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Investigation of antineoplastic activities using Panc-1 and Hepg-2 cell lines on *Tamarindus indica* bark

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Abstract
Cancer is a growing health problem around the world and is the second leading cause of death after heart disease. According to a recent report by the International Agency for Research on Cancer (IARC), in 2008 there were 12.7 million new cancer cases throughout the world. There are now more than 10 million cases of cancer per year worldwide, including a group of more than 100 diseases such as cancer of the liver, lung, stomach, colon, breast, and so forth. The most rational way to affect carcinogenesis is by interfering with modulation steps (initiation, promotion, and progression) as well as the associated signal transduction pathways. There are numerous physiological and biochemical carcinogens, for example, ultraviolet and ionizing radiation, asbestos and tobacco smoke, infections by virus, bacteria, parasites, and contamination of food by mycotoxins (e.g., aflatoxins causing liver cancer). This research investigates the antineoplastic and antioxidant effects of the ethanolic extract of *Tamarindus indica* bark against PANC-1 and HepG2 Cell lines. The study elucidates the underlying physiological mechanisms contributing to anticancer and antioxidant activity, including the role of Cell lines. Through *in vitro* antioxidant assays, specifically the DPPH method, the extract demonstrated significant free radical scavenging activity, comparable to ascorbic acid. Histological evaluations confirmed the extract's antineoplastic properties, suggesting its potential as a natural therapeutic agent. The findings underscore the importance of exploring plant-based treatments for managing disease, highlighting *Tamarindus Indica's* bioactive compounds such as flavonoids and tannins as contributors to its medicinal efficacy. This research demonstrates us about the underneath proof of antineoplastic and antioxidant properties of *Tamarindus Indica* bark.

Keywords: Anticancer, Antioxidant, *Tamarindus Indica*, PANC-1, HepG2

Introduction

Cancer is one of the dangerous diseases of the 20th century occur in humans, spreading Fastly in 21st century and presently there is lots of new anticancer agents was discovered from natural products or plants.

Tamarind (*Tamarindus indica*) is an introduced plant, naturalized in India. The fruit of the tamarind tree or 'Assam tree' is known as *'imli'* or 'Indian date'. The sticky acidic pulp of tamarind fruit has been used as a food ingredient and medicine for many years. The edible fruits, and especially the pulp, can be eaten raw or used as sherbet or as an ingredient in curries, pickles, etc. The seeds contain starch and are eaten raw or cooked in times of scarcity and used in cloth mills. The Tamil practitioners used it with other ingredients to treat jaundice. The old pulp is preferred in medicine. The hakims use the pulp in preparation of churna (powder) of various mixtures, which is used as an appetizer [1]. Cancer is a growing health problem around the world and is the second leading cause of death after heart disease. There are now more than 10 million cases of cancer per year worldwide, including a group of more than 100 diseases such as cancer of the liver, lung, stomach, colon, breast, and so forth [2]. There are numerous physiological and biochemical carcinogens, for example, ultraviolet and ionizing radiation, asbestos and tobacco smoke, infections by virus (e.g., hepatitis B virus causing liver cancer and human papilloma virus causing cervical cancer), bacteria (Helicobacter pylori causing gastric cancer) [3] There is a general call for new drugs that are highly effective, possess low toxicity, and have a minor environment impact. Novel natural products offer opportunities for innovation in drug discovery [4].

In fact, natural products play, a major role in cancer prevention and treatment. A considerable number of antitumor agents currently used in the clinic are of natural origin. For instance, over half of all anticancer prescription drugs approved internationally between the 1940s and 2006 were natural products or their derivatives [5].

Natural compounds isolated from medicinal plants, as rich sources of novel anticancer drugs, have been of increasing interest since then. Traditional medicinal herbs have been used for pharmaceutical and dietary therapy for several millennia in East Asia, for example, in China, Japan, India, Thailand, and are currently widely used in cancer therapy.

The cancer-protective effects elicited by these dietary compounds are believed to be due to the induction of cellular defense systems including the detoxifying and antioxidant enzymes system as well as the inhibition of anti-inflammatory and anticancer growth signaling pathways culminating in cell cycle arrest and/or celldeath [6]. It is estimated that more than 5,000 individual phytochemicals have been identified in fruits, vegetables, grains, and other plants, mainly classified as phenolics, carotenoids, vitamins, alkaloids, nitrogen-containing compounds, organosulfur compounds and essential oils [7]. It has been mentioned that antioxidant activity of plants might be due to their phenolic compounds. Flavonoids are a group of polyphenolic compounds with known properties which include free radical scavenging, inhibition of hydrolytic oxidative enzymes [8].

Antioxidant activity

Oxidation

Oxidation is one of the destructive processes, where in it breaks down and damage various molecules. Oxygen via its

transformation produces reactive oxygen species (ROS) such as super oxide, hydroxyl radicals, and hydrogen peroxide. They provoke uncontrolled reactions. Molecular oxygen is an essential component for all living organisms, but all aerobic species suffer from injury if exposed to concentration more than 21%. Free radicals attack and induce oxidative damage to various biomolecules including proteins, lipids, lipoproteins, and DNA. The body possess several defense systems comprising enzymes and radical scavengers. Some of them constitute the repair systems for biomolecules that are damaged by the attack of free radicals.

Sources of oxidants in the body [9]

1. Cyclooxygenation
2. Lipooxygenation
3. Lipid per oxidation
4. Re perfusion of ischaemic organs
5. Metabolism of xenobiotics including alcohol and cigarette smoking
6. Ultra violet and ionizing radiation

Free radicals and damage produced by them [10, 11]

1. Hydroxyl free radical (OH.)
2. Super oxide free radical (O.)
3. Hydrogen peroxide (H_2O_2)
4. Singlet Oxygen (O_2)
5. Peroxyl free radical (RO-O)

Free radicals are oxidants, which causes oxidation reactions in the body. The oxidative degradation of poly unsaturated fatty acids cell membranes is induced by free radicals. This causes biological membranes to lose their integrity and functions, as well as destabilizing membrane receptors. Damage to cellular components is also a result of reactive aldehyde formation. Enzymes are inactivated when proteins are attacked, but DNA strands are broken when nucleic acids are damaged. As a result, free radicals harm the cells.

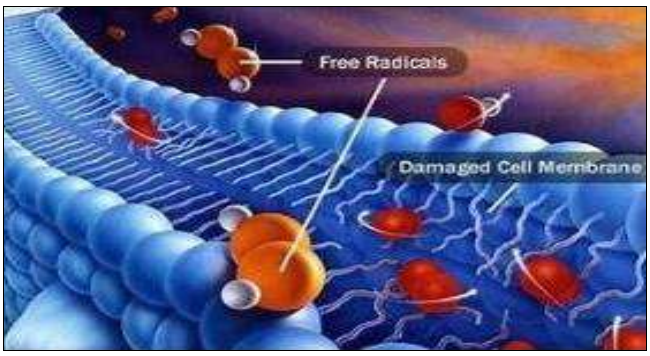


Fig 1: Cell membrane damaged due to free radicals

Free radical scavenger or antioxidants [12, 13, 14, 15]

A substance which at low concentration can prevent or delay the oxidation of an oxidizable substrate are known as free radicals' scavengers. Chemically, oxidation is removal of electrons and reduction is gain of electrons; oxidation is always accompanied by reduction. Antioxidants are commonly found in dietary supplements and have been studied for their ability to protect diseases like cancer, coronary heart disease. Although early research suggested that antioxidant supplements could help health, later big clinical trials using a limited number of antioxidants found no benefit and even revealed that excessive supplementation with some putative antioxidants could be hazards. To prevent free radical formation and eventually tissue damage our body has an array of antioxidant defense system which allows us to cope with the "oxidative stress "inflicted by them.

Endogenous antioxidants

- Superoxide dismutase (SOD)
- Glutathione reduced form
- Carotenoids
- Melatonin α -Tocopherol (Vit. E)
- Ascorbic acid (Vit. C)

Exogenous antioxidants

- Vitamin C (ascorbic acid/ascorbate)
- Vitamin E (tocopherols, tocotrienols)
- Carotenoids (α-carotene, β-carotene)
- Polyphenols (flavanols, isoflavones)
- Trace elements (zinc, selenium)

Trace Elements and Minerals: Selenium is a trace element, which enhances the antioxidant activity of Vitamin E. It also promotes synthesis of glutathione peroxidase as well as stimulates the immune system of the body and therefore is most favoured ingredient in various antioxidant formulations. Manganese, Zinc, Copper and Chromium are good source of generating superoxide dismutase, glutathione peroxidase and catalase enzyme.

Vitamin C: Vitamin C, also known as ascorbic acid (enantiomer, L-ascorbic acid), is an antioxidant and hydro soluble vitamin. It is an electron donor, which explains why it acts as a reducer, directly neutralizing or reducing the damage caused by electronically dis equilibrated and instable reactive species known as free radicals (FR).

Anticancer activity

There are millions of plants available in the world with greater importance. The compounds isolated from various parts of plants play a vital role in treatment of various diseases and have received good attention in recent years due to their different pharmacological properties including cytotoxic and anticancer activity. Plants play a vital place in the treatment of cancer. It is estimated that plant derived compounds one or the other way constitute more than 50% of anticancer agents [16].

The anticancer drugs either kill cancer cells or modify their growth. However, selectivity of majority of drugs is limited and they are one of the most toxic drugs used in therapy. Treatment of malignant diseases with drugs is a rather recent development- started after 1940 when nitrogen mustard was used, but progress has been rapid, both in revealing pathobiology of the diseases and in discovery of new drugs. The latest innovations target growth factors, specific signaling pathways, angiogenesis, tumour antigens, immune therapies, etc. to introduce a different spectrum of drugs. In addition, attempts have been made to define optimal combinations, treatment strategies and patient support measures [17]. Cell culture is practiced extensively throughout the world today. The techniques required to allow cells to grow and be maintained outside the body have been developed throughout the 20th century. In the 50 years since the publication of the first human cancer cell line, HeLa (1), thousands of cell lines representing most of the spectrum of human cancer have been derived. These have

provided tools to study in depth the biochemistry and molecular biology associated with individual cancer types and have helped enormously in our understanding of normal as well as cancer cell physiology. Although some caution is required in interpreting data obtained by studying cells *in vitro*, it has allowed investigation of a complex disease such as cancer to be simplified to its component parts [18].

Primary culture, i.e., the initial culture established from an individual, represents the situation most closely related to the original tissue. The primary material used may be either a fragment, for example an explant, that can be made to attach to the substrate wherein cells can migrate and grow directly from the fragment, or tumor material that can be broken up by mechanical or enzymatic means into single cells or clusters of cells. The source of the material can have an impact on the efficiency of this process, with cultures being more easily established from primary ascitic or pleural effusions already containing cells in suspension than from solid tumors. Enzymes routinely used for disaggregation include trypsin and collagenase. Many of the cell types within the initial cell mix may not adhere to a substrate readily or grow under the culture conditions, and the balance of cell types in culture may change rapidly with time as the fast-growing cells outgrow the slower or nonproliferating cell types. This loss of heterogeneity has both advantages and disadvantages. With selection to produce a more homogeneous cell population, if the predominant emerging cell type obtained is the one of interest, then this might be considered helpful and desirable. For the development of cell lines, this is necessary to allow a pure population to emerge. The disadvantage of selective growth is that the heterogeneity and diversity of the multicellular tumor is lost with the subsequent absence of key intracellular interactions [19].

Cancer Cell Lines

The development of a culture beyond the primary culture results in a "cell line." The importance of a cell line lies in its ability to provide a renewable source of cell material for repeat studies. Cell line models should reflect the properties of their original cancers,

e.g., maintenance of histopathology when transplanted into immunodeficient mice, genotypic and phenotypic characteristics, gene expression and drug sensitivity [20]. However, as it is frequently fast-growing cell lines from poorly differentiated tumors that are generally selected for growth *in vitro*, the cell lines in widespread use may not necessarily always reflect those found in the majority of the clinical disease. Virtually all types of cancer cells can now be grown in culture.

Cancer Cell Collections

Cancer cell lines are widely available through a number of large cell banks. The largest of these are listed in and in addition there are many other national collections that are often government sponsored and nonprofit making. The World Federation for Culture Collection has 469 culture collections in 62 countries (http:// www.wfcc.info), although not all hold cancer cell cultures. The number of more specialized banks concentrating on specific cancer types is also expanding rapidly and these are most easily identified through worldwide web searches [21]. It is recognized that if a cell line can be obtained from a reputable bank, then that is the best source. These provide guarantees of authentication and freedom from contamination that may not be the case when transferring between laboratories. The latter transfer often spreads microbial contamination (especially mycoplasma) and increases the opportunities for mix-ups. Sometimes, however, an academic laboratory may be the only source of a unique cell line.

Methods and Marerials

Plant Collection and Authentication

The fresh bark of *Tamarindus Indica* was collected from the Davanagere district, Karnataka state. The plant was authenticated by taxonomist Dr. Haleshi.C.Asst. Professor at Davangere university, and dried under shed in Bapuji Pharmacy college, Davangere, Karnataka, India.

Preparation of the Extracts

The coarse powdered material was subjected to Soxhlet extraction with ethanol (95%). The temperature (70-80°C) was maintained on an electrical heating mantel with thermostat control. Appearance of Golden yellow colored solvent in the siphon tube was taken as the end point of extraction. The ethanolic extract was evaporate to dryness and extract was concentrated to get powdered form and preserved in the desiccator. The obtained extract was subjected to phytochemical investigation and pharmacological activities.

Phytochemical Investigation of Extract [22, 23]

Phytochemical analyses have confirmed the presence of notable secondary metabolites for ethanolic extract, specifically flavonoids, alkaloids, glycosides, and tannins.

Evaluation of Antioxidant Activity by *In-Vitro* Method: Principle of DPPH (α, α-diphenyl-β-picryl hydrazyl) [24]

The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay is a widely used method to evaluate the antioxidant activity of compounds by measuring their ability to donate electrons or hydrogen atoms to neutralize free radicals. The principle is based on the reduction of the DPPH radical, a stable free radical with a characteristic deep violet color, into a colorless or pale-yellow compound in the presence of an antioxidant. The decrease in absorbance at 520 nm, due to the scavenging of the DPPH radical by an antioxidant, is measured spectrophotometrically.

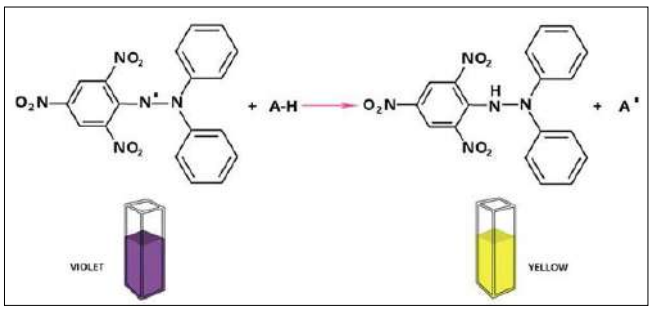


Fig 2: Principle of DPPH

Procedure [25]

DPPH Preparation: Reagent 2, 2-diphenyl 1-picryl hydrazyl solution (DPPH,0.1mM): 4mg of DPPH was dissolved in 100 ml of methanol.

Preparation of standard solutions: (Stock) Ascorbic acid (100mg) is dissolved in 100ml of freshly distilled Methanol which gives 1000 µg/ml concentration. Prepare a different concentration of 50, 100, 200, 400, 600 µg from above stock. Add 0.5, 1, 2, 4, 6 ml from the stock solution (1000 µg/mL) to the 10 ml Volumetric flask. And add 3ml of DPPH Solution to the above 10 ml volumetric flask which contains standard solution, made up to the mark with methanol.

Preparation of Ethanolic extract of *Tamarindus Indica bark* solutions: *TI* (100mg) is dissolved in 100ml of freshly distilled Methanol which gives 1000 µg/ml concentration. Prepare a different concentration of 50, 100, 200, 400, 600 µg from above stock. Add 0.5, 1, 2, 4, 6 ml from the stock solution (1000 µg/mL) to the 10 ml Volumetric flask. And add 3ml of DPPH Solution to the above 10 ml volumetric flask which contains standard solution, made up to the mark with methanol. Prepare a control by adding 3ml of DPPH and add methanol solvent. Blank=Methanol. Incubation period of 30min was allowed at room temperature in dark place to complete any reaction that is to be occurred. Then absorbance was measured by UV spectrophotometer at λ_{max} 520 nm against blank. Ascorbic acid used as standard free radical scavenger activity of extract was compared with it. Activity of the sample was calculated by the formula.

$$\% \text{ Scavenging activity} = \frac{\text{Absorbance of control} - \text{Absorbance of extract}}{\text{Absorbance of control}}$$

Background of the study [26]

MTT assay is a colorimetric assay used for the determination of cell proliferation and cytotoxicity, based on reduction of the yellow-coloured water-soluble tetrazolium dye MTT to formazan crystals. Mitochondrial lactate dehydrogenase produced by live cells reduces MTT to insoluble formazan crystals, which upon dissolution into an appropriate solvent exhibits purple colour, the intensity of which is proportional to the number of viable cells and can be measured spectrophotometrically at 570nm (Alley, M. C *et al*., 1986, Mosmann *et al*., 1983).

Materials [27]

1. Cell line: HepG2: Human hepatocellular carcinoma and PANC-1: Human pancreatic cell line.
2. Cell culture medium: Mc Coy's 5A medium (#AL057S, HIMEDIA)
3. Foetal Bovine Serum (#RM10432, Himedia)
4. Antibiotic Antimycotic Solution-Penicillin & Streptomycin (#A001A, Himedia)
5. Trypsin-EDTA solution (#TCL155, Himedia)
6. D-PBS (#TL1006, Himedia)
7. DMSO (#PHR1309, Sigma)
8. MTT Reagent (# 4060, Himedia)
9. Quercetin (#Q4951, sigma)
10. T25 flask (#12556009, Biolite - Thermo)
11. 96-well plate for culturing the cells (Corning, USA)
12. 1.5 ml centrifuge tubes (TARSON)
13. 50 ml centrifuge tubes (# 546043 TARSON)
14. Adjustable pipettes (2-10µl, 10-100µl, and 100-1000µl), multichannel pipettes and a pipettor (#Eppendorf).
15. 10 to 1000 µl tips (TARSON)

Equipments [28]

1. Centrifuge (Remi: R-8ºC).
2. Pipettes: 2-10µl, 10-100µl, and 100-1000µl.
3. Inverted microscope (Biolinkz, India)
4. 37°C incubator with humidified atmosphere of 5% CO_2 (Healforce, China)
5. 96well microplate reader (ELX-800, BioTek, USA)

Assay controls [29]

1. Medium control (medium without cells)
2. Negative control (medium with cells but without the experimental drug/compound)
3. Positive controls (medium with cells and 25 µg/ml of Quercetin)

Note: Extracellular reducing components such as ascorbic acid, cholesterol, alphatocopherol, dithiothreitol present in the culture media may reduce the MTT to formazan. To account for this reduction, it is important to use the same medium in control as well as test wells.

Steps followed [30]

1. Seed 200µl cell suspension for adherent in a 96-well plate at required cell density (20,000 cells per well), without the test agent. Allow the cells to grow for about overnight.
2. Add appropriate concentrations of the test agent (Mentioned in the results - Excel sheet).
3. Incubate the plate for 24hrs at 37°C in a 5% CO_2 atmosphere.
4. After the incubation period, takeout the plates from incubator, and remove spent media only for adherent cell line and add MTT reagent to a final concentration of 0.5mg/mL of total volume.
5. Wrap the plate with aluminium foil to avoid exposure to light.
6. Return the plates to the incubator and incubate for 3 hours. (Note: Incubation time varies for different cell lines. Within one experiment, incubation time should be kept constant while making comparisons.)
7. Add 100 microlitres of DMSO. gentle stirring in a gyratory shaker will enhance dissolution. Occasionally, pipetting up and down may be required to completely dissolve the MTT formazan crystals especially in dense cultures.
8. Read the absorbance on a spectrophotometer or an ELISA reader at 570nm and 630nm uses a reference wavelength.
9. The IC_{50} value was determined by using linear regression equation i.e., Y =Mx+C and Logarithmic equation i.e., Y=Mln(x)+C
10. Here, Y = 50, M and C values were derived from the viability graph

Column chromatography

Column chromatography is simple and the most popular separation and purification technique. Both solid and liquid samples can be separated and purified by column chromatography. Column chromatography consists of a stationary solid phase that adsorbs and separates the compounds passing through it with the help of a liquid mobile phase. On the basis of their chemical nature, compounds get adsorbed and elution is based on differential adsorption of a substance by the adsorbent. Various stationary phases, such as silica, alumina, calcium phosphate, calcium carbonate, starch, and magnesia, and different solvent compositions based on the nature of compounds to be separated and isolated, are used in column chromatography.[114] Optimization of the method is an important task in the separation of different groups of compounds in extracts. In column chromatography, a cylindrical glass tube, which is plugged at the bottom by a piece of glass wool or porous disc, is filled with slurry (adsorbent) and a suitable solvent. Samples to be separated are mixed with silica and introduced at the top of the column and allowed to move with the solvent. With polarity differences, compounds are adsorbed at different regions and desorbed with suitable solvent polarity. The compound of higher adsorption ability will be adsorbed at the top and that with the lower one will be at the bottom. By adding the solvent at the top, compounds get desorbed and pass through the column and this process is called elution.

Results

Phytochemical Investigation of Extract

Table 1: Phytochemical investigation of *TI*

Sl. No	Phytochemicals	Results
01.	Carbohydrates	Present
02.	Proteins	Absent
03.	Tannins	Present
04.	Tri-terpenoids	Present
05.	Flavonoids	Present
06.	Saponins	Absent
07.	Steroids	Absent
08.	Glycosides	Present
09.	Alkaloids	Present

Anti-oxidant activity

Free radicals are constantly produced, causing substantial damage to tissue and bio molecules, which can lead to a variety of diseases. *Tamarindus Indica* extract is used as an alternative source of medicine to treat oxidative stress-related diseases. The antioxidant activity of Bark portion of *Tamarindus Indica* (*TI*) extract was evaluated using the DPPH method in this work. At a concentration of 600 µg/ml, an ethanolic extract of *Tamarindus Indica* Bark showed strong DPPH radical inhibition (90.6%). In a DPPH *in-vitro* experiment, sequentially produced Et-OH extracts revealed high antioxidant activity. As a result, that which has a high antioxidant activity.

Calculation

IC50 value(x) of fraction TI-H showing antioxidant activity by DPPH Method was calculated by slope from the above graph using formula,

$Y = Mx + C$

$50 = 0.0777x + 42.495$

$0.077x = 42.495 – 50$

$X = -7.505/0.077$

$X = 97.4$ µg/ml

Therefore, IC50 value of fraction TI-H was found to be 97.4 µg/ml.

Table 2: Antioxidant activity of Bark part of *Tamarindus Indica* by DPPH method

Sl. No.	Concentration	Absorbance at 520nm	% DPPH Scavenging
1.	Control	0.964	0.0000
Standard (Ascorbic acid)			
1	50µg/ml	0.527	45.4
2	100µg/ml	0.389	59.6
3	200µg/ml	0.348	64.02
4	400µg/ml	0.180	81.3
5	600µg/ml	0.089	90.6
Ethanolic Extract (TEST)(*TI)*			
1	50µg/ml	0.58	39.8
2	100µg/ml	0.439	54.5
3	200µg/ml	0.377	60.9
4	400µg/ml	0.246	74.5
5	600µg/ml	0.128	86.7

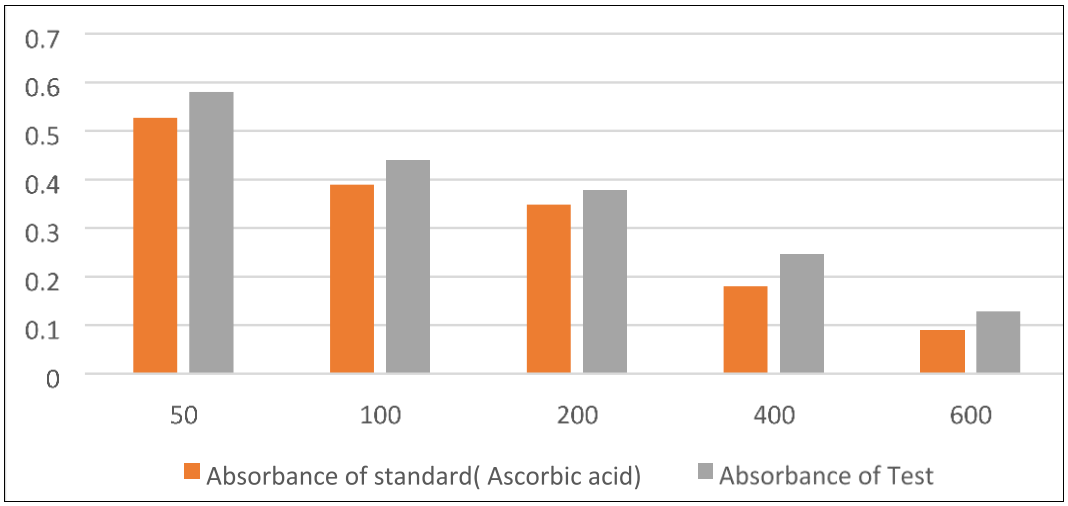


Fig 3: Absorbance of Standard and test compound

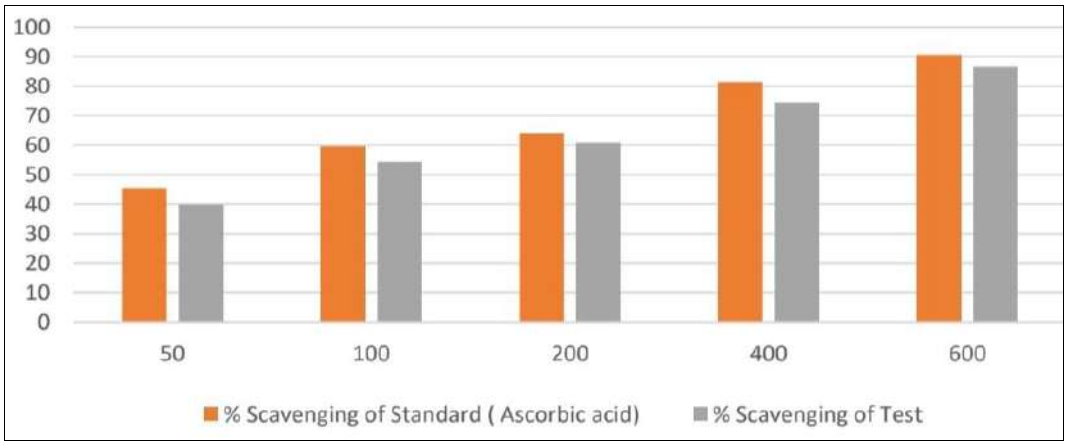


Fig 4: % Scavenging of standard and test compound

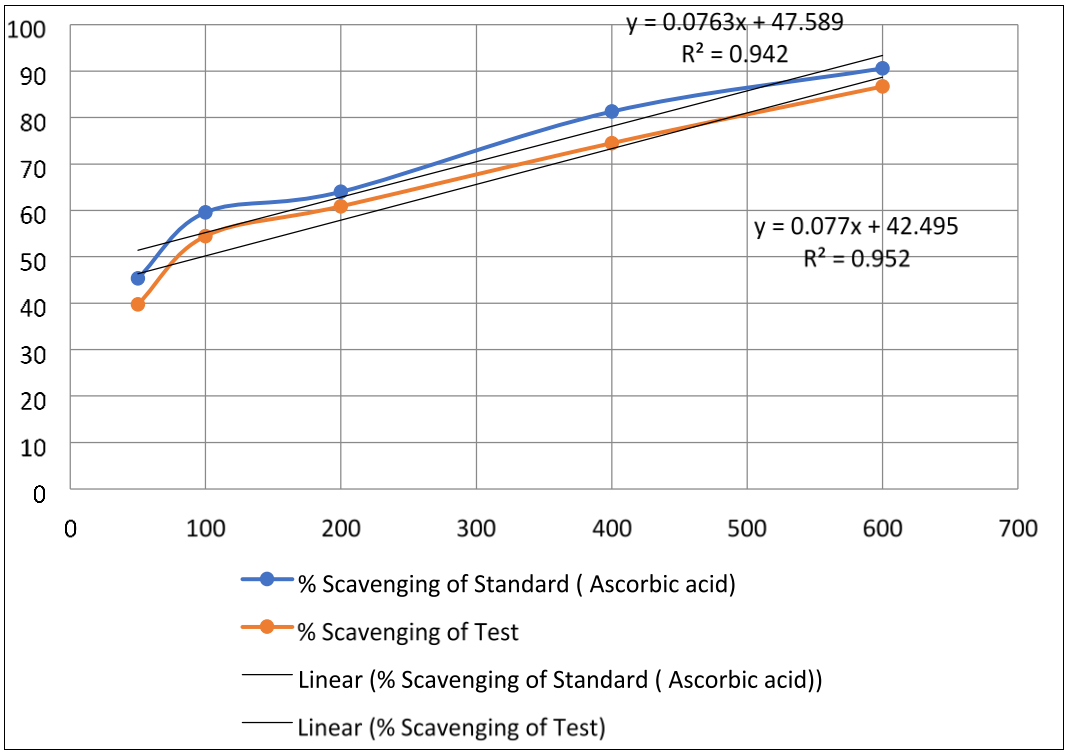


Fig 5: Linear graph of percentage scavenging of standard and test compound**.**

Standard (Ascorbic acid)	R^2=0.942	y=0.0763+47.589	31.59
Test (*TI*)	R^2=0.952	y=0.0777+42.495	97.4

Table 3: Column Chromatography of the ethanolic extract using silica gel (100-200 mesh) (each fraction of 100 ml)

Sl. No	Fraction Name	Elute	Number of Fraction	On evaporation
1	TI-A	Petroleum ether	1	No residue
2	TI-B	Chloroform	1	No residue
3	TI-C	Chloroform: Ethyl Acetate 50:50	1	Greenish residue
4	TI-D	Ethyl Acetate: Chloroform 60:40	1	Light brown residue
5	TI-E	Ethyl Acetate: Ethanol 50:50 60:40 70:30	3	Brown residue, Dark brown residue, Dark brownish residue
6	TI-F	Ethanol: Ethyl Acetate 50:50 60:40	2	Amber residue, Reddish residue

Anticancer activity

In this study, given test compound is evaluated to analyse the cytotoxicity effect on HepG2 and PANC-1 cell line. The concentrations of the test compound used to treat the cells are as follows:

Calculation

To calculate the IC_{50} value from the graph, the equation Y=Mx + C is used.

Y = Mx + C, which is provided in the graph as;

Y = -0.1666+103.67

The IC_{50} value corresponds to the concentration of the drug (x) at which the cell viability (y) is 50%.

50 = -0.1666 + 103.67

0.1666x = 103.67 – 50

0.1666x = 53.67

X= 53.67/0.1666

X= 322.14 µg/ml

The IC_{50} value, based on the graph is approximately 322.14 µg/ml. This indicates the concentration at which 50% cell viability is observed for the drug.

Calculation

To calculate the IC_{50} value from the graph, the equation Y=Mx + C is used.

X= Mx + C, which is provided in the graph as;

X= -0.1752+99.88

The IC_{50} value corresponds to the concentration of the drug (x) at which the cell viability (y) is 50%.

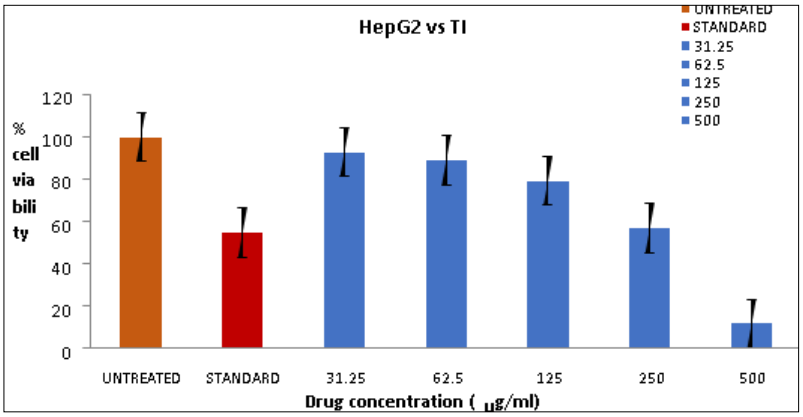
50 = -0.1752 + 99.88

0.1752x = 99.88 – 50 0.1752x = 49.88

X = 49.88/0.1752

X = 284.70 µg/ml

The IC_{50} value, based on the graph is approximately 284.70µg/ml. This indicates the concentration at which 50% cell viability is observed for the drug.



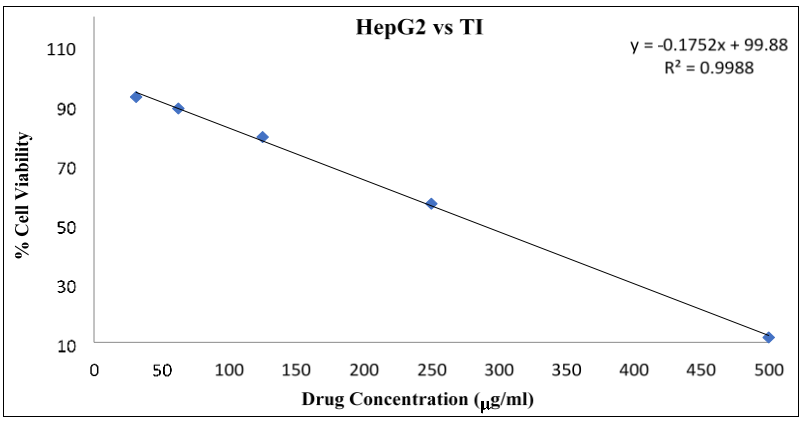


Fig 6: Mean % cell viability of HepG2 cell line after exposing to test compound for 24hrs

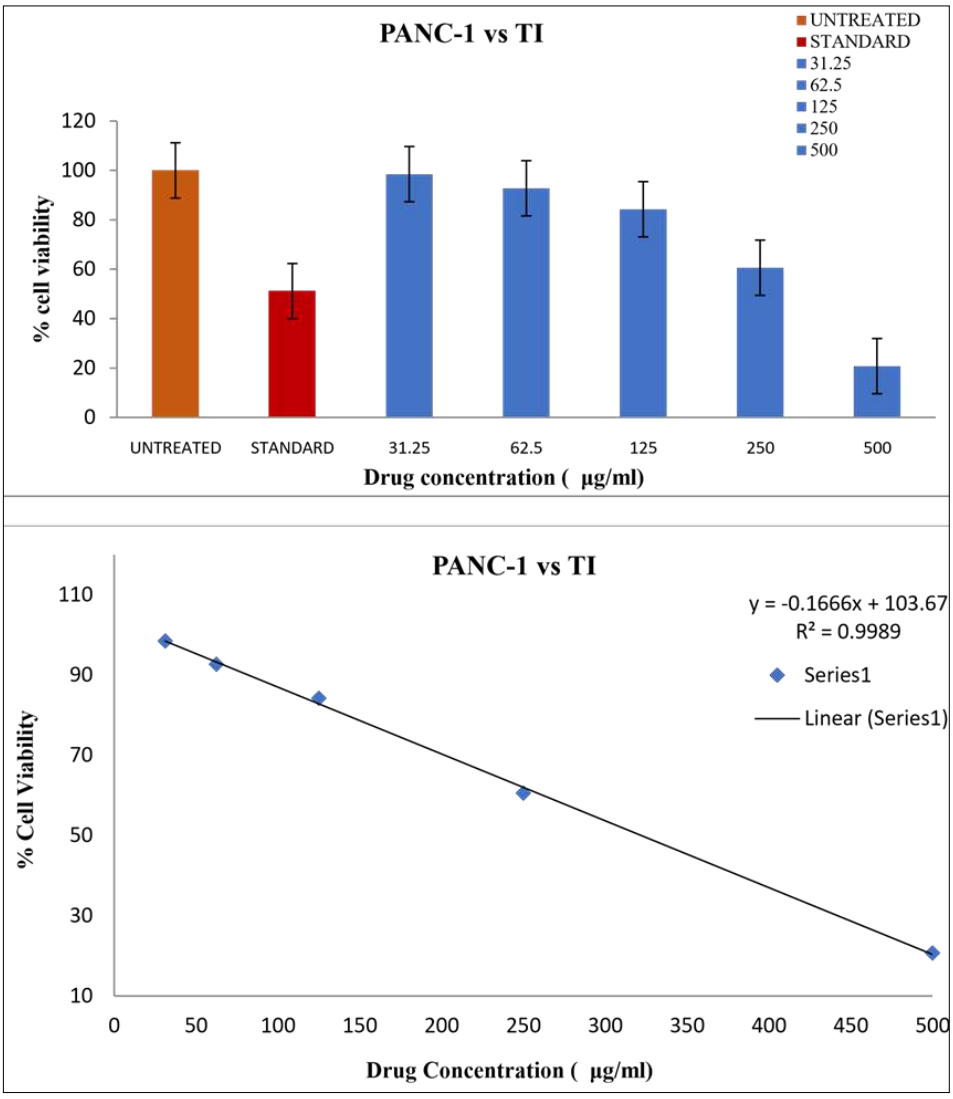


Fig 7: Mean % cell viability of PANC-1cell line after exposing to test compound for 24hrs.

Table 4: Evaluation of Anticancer activity of ethanolic extract of *Tamarindus Indica* bark against HepG-2 Cell lines

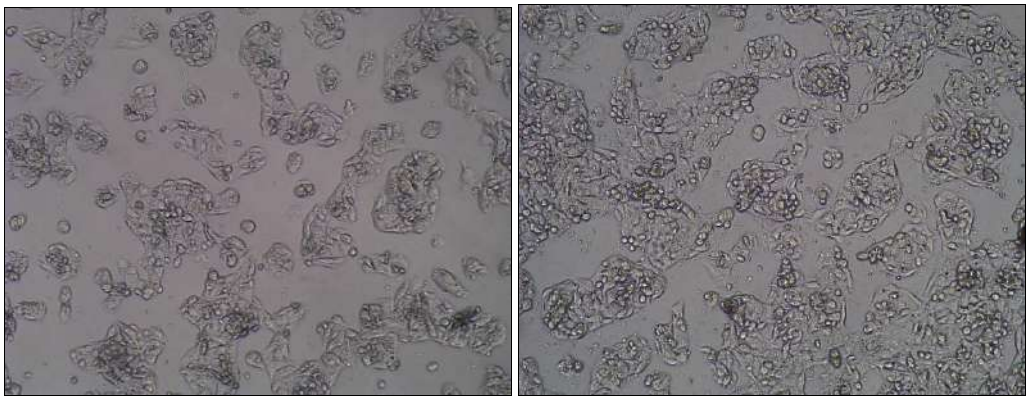
Concentration Unit: µg/ml		**Incubation:24hrs**						
Concentration	**Blank**	**Untreated**	**Standard**	**31.25**	**62.5**	**125**	**250**	**500**
Abs Reading 1	0.022	1.665	0.926	1.562	1.501	1.353	0.947	0.2
Abs Reading 2	0.024	1.686	0.935	1.553	1.485	1.314	0.975	0.226
Mean Abs	0.02	1.6755	0.9305	1.5575	1.493	1.3335	0.961	0.213
Mean Abs (Sample-Blank)		1.6555	0.9105	1.5375	1.473	1.3135	0.941	0.193
STANDARD DEVIATION		0.014849242	0.006363961	0.006363961	0.01131371	0.02757716	0.019799	0.0183848
STANDARD ERROR		0.0105	0.0045	0.0045	0.008	0.0195	0.014	0.013
Cell Viability %		100	54.99848988	92.87224404	88.9761401	79.3415886	56.840834	11.658109

IC50 VALUE= ~ 284.7 µg/ml

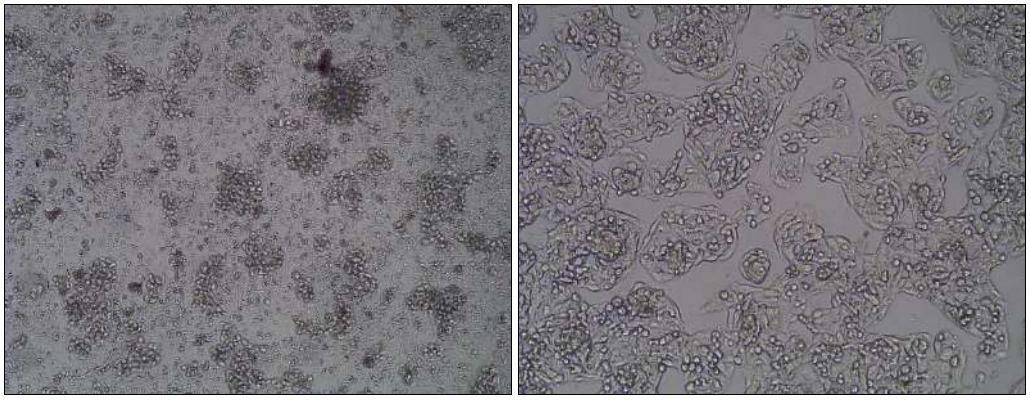
Table 5: Evaluation of Anticancer activity of ethanolic extract of *Tamarindus Indica* bark against PANC-1 Cell lines.

Concentration Unit: µg/ml		**Incubation:24hrs**						
Concentration	**BLANK**	**UNTREATED**	**STANDARD**	**31.25**	**62.5**	**125**	**250**	**500**
Abs Reading 1	0.035	1.729	0.892	1.696	1.564	1.453	1.059	0.402
Abs Reading 2	0.02	1.727	0.895	1.709	1.645	1.466	1.051	0.347
Mean Abs	0.02	1.728	0.8935	1.7025	1.6045	1.4595	1.055	0.3745
Mean Abs (Sample-Blank)		1.708	0.8735	1.6825	1.5845	1.4395	1.035	0.3545
STANDARD DEVIATION		0.001414214	0.00212132	0.009192388	0.05727565	0.00919239	0.0056569	0.0388909
STANDARD ERROR		0.001	0.0015	0.0065	0.0405	0.0065	0.004	0.0275
Cell Viability %		100	51.14168618	98.50702576	92.7693208	84.2798595	60.59719	20.755269

IC50 VALUE= ~ 322.14 µg/ml

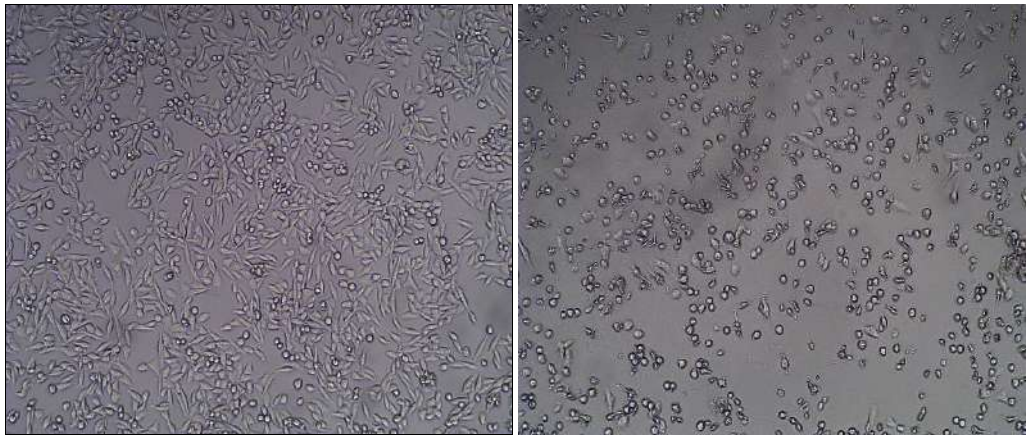


a) Standard **b)** Lower dose (31.25 µg/ml)

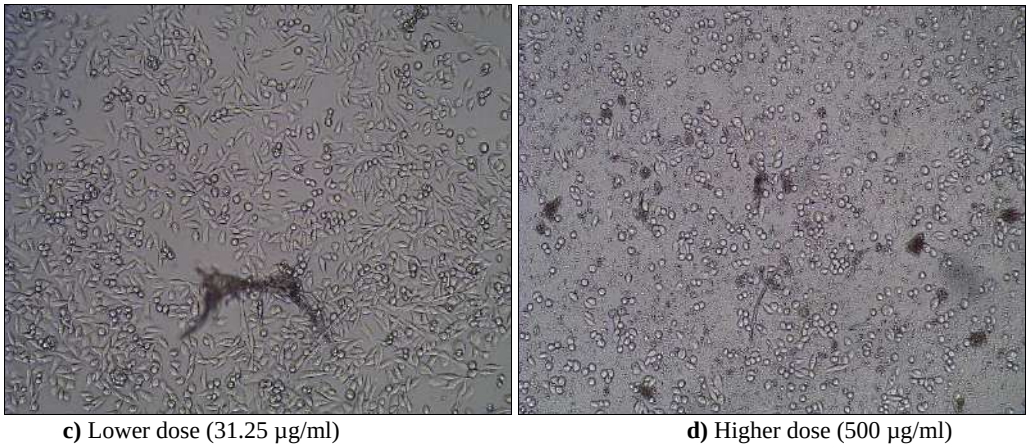


c) Higher dose (500 µg/ml) **d)** Untreated

Fig 8: Histopathological images of HepG2 Cell lines



a) Standard **b)** Lower dose (31.25 µg/ml)



c) Lower dose (31.25 µg/ml) **d)** Higher dose (500 µg/ml)

Fig 9: Histopathological images of PANC-1 Cell lines

Discussion

The activity was performed using DPPH radical scavenging activity. The reduction capacity of this radical was determined by decrease in its absorbance at λ_{max} 520 nm induced by ethanolic extract of *Tamarindus Indica (TI)* exhibits potential antioxidant activity. It produces hydrazine by converting the unpaired electrons to paired electrons due to the hydrogen donating ability of the *TI* extract. The concentration of 600 µg/ml ethanolic extract of *Tamarindus Indica (TI)* showed maximum inhibition (86.7%) and the dose of 400 µg/ml showed significant inhibition (74.6%) respectively. However, in this study the percentage of scavenging inhibition of *Tamarindus Indica (TI)* in DPPH radical scavenging assay was higher in comparison with standard compound. The presence of antioxidants (flavonoids) of secondary metabolites are responsible to scavenging of free radicals. In general, effective radical scavenging was the 3,4 – ortho dihydroxy configuration in ring 3 and 4 carbonyl groups giving a catechol like structure in ring C is also beneficial for antioxidant activity of flavonoids. In addition to this the TI shows potent cytotoxicity results showed that the fraction TI-F exerted notable cytotoxic effects on both PANC- 1 and HepG2 cell lines, with IC_{50} Values of 322.14 µg/ml for PANC-1 cells and 284.7 µg/ml for HepG2 cells. These results suggest that the extract has selective cytotoxic activity and could potentially serve as a natural source for anticancer agents. Overall, the findings of this study indicates that the *TI*-F fractions of *Tamarindus Indica* bark extracts possesses promising antioxidant and anticancer properties, which may be attributed to its rich phytochemical profiles.

Conclusion

The present study was aimed to access antioxidant and anticancer activities of ethanolic extracts of *Tamarindus indica* through MTT assay of HepG2 and PANC-1 cell lines. The plant contain two important secondary metabolites (flavonoids and phenolic compounds) are responsible for the antioxidant property. The extract of the major important constituents the flavonoids able to act as multitarget compounds affecting several molecular network activities and potentially providing preventive and curative effects by exhibiting as antioxidant property.

Disclosure

The authors report no conflicts of interest in this work.

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