolved in water, warmed, and filtered. The filtrate was then tested with the following reagents:

a) Ferric chloride test

A 5% w/v solution of ferric chloride in 90% alcohol was prepared. Adding a few drops of this solution to the filtrate resulted in a dark green or deep blue color, indicating the presence of tannins.

b) Lead acetate test

Adding a 10% w/v solution of basic acetate to the test filtrate resulted in a precipitate, indicating the presence of tannins.

c) Potassium dichromate test

The addition of potassium dichromate solution to the test filtrate did not produce a dark color, indicating the absence of tannins.

Test for Flavonoids (Shinoda test)

A small amount of the test extract was dissolved in 5 ml of 95% ethanol, treated with a few drops of concentrated hydrochloric acid and 0.5 g of magnesium metal. The appearance of a pink, crimson, or magenta color within a minute or two indicated the presence of flavonoids.

Test for phytosterols

Small amounts of each extract were dissolved in 5 ml of chloroform, and various tests were conducted to detect phytosterols.

a) Salkowaski test

A few drops of concentrated sulfuric acid were added to 1 ml of the prepared chloroform solution. The development of a green color indicated the presence of phytosterols.

b) Liebermann Burchard Test

The chloroform solution was heated with a few drops of concentrated sulfuric acid and 1 ml of acetic anhydride. The appearance of a green color indicated the presence of phytosterols.

Test for saponins

Foam Test

A few milligrams of the test extract were shaken vigorously with sodium bicarbonate and water in a test tube. The formation of a stable, honeycomb-like froth indicated the presence of saponins [6-7].

Pharmacological Studies

Evaluation of anticancer activity of *Tamarindus indica*

The experimental protocol was submitted and conducted at The Maratha Mandal Dental College in Belgaum, Karnataka.

MTT Assay.

Cell line used: A 431

Dulbecco`s Modified Eagle Media (DMEM) with low glucose -Cat No-11965-092 (Gibco, Invitrogen)

Fetal bovine serum (FBS) - Cat No -10270106 (Gibco, Invitrogen)

Antibiotic – Antimycotic 100X solution (Thermofisher Scientific)-Cat No-15240062

Positive control – Doxorubicin

Negative Control- Untreated Cells with media

MTT 3-(4, 5-dimethythiazol-2-yl)-2, 5-diphenyl tetrazolium bromide: 5mg in one ml of PBS

Protocol:

Cells were seeded in a 96-well microplate and incubated overnight at 37ºC with 95% humidity and 5% CO2. Samples were added at various concentrations (100, 50, 25, 12.5, 6.25, and 3.125 µg/ml) and incubated for an additional 48 hours. The wells were washed with PBS, and 20 µL of MTT solution was added to each well. After 4 hours of incubation at 37ºC, 100 µL of DMSO was added to dissolve the formazan crystals, and absorbance was measured at 570 nm using a microplate reader [8-10].

Formula: Surviving cells (%) = Mean OD of test compound /Mean OD of Negative control ×100

Results

Collection of selected plants.

Tamarindus indica is widely cultivated throughout India. Specimens were obtained from a local nursery near Nagpur.

Authentication of Selected Medicinal Plants from Competent Authority

Tamarindus indica plants were collected from a local nursery near Nagpur. Authentication of the plant material was carried out with the help of Dr. N. M. Dongarwar from the Department of Botany at R. T. M. Nagpur University, who assigned specimen number 212 to the authenticated samples.

Preliminary phytochemical screening from ethanolic extracts of *Tamarindus Indica.*

Table 1.1 Preliminary phytochemical screening of the alcoholic extract of *Tamarindus indica* plant

Sr. No.	Plant Constituent	Test/ Reagent	Inference
01.	Sugars	Molisch Test Fehling's Test	+ +
02.	Proteins	Millon's Test Xanthoprotein Test	+ +
03.	Amino acids	Ninhydrin Test	_
04.	Alkaloids	Dragendorff's reagent Mayer's reagent Wagner's reagent	+ + +
05.	Glycosides	Legal's Test Borntrager's Test	+ +
06.	Tannins	($FeCl_3$)Ferric chloride Test Lead acetate Test Potassium dichromate Test	+ + +
07.	Flavonoids	Shinoda Test	_
08.	Phytosterols	Salkowaski Test Li+ebermann Test	_ _
09.	Saponins	Foam Test	_

'+'indicates: present; '-' indicates: absent

Determination of anticancer activity of nanosuspension (nanoparticles) of plants extracts by In-vitro method

Cytotoxic activity carried by MTT assay

Materials

A 431

Dulbecco`s Modified Eagle Media (DMEM) with low glucose -Cat No-11965-092 (Gibco, Invitrogen)

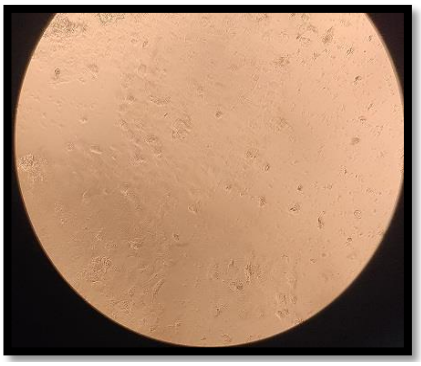
Fetal bovine serum (FBS) - Cat No -10270106 (Gibco, Invitrogen)

Antibiotic – Antimycotic 100X solution (Thermofisher Scientific)-Cat No-15240062

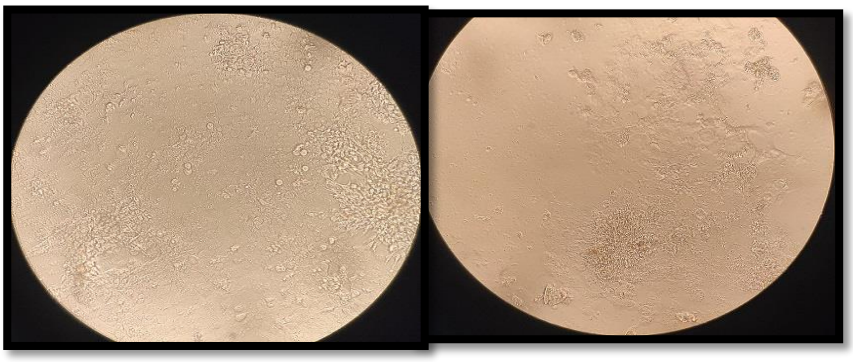
Positive control – Doxorubicin

Negative Control- Untreated Cells with media

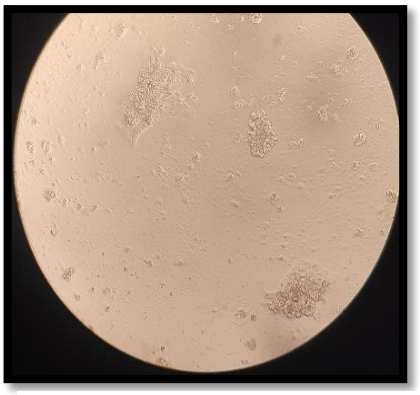
Formula: Surviving cells (%) = Mean OD of test compound /Mean OD of Negative control ×100



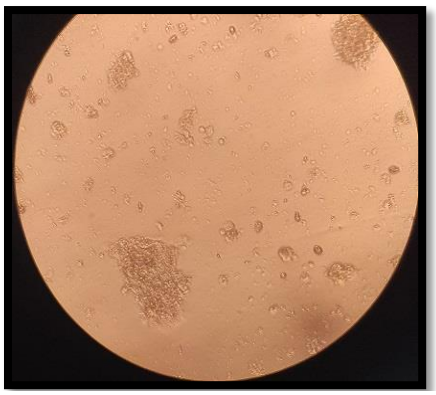
A. Cell Viability of A431 3.125 µg



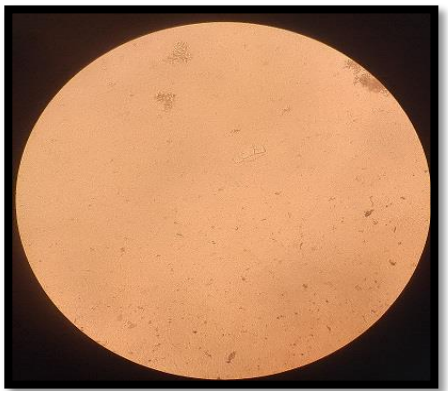
B. Cell Viability of **A431** 6.2 µg C. Cell Viability of A431 12.5 µg



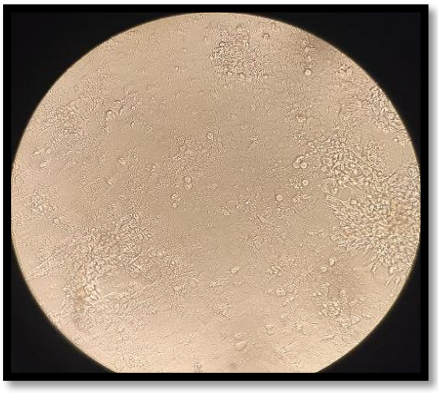
D. Cell Viability of A431 **A431** 25 µg



E. Cell Viability of A431 **A431** 50 µg



F. Cell Viability of A431 100 µg



G. Cell Viability of A431 Negative control

Fig. 1. CELL VIABILITY OF A431

Table 1.2. Cell Viability of A431 for *Tamarindus indica* Plant

CELL VIABILITY OF A431						
CONCENTRATION (µg/ml)	*Tamarindus indica* Extract			Doxorubicin		
100	24.51	24.42	24.69	13.15	13.25	13.35
50	28.58	28.76	28.41	32.23	32.33	32.43
25	34.69	34.60	34.87	43.88	43.67	43.78
12.5	53.36	53.54	53.01	44.58	44.28	44.48
6.2	63.27	63.63	63.19	45.58	45.38	45.48
3.125	71.33	71.15	71.59	46.39	47.12	46.48
Negative Control	100			100		
IC_{50} value (%)	23.65			2.54		
Standard deviation	0.08			0.05		

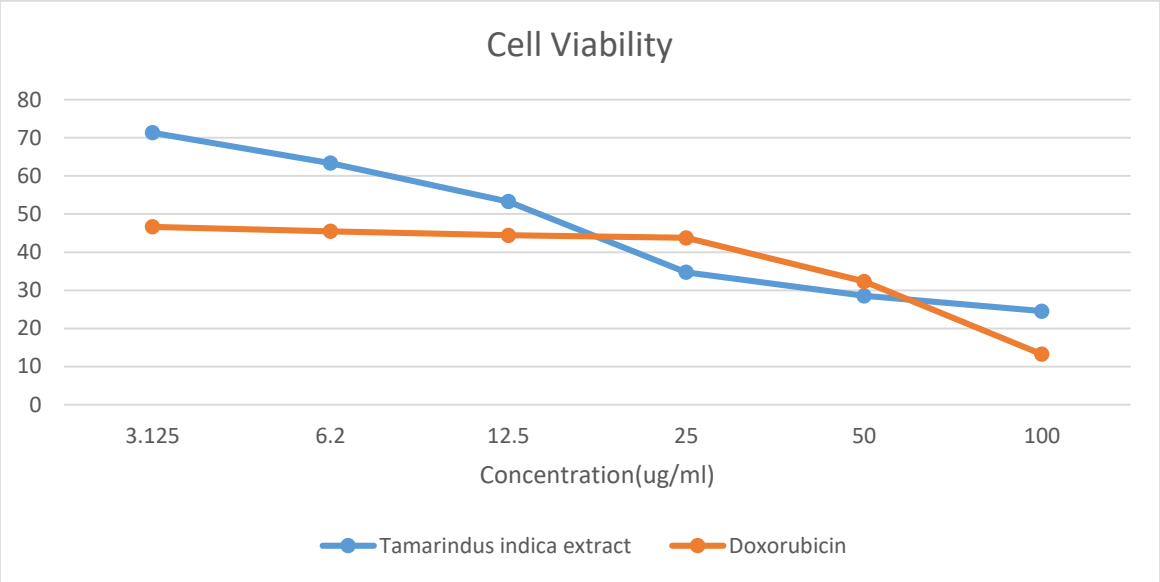


Fig 2. Cell Viability Graph

DISCUSSIONS

Tamarindus indica plant samples were sourced from a local nursery in Nagpur. To verify the authenticity of the plant material, herbarium sheets were utilized, and the validation was performed by Dr. N. M. Dongarwar from the Department of Botany at R. T. M. Nagpur University. The validated specimen was assigned the herbarium sheet number 212 for *Tamarindus indica.*

The initial phytochemical screening of ethanolic extracts from *Tamarindus indica* indicated the presence of various bioactive compounds, including sugars, proteins, alkaloids, glycosides, and tannins. These components were identified through a series of standard phytochemical tests.

To evaluate the anticancer potential of the plant extract, an MTT assay was conducted using A431 cell lines. The ethanolic extracts demonstrated significant anticancer activity, with an IC50 value of 23.56%, suggesting a promising effect in the study.

Conclusion

Tamarindus indica was sourced from a local nursery in Nagpur and subsequently authenticated. Phytochemical analysis of the plant's ethanolic extracts confirmed the presence of sugars, proteins, alkaloids, glycosides, and tannins. The extracts were then tested for anticancer properties using an MTT assay on A431 cell lines, resulting in a promising IC50 value of 23.65%. The findings underscore the significant potential of *Tamarindus indica's* ethanolic extracts in anticancer research.

Acknowledgement

We express our gratitude to Sonekar College of Pharmacy for providing the necessary infrastructure, chemicals, and equipment essential for conducting this study. We also extend our thanks to Maratha Mandal's Central Research Laboratory, Belgaum, for conducting the MTT assay.

ABBREVATIONS

PBS	Phosphate-buffered saline
DMSO	Dimethyl sulfoxide
FBS	Fetal bovine serum
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide assay
µg	Microgram
µL	Microliter

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