RESEARCH ARTICLE

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Cytotoxic Activity of the Ethyl Acetate Extract of Iraqi Carica papaya Leaves in Breast and Lung Cancer Cell Lines

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Abstract

The aim of this study was to evaluate the cytotoxic effect of the ethyl acetate fraction of Iraqi Carica papaya (*C. papaya*) in breast and lung cancer cell lines, MCF-7 and A549, respectively. **Methods:** The ethyl acetate extract of Iraqi *C. papaya* leaves was prepared and tested for its phytochemical constitution. The 3-(4,5-dimethylthiazoline-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was performed in breast (MCF-7) and lung (A549) cells lines that were treated with different concentrations of ethyl acetate extract (3.125,6.25,12.5, 25, 50, and 100µg/ml). After 72 hrs of treatment, cell viability was evaluated. **Results:** The ethyl acetate extract of *C. papaya* showed considerable cytotoxic activity in the MCF-7 and A549 cell lines. The activity was dose-dependent; The half-maximum inhibitory concentration (IC_{50}) values were 22.74µg/ml and 8.674 µg/ml in the MCF-7 and A549 cell lines, respectively. **Conclusion :** The ethyl acetate fraction of Iraqi *C. papaya* leaves has potential anticancer activity in lung and breast cancer.

Keywords: Anticancer- A549- breast cancer- carica papaya- lung cancer- MCF-7.

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Introduction

Cancer is the leading cause of death worldwide and is accompanied by significant morbidity, putting enormous strain on society (Siegal, Miller, and Jemal, 2014). Cancer is the second biggest cause of mortality worldwide, accounting for approximately 1 in every 6 deaths, according to the World Health Organization (WHO) (Al-Hussaniy, 2022a). Each year, the WHO estimates that 14.1 million people are diagnosed with cancer and (10) 10 million people pass away from the disease (Mathur et al., 2020). Surgery, chemotherapy, and radiation therapy are the current standard treatments for treatment; however, they all have substantial side effects and a poor prognosis (Bhattacharya, 2022; Chen et al., 2020; Al-Hussaniy et al, 2022b).

Plants and phytochemicals have been extensively studied as potential anticancer agents because they are safe and non-toxic to a great extent and readily available. Currently, a handful of plant products are used for cancer treatment. However, many plant products have revealed very promising anticancer activities in vitro studies that need to be evaluated by in vivo and clinical studies(Greenwell and Rahman, 2015).

Carica papaya (*C. papaya*) L., also known as paw, belongs to the Caricaceae family. It is used as food and as a medicinal plant for the treatment of many diseases all over the world (Nariya and Jhala, 2017). Different parts of Carica papaya, such as leaf, bark, roots, latex, fruit, flower, and seed, have a wide range of reported therapeutic use in traditional medicine. *C. papaya* has been shown to have anthelmintic, antiprotozoan, antibacterial, antifungal, antiviral, anti-inflammatory, antihypertensive, hypoglycemic and hypolipidemic, wound healing, anticancer, free radical scavenging, antisickling, neuroprotective, diuretic, abortifacient, and antifertility properties (Nguyen et al., 2013).

The *C. papaya* leaves have been evaluated in 3 stages (green, yellow and brown) and found to be rich in pseudo carpaine, carpaine,dehydrocarpaine I and II, choline, carposide, vitamin C, vitamin E, vitamin B1, vitamin B2 and minerals such as calcium, magnesium, sodium, potassium, manganese, and iron. In traditional medicine, the leaves of *C. papaya* are used to cure a broad variety of illnesses, including malaria, dengue fever, and jaundice, and for their immunomodulatory and antiviral activity (Yogiraj et al., 2014b).

In Australia, people consume *C. papaya* leaf juice for their alleged anticancer activity (10 ,6). A patent by Morimoto and Dang has referred to several studies that pointed to increased survival rates of cancer patients who were consuming aqueous *C. papaya* leaf extract (Morimoto and Dang, 2008). Furthermore, Nguyen et al., (2016) have reported that the lyophilized extract of the juice of *C.*

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papaya leaves has cytotoxic activity in the oral squamous cell carcinoma cell line (SCC25). At the same time, Pandeyet al., (2017) reported that the juice of lyophilized *C. papaya* leaves has selective anti-proliferative activity on the prostate cancer cell line (PCa).

This study aimed to investigate the cytotoxic activity of the ethyl acetate fraction of Iraqi *C. papaya* leaves in breast cancer(MCF-7) and lung cancer(A549) cell lines.

Materials and Methods

Collection of Plant Material

C. papaya L. (Fresh leaves) were collected from a local botanical garden in Baghdad in June 2021. A specialist scientist from the College of Science, University of Baghdad, Baghdad, Iraq, has performed the botanical identification of the leaves.

Sample Preparation

The leaves were cleaned, shade dried at room temperature, and then ground to a fine powder. One hundred and twenty grams of powdered *C. papaya* leaves were weighed in a flask, then defatted with hexane for 72 h to remove chlorophyll and waxy material, which was left to dry at room temperature; then packed in the thimble of the soxhlet apparatus and extracted with 3L of 85% ethanol for 20 h. After filtering, the solvent was removed using a low-pressure rotary evaporator and left to dry. The extract was then suspended in 600 ml of distilled water and then partitioned using the liquid-liquid technique using the separatory funnel using 600 ml of chloroform, ethyl acetate, and n-butanol. This process was repeated three times. The ethyl acetate fraction evaporated to dryness, and the product was weighed and assigned for further analysis.

Preliminary Phytochemical Examination

The preliminary phytochemical investigation was carried out using general and specific tests to detect or confirm the presence of alkaloids, phenolic acid, flavonoids, tannins, saponin, glycosides, terpenoids, coumarins, and sterols (Morsy, 2014, Ibrahim et al., 2019).

Alkali Detection

To 2 ml of ethyl acetate fraction in a test tube, we added 2-3 drops of Dragendoff's reagent. An orange-red precipitate with turbidity indicated the presence of alkaloids.

Phenolic Acid Detection

10 mg of ethyl acetate extract was dissolved in 10 ml of distilled water, and the resulting filtrate was combined with 3 ml of 5%ferric chloride solution ($FeCl_3$). A dark green or blue-black precipitate is formed in the presence of phenolic acid.

Flavonoid Detection

To 1ml of ethyl acetate fraction, 2ml of 2% sodium hydroxide (NaOH) solution, and a few drops of diluted hydrochloric acid (HCl), an intense yellow color, becomes colorless by the addition of the diluted acid in the presence of flavonoids.

Tannins Detection

25 mg of ethyl acetate extract was dissolved in 10 ml of distilled water and 3 drops of 1% $FeCl_3$ solution; a blue-green color indicates the presence of tannins.

Saponin detection

25 mg of ethyl acetate extract was dissolved in about 10 ml of distilled water, stirred for 15 seconds, and allowed to stand for 15; persistent foaming indicates the presence of saponins.

Cardiac glycoside detection

In 1 ml of ethyl acetate extract,1.5 ml of glacial acetic acid and 1 drop of 5% $FeCl_3$ were added. Then 1 ml of concentrated sulfuric acid (H_2SO_4)was added along the side of the test tube; A brown ring at the interface indicates the presence of deoxy sugar.

Terpenoids Detection

To 5 ml of ethyl acetate extract, 2 ml of chloroform was added, followed by the careful addition of 3 ml of concentrated H2SO4. Reddish brown color indicates the presence of terpenoids

Coumarins detection

A spot of ethyl acetate was placed on a silica gel chromatography plate, left to dry under UV light observation, and then sprayed with 1% potassium hydroxide (KOH) reagent. A blue-green fluorescence indicates the presence of coumarins.

Steroids Detection

0.5g of ethyl acetate extract was treated with 10 ml of chloroform, then 10 drops of acetic anhydride and 2 drops of concentrated H_2SO_4 were added. The green solution indicates the presence of a steroidal nucleus.

Chemicals and reagents

Trypsin/EDTA(Ethylenediaminetetraacetic acid), RPMI 1640 medium, and fetal bovine serum were purchased from Capricorn Scientific (Ebsdorfergrund, Germany). Dimethylsulfoxide (DMSO) was purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). 3-(4, 5-dimethylthiazol-2-yl)-2, 5 diphenyl tetrazolium bromide (MTT) was purchased from Bioworld Technology (Dublin, OH, USA). Dulbecco's modified Eagle's medium (DMEM), glutamine, fetal bovine serum (FBS), and sodium pyruvate were purchased from Elabscience Biotechnology(Houston, TX, USA). Ferric chloride($FeCl_3$), hydrochloric acid(HCl), sodium hydroxide(NaOH), potassium hydroxide (KOH), sulfuric acid (H_2SO_4), glacial acetic acid, chloroform, acetic anhydride, ethanol, and ethyl acetate were purchased from Alphachemika(Mumbai, Maharashtra, India).

Cell line culture

Cell lines were grown in Dulbecco's modified Eagle's medium (DMEM), supplemented with 2 mM/l glutamine purchased, 10% FBS, 1.0 mM sodium pyruvate,

penicillin, and streptomycin. Cells were subcultured after trypsinization with 0.25% in 0.5 mM EDTA and incubated at 37°C in a humidified atmosphere with 5.0% CO_2.

Cytotoxicity Assays

To evaluate the cytotoxicity of the ethyl acetate fraction of Iraqi *C. papaya* leaves, the MTT cell viability assay was performed. The MTT assay is generally performed to assess cell viability and calculate the half-maximal inhibitory concentration (IC_{50}) of agents with potential cytotoxic effects. MTT is a simple, fast, and cost-efficient in vitro assay that quantifies viable cells by their ability to reduce the yellow tetrazolium salt to purple formazan. (Tolosa, Donato and Gómez-Lechón, 2015, Al).

Cell lines were seeded at 1×10^4 cells/well, left to adhere overnight in a CO2 incubator, and achieved a confluent monolayer. Cells were then treated with different doses (3.15, 6.25, 12.5, 25, 50 and 100 µg/ml) of the ethyl acetate fraction of Iraqi *C. papaya* leaves and incubated for 72 h. After incubation, the medium was removed and 28 µl of 2 mg/ml solution of MTT was added to each well and incubated for 1.5 h at 37°C. MTT solution was then removed, and 130 µl DMSO was added to each well in order to dissolve the resultant purple colored formazan crystals and followed by 37°C incubation for 15 min with continuous shaking (Abdullah, Al-Shammari and Lateef, 2020).

Finally, the absorbance was measured by a microplate reader (SPL Life Sciences Co. Ltd., Korea)at 492 nm. The assay was performed in triplicate. The percentage of viable cells was determined by comparing the absorbance of cells treated with extracts with that of untreated control cells. The inhibition rate of cell growth (the percentage of cytotoxicity) was calculated using the following equations (Al-Shammari et al., 2019):

$$\text{Percent cell viabilty} = \frac{\text{Average absorbance of treated cells}}{\text{Average absorbance of untreated cells}} \times 100 \quad (1)$$

$$\text{Percent cell inhibition} = 100 - \text{Percent cell viability} \quad (2)$$

Statistical Analysis

GraphPad Prism 6 was used for the statistical analysis of the collected data. (Al-Taee and Al-Shammari, 2019) and were presented as the mean ± SD of triplicate measurements (Al-Ziaydi et al., 2020; Naji et, al., 2022). The one-way analysis of variance (ANOVA) test was used to compare the mean percentage of inhibition between different doses of treatment. Regression analysis was used to calculate the IC_{50} value from the percentage of inhibition. $P<0.05$ were considered significant.

Results

The preliminary qualitative phytochemical screening of the ethyl acetate fraction of the Iraqi *C. papaya* leaves showed the presence of phenolic acids, flavonoids, tannins, saponins, and glycosides. As shown in Table 1.

The percentage of cell inhibition induced by treating cell lines with different doses of the ethyl acetate extract of Iraqi *C. papaya* leaves in the MCF-7 and A549 cell lines were dose-dependent (Figure 1). The IC_{50} of the

Table 1. Phytochemical Screening of Iraqi *C. papaya* Leaves

Phytochemical	Result
Alkaloids	–
Phenolic acids	+
Flavonoids	+
Tannins	+
Saponins	+
Glycosides	+
Terpenoids	–
Coumarins	–
Steroids	–

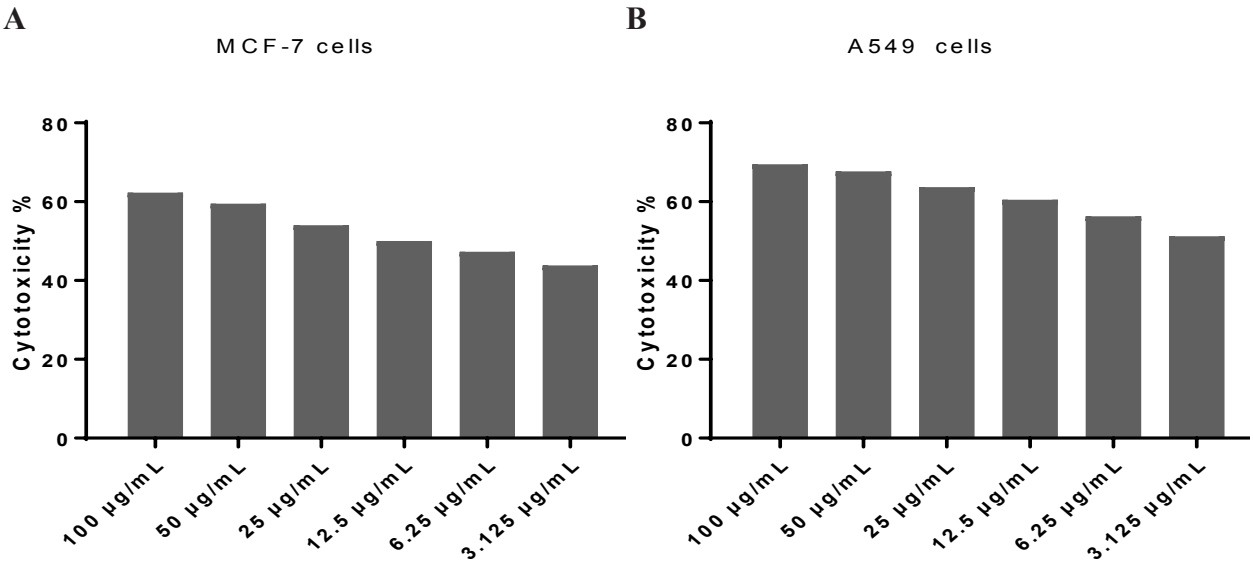

Figure 1. The Percentage of Cell Inhibition Per Different Doses of the Ethyl Acetate Extract of the Iraqi *C. papaya* leaves in A. MCF-7 cell line and the B. A549 cell line.

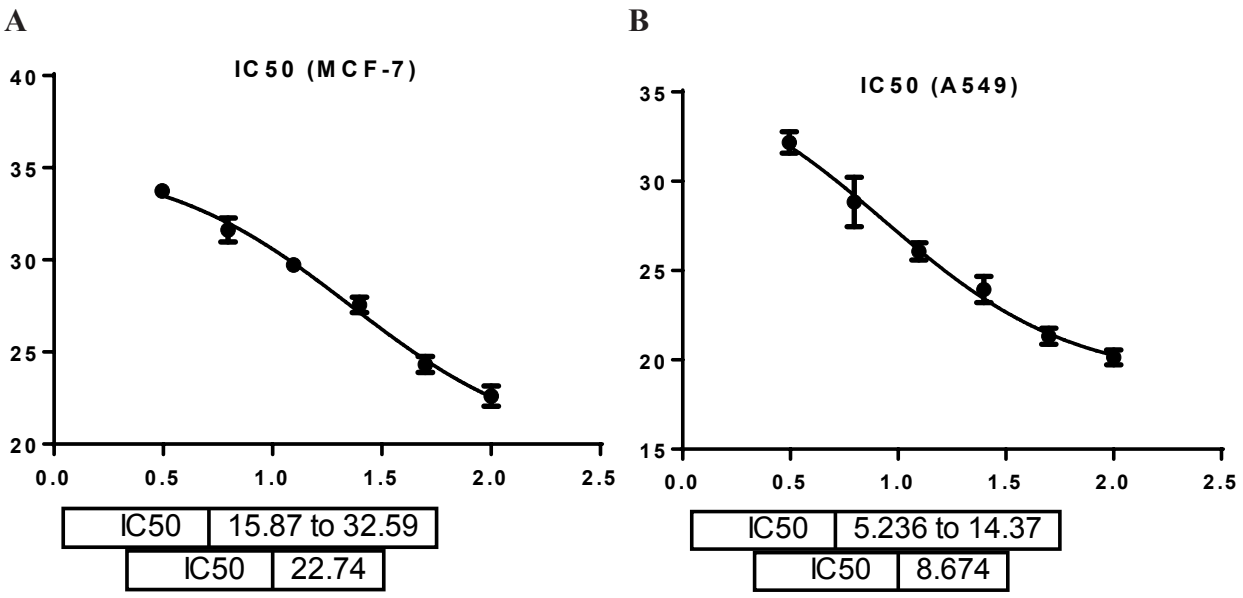

Figure 2. Reduction of Cell Viability Per Different Doses of the Ethyl Acetate Extract of the Iraqi *C. papaya* Leaves in A. MCF-7 cell line and the B. A549 cell line.

C. papaya leaves was 22.74 µg/ml in MCF-7; it was 8.674 µg/ml in A549 cells (Figure 2).

The cell viability of MCF-7 and A549 cell lines treated with a dose of the ethyl acetate extract of *C. papaya* leaves equal to the respective IC_{50}, compared to untreated cells, is shown in Figures 3 and 4, respectively.

Discussion

The chemical constituents of herbs responsible

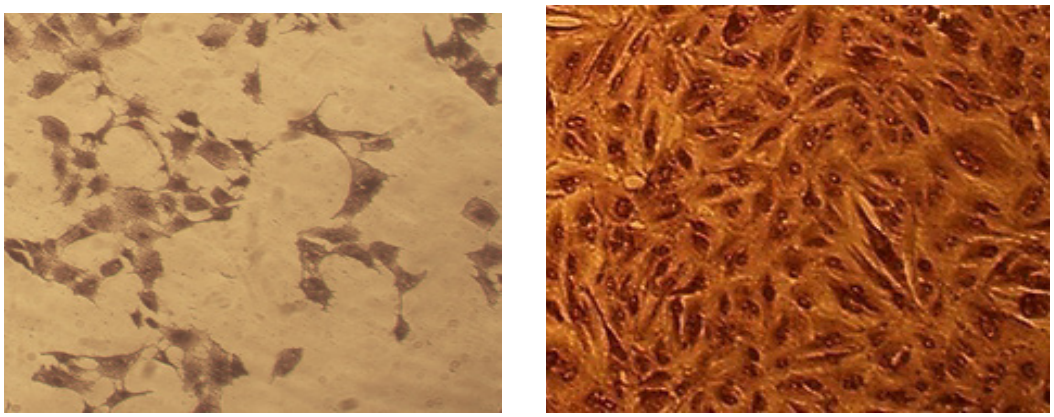

Figure 3. Viability of MCF-7 Cells Treated with IC_{50} of the Ethyl Acetate Fraction of the Iraqi *C. papaya* Leaves (left) and Untreated Cells (right).

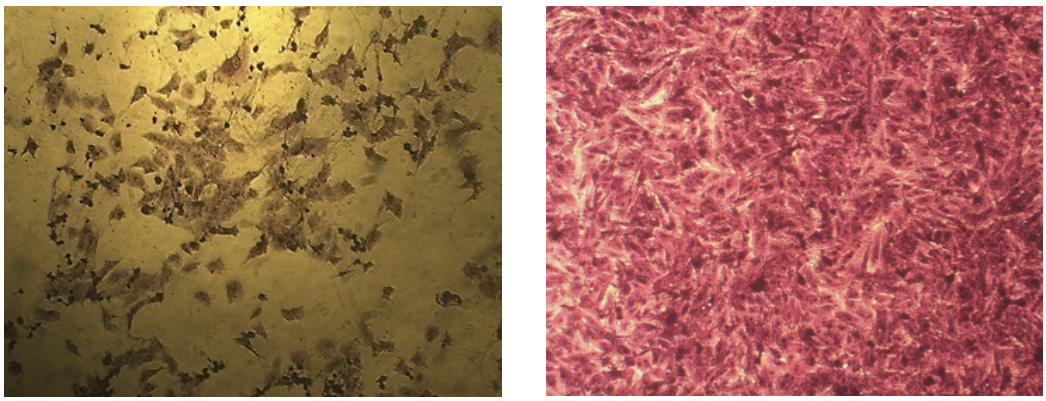

Figure 4. Viability of A549 Cells Treated with IC_{50} of the Ethyl Acetate Fraction of Iraqi *C. papaya* leaves (left) and untreated cells (right).

for their beneficial pharmacological and physiological activities in humans are the reason for the high medicinal value of herbs. Evidence from *C. papaya* investigations indicates that the entire plant contains many secondary metabolites.(Ayoola and Adeyeye, 2010).

Preliminary phytochemical screening in the present study showed that the ethyl acetate extract of Iraqi *C. papaya* leaves contains several phytochemicals: phenolic acid, flavonoids, saponins, tannins, and glycosides (Table 1). This occur following Wadekar et al., (2021).

Even though *C. papaya* leaves have been used as an anticancer agent; only a few studies have examined the effect of leaf extracts on cancer cells or investigated the mechanism of action in this regard (Nguyen et al., 2016). In the present study, the ethyl acetate extract of Iraqi *C. papaya* leaves showed a significant cytotoxic effect against two cancer cell lines, breast (MCF-7) and lung (A549) cells. However, the IC_{50} of *C. papaya* leaves extract was lower in A549 cells than in MCF-7 cells, indicating higher cytotoxic activity in lung cancer cells.

Astuti et al. have reported that aqueous *C. papaya* leaf extract induced apoptosis and inhibited the proliferation of human breast cancer cells (Astuti and Murdiati, 2017).

The reported cytotoxic effect of *C. papaya* can be attributed to the phytochemical component present in the extract. Flavonoids and phenolic acid have been shown to have potent free radical scavenging activity by modulating the activity of enzymes involved in reactive oxygen species scavenging, contributing to cell cycle arrest, inducing apoptosis and autophagy, inhibiting of angiogenesis and inhibiting of proliferation and metastasis of cancer cells (Roleira et al., 2015; Kopustinskiene et al., 2020; Abotaleb et al., 2018). Vuonget et al., (2015) used optimized extraction conditions to extract saponins and the total phenolic constituent from *C. papaya* leaves. They found the extract has significant antioxidant, free radical scavenging, iron-reducing capacity, and cytotoxic activity against two human pancreatic cell lines. Saponins and tannins have been reported to interfere with DNA replication and cancer cell proliferation; and have been used for the prevention and treatment of cancer (Elekofehinti et al., 2021; Yildirim and Kutlu, 2015; Li, Wang, and Liu, 2003). Finally, glycosides isolated from different medicinal plants have been reported to have numerous anticancer activities against several cancer cell lines (Khan et al., 2019; Menger et al., 2012; Ngozi et al., 2010).

In conclusion, the ethyl acetate extract of Iraqi *C. papaya* leaves reduced the viability of human breast and lung cancer cells. This effect may be attributed to the phytochemical constituents documented by the preliminary phytochemical screening. Further study is required to establish the specific constituent(s) responsible for the inhibition of the proliferation of cancer cells, as well as, the exact mechanism(s)in this regard.

Author Contribution Statement

Sura Basim, draft writing and making whole work; Ali A. Kasim, supervision, proofreading, data analysis.

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Ethical Approval

We was received an ethical approval from ethical committee collage of pharmacy university of Baghdad number (1238)2021.

Conflict of interest

None.

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