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ANTINEOPLASTIC PROPERTIES OF ETHANOLIC EXTRACT OF *FICUS INFECTORIA* (ROXB.) LEAVES ON HUMAN PANCREATIC CARCINOMA CELL LINE

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ABSTRACT:

Ficus infectoria (Roxb.) is an Indian medicinal plant which belongs to the family 'Moraceae'. The main objective of the research investigation study has been focused to evaluate the cytotoxicity activity of ethanolic extract of *Ficus infectoria* (Roxb.) leaves on Human pancreatic carcinoma cell line. The preliminary phytochemical investigation of test sample has been conducted to identify the presence of various pharmacologically useful active phytoconstituents. The anti-cancer activity of test sample (EEFIL) has been subjected to MTT assay on Human pancreatic carcinoma cell line (PANC-1) against standard (Doxorubicin) respectively. Henceforth, results have been shown that the phytochemical analysis of test sample has been confirmed the presence of flavonoids, tannins, phenolic compounds and steroids by showing positive report respectively. The test sample and standard have been shown to be toxic at 1000µg/mL and 20µg/mL on PANC-1 cell line respectively. Similarly, the cytotoxic effect of test sample and standard have been exhibited the IC_{50} values of 671.0224µg/mL and 15.49µg/mL on PANC-1 cell line respectively. In conclusion, on the basis of these results obtained from this cytotoxicity study by using MTT assay has been concluded that, the test sample has been exhibited the cytotoxic effect at IC_{50} value of 671.0224µg/mL on PANC-1 cell line.

KEYWORDS: Anticancer, *Ficus infectoria* (Roxb.), test sample (EEFIL), standard (Doxorubicin), PANC-1, IC_{50}.

INTRODUCTION:

Cancer:

Cancer is a disease which is characterized by uncontrolled proliferation of cells that have been transformed from the normal cells of the body. In other words, Neoplasm is a mass of tissue formed, as a result of abnormal, excessive, uncoordinated, autonomous and purposeless proliferation of cells. Cancer (malignant neoplasm) is a class of diseases in which a group of cells display uncontrolled growth, invasion and sometimes metastasis. These three malignant properties of cancers differentiate them from benign tumors which are self-limited and do not invade or metastasize.[1] Cancer can affect people at all ages with the risk for most types increasing with age. Cancers are primarily an environmental disease with 90-95% of cases due to lifestyle and environmental factors and 5-10% due to genetics. Common environmental factors leading to cancer death includes; tobacco (25-30%), diet and obesity (30-35%), infections (15-20%), radiation, stress, lack of physical activity and environmental pollutants.[2]

Pancreatic cancer:

The pancreas is a vital organ of gastrointestinal system usually in abdomen which is located behind the lower portion of stomach which produces unique enzymes that helps in breakdown of food into small fragments. Pancreatic cancer typically begins at the area which secretes digestive enzymes and usually starts in the tissues of the pancreas. Pancreas can develop both malignant and non-cancerous tumours among other growths.[3] The cells that lining ducts that expels digestive enzymes from the pancreas, where the most prevalent type of pancreatic cancer first develops. Rarely pancreatic cancer found in its earliest stages when it is most treatable. This is due to fact that symptoms frequently don't appear until disease has spread to other organs. When cells in pancreas experience DNA changes (mutations), results in pancreatic cancer. The cells are instructed by these mutations to grow uncontrollably and to live on after normal cells would expire. A tumour may develop from these cells as they gather. If untreated pancreatic cancer cells have the potential to invade surrounding organs, blood vessels and distant regions of the body.[4]

Most pancreatic cancer begins in the cells that line ducts of the pancreas. This type of cancer is called pancreatic

adenocarcinoma or pancreatic exocrine cancer. Less frequently cancer can be formed in the hormone producing cells or the neuroendocrine cells of the pancreas. These types of cancer are called pancreatic neuroendocrine tumors, islets cell tumors or pancreatic endocrine cancer. Different pro-and anti-inflammatory mediators generated from pancreas during inflammation increases genomic damage and cellular proliferation which ultimately results in pancreatic cancer.[5] Tumour like masses of the exocrine pancreas includes; congenital cystic disease and pancreatic pseudocysts. True pancreatic tumours are classified into benign pancreatic tumours (e.g. serous cystadenoma, fibroma, lipoma and adenoma) and malignant pancreatic tumours (i.e., carcinoma of the pancreas). Out of all these, only two pancreatic lesions-pseudocyst and carcinoma of the pancreas are common.[6]

Cytotoxicity and Cell viability:

Cytotoxicity is the quality of being toxic to cells. Cytotoxicity can be monitored by using MTT or MTS assay. MTT assay measures the reducing potential of the cell by using a colorimetric reaction. Viable cells will reduce the MTT reagent to a coloured formazan product. The estimation of number of viable or live cell in a cell culture tissue or multiwall plate is known as cell viability.[7,8]

Plant derived anti-cancer drugs in clinical development:

The plant kingdom are the sources of secondary metabolites which have been investigated for their anti-cancer activity, leading to the development of new clinical drugs. Thus, research has been developed into investigating the potential properties and uses of terrestrial plants extracts for the preparation of potential nanomaterial-based drugs for cancer diseases.[9] Many plant species are already being used to treat or prevent development of cancer. Multiple researchers have been identified the plant species which have been demonstrated anti-cancer properties with a lot of focus on those that have been used in herbal medicine in developing countries.[10-14] Natural products have been well documented to play critical roles in modern drug development, particularly for anti-cancer agents.[15] A large number of natural products derived-compounds in various stages of clinical development have been demonstrated the viability and importance of using natural estimated that 4000 plants have been traditionally used. Folk and herbal medicines representing of about 75% the medicinal needs for the treatment of pancreatic diseases.[16]

Globally, cancer is a disease which severely effects the Human population. There is a constant demand for new therapies to treat and prevent this life-threatening disease. Scientific and research interest is drawing its attention towards naturally-derived compounds, as they are considered to have less toxic side effects compared to current

treatments, such as chemotherapy. New technologies include; nanoparticles for nano-medicines which aim to enhance anti-cancer activities of plant-derived drugs by controlling the release of the compound and investigating new methods for administration. Thereby, the compounds which are characteristic to the plant kingdom and are necessary for plant survival and housekeeping of the organism are being investigated for their ability to inhibit growth and initiate apoptosis of cancerous cells.[17,18]

Plant derived anti-cancer agents in drug discovery:

The search for anti-cancer agents from plant sources have been started in 1950s, with the discovery and development of Vinca alkaloids (Vinblastine & Vincristine) and isolation of the cytotoxic Podophyllotoxins. These discoveries have been prompted the United States National Cancer Institute (NCI), to initiate an extensive plant collection program in 1960s and it has been focused mainly in temperate regions. This led to the discovery of many novel chemotypes showing a range of cytotoxic activities, but their development into clinically active agents have been spanned a period of some 30 years from 1960s to 1990s. The current plant drug discovery relies heavily on bioactivity guided fractionation, which has been resulted in the isolation of many important anti-cancer drugs, such as Paclitaxel and Camptothecin. The National Cancer Institute (USA) has been collected about 35,000 plants from 20 countries and it has been screened around 1,14,000 extracts for their anti-cancer activity. Out of the 92 anti-cancer drugs, commercially available prior to 1983 in US and among worldwide has been approved anti-cancer drugs between 1983 and 1994, 60% are of natural origin. The discovery of Paclitaxel from pacific yew *Taxus bravifolia* and Camptothecin from Chinese ornamental plant *Camptotheca acuminate* have been opened a new horizon for the treatment of carcinogenesis. There are more than 2,70,000 higher plants existing on this Earth, but a small portion so far has been explored phytochemically. So, it can be anticipated that plants can provide potential bio-active compounds for the development of new leads to combat cancer disease.[19] About 3000 plants species that have been reportedly used in the treatment of cancer. Plant derived compounds have been played an important role in the development of several clinically useful anti-cancer agents includes; Vincristine, Vinblastine, Camptothecin derivatives, Topotecan, Irinotecan and Paclitaxel. Some promising new agents are in clinical development based on selective activity against cancer related molecular targets, including Flavoperidol and Combrestatin A_4 phosphate.[20]

Numerous screenings of Indian medicinal plants have been carried out for cytotoxicity activity. Many a times, carcinogenesis is associated with the generation of reactive oxygen species (ROS), results from cell metabolism

as well as extracellular process.[21] Although these ROS possess a necessary physiological function in homoeostasis; but when produced in excess, they play role in pathogenesis of cancer. ROS exert detrimental effects, such as damaging of cell macromolecules i.e., DNA, lipid and proteins. Among these targets, lipid peroxidation is particularly damaging, because it leads to facile propagation of free radical reactions. It's very interesting to observe that, some compounds with highest anti-oxidant property have been shown highest cytotoxic property as well as anti-tumor promoting property.[22]

Ficus infectoria (Roxb.) is one such Indian medicinal plant has being used in folk medicine for treating various Human diseases and disorders. The plant has been reported to cure Human pancreatic carcinoma has not been evaluated pharmacologically. The leaf part of this plant has been selected; based on their traditional usage, therapeutic evidence and previous scientific data, which would provide us new molecule to fight Human pancreatic carcinoma. Henceforth, the current research investigation study has been focused to screen *Ficus infectoria* (Roxb.) leaves for their in-vitro cytotoxicity activity on Human pancreatic carcinoma cell line.

PLANT PROFILE:

TAXONOMICAL CLASSIFICATION:[23-25]

Scientific name : *Ficus infectoria* (Roxb.).

Synonyms : *Ficus lacor* Buch.Ham, *Ficus lucescens* Blume, *Ficus virens*.

Common names : Spotted white fig, pilkhan, sacred fig tree.

Kingdom : Plantae (Plants).

Phylum : Tracheophyta.

Class : Magnoliopsida (Dicotyledons).

Order : Rosales.

Family : Moraceae.

Genus : *Ficus*.

Species : *Ficus infectoria* (Roxb.).

VERNACULAR NAMES:[23-25]

Kannada : Basseri, juvvi, karibassari.

Hindi : Kahimal, kaim, koel, pilkhan, pakar.

Marathi : Bassari, dhedumbara, gandhaumbara.

Tamil : Jovi, kallal, suvi, kurugatti.

Telugu **:** Badijuvvi, jati.

Malayalam **:** Bakri, chakkila, chela.

MORPHOLOGY:[23-25]

PLANT: *Ficus infectoria* (Roxb.) is a plant species that belongs to the Moraceae family. It is commonly known as 'white fig'. It is a monoecious, large perennial, spreading, huge deciduous and fast-growing closely foliaceous trees. All parts of this plant are acrid, pungent and cooling.

GEOGRAPHICAL DISTRIBUTION: It is distributed in Nanjungud, Mysore, Shivamogga, Mangalore, Dharwad, Hassan, Karnataka, Maharashtra, New Delhi, Uttar Pradesh, Kerala, Tamil Nadu, West Bengal and Assam, India.

LEAVES: The leaves are membranous, coriaceous, glabrous, ablong ovate-lanceolate, abrupt shortly acuminate with entire sub-undulate margins; base usually rounded, slightly subcordate or truncate, narrowed or acute; petioles are 3.8-5.7cm long, indistinctly joined with the blade; stipules about 13mm long broadly ovate, acute, pubescent, receptacles axillary in pairs, unisexual, sessile and globose.

ETHNOBOTANICAL IMPORTANCE:[23-27]

Ficus infectoria (Roxb.) plant has been used in traditional system of medicine such as Ayurveda and Unani for the treatment of gastric ulcer, skin ulcer, bone fracture, leprosy, tuberculosis, wounds, gastric problems, vertigo, dysentery, inflammation, burning sensation, acne, typhoid, skin diseases, pain, menstrual disorders, neuropsychiatric disorders, stomach disorders, blood diseases and vaginal diseases respectively.

PHYTOCHEMICAL PROFILE:[23-27]

The aerial parts of *Ficus infectoria* (Roxb.) contains bio-active phytochemical constituents, such as flavonoids, phenolic compounds, tannins, saponins, steroids, terpenoids, glycosides, carbohydrates, alkaloids, proteins and amino acids respectively.

Fig 1 & 2: Photograph showing the leaves and fruits of *Ficus infectoria* (Roxb.).

Fig 1: Leaves

Fig 2: Fruits

Fig 3: Photograph showing the whole tree of *Ficus infectoria* (Roxb.).

Table 1: List of Indian medicinal plants showing anti-cancer activity on Human pancreatic carcinoma cell lines by using MTT assay.

Sl. No.	Botanical name of the plant	Family	Plant part used	References
01	*Acacia chundra*	Fabaceae	Bark	Mujeebulla R H *et al.*,(2023)[28]
02	*Callistemon subulatus*	Myrtaceae	Leaves	Awale S *et al.*,(2022)[29]
03	*Ravenala madaga scariensis*	Strelitziaceae	Leaves	Priyadarsini S *et al.*,(2020)[30]
04	*Calotropis gigantea*	Asclepiadaceae	Flowers	S Bhagavathy *et al.*,(2015)[31]
05	*Rauwolfia vomitoria*	Apocynaceae	Roots	Qi chen *et al.*,(2014)[32]

MATERIALS AND METHODS:

Collection, identification and authentication of *Ficus infectoria* (Roxb.) leaves:

Ficus infectoria (Roxb.) leaves have been collected and procured from Sri Dhanvantri Arogyashram Trust, Nanjungud, Mysore. It has been identified and authenticated by Dr. Haleshi.C, Assistant Professor, Head of Department of Botany, Davangere University, Shivagangotri, Davangere, Karnataka, India.

Drying and powdering of *Ficus infectoria* (Roxb.) leaves:

About 2kg of *Ficus infectoria* (Roxb.) leaves have been shade dried for 20-25 days at room temperature and then powdered by using a dry grinder and finally passed through sieve no.40 to obtain coarse powder.

Preparation of test sample extract (EEFIL):

The air-dried coarsely powdered plant material (127gm) has been subjected to soxhlation extraction method in a soxhlet extractor by using ethanol in 8 batches of 31.77 gm each for 4 days as per standard procedure. The temperature (70-80°C) has been maintained on an electrical heating mantel with thermostat control. Appearance of colourless solvent in siphon tube has been taken as the end point of extraction (approximately 34 cycles). The above process has been repeated for several times, until the sufficient amount of extract has been produced. It has been filtered and concentrated. The solvent has been removed completely over the water bath and then dried. Finally, the test extract so obtained has been weighed, labelled and yield has been calculated in terms of gram percent of the weight of powdered plant material taken. It has been stored in air-tight container and preserved in

a refrigerator for further use. The physical characteristics of test sample (EEFIL) has been recorded. Then, it has been subjected to phytochemical analysis and pharmacological screening, based on the presence of active constituents.[33]

The percentage yield of test sample (EEFIL) has been calculated by using following formula:

$$\text{Percentage yield} = \frac{\text{Weight of the dry extract obtained after extraction}}{\text{Weight of the dry powder subjected to extraction}} \times 100$$

Preliminary phytochemical analysis of test sample extract (EEFIL):

Accurately weighed 4gm of test sample extract (EEFIL) has been dissolved in 300ml of ethanol. Then, it has been subjected to following phytochemical analysis to detect the phytochemical constituents, such as flavonoids, tannins, phenolic compounds, saponins, steroids, alkaloids, glycosides, carbohydrates, proteins and amino acids as per standard procedure.[34,35]

RESEARCH METHODOLOGY:

EVALUATION OF CYTOTOXICITY ACTIVITY ON HUMAN PANCREATIC CARCINOMA CELL LINE (PANC-1):

Outline of the method:

The objective of this cytotoxicity study has been assessed for clear and concise instructions on performing the test sample (EEFIL) against standard (Doxorubicin) for their in-vitro cytotoxicity activity on Human pancreatic carcinoma cell line (PANC-1) by using MTT assay at Radiant Research Services. Pvt. Ltd. Bengaluru, Karnataka, India.

Table 2: List of chemicals and materials used in cell culture.[36]

Sl.No.	Name of chemicals and materials	Manufacturer (Country)
01	Cancer cell line (PANC-1)	NCCS (Pune, India)
02	Cell culture medium (MEM, DMEM)	Gibco (New York, Italy)
03	Fetal Bovine Serum (FBS)	Gibco (New York, Italy)
04	Phosphate Buffered Saline (PBS)	HiMedia (India)
05	MTT reagent	HiMedia (India)
06	DPBS	HiMedia (India)
07	DMSO	SRL (Mumbai, India)
08	Antibiotics	HiMedia (India)
09	Trypsin-EDTA	HiMedia (India)
10	Pipettes and a pipettor	Benchtop (USA)
11	96-well microtitre plates	Corning (USA)

Table 3: List of equipments and instruments used in cell culture.[36]

Sl. No.	Name of equipments and instruments	Manufacturer (Country)
01	Bio-safety cabinet	Ascension (India)
02	Automated micro plate reader	Biotech (USA)
03	Inverted tissue culture microscope	Nikon (Japan)
04	37°C incubator with humidity of 5% CO_2	NUAIRE (USA)
05	-20°C Deep Freezer	Vest frost (Denmark)

Cell line and Cell culture medium:[36]

Human pancreatic carcinoma cell line (PANC-1) has been procured from National Centre for Cell Science (NCCS, Pune, India). Stock cells of PANC-1 have been cultured in MEM supplemented with 10% inactivated Fetal Bovine Serum (FBS), Penicillin (100 IU/mL), Streptomycin (100µg/mL) and Amphotericin-B (5µg/mL) in a humidified atmosphere of 5% CO_2 at 37°C until confluent. The cells have been dissociated with cell dissociating solution i.e., Trypsin Phosphate Versene Glucose solution (TPVG-0.2% Trypsin, 0.02% EDTA, 0.05% glucose in PBS). The stock cultures have been grown in 25cm^2 culture flasks and the viability of the cells have been checked and centrifuged. The cytotoxicity studies have been carried out in 96well microtiter plates and incubated at 37°C for 24hrs in 5% CO_2 atmosphere.

Preparation of test compounds for cytotoxicity screening:[36]

- 10mg of test substance (EEFIL) has been accurately weighed and separately dissolved in MEM medium supplemented with 2% inactivated FBS to obtain a stock solution of 10mg/mL concentration.
- Serial two-fold dilutions have been prepared from stock solution and the initial concentration ranging from 1000µg/mL, which has been prepared by adding 100µL of stock solution to 900µL of 2% serum containing media and from that 500µL of media has been taken and added to the next well containing 500µL of 2% media.
- Furthermore, serial two-fold dilutions have been prepared upto 8 different concentrations ranging from 1000-7.8µg/mL to prepare lower concentrations for cytotoxicity testing.

Determination of cell cytotoxicity by using MTT assay:[36]

1. The monolayer cell culture has been trypsinized and cell count has been adjusted to 1,00,000 cells/mL by using MEM media containing 10% FBS.
2. To each well of 96well microtiter plates, 0.1mL of diluted cell suspension has been added.
3. After 24hrs, when a partial monolayer has been formed, supernatant has been flicked off and the monolayer has been washed once with DPBS medium.
4. The different concentrations of test compounds have been added on to the monolayer in microtiter plates and untreated cells have been maintained as a cell control for comparison.
5. Then, plates have been incubated for 24hrs at 37°C in 5% CO_2 atmosphere and then microscopic examination has been carried out and finally observations have been noted.
6. After 24hrs of incubation, test solutions in the wells have been discarded and 100µL of MTT diluted with DPBS has been added to each well.
7. The plates have been gently shaken and re-incubated for 3hrs at 37°C in 5% CO_2 atmosphere.
8. After 3-4hrs, supernatant culture medium has been carefully removed and 100µL of DMSO has been added to each well.
9. Then, plates have been gently shaken to solubilize the formed formazan and culture plate has been placed on a microplate reader.

10. The absorbance has been measured by using a microplate reader at a wavelength of 570nm and the effect of each concentration has been determined in triplicate.

11. The percentage cell growth inhibition has been calculated by using following formula:

$$\% \text{ cell growth inhibition} = \frac{A_0 - A_1}{A_0} \times 100$$

Whereas;

A_0 = Absorbance of control sample (nm).

A_1 = Absorbance of test/standard sample (nm).

RESULTS:

Preparation of test sample extract (EEFIL):

The physical characteristics of test sample has been recorded. The percentage yield of test sample extract (EEFIL) has been reported as shown in table 4.

Table 4: Table showing the physical characteristics and percentage yield of test sample extract (EEFIL).

Sl.No.	Parameters	Description
01	Name of extract	Ethanolic extract (EEFIL)
02	Method of extraction	Soxhlation
03	Weight of the dry powder (in gm)	127gm
04	Weight of the dry extract (in gm)	12.78gm
05	Percentage yield (%)	10.06%
06	State	Semi-solid
07	Colour	Dark brownish green
08	Odour	Characteristic
09	Consistency	Thick
10	Texture	Smooth
11	Homogeneity	Good

Preliminary phytochemical analysis of crude extract of *Ficus infectoria* (Roxb.) leaves:

The qualitative phytochemical analysis study of test sample has been confirmed that, the test sample (EEFIL) contains flavonoids, tannins, phenolic compounds and steroids by showing positive report as shown in table 5.

Table 5: Table showing the effect of phytochemical analysis of crude extract of *Ficus infectoria* (Roxb.) leaves.

Sl.No.	Phytochemical constituents	Qualitative phytochemical tests	Report
01	Flavonoids	a) Shinoda test	+
		b) Lead acetate test	+
		c) Ferric chloride test	+
		d) Alkaline reagent test	+
02	Tannins and phenolic compounds	a) Ferric chloride test	+
		b) Lead acetate test	+
		c) Gelatin test	+
		d) Dilute Nitric acid test	+
03	Steroids	a) Libermann-Burchard test	+
		b) Salkowaski test	+
		c) Libermann test	+
04	Tri-terpenoids/Triterpenes	a) Salkowaski test	+
		b) Libermann-Burchard test	+
05	Saponin glycosides	a) Foam test	+
06	Alkaloids	a) Mayer's test	-
		b) Hager's test	-
		c) Wagner's test	-
		d) Dragendorff's test	-
07	Carbohydrates	a) Benedict's test	-
		b) Barfoed's test	-
		c) Fehling's test	-
		d) Molisch's test	-
08	Glycosides	a) Keller-Kiliani test	-
		b) Bromine water test	-
		c) Baljet test	-
		d) Legal test	-
09	Proteins and amino acids	a) Biuret test	-
		b) Millon's test	-
		c) Ninhydrin test	-

Note: + ve = present & - ve = absent

Anti-cancer activity:

From this cytotoxicity study, the test sample (EEFIL) and standard (Doxorubicin) have been evaluated to analyze the cytotoxic effect on Human pancreatic carcinoma cell line (PANC-1) by using MTT assay. The percentage cell growth inhibition of test sample and standard have been calculated by using IC_{50} values and measures how much concentration of a particular drug is needed to inhibit the effectiveness of a compound in inhibiting biological function to exhibit cytotoxic property. Henceforth, PANC-1 cells have been treated with test sample (EEFIL) and standard (Doxorubicin) in a range of concentrations from 1000-7.8µg/ml and 20-8µg/ml as shown in below tables and figures.

Table 6: Table showing the effect of cytotoxicity activity of test sample (EEFIL) in terms of percentage cell growth inhibition on Human pancreatic carcinoma cell line (PANC-1).

PANC-1						
Sl. No.	**Group**	**Conc (µg/mL)**	**Average absorbance @ 570nm**	**% cell growth inhibition after treatment**	**% cell viability after treatment**	**IC_{50} (µg/mL)**
01	Test (EEFIL)	1000	0.728	60.42 ± 4.01	39.58 ± 4.01	671.022
		500	0.957	47.99 ± 3.89	52.01 ± 3.89	
		250	1.318	28.34 ± 2.99	71.66 ± 2.99	
		125	1.491	18.95 ± 2.59	81.05 ± 2.59	
		62.5	1.657	9.90 ± 3.37	90.10 ± 3.37	
		31.25	1.720	6.48 ± 3.81	93.52 ± 3.81	
		15.625	1.743	5.24 ± 3.13	94.76 ± 3.13	
		7.8	1.818	1.17 ± 0.83	98.83 ± 0.83	
02	Control	00	1.84	1.09 ± 1.41	98.91 ± 1.41	>1000

Note: Data have been analyzed by using one way ANOVA, followed by Dunnett's pairwise comparison. The values have been expressed as mean ± SD.

Fig 4: Histogram showing the effect of cytotoxicity activity of test sample (EEFIL) on Human pancreatic carcinoma cell line (PANC-1).

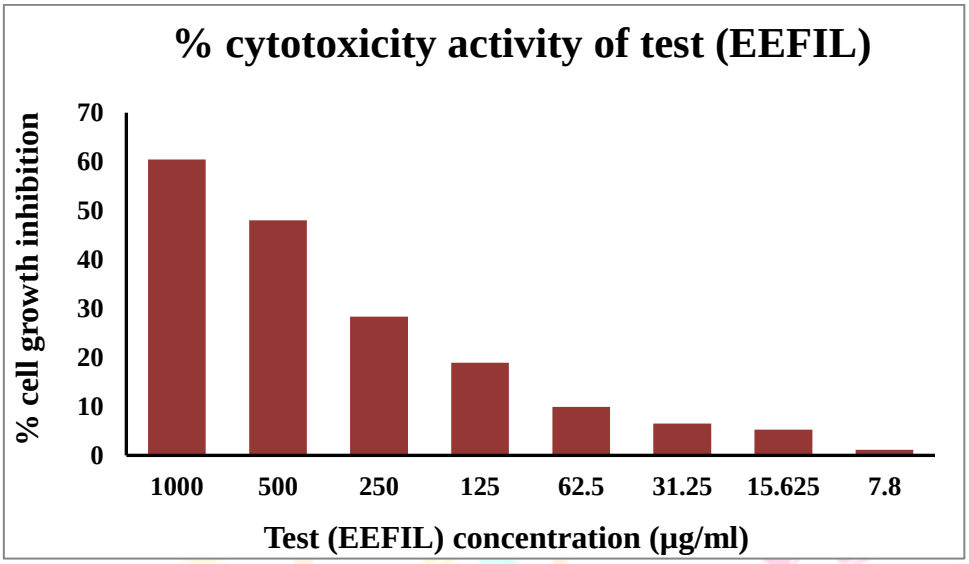


Table 7: Table showing the effect of cytotoxicity activity of standard (Doxorubicin) in terms of percentage cell growth inhibition on Human pancreatic carcinoma cell line (PANC-1).

PANC-1						
Sl. No.	**Group**	**Conc (µg/mL)**	**Average absorbance @ 570nm**	**% cell growth inhibition after treatment**	**% cell viability after treatment**	**IC_{50} (µg/mL)**
01	Control	00	0.61	1.09 ± 1.41	98.91 ± 1.41	>1000
02	Standard (Doxorubicin)	20	0.255	58.20 ± 1.07	41.80 ± 1.07	15.49
		18	0.282	53.66 ± 0.28	46.34 ± 0.28	
		16	0.304	50.16 ± 1.23	49.84 ± 1.23	
		14	0.317	47.98 ± 1.14	52.02 ± 1.14	
		12	0.338	44.59 ± 1.51	55.41 ± 1.51	
		10	0.361	40.71 ± 0.66	59.29 ± 0.66	
		8	0.386	36.72 ± 0.35	63.28 ± 0.35	

Note: Data have been analyzed by using one way ANOVA, followed by Dunnett's pairwise comparison. The values have been expressed as mean ± SD.

Fig 5: Histogram showing the effect of cytotoxicity activity of standard (Doxorubicin) on Human pancreatic carcinoma cell line (PANC-1).

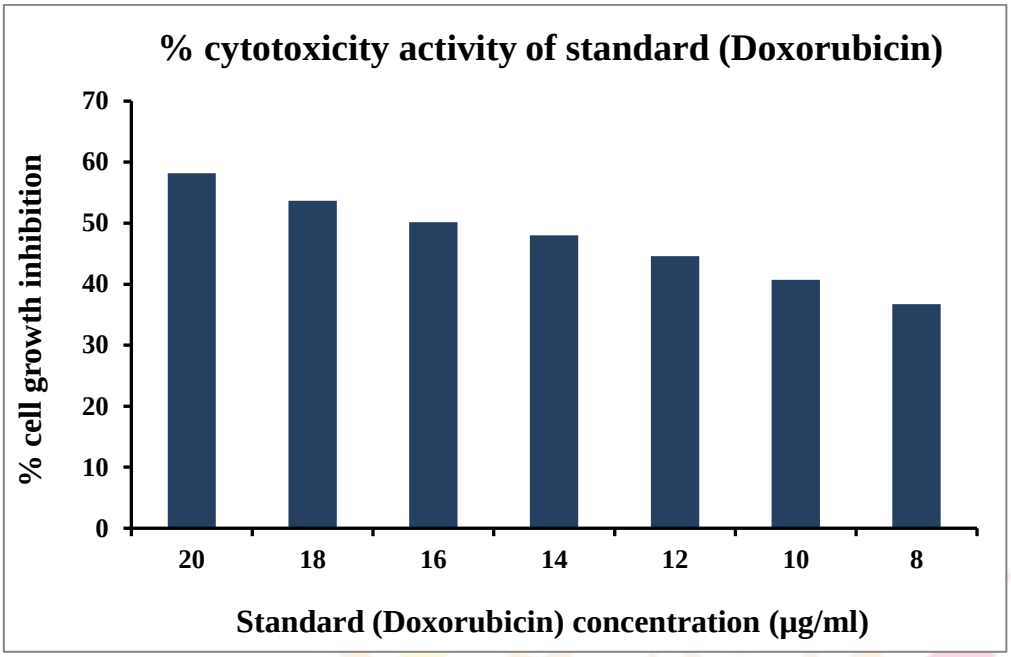
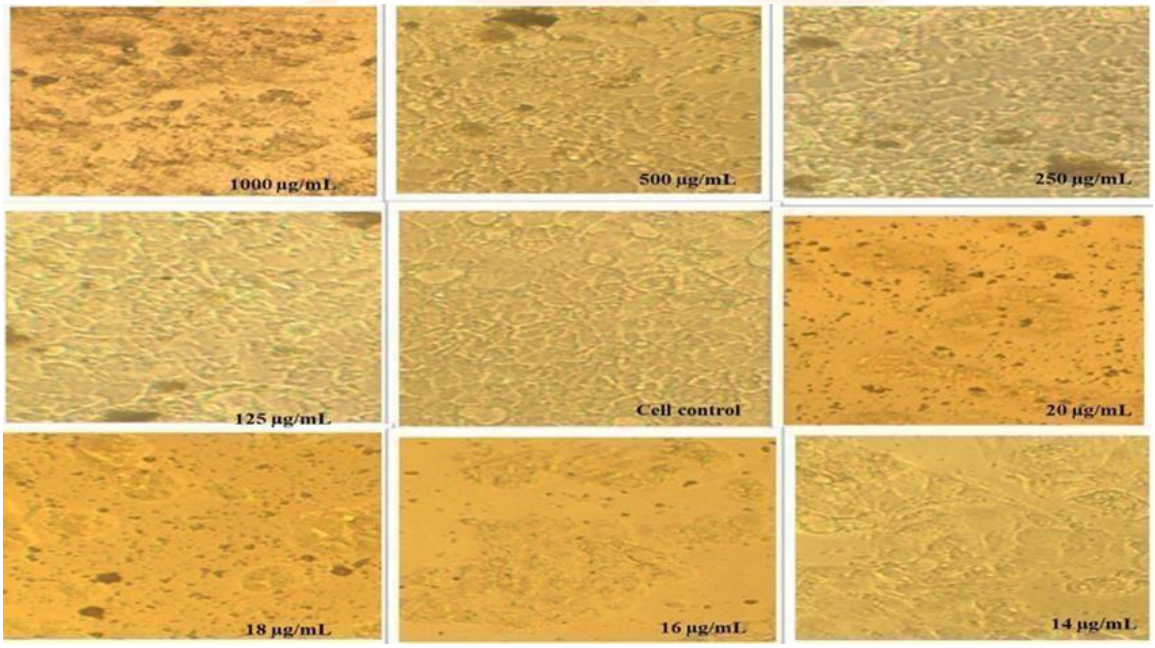


Fig 6: Microscopic image showing the effect of cell viability of different concentrations of test sample (EEFIL) and standard (Doxorubicin) on Human pancreatic carcinoma cell line (PANC-1).



DISCUSSION:

In current study, it has been undertaken to screen *Ficus infectoria* (Roxb.) leaves for their cytotoxicity activity. The literature survey has been revealed that, the phytochemical profile and pharmacological activities of this plant part is incomplete, by referring indexed journal and other information sources. Keeping the native knowledge and the above-mentioned literature information, *Ficus infectoria* (Roxb.) plant has been selected

for present study to screen a leaf part for the category of cytotoxicity activity.

The test sample (EEFIL) has been obtained by soxhlation extraction method for the most effective once, on inhibiting the cell proliferation. Then, it has been subjected to preliminary phytochemical analysis tests and the outcome of these phytochemical analysis tests of test sample has been confirmed the presence of flavonoids, tannins, phenolic compounds and steroids by showing positive report respectively.

MTT assay has been employed to test the cytotoxic effect of selected concentrations by measuring the metabolic activity through a colorimetric determination. This assay has been usually carried out to detect the cells with constant mitochondrial activity, thereby an increase or decrease in the number of viable cells is linearly related to mitochondrial activity. In MTT assay, dissolved MTT has been converted to an insoluble purple formazan by using mitochondrial lactate dehydrogenase enzyme of viable cells.

Henceforth, MTT assay has been performed on Human pancreatic carcinoma cell line (PANC-1) for evaluating the cytotoxicity of test sample (EEFIL). Thereby, the test sample (EEFIL) and standard (Doxorubicin) have been assayed for their in-vitro cytotoxicity study on Human pancreatic carcinoma cell line (PANC-1) by exposing the cells to different concentrations ranging from 1000-7.8µg/mL and 20-8µg/mL respectively. The test sample and standard have been shown to be toxic at 1000µg/mL and 20µg/mL on PANC-1 cell line respectively. Whereas, the cytotoxic effect of test sample and standard have been exhibited the IC_{50} values of 671.0224µg/mL and 15.49µg/mL on PANC-1 cell line respectively. Similarly, the comparison of cytotoxic effect of test sample (EEFIL) and standard (Doxorubicin) have been determined in terms of percentage cell growth inhibition after treatment and it has been found to be 60.42 ± 4.01% and 58.20 ± 1.07% at higher concentration of 1000µg/mL and 20µg/mL on PANC-1 cell line respectively.

Henceforth, these results have been suggested that, the cytotoxic effect of test sample (EEFIL) on Human pancreatic carcinoma cell line (PANC-1) has been evaluated. Thus, the test sample (EEFIL) has been subjected to isolate the targeted Human pancreatic carcinoma cell line (PANC-1) to establish molecular level of mechanism of action. Therefore, on the basis of these results exhibited by this cytotoxicity study, *Ficus infectoria* (Roxb.) leaves has been major bio-active phytochemicals of therapeutic importance. It has been concluded that, *Ficus infectoria* (Roxb.) is an unexplored plant in the field of medicine. Thus, *Ficus infectoria* (Roxb.) leaves can be considered as a low cost, potent and herbal medicine for pancreatic disease.

CONCLUSION:

The present research investigation study has been targeted on the evaluation of in-vitro cytotoxicity activity of ethanolic extract of *Ficus infectoria* (Roxb.) leaves on Human pancreatic carcinoma cell line. This study has been reported that, the scope for exploration of extensive phytochemicals of test sample (EEFIL) contains major secondary metabolites, such as flavonoids, tannins, phenolic compounds and steroids respectively. Henceforth, it has been concluded that *Ficus infectoria* (Roxb.) leaves has been major bio-active phytoconstituents which are responsible for cytotoxic property which might be present in this test sample. Further, phytochemical explorations are needed to characterize the precise molecule which are responsible for anti-cancer activity. On the basis of these results exhibited by this cytotoxicity study by using MTT assay has been suggested that, the test sample (EEFIL) and standard (Doxorubicin) have been shown to be toxic at 1000µg/mL and 20µg/mL on PANC-1 cell line respectively. Whereas, the test sample (EEFIL) and standard (Doxorubicin) have been exhibited the cytotoxic effect at IC_{50} values of 671.0224µg/mL and 15.49µg/mL on PANC-1 cell line respectively. Although, the mechanism of action which might be presence of flavonoids, tannins and phenolic compounds. Hence, these compounds are known to scavenge the formation of free radicals have great potential in ameliorating diseases, such as cancer. Based on the evidence of these investigation, it has been concluded that *Ficus infectoria* (Roxb.) leaves has multiple medicinal property, particularly as anti-cancer activity. This indicates that, the test sample (EEFIL) may be used as an effective cytotoxic property.

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