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Evaluation of cytotoxic activity of Ficus carica latex extract on cancer cell line (Caco-2) and HDFn cells: MTT-based cytotoxicity and GC-MS characterization

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Abstract
The present study aimed to investigate the therapeutic effects of fig latex extract on Caco-2 and HdFn cancer cell lines. The toxicity of fig latex was determined using the MTT assay against Caco-2. The active chemical compounds in the latex were also identified using gas chromatography-mass spectrometry. According to the data, fig latex showed selective activity in inhibiting the growth of Caco-2 cells compared to HdFn cells, with an IC50 value of 92.03 µg/mL for Caco-2 cells and 122.5 µg/mL for HdFn cells. Fig latex-treated Caco-2 cells also showed a significantly higher inhibitory effect compared to HdFn cells. Thus, our results suggest that fig latex may be a promising treatment for colon cancer in humans.

Keywords: Ficus carica latex, Caco-2 cells, HdFn cells, MTT assay, GC-MS analysis

Introduction

Cancer is a huge global health concern that poses a serious threat to human life. More than half of all cancer fatalities are caused by the six most common cancer types: lung, colorectal, liver, stomach, female breast, and oral squamous cell carcinoma (OSCC). An unfavorable prognosis results from the increased likelihood of cancer spread and recurrence. Early prevention and focused therapy to lower mortality depend on a better understanding of the molecular process behind the growth of malignant cancer*(* Liu *et al*.,2025) [9]. Immune cell and cancer metabolism is a key factor in cancer development, spread, and metastasis. Cancer cells often utilize metabolic reprogramming to meet their energy and biosynthetic needs to maintain their rapid growth and proliferation.*(* Jiang *et al*.,2025) [6]. One of the most significant illnesses in the modern world, cancer claimed almost ten million lives in 2020. One of the most prevalent forms of cancer is colorectal cancer (CRC). In 2020, CRC came in third place with around 1.93 million new cases and 916 000 fatalities. (Yeniocak *et al*.,2025) [13]. There are many factors that may lead to the development of colorectal cancer, including advanced age, diet, family history, smoking, and alcohol consumption. Mutations, particularly chromosomal instability, are a major risk factor for this type of cancer*(* Khajeh *et al*.,2024) [8]. Conventional treatments, including radiation, chemotherapy, and surgery, are often limited in effectiveness and associated with side effects and drug resistance) Liu *et al*.,2025) [9]. The use of natural chemicals as anticancer medicines is a recognized treatment strategy because of their minimal cytotoxicity and accessible availability. There are a lot of anticancer drugs made from natural substances in the pharmaceutical sector nowadays. (Yeniocak *et al*.,2025) [13]. In the current age of drug development, natural products have offered an unmatched source of anticancer medications. Natural products, together with their derivatives and analogues, are essential in the treatment of cancer because they alter several signaling pathways and the cancer microenvironment. (Hashem *et al*.,2022) [4]. In the Moraceae family, (Ficus carica L.) are members of the genus Fig. Bioactive substances found in fig latex include terpenoids, phytosterols, fatty acids, organic acids, amino acids, and proteases. Every one of the bioactive substances found in fig latex has antibacterial, antioxidant, antidiabetic, and anticancer qualities. *(*Zhang *et al*.,2024) [14]. Ficus carica has a lot of polyphenols. This fruit's primary phenolic components include quercetin and quercetin-related compounds, which are known to have anticancer effect against a variety of cancer types. (Dekdouk *et al*.,2024) [3].

Material and Methods

- **Plant Collection**

The fig was gathered in July and August of 2024 from *Ficus carica* from Kirkuk, Iraq. Before being utilized, 100 milliliters of fig latex were removed from the plant and kept at 4C in a sterile glass bottle.

- **Equipment and Apparatus**

Throughout the study, the following tools were used: Inverted Microscope Olympus (Japan), CO2 incubator Gallenkamp (England), Micro-titer Plate Reader (ELISA Reader) Bio-Rad (Germany), Haemocytometer Sigma (USA), Autoclave Bio lab (Korea), Microtiter Plate (Tissue Culture) Hi Media (Hindi), Tissue Culture Flasks Hi Media (Hindi), Gas chromatography spectroscopy (Agilent-USA).

- **Cell Preparation**

The growth media was drained and the cell plate cleaned twice with sodium phosphate buffer once the cultivated cell lines had established a confluent monolayer in the flask. Following trypsinization, cells were collected by adding two to three milliliters of trypsin/EDTA solution, and they were then incubated for one to two minutes at 37°C until they separated. The cells were diluted in RPMI-1640 complete media to 7.5 x 104 cells mL-1. Each well of a 96-well plate received 100 µl aliquots of the cell suspension. The cells were kept in 5% CO2 at 37°C for the whole night.

- **Chemical and Biological materials used in this study**

EDTA, Dimethyl sulfoxide (DMSO), Trypsin, Fetal bovine Serum, RPMI-1640 Media, Phosphate Buffer Saline, Ascorbic Acid, Sodium bicarbonate (Sigma (USA), NaH2PO4, Na2HPO4, Crystal Violet Analar (England), Benzyl Penicillin, Streptomycin, Amphotericin Ajenta Pharm (India).

- **Cell Lines**

Two cell lines were utilized in this study

1. **Caco-2 cells:** human colon cancer cell line as an intestinal epithelial model.
2. **HdFn cells:** Neonatal foreskin was utilized to extract human dermal fibroblasts, which were then employed as a standard control cell line.

- **Culture Media and Solutions**

PBS buffer was prepared in accordance with standard procedure and autoclaved; trypsin-EDTA solution was used for cell detachment, and trypan blue staining was used to evaluate cell viability. RPMI-1640 was supplemented with 10% fetal bovine serum, 1% penicillin-streptomycin (10^6 IU/mL), and 1% sodium bicarbonate (4.4 g/100 mL).

Data analysis

A one-way analysis of variance ANOVA (Tukey Test) was performed to test whether group variance was significant or not, statistical significance was defined as * $p \leq 0.05$ and ** $p \leq 0.01$. All experiments were performed in triplicates and data were expressed as mean± standard deviation and statistical significances were carried out using Graph Pad Prism version 8 (Graph Pad Software Inc., La Jolla, Co).

Gas Chromatography-Mass Spectrometry (GC-MS)

According to GC mass analysis at retention time between 0-50 minutes, the results of the obtained compounds, which were determined by the National Institute of Standards and Technology (NIST), show the main chemical compounds in the latex of the Ficus carica plant, as shown in Table (1) and Figure (1).

Table 1: Phytochemical Compoundes of *Ficus Carica* Plant.

No.	RT (min)	Area	Name	Quality	CAS Number
1	14.386	21.63	Dodecamethylcyclohexasiloxane	86	000540-97-6
2	18.023	16.22	Pentasiloxane, dodecamethyl-	42	071579-69-6
3	21.303	5.11	Hexasiloxane, tetradecamethyl-	37	000000-00-0
4	24.151	1.38	Cyclononasiloxane, octadecamethyl-	83	109007-87-6
5	46.608	8.27	Cyclocasiloxyane, eicosamethyl-	49	019095-24-0
6	47.531	33.76	Stannane, tetrapropyl-	56	002176-98-9
7	49.534	13.64	Cyclodecasiloxane, eicosamethyl-	41	019095-24-0

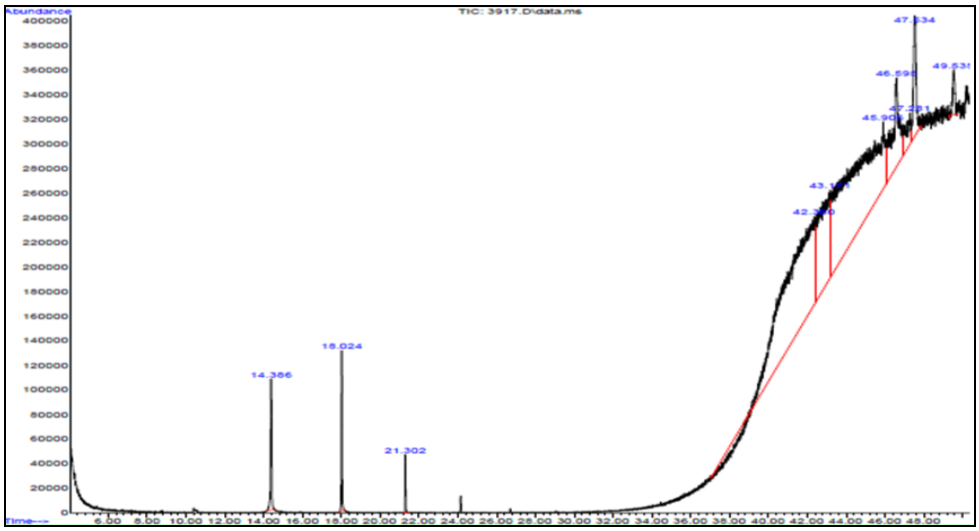

Fig 1: GC _MS chromatogram of Fig Latex extract showing the retention times of major bioactive compoundes.

The cytotoxic effect of Ficus carica plant latex on HdFn and CaCo-2 cell line

The findings showed that the anticancer activity of ficus carica latex against CaCo-2 cells was concentration-dependent, with cell viability sharply declining as the concentration rose. At 25, 50, 100, 200, and 400 µg/mL, the viability percentages were 94.94±2.34%, 84.02±0.83%, 61.84±4.38%, 55.56±2.03%, and 41.28±2.58%, respectively. Ficus carica latex, on the other hand, had a comparatively better cell viability at all tested dosages, demonstrating a lesser cytotoxic effect on normal HdFn cells. At the same concentrations, the viability rates for HdFn cells were 96.18±0.23%, 94.29±2.97%, 85.64±3.32%, 80.21±3.11%, and 72.65±1.95%. as shown in table (1-2). According to these findings, *Ficus carica* latex selectively kills malignant CaCo-2 cells while causing little harm to healthy fibroblast cells (HdFn). This is a good property for possible anticancer drugs. This pattern is further supported by the computed IC_{50} values, which, as shown in Figure (1-2), reveal an IC_{50} of 92.03 µg/mL for CaCo-2 cells and 122.5 µg/mL for HdFn cells. This suggests that a lower quantity of Ficus carica latex is needed to inhibit the proliferation of CaCo-2 cells relative to the HdFn cells.

Table 2: The cytotoxic effect of Ficus carica on HdFn and CaCo-2 cell line

Concentration µg mL^{-1}	Mean viability (%) ± SD	
	HdFn	CaCo-2
400	72.65±1.95	41.28±2.58
200	80.21±3.11	55.56±2.03
100	85.64±3.32	61.84±4.38
50	94.29±2.97	84.02±0.83
25	96.18±0.23	94.94±2.34

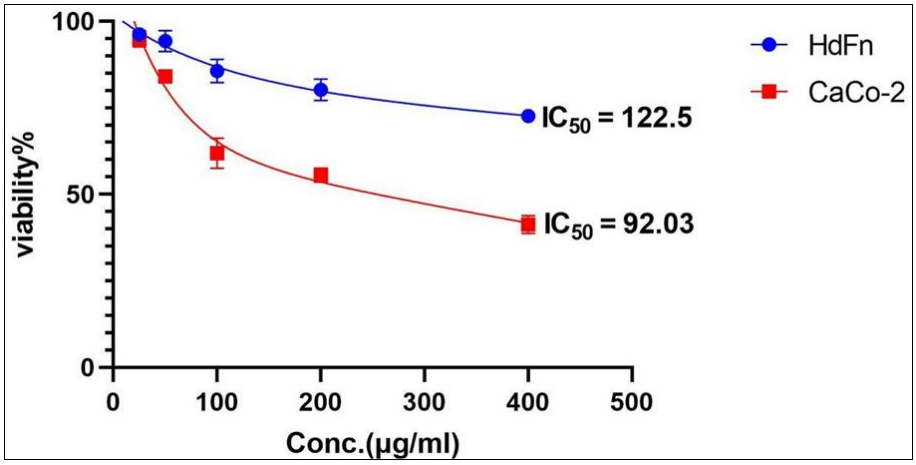

Fig 2: The cytotoxic effect of *Ficus carica* plant latex on HdFn and CaCo-2 cell line.

Disscusion

Medicinal plants are frequently used as a source for new medications that treat a range of illnesses. According to the findings of a study by (Abdel-Aty *et al.* ,2019) [1], fig latex extracts (FCE) include a range of phytochemicals that may have therapeutic use. It was discovered by gas chromatography-mass spectrometry (GC-MS) study that the majority of the phytochemicals present in fig latex had potential medicinal uses. Strong anti-tumor, anti-cancer, and anti-apoptotic therapeutic activities are exhibited by these specific compounds. (Hossain *et al*., 2021) [5] did a research to find out what the substance dodecamethylcyclohexasiloxane (D6) does. They found that it has an influence on the activity of several enzymes and genes that are linked to it. A research by (Mopuri *et al*., 2018)**,** used gas chromatography-mass spectrometry to look into *F. carica* fruits and found a lot of biologically active substances that help stop a lot of ailments. According to the study, these substances have antioxidant qualities that help shield cells from free radicals, which can lead to a number of reactions that eventually result in cancer. The majority of the volatile cyclic methyl siloxane compounds found in a research by (Rasyid, A., & Putra, M. Y. 2023) [11]. shown potent antibacterial and antioxidant action. These compounds included cyclohexasiloxane and dodecamethyl-cyclopentasiloxane. Our findings are in line with a research by (Kasim *et al*., 2022) [7]. that used GC_MS to examine plant chemicals and found that several of them, including octadecamethyl cyclonasiloxane and eicosamethyl cyclodecasiloxane cyclodexamethyl, exhibited antioxidant activity. According to a research by (Cabanlit *et al*.,2025), fig latex is important because of its biochemical makeup. Its antibacterial and antioxidant qualities are strengthened by its phenolic and flavonoid levels. Latex flavonoids also have cytotoxic and anti-inflammatory properties. By preventing cell cycle arrest and triggering apoptosis, some latex compounds also have anticancer action, which may have medicinal uses. Terpenes, which are abundant in F. carica plants, have been shown in several studies to have anti-inflammatory properties. Active chemicals were found in the leaves of F. carica, according to a recent study. Both an antioxidant pharmacological activity and a great anti-inflammatory impact were demonstrated by these active substances. By altering a number of dysregulated signaling cascades involved in angiogenesis, invasion, metastasis, autophagy, cell proliferation, and cell cycle regulation, F. carica has been shown to employ multitargeted pathways to prevent the onset and spread of cancer. Therapeutic foods have proven essential in the treatment of human diseases, particularly cancer. Because of its inexpensive cost, wide variety of pharmacological activities, long history of use, and accessibility, F. carica is a potential antineoplastic medication. F. carica affects several cancers, including breast cancer (Morovati *et al*., 2022) [10]**.** In a related study, (Soltana *et al*., 2019) [12], showed that fig latex extract was efficient in causing the HCT-116 colorectal cancer cell line to undergo apoptosis. The highest anti-proliferation action was shown by latex extracts. Additionally, it has been demonstrated that fig extracts are strong apoptosis inducers, indicating that they may have therapeutic use.

Conclusion

Gas chromatography-mass spectrometry (GC-MS) demonstrated that fig latex contains several bioactive phytochemicals that may have anticancer effects. According to the results, an MTT assay demonstrated selective cytotoxicity of fig latex against CaCo-2 colon cancer cells. The latex extract was more potent and effective against CaCo-2 colon cancer cells (IC_{50} = 92.03 µg/mL) than against normal HDFn fibroblast cells (IC_{50} = 122.5 µg/mL). The increased cytotoxic activity was associated with higher extract concentrations, suggesting that latex chemicals are responsible for this effect. These results demonstrate the potential of fig latex as a natural anticancer source. Further studies are needed to isolate and characterize the active components, evaluate their efficacy against cancer cells, study their mechanisms of action, and assess their therapeutic potential in cancer treatment.

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