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Research Article

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Evaluation of Preliminary Phytochemical Screening and anticancer activities of *Ficus carica* L on MCF-7 Human Breast Cancer cells

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Abstract

The most common cause of cancer-related deaths in women worldwide is breast cancer, underscoring the critical need for effective treatments. The *Ficus carica*, commonly known as the common fig, has shown impressive abilities to inhibit tumor formation and slow the growth of cancerous cells by regulating multiple signalling systems and their interactions, involving various essential cell signalling molecules. Building on these promising findings, the main goal of the current study is to evaluate the preliminary phytochemical screening and cytotoxic effect of *F. carica* fruit extract on its potential anti-cancer activities. Additionally, the study aims to observe and record any changes in the appearance of MCF-7 breast cancer cell lines in response to the fruit extract. This comprehensive investigation seeks to provide valuable insights into the potential therapeutic effects of *F. carica* in the context of breast cancer treatment. The preliminary phytochemical screening was done to reveal the bioactive constituents of *F. carica*. Qualitative phytochemical studies exhibited the presence of alkaloids, saponins, flavonoids, terpenoids, steroids, tannins, glycoside and protein compounds. The cytotoxic effect of the ethanolic fruit extract of *F. carica* on MCF-7 cell lines was investigated in a laboratory study. The assessment involved utilising the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay to measure the cell viability in response to the extract. Additionally, observable changes in the cell morphology of MCF-7 cells were noted upon exposure to the extract. The results demonstrated that the ethanolic fruit extract of *F.carica* with different concentrations (1000 µg/ml, 500 µg/ml, 250 µg/ml, 125 µg/ml, 62.5 µg/ml, 31.2 µg/ml, 15.6 µg/ml, 7.8 µg/mL) had shown a dose- dependent significant cytotoxic activity of 17.83%, 24.85%, 31.68%, 38.70%, 46.29%, 53.32%, 60.34% and 67.36%, respectively, 47.14% of cell

viability was observed at 31.2 µg/ml concentration of ethanolic fruit extract of *F. carica*. From this study, it can be concluded that the methanolic fruit extract of *F. carica* showed significant anticancer activity against MCF-7 cell lines and the ethanolic fruit extract of *F. carica* could be used for the treatment of breast cancer.

Keywords: Breast cancer, *Ficus carica*, Ethanol extract, MCF 7 cells.

Introduction

Natural compounds found in plants are an important and abundant source of potential anti-cancer drugs, with ongoing efforts to unlock their therapeutic potential. Research indicates that medicinal herbs may offer a potential breakthrough in cancer treatment, providing hope for the development of new therapy options. However, there is a growing concern about the increasing resistance of breast cancer cells to chemotherapy drugs, presenting significant challenges in the fight against the disease. Among women's diseases, breast cancer has a significantly greater incidence than any other cancer [1]. The most common types of breast cancer are triple-negative breast cancer (TNBC), human epidermal growth factor receptor-2-positive (HER2+), and estrogen receptor-positive (ER+) breast cancer. TNBC is particularly challenging to treat because it lacks the specific proteins targeted by some treatments. In breast cancer, important molecules such as GATA3, p53, Bax, p21, ELF5, and cyclin-dependent kinases (CDKs) are closely linked to the growth and death of cancer cells. CDKs play a crucial role in modifying the cell cycle, repairing damaged DNA, and triggering cell death. Metastasis, the dissemination of cancer from its primary site to other parts of the body, is the primary cause of death and involves various molecules including, MPP2, TIMP1, and TIMP2. Based on where the tumor is located in the breast, it may be in situ, meaning that the cancer cells replace the epithelial cells in the mammary gland ducts, and it may grow to form lobules without spreading to the surrounding tissues. Additionally, it can be more prevalent and aggressive, with the propensity to spread outside of the ducts and into the surrounding breast tissues [2]. Natural products are full of many different kinds of molecules and unique substances, many of which may be novel. Among the most prevalent and useful natural products, plants also include minerals and secondary metabolites. These include substances that have been shown to have antioxidant, antibacterial, and anticancer properties, such as acyl lipids, sterols, and flavonoids [3].

A prominent member of the Moraceae family, which has long been domesticated by humans, is the fig, *Ficus carica*. Although *F. carica* originated in the Middle East and West Asia, it has since spread to many other parts of the world. *F. carica* products are used extensively as food and medicine to treat a variety of illnesses [4]. For instance, fig fruit extract shows modest cytotoxic actions against human cancer cell lines, such as MCF7, HepG2, and U2OS,3 and antiproliferation against cervical cancer cell lines. It also has hypotensive and antihypertensive effects in glucose-induced hypertensive rats [5]. Fig leaves, fruit, and latex are rich sources of compounds with potential anticancer properties. Of particular interest are the compounds bergapten and psoralen [6]. Bergapten has been found to exhibit inhibitory effects on liver cancer cell lines, stomach cancer cell lines, and NPC cells. Its mechanism of action involves direct cell killing, cell cycle arrest, and induction of apoptosis. These findings suggest bergapten's potential as a multi-targeting agent in cancer treatment. On the other hand, psoralen has demonstrated the ability to inhibit the proliferation and migration of MCF7/ADR cells. Moreover, it has shown promise in inhibiting breast cancer cell growth within the bone microenvironment and in modulating the function of osteoblasts and osteoclasts in tumor-bearing mice [7]. These insights underscore the potential of psoralen as a multifaceted therapeutic agent with implications for breast cancer and bone metastasis treatment. Several studies have reported that some species of

Ficus possess pharmacological activities, such as antioxidant, antimicrobial [8], antiviral, antiinflammatory, antiparasitic, antidiabetic, and antiproliferative activities [9]. Although the mechanisms of fig action on human health have not been fully elucidated, the ubiquity of polyphenols and their high flavonoid content suggest a strong anticancer potential [10]. The present study was carried out to evaluate the preliminary phytochemical screening and cytotoxic effects of ethanolic fruit extract of *F. carica* potential on MCF7 breast cancer cells *in vitro*.

Materials and Methods

Plant material and extraction:

A number of bioactive compounds have been revealed from *Ficus carica* that are found to be responsible for the pharmacological potential. The entire *Ficus carica* L. fruit was subjected to a thorough extraction process using ethanol, known as the Soxhlet extraction method, which involves continuous hot extraction. Once the extraction process was complete, the resulting extracts were carefully evaporated to dryness under vacuum conditions. The dried extracts obtained from this process were used in an in-vitro cytotoxicity assay to assess their impact on MCF7 breast cancer cells. This assay specifically utilised the MTT assay method [11].

Cell culture condition and treatment

The MCF7 breast cancer cell line, a widely used model for studying breast cancer, was obtained from the National Centre for Cell Sciences in Pune (NCCS). The cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM), a commonly used cell culture medium, supplemented with 10% Fetal Bovine Serum, penicillin (100 U/ml), and streptomycin (100 µg/ml) in a controlled environment with a humidified atmosphere containing 5% CO_2 at a temperature of 37°C Stock solutions of *Ficus carica* L. fruit ethanolic extract, a natural product with potential bioactive properties, were prepared at a concentration of 1 mg/ml in DMSO (dimethyl sulfoxide) and stored at -20ºC until use. This extract is known for its potential anticancer properties and was thus chosen for use in the study.

Cancer cell proliferation analysis

An MTT assay was used to examine the growth of cancer cells. In 24-well plates, MCF7 cells (1 x 105/well) were plated and incubated at 37°C with 5% CO_2. The different sample concentrations were introduced as the cell reached confluence, and it was then incubated for a full day. Following incubation, the sample was taken out of the well and cleaned using either DMEM without serum or phosphate buffered saline (pH 7.4). 0.5% 3-(4, 5-dimethyl-2-thiazolyl)-2, 5-diphenyl--tetrazolium bromide (MTT) was added to 100µl/well (5 mg/ml) and allowed to incubate for 4 hours. After the cell samples were subjected to an incubation period, we carefully added one millilitre of DMSO to each well to ensure uniform mixing. Following this, we used a UV spectrophotometer to measure the absorbance at 570 nm, with DMSO acting as the blank for accurate readings. The data obtained from these measurements were then used to generate a graphic representation illustrating the concentration required to achieve a 50% inhibition (IC_{50}). Additionally, we calculated the percentage of viable cells by utilising the following formula:

% Cell viability = A 570 of treated cells/A570 of control cells × 100

Plotting of graphs is done with the sample concentration on the X-axis and the percentage of cell viability on the Y-axis. Each assay includes a cell control and a sample control to compare the results of the complete cell viability evaluations.

Results

The ethanolic fruit extract of *F. carica* was prepared by Soxhlet extraction. The preliminary phytochemical screening was done to reveal the bioactive constituents of *F. carica*. Qualitative

phytochemical studies exhibited the presence of alkaloids, saponins, flavonoids, terpenoids, steroids, glycoside, tannins and protein compounds, as outlined in Table 2. The study examined the potential anticancer effect of ethanolic fruit extracts from *Ficus carica* on the MCF7 breast cancer cell line. To assess the impact of *F. carica* extracts, the cell viability of MCF7 breast cancer cells was measured using the MTT test. The study used ethanolic *F.carica* fruit extracts with IC_{50} values of 1000 µg/ml, 31.2 µg/ml, and 7.8 µg/ml during a 24 to 72 hours treatment period, as depicted in Figure 1. The results showed that as the concentration of the extract increased, the viability of the breast cancer cells decreased over time, as depicted in Figure 2. Additionally, the study revealed that *F. carica* inhibited 50% of the MCF7 cell population at a biological concentration of 31.2 µg/ml, as outlined in Table 2.

Table 1: Preliminary Phytochemical analysis of Ethanolic extract of *F.carica*

S. No	Test	Ethonolic Extract of *F.carica*
1	Alkaloids	Positive
2	Terpenoids	Positive
3	Saponins	Positive
4	Flavonoids	Positive
5	Terpenes	Positive
6	Tannins	Positive
7	Glycosides	Positive
8	Steriods	Positive
9	Protein	Positive

Table 2: Cytotoxicity activity of *F. carica* ethanolic extract against MCF7 breast Cell line at different concentrations by MTT assay

Concentration (µg/ml)	Cell Viability (%)
1000	17.83
500	24.85
250	31.68
125	38.70
62.5	46.29
31.2	**53.32**
15.6	60.34
7.8	67.36
Control	100

Figure 1: Cytotoxicity activity of *F. carica* ethanolic extract against MCF7 Breast Cell line at different concentrations by MTT assay.

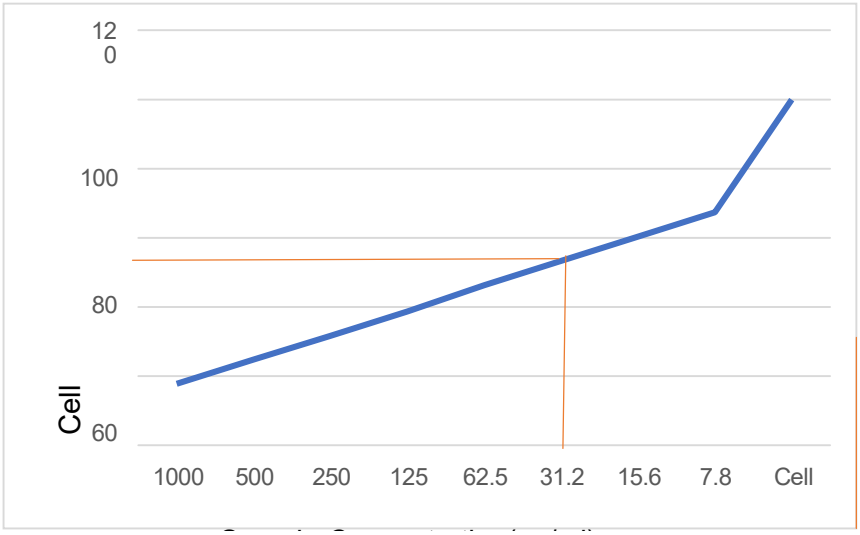
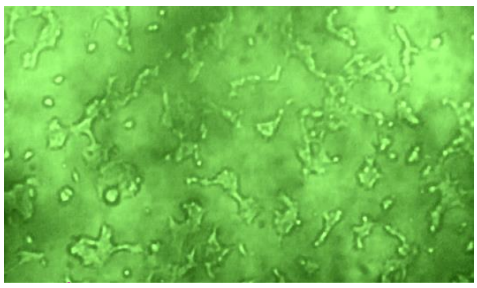
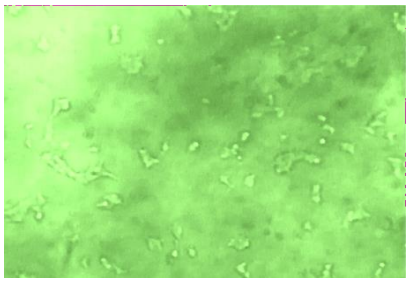


Figure 2: Cytotoxicity activity of *F. carica* ethanolic extract against the MCF7 Cell lines

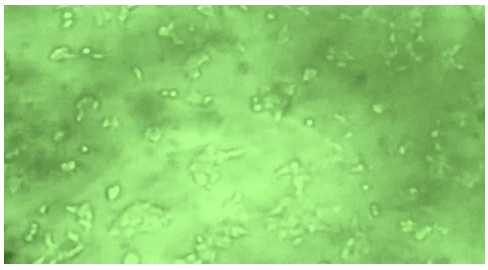
MCF 7 (Control)



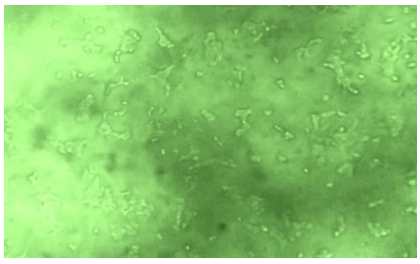
Conc.1000 µg/ml



Conc.31.2 µg/ml



Conc.7.8 µg/ml



Discussion

Herbal remedies, particularly those that are edible, are valuable adjunctive alternative therapies for the prophylaxis of numerous illnesses, including cancer [12]. As a result, researchers studying cancer have been interested in them; several herbal remedies are currently being used in clinical settings and have shown promising results [13]. The results of several scientific studies indicate that a wide variety of physiologically active substances are present in the extracts made from herbs. Together with few side effects, these parts alone or in combination

have shown strong anti-tumor activities in both lab tests and living things [14]. Extracts from the stem, bark, fruit, leaves, and latex of the fig tree have been found to possess anti-tumor properties.

In this study, it was reported that treatment with *F. carica* fruit ethanolic extract inhibits the proliferation of MCF7 cells, which are breast epithelial cancer cell lines. The inhibition percentage with regard to cytotoxicity was found to be 53.32% at 31.2 µg/ml (Table: 4). This result suggests that the fruit extract of *F. carica* has specific growth-inhibitory effects on breast cancer cells. A number of bioactive compounds have been revealed from *Ficus carica* that are found to be responsible for the pharmacological potential. Thus, the preliminary phytochemical screening was done to reveal the bioactive constituents of *F. carica*. Qualitative phytochemical studies exhibited the presence of alkaloids, saponins, flavonoids, terpenoids, steroids, glycoside tannins and protein compounds.

In agreement with the current result, previous studies have found that fig leaves, fruit, and latex all contain anticancer components [15]. According to earlier studies, fig fruit extracts have modest cytotoxic effects on MCF7, HepG2, and U2OS cells. Additionally, the growth of some cancer cell lines, such as the Raji B-cell lymphoma and stomach cancer lines, is inhibited by fig latex. Moreover, research has shown that *F. carica* leaf extract can upregulate the expression of genes linked to apoptosis, including TP53, TP21, and BAX, and exhibits an antiproliferative action on TNBC cell MDA-MB-231 [16].

Results from another investigation showed that *Ficus carica* fruit crude extracts had an antiproliferative effect on breast cancer cells that was positive for the estrogen/progesterone receptor. It is thought that the bioactive substances in the crude extracts are what cause this effect. Furthermore, the study also indicated that fruit extracts from *Ficus carica* may have the ability to protect estrogen/progesterone receptor-positive breast cancer cells against chemotherapeutic agents [17-18].

A study conducted by Srivastava *et al*. revealed that when quercetin, which is the primary phenolic compound found in *F. carica*, was administered to breast cancer cell cultures, it triggered a reaction in the DNA of the cancer cells. This accumulation of reactions can lead to a process called apoptosis, which is the programmed cell death of cancer cells. Furthermore, this apoptotic process was found to be associated with the activities of p53 and p21, which are important proteins in regulating cell growth and division. Quercetin was observed to stimulate the expression of p21 and suppress the expression of cyclin D1, which is a protein that plays a key role in regulating the cell cycle [19-20]. It can be suggested that these fruits contain compounds that can help in the prevention and treatment of cancers that also include breast cancer and more research should be done to identify the particular bioactive substances in the fruit extracts and clarify how they work.

Conclusion

The preliminary phytochemical analyses revealed the presence of several compounds that possess the potential efficiency of *F. carica*. Hence, perhaps it can be encouraged to be this fruit as an alternative to current medicines. The investigation results demonstrate that the ethanolic fruit extract of *F. carica* has shown significant potential as a cytotoxic agent against MCF-7 breast cancer cell lines. This promising finding suggests that the extract could be a valuable asset in the treatment and management of breast cancer. Furthermore, the study aims to delve deeper into the bioactivity-guided fractionation of the bioactive anticancer compounds present in the ethanolic fruit of *F. carica*, in addition to conducting further *in vivo* research. These findings provide substantial support for the traditional Indian medical system's historical utilisation of *F. carica* ethanolic fruit extract as an effective anticancer remedy.

Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this article.

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