In Vitro Evaluation of The Anti-Proliferative Effect of Quercus Infectoria Crude Extracts on MCF-7 Cell Line

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ABSTRACT

Background: Breast cancer ranks as the most prevalent cancer affecting women globally, occurring both developed and developing nations. In 2018, Breast cancer came in second place among the most common cancers, while came in the fifth place among the most common causes of cancer death. Chemotherapy, the primary method in cancer treatment, utilizing specific medications to manage the spread of cancer cells. However, these drugs have been known to exhibit adverse effects on non-cancerous cells. Consequently, there is a pressing need to create new anticancer medications that selectively target cancer cells.

Aim: In this context, plant-derived natural products are anticipated to provide promising candidates for the specific anticancer medications. Therefore, this study aimed to evaluate the anti-proliferative effect of Gall Oak (*Quercus infectoria* Oliv.) as an alternative medicine on human breast MCF-7 cancer cell line.

Materials and Methods: Oak galls were extracted using three solvents (80% Ethanol, Ethyl Acetate and Petroleum Ether 40° - 60°), which differ in polarity, using SER 148 Solvent Extractor. Colorimetric detection was conducted to determine the phytochemical constituents of the extracts. The cytotoxic activity of the extracts using Crystal Violet stain on MCF-7 cell line was evaluated. Eight concentrations of each extract were prepared (0.78, 1.56, 3.125, 6.25, 12.5, 25, 50 and 100 µg/ml). With three replicates for each concentration, optical density (OD) of cell growth was read by a multi-detection plate reader at a transmitting wavelength of 492 nm.

Results and conclusion: The results of the study indicate that the best inhibitory activity of the oak galls petroleum ether extract on MCF-7 cell line was found at 25 µg/ml concentration after 48 hours of treatment. This could potentially be attributed to the existence of many phenolic compounds especially quercetin, a flavonoid group antioxidant that has strong anticancer properties.

Keywords: *Quercus infectoria*, plant extract, phytochemical analysis, breast cancer, MCF-7, cell line, antiproliferative, cytotoxicity.

التأثير المضاد للتكاثر للمستخلصات الخام Quercus infecttoria

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الخلاصة

الخلفية: يحتل سرطان الثدي المرتبة الأولى كأثر أنواع السرطان شيوعا لدى النساء عالميا، حيث يحدث في الدول المتقدمة والنامية على حد سواء. في عام ٢٠١٨، جاء سرطان الثدي في المرتبة الثانية بين أكثر أنواع السرطان شيوعا، بينما جاء في المرتبة الخامسة بين أكثر الأسباب شيوعا لوفاة السرطان. العلاج الكيميائي، الأسلوب الرئيسي في علاج السرطان يستخدم أدوية معينة للتحكم في إنتشار خلايا السرطان. ومع ذلك، كان من المعروف أن هذه الأدوية تظهر تأثيرات سلبية على الخلايا غير السرطانية. ونتيجة لذلك، هناك حاجة ملحة لإيجاد أدوية جديدة مضادة للسرطان تستهدف إنتقائيا خلايا السرطان.

الهدف: في هذا السياق، يتوقع ان توفر المنتجات الطبيعية المستمدة من النباتات مرشحات واعدة للأدوية المضادة للسرطان الانتقائية. لذا، كان هدف هذه الدراسة تقييم التاثير المضاد للتكاثر لعفص البلوط (*Quercus infectoria* Oliv.) كدواء بديل على خط خلايا سرطان الثدي البشري MCF-7.

المواد وطرق العمل: تم إستخلاص عفص البلوط بإستخدام ثلاثة مذيبات (٨٠% إيثانول، أسيتات الأثيل والإيثر البترولي ٤٠° - ٦٠°)، ذات قطبية مختلفة، بإستخدام جهاز (Solvent Extractor SER 148). تم إجراء إختبار الكشوفات الكيميائية لتحديد المكونات النباتية الكيميائية للمستخلصات. تم تقييم النشاط السام للمستخلصات على خط خلايا MCF-7 بإستخدام صبغة البنفسجي البلورية. تم تحضير ثماني تراكيز من كل مستخلص (٠.٧٨ و١.٥٦ و٣.١٢٥ و٦.٢٥ و١٢.٥ و٢٥ و٥٠ و١٠٠ ميكروغرام/مل). مع ثلاث مكررات لكل تركيز، تم قراءة الكثافة البصرية (OD) لنمو الخلية بواسطة جهاز Multi-detection plate reader عند الطول الموجي ٤٩٢ نانومتر.

النتائج والإستنتاج: تشير نتائج الدراسة إلى أن أفضل نشاط مثبط لمستخلص الإيثر البترولي لعفص البلوط على خط خلايا MCF-7 تم العثور عليه في تركيز ٢٥ ميكروغرام/مل بعد ٤٨ ساعة من المعالجة. يمكن أن يعزى ذلك إلى وجود العديد من المركبات الفينولية، وخاصة الكيرسيتين، وهو مضاد أكسدة من مجموعة الفلافونويدات وله خصائص قوية مضادة للسرطان.

الكلمات المفتاحية : Quercus infectoria، MCF-7، السمية الخلوية.

INTRODUCTION

Epithelial cells found in the glands' terminal lobules are usually where breast cancer starts. Breast cancer comes in two primary varieties: non-invasive and invasive (common) [1-3]. Breast tumors account for about 25% of all cancer occurrences and are the most common kind among women. They are also the second most commonly diagnosed malignancy globally. In most countries, it is the most prevalent cancer to be diagnosed, and it is the second leading cause of death for women, behind lung cancer [4-6].

During 2020 it has been noticed that about 685,000 deaths worldwide from breast cancer, while an estimated 2.26 million new cases of the disease were reported. With between 14-42% of all cancers identified in Arab females, breast tumors are thought to be the most common malignancy in the Arab world,

including Iraq [4,7-9]. As part of the ongoing advancements in the treatment of breast cancer, alternative medicines such nanotechnology, viral therapy, and the use of plant extracts as anti-cancer agents are being investigated [9]. The plant gall oak, botanically known as *Quercus infectoria* belong to Fagaceae Family, grows as a shrub or small tree in Greece, Asia Minor, Syria and Iran [10]. The galls arise on young branches of this tree as a result of the attack by the gall Oak wasps, Andricus spp. which is the most spread insects on Oak trees in north of Iraq [11]. It has been used since ancient time to treat the inflammatory disease. Traditionally, it is used for treatment of toothache and gingivitis, skin disorders, anti-bleeding, haemorrhoids, breast and vaginal firming creams and to treat prolapse of rectum [12-14]. Furthermore, pharmacological tests have demonstrated that the galls of Q. infectoria possess antioxidant property [15,16], tyrosinase inhibitory activity [17], astringent [18], antidiabetic [19], antitremorine [20], analgesic activity [21], antiviral, antifungal [22], antibacterial [23], anti-Candida [24], anti-dental pathogens [25], anti-leishmanial activity [26], chemo-preventive effect [27] and enhancement of bone formation [28]. Gallic acid, syringic acid, ellagic acid, β-sitosterol, amentoflavone, hexamethyl ether, isocryptomerin, methyl betulate, methyl olenate, and hexagalloyl glucose are among the many components of Q. infectoria galls [29]. The purpose of this study is to assess how crude extracts from oak (Quercus infectoria) galls affect the MCF-7 (human breast adenocarcinoma) cell line's ability to proliferate.

MATERIALS AND METHODS

Collection and Preparation of plants for extraction:

Oak galls were brought from Agriculture and Forestry College/University of Duhok. Plants classification was carried out at Agriculture and Forestry College/University of Mosul by Dr. Haees AL-Jowary. Oak galls were carefully cleaned with tap water and air-dried at shade at (25 °C) for 10 days. Before extraction, oak galls were grounded into powder via an electrical grinder and kept until use at 4 °C.

Oak galls extracts Preparation:

The extraction was preceded at the central laboratory of Agriculture and Forestry College/University of Mosul. Solvent Extractor SER 148 (VELP® Scientifica) was used, solvent/solid ratio of (10:1) where 10g of plant powder was extracted with 100 ml of the solvent [30]. Three solvents (80% Ethanol, Ethyl Acetate, and Petroleum Ether 40° - 60°), which differ in polarity, were used. The extracts were completely freeze-dried using lyophilizer (Lyovac GT2) (Labexchange®), After that, they were weighed, put into glass jars that were amber in color, and stored at 15°C until needed. Using the following formula [31], the extraction yield (%) was calculated from the weight of the plant powder and the extract:

$$\text{yield of Extraction } (\%) = \frac{\text{Extract weight } (g)}{\text{plant powder weight } (g)} 100\% \ldots\ldots (1)$$

Preparation of Stock Solution:

Two stock solutions were made for every extract. To create Stock (1) (0.05 g/ml), 0.05 g of extract was dissolved in 1 ml of solvent (phosphate buffer saline PBS was used to dissolve both ethanol and ethyl acetate extracts, and 10% dimethyl sulfoxide 10% DMSO was used to dissolve petroleum ether extract). Stock (1) was converted to Stock (2) (0.001 g/ml) using the Dilution Equation:

$$C_1V_1 = C_2V_2 \ldots\ldots (2)$$

where: V1 stands for starting volume, C1 for starting concentration (Stock (1)), C2 for final concentration (Stock (2)), and V2 for final volume.

Phytochemical Analysis:

The colorimetric detection of phytochemical constituents was carried out at the Ministry of Industry and Minerals / Iben Al-Betar Research Centre / Corporation of Research and Industrial Development / Iraq, using chemical methods as below:

- **Detection of Tannins:**

A favorable outcome is indicated by the production of a white precipitate when Lead Acetate Trihydrate (1%) was used ,as per reference [32].

- **Detection of Glycosides:**

A favorable outcome with Fehling's reagent is shown by the production of a red precipitate, as per reference [32].

- **Detection of Carbohydrates:**

A positive outcome using Molisch's reagent is shown by the creation of a blue ring, as per reference [33].

- **Detection of Resins:**

A favorable outcome is indicated by the production of a white precipitate when Lead Acetate Trihydrate (1%), as per reference [33].

- **Detection of Phenols:**

A positive outcome is indicated by the emergence of a blue or green tint when applying 1% ferric chloride, according to [33].

- **Detection of Alkaloids:**

Using Wagner's reagent, turbidity formation signifies a successful outcome, according to [33].

- **Detection of Coumarins:**

A test tube containing a tiny amount of extract was cooked in a boiling water bath for a few minutes, as per [34], and the filter paper was moistened with a diluted solution of sodium hydroxide (2N). The filter paper was then subjected to a UV light source. A favorable result is indicated by the development of a greenish-yellow glow.

- **Detection of Flavonoids:**

Using the alcoholic potassium hydroxide (5N) reagent, [34] states that the production of a yellow precipitate denotes a successful outcome.

- **Detection of Terpenes and Steroids:**

A little amount of chloroform was used to dissolve 1g of extract, and then an acetic anhydride drop and a strong sulfuric acid drop were added, per [35]. A positive response to terpenes is indicated by the formation of a brown color; a positive response to steroids is indicated, after some time, by the appearance of a dark blue color.

- **Detection of Proteins:**

A positive outcome when applying the Biuret reagent is indicated by the emergence of a violet color [35].

- **Detection of Saponins:**

Using aqueous Mercuric chloride (5%) reagent, [35] states that the production of a white precipitate denotes a successful outcome.

Culture of Cell Line

Maintaining of cells:

The Iraqi Center for Cancer and Medical Genetic Researches (ICCMGR)/Baghdad was the source of MCF-7 (Michigan Cancer Foundation-7). The present investigation employed passage 55. The cells were cultivated in Minimum Essential Medium (MEM, USBiological Life Sciences) supplemented with 10% Fetal Bovine Serum (FBS, Capricorn Scientific GmbH), 100 mg/L Penicillin-Streptomycin (Capricorn Scientific GmbH), and 2.5 µg/ml Amphotericin B (Capricorn Scientific GmbH). The cells were then incubated at 37 °C in an incubator with CO_2 atmosphere. Every two days, the medium was replaced, and the cells were subcultured when they reached 70–80% confluency. Trypsin-EDTA (Ethylene diamine tetra-acetic acid) (US Biological Life Sciences) was used to detach the cells in the flask for the subculture. Viable cells were then counted using an improved Naubauer counting chamber and Trypan blue stain; dead cells stain blue when viewed under a light microscope at magnifications of 10X and 40X. Next, we calculated the number of cells per unit volume (cells/ml) using the following formula.

$$\mathbf{C} = \mathbf{N} \times \mathbf{D} \times \mathbf{10^4} \ldots\ldots (3)$$

where N is the total number of viable cells enumerated, D is the dilution factor (D=10), and C is the number of viable cells per milliliter (cells/milliliter) [36].

Cytotoxicity assay:

This procedure was carried out in an aseptic environment in accordance with [36,37]. Eight concentrations (100, 50, 25, 12.5, 6.25, 3.125, 1.56, & 0.78 µg/ml) were made for each extract by employing the Dilution Equation (2) [38] to dissolve a given volume of stock (2) in serum-free MEM medium as a solvent. After trypsinizing a 70%–80% confluent monolayer culture of the cell line and resuspending it in the whole growth media, 200µl of the cells (1x104 cells/well) were planted in a 96-well microtitration plate with a flat bottom. Only medium has been inserted into the wells along the plate's edge to reduce the edge effect. To obtain a confluent monolayer, the plates were covered with parafilm and cultured for 24 hours at 37°C in a CO_2 incubator. The media was decanted after a 24-hour period, and the cells were subsequently treated with successive dilutions of each extract. For each of the three extracts' concentrations, three duplicates were employed. Following exposure to each extract for 24, 48, and 72 hours, the medium was withdrawn, and 100 µl of 10% crystal violet stain was applied to each well. The plate was then incubated for 20 minutes at 37°C, and then it was gently rinsed with tape water and allowed to dry. Lastly, a Multi-detection plate reader (BMG LABTECH) was used to measure the optical density (O.D.) of the living cells in each well at a transmission wavelength of 492 nm [36].

Statistical analysis:

The mean ± standard error (SE) was used to express the data. Gen Stat (version 12) was used to determine the statistical significance of the data using the Least Significant Difference (LSD) and Two-way ANOVA. P-values were deemed statistically significant at the level of $P < 0.05$.

RESULTS

Oak (Quercus infectoria) galls extraction:

Three types of solvents (80% Ethanol, Ethyl Acetate and Petroleum Ether) were used to obtain extracts from oak galls. Table (1) shows that QI (Quercus infectoria) Ethanol extract, brown crystals, weighed 2.3435 gram giving 23.453 % yield, while QI Ethyl Acetate extract, yellow powder, weighed 2.0716 gram giving 20.716 % yield, and finally, QI Petroleum Ether extract, olive green oily liquid, weighed 1.2984 gram giving 2.164 % yield. The weight of sample of both Ethanol and Ethyl Acetate extracts was 10 grams while it was increased to 60 grams for Petroleum Ether extract because of the low yield it gave when using 10 grams.

Table (1) characterization of Oak galls extracts

Solvent	Color	Texture	Weight of sample (g)	Weight of extract (g)	Yield (%)
80% Ethanol	Light Brown	Crystals	10 g	2.3435 g	23.453 %
Ethyl Acetate	Light Yellow	Powder	10 g	2.0716 g	20.716 %
Petroleum Ether	Deep Olive Green	Thick oily liquid	60 g	1.2984 g	2.164 %

Phytochemical analysis of Oak (Quercus infectoria) galls extracts:

Numerous chemical analyses were carried out to determine whether active ingredients were present in each extract and, conversely, how solvents affected the extracts (Table 2). The results showed that QI Ethanol extract contains carbohydrates, coumarins, flavonoids, glycosides, saponins and tannins. While it lacked alkaloids, phenols, proteins, resins, steroids and terpenes. As for QI Ethyl Acetate extract, it contained carbohydrates, coumarins, flavonoids, glycosides, phenols, saponins and tannins and lacked alkaloids, proteins, resins, steroids and terpenes. Finally, QI Petroleum Ether extract, it contained carbohydrates, coumarins, flavonoids, glycosides, saponins and tannins and lacked alkaloids, phenols, proteins, resins, steroids and terpenes.

Table (2) results of extracts' chemical identification

Test	Extracts		
	80% Ethanol	Ethyl Acetate	Petroleum Ether
Alkaloids Test	-	-	-
Carbohydrates Test	+	+	+
Coumarins Test	+	+	+
Flavonoids Test	+	+	+
Glycosides Test	+	+	-
Phenols Test	-	+	-
Proteins Test	-	-	-
Resins Test	-	-	-
Saponins Test	+	+	+
Steroids Test	-	-	-
Tannins Test	+	+	+
Terpenes Test	-	-	-

Cytotoxic effect of Quercus infectoria galls Ethanol crude extract on MCF-7 Cell Line In vitro after 24, 48 and 72 hrs. treatment:

After a 24-hour treatment, statistical analysis in this study revealed that, when compared to the control (PBS) (0.281±0.019), the MCF-7 cell line's proliferation increased considerably at all concentrations (0.78, 1.56, 3.125, 6.25, 12.5, 25, 50 and 100) (0.368±0.048, 0.377±0.009, 0.554±0.028, 0.554±0.069, 0.454±0.013, 0.421±0.032, 0.356±0.048, 0.372±0.023) respectively (figure 1) (Table 3). The concentrations of 6.25 µg/ml (0.219±0.061), 50 µg/ml (0.260±0.018), and 100 µg/ml (0.373±0.021) were found to significantly enhance the number of cells after a 48-hour therapy. In contrast to the control (PBS) (0.125±0.012), the other doses of 0.78, 1.56, 3.125, 12.5, and 25 µg/ml had no influence on the growth of the cell line (0.152±0.014, 0.143±0.005, 0.156±0.019, 0.159±0.009 and 0.170±0.009) respectively (figure 2) (Table 3). When treated with quantities of 25 µg/ml (0.188±0.025), 50 µg/ml (0.200±0.048), and 100 µg/ml (0.308±0.068) after 72 hours, the statistical analysis showed a clear increase in cells when compared with the control (PBS) (0.091±0.014) (figure 3) (Table 3).

Table 3: Average ± Standard Error for the impact of various concentrations of Quercus infectoria galls ethanol crude extract on MCF-7 cell line proliferation In vitro following treatment for 24, 48, and 72 hours: (O.D. observation).

Concentration	Exposure period		
	24 hours	48 hours	72 hours
	Mean± SE	Mean± SE	Mean± SE
Control	0.281±0.019	0.125±0.012	0.091±0.014
0.78 µg\ml	0.368±0.048	0.152±0.014	0.115±0.012
1.56 µg\ml	0.377±0.009	0.143±0.005	0.106±0.008
3.125 µg\ml	0.554±0.028	0.156±0.019	0.096±0.008
6.25 µg\ml	0.554±0.069	0.219±0.061	0.106±0.014
12.5 µg\ml	0.454±0.013	0.159±0.009	0.135±0.008
25 µg\ml	0.421±0.032	0.170±0.009	0.188±0.025
50 µg\ml	0.356±0.048	0.260±0.018	0.200±0.048
100 µg\ml	0.372±0.023	0.373±0.021	0.308±0.068
Effect	Conc.	Time	Conc. & time
L.S.D (0.05)	0.052	0.029	0.091

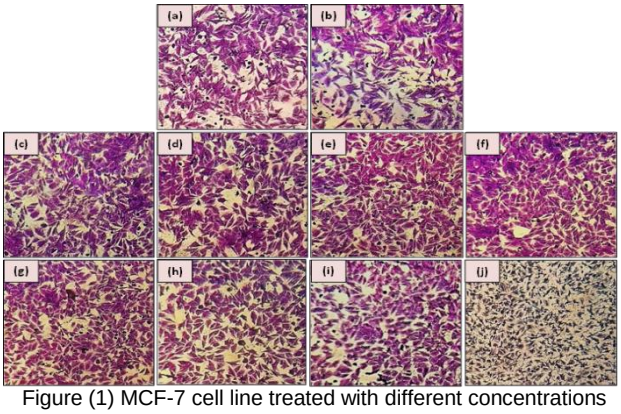

Figure (1) MCF-7 cell line treated with different concentrations of oak gall 80% Ethanol crude extract after 24 hrs. (40X) (a) first control (untreated cells) (b) second control (treated with 50% Ethanol) (c) 0.78 µg/ml (d) 1.56 µg/ml (e) 3.125 µg/ml (f) 6.25 µg/ml (g) 12.5 µg/ml (h) 25 µg/ml (i) 50 µg/ml (j) 100 µg/ml

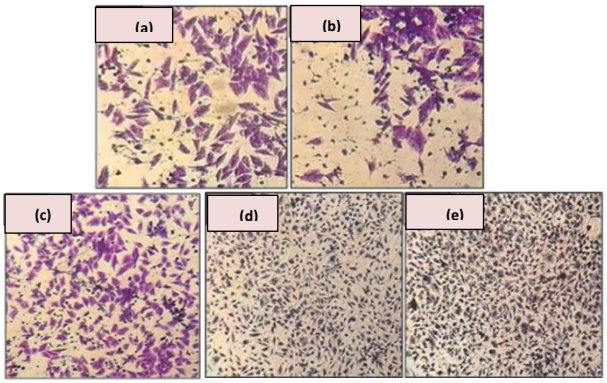

Figure (2) MCF-7 cell line treated with different concentrations of oak gall 80% Ethanol crude extract after 48 hrs. (40X) (a) first control (untreated cells) (b) second control (treated with 50% Ethanol) (c) 6.25 µg/ml (d) 50 µg/ml (e) 100 µg/ml

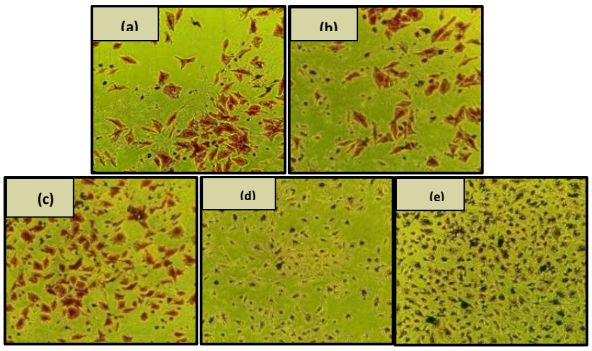

Figure (3) MCF-7 cell line treated with different concentrations of oak gall 80% Ethanol crude extract after 72 hrs. (40X) (a) first control (untreated cells) (b) second control (treated with 50% Ethanol) (c) 25 µg/ml (d) 50 µg/ml (e) 100 µg/ml

Cytotoxic effect of Quercus infectoria galls Ethyl Acetate crude extract on MCF-7 Cell Line In vitro after 24, 48 and 72 hrs. treatment:

When compared to the control (PBS) (0.241±0.029), the current results showed a clear increase in cells after 24 hours for all concentrations tested, ranging from 0.78 µg/ml to 50 µg/ml (0.398±0.027, 0.404±0.104, 0.380±0.045, 0.454±0.090, 0.410±0.078, 0.462±0.064, 0.328±0.059) respectively, with the exception of 100 µg/ml, which showed no impact (figure 4) (Table 4). Following a 48-hour period, the statistical examination of the data revealed a clear rise in cell proliferation for doses of 12.5 µg/ml (0.286±0.153), 50 µg/ml (0.223±0.012), and 100 µg/ml (0.314±0.011). In contrast, all other concentrations, (0.78, 1.56, 3.125, 6.25 and 25) (0.166±0.025, 0.169±0.046, 0.114±0.013, 0.129±0.008, 0.188±0.006) respectively, did not substantially affect the proliferation of cells when compared to the control (PBS) (0.132±0.009) (figure 5) (Table 4). Following a 72-hour treatment, the statistical analysis using the least significant differences (L.S.D.) of the results showed that, in comparison to the control (PBS) (0.091±0.014), cells proliferated significantly more when treated with concentrations of 25 µg/ml (0.189±0.021), 50 µg/ml (0.176±0.018), and 100 µg/ml (0.184±0.008); all other concentrations, namely 0.78, 1.56, 3.125, 6.25, and 12.5 µg/ml (0.094±0.012, 0.089±0.005, 0.101±0.012, 0.109±0.009 and 0.136±0.008) respectively, were ineffective (figure 6) (Table 4).

Table 4: Mean ± Standard Error for the Impact of Various Concentrations of Quercus infectoria galls Ethyl Acetate Crude Extract on MCF-7 Cell Proliferation In vitro following treatment for 24, 48, and 72 hours: (O.D. observation).

Concentration	Exposure period		
	24 hours	48 hours	72 hours
	Mean± SE	Mean± SE	Mean± SE
Control	0.241±0.029	0.132±0.009	0.091±0.014
0.78 µg\ml	0.398±0.027	0.166±0.025	0.094±0.012
1.56 µg\ml	0.404±0.104	0.169±0.046	0.089±0.005
3.125 µg\ml	0.380±0.045	0.114±0.013	0.101±0.012
6.25 µg\ml	0.454±0.090	0.129±0.008	0.109±0.009
12.5 µg\ml	0.410±0.078	0.286±0.153	0.136±0.008
25 µg\ml	0.462±0.064	0.188±0.006	0.189±0.021
50 µg\ml	0.328±0.059	0.223±0.012	0.176±0.018
100 µg\ml	0.259±0.022	0.314±0.011	0.184±0.008
Effect	**Conc.**	**Time**	**Conc. & time**
L.S.D (0.05)	**0.076**	**0.042**	**0.132**

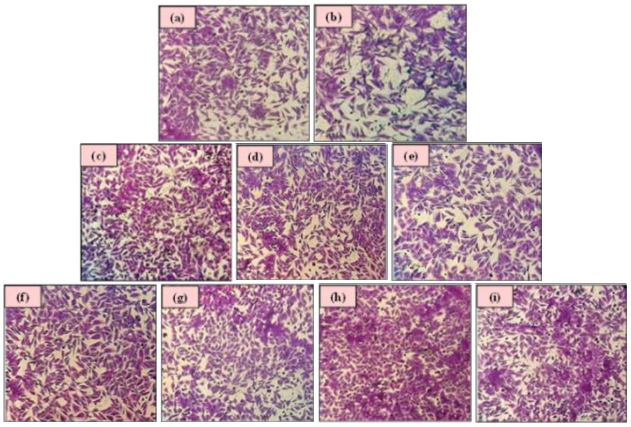

Figure (4) MCF-7 cell line treated with different concentrations of oak gall Ethyl Acetate crude extract after 24 hrs. (40X) (a) first control (untreated cells) (b) second control (treated with PBS) (c) 0.78 µg/ml (d) 1.56 µg/ml (e) 3.125 µg/ml (f) 6.25 µg/ml (g) 12.5 µg/ml (h) 25 µg/ml (i) 50 µg/ml

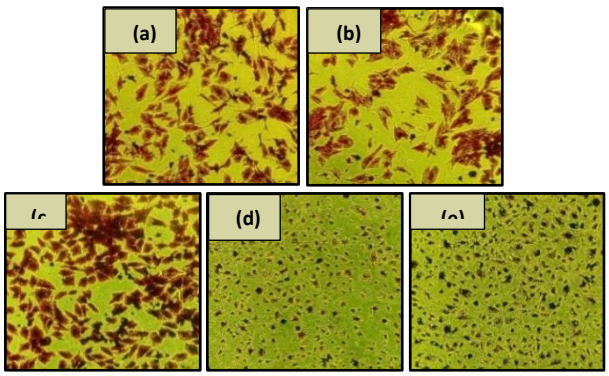

Figure (5) MCF-7 cell line treated with different concentrations of oak gall Ethyl Acetate crude extract after 48 hrs. (40X) (a) first control (untreated cells) (b) second control (treated with PBS) (c) 12.5 µg/ml (d) 50 µg/ml (e) 100 µg/ml

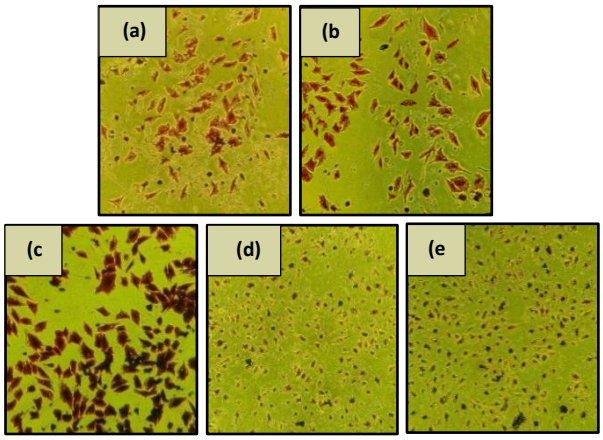

Figure (6) MCF-7 cell line treated with different concentrations of oak gall Ethyl Acetate crude extract after 72 hrs. (40X) (a) first control (untreated cells) (b) second control (treated with PBS) (c) 25 µg/ml (d) 50 µg/ml (e) 100 µg/ml

Cytotoxic effect of Quercus infectoria galls Petroleum Ether crude extract on MCF-7 Cell Line In vitro after 24, 48 and 72 hrs. treatment

Statistical analysis of the results, after 24-hour treatment revealed obvious increase in proliferation of tumor cells when treated with concentrations 3.125 µg/ml (0.363±0.015) and 6.25 µg/ml (0.329±0.063), while all the remaining concentrations (0.78, 1.56, 12.5, 25, 50 and 100) (0.265±0.026, 0.286±0.010, 0.294±0.013, 0.317±0.035, 0.307±0.023 and 0.302±0.045) were non-significant as compared with the control (10% DMSO) (0.284±0.066) (figure 7) (Table 5). Following a 48-hour period, the outcomes demonstrated that, in comparison to the control (10% DMSO) (0.229±0.009), quantities of 25 and 100 µg/ml of galls petroleum ether crude extract were most successful in suppressing the proliferation of the MCF-7 cell line (0.182±0.001 and 0.183±0.012) respectively, while the remaining concentrations (0.78, 1.56, 3.125, 6.25, 12.5 and 50) (0.202±0.017, 0.225±0.024, 0.224±0.031, 0.208±0.021, 0.252±0.022, 0.237±0.011) respectively were non-significant (Figure 8) (Table 5). When treated with doses ranging from 0.78 to 25 µg/ml (0.190±0.004, 0.170±0.006, 0.201±0.020, 0.205±0.012, 0.185±0.010 and 0.181±0.010) respectively, the current results showed a noticeable rise in cells after 72 hours, while the concentrations of 50 µg/ml (0.141±0.003) and 100 µg/ml (0.145±0.017) had no influence on the proliferation of MCF-7 tumor cells when compared to the control (figure 9) (Table 5).

Table (5) Mean ± SE for the effect of different conc. of *Quercus infectoria* galls Petroleum Ether crude extract on the proliferation of MCF-7 cell line *In vitro* after 24, 48 and 72 hrs. treatment: (observation of O.D).

Concentration	Exposure period		
	24 hours	48 hours	72 hours
	Mean± SE	Mean± SE	Mean± SE
Control	0.284±0.066	0.229±0.009	0.119±0.002
0.78 µg\ml	0.265±0.026	0.202±0.017	0.190±0.004
1.56 µg\ml	0.286±0.010	0.225±0.024	0.170±0.006
3.125 µg\ml	0.363±0.015	0.224±0.031	0.201±0.020
6.25 µg\ml	0.329±0.063	0.208±0.021	0.205±0.012
12.5 µg\ml	0.294±0.013	0.252±0.022	0.185±0.010
25 µg\ml	0.317±0.035	0.182±0.001	0.181±0.010
50 µg\ml	0.307±0.023	0.237±0.011	0.141±0.003
100 µg\ml	0.302±0.045	0.183±0.012	0.145±0.017
Effect	Conc.	Time	Conc. & time
L.S.D (0.05)	0.04	0.022	0.07

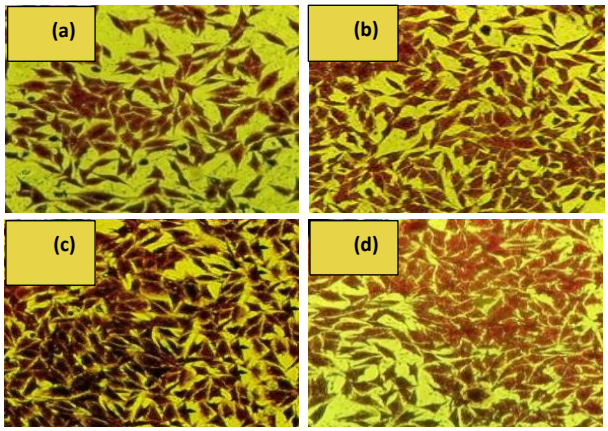

Figure (7) MCF-7 cell line treated with different concentrations of oak gall Petroleum Ether crude extract after 24 hrs. (40X) (a) first control (untreated cells) (b) second control (treated with PBS) (c) 3.125 µg/ml (d) 6.25 µg/ml

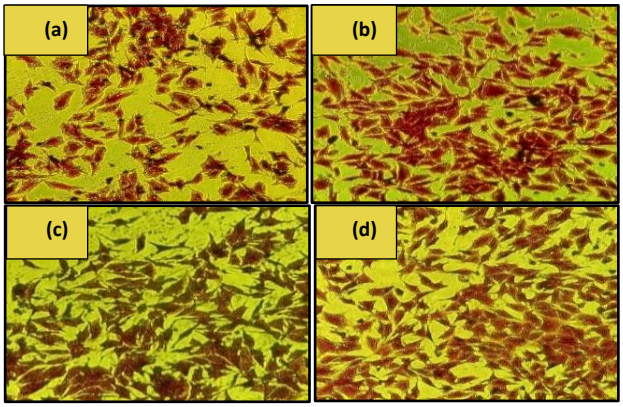

Figure (8) MCF-7 cell line treated with different concentrations of oak gall petroleum ether crude extract after 48 hrs. (40X) (a) first control (untreated cells) (b) control (treated with 10% DMSO) (c) 25 µg/ml (d) 100 µg/ml

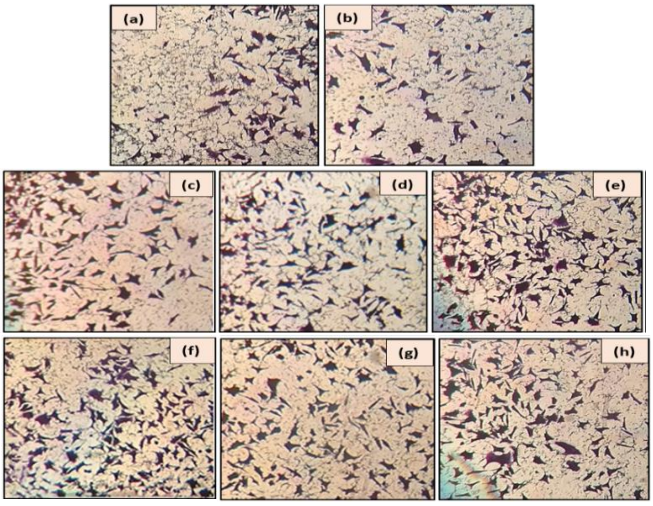

Figure (9) MCF-7 cell line treated with different concentrations of oak gall Petroleum Ether crude extract after 72 hrs. (40X) (a) first control (untreated cells) (b) second control (treated with PBS) (c) 0.78 µg/ml (d) 1.56 µg/ml (e) 3.125 µg/ml (f) 6.25 µg/ml (g) 12.5 µg/ml (h) 25 µg/ml

DISCUSSION

Oak (Quercus infectoria) galls extraction and phytochemical analysis:

The extraction results showed that the type of solvent has a clear effect on the color and texture of the extract, in addition to its effect on the yield. The color of the extract changed from brown when 80% Ethanol was used as a solvent to yellow when using ethyl acetate, then change again to olive green when using Petroleum Ether as a solvent. The texture varied from powder for 80% Ethanol and Ethyl Acetate extracts to thick oily liquid for Petroleum Ether extract. The extraction yield was clearly affected by the solvent polarity, where the yield directly proportional with solvent polarity [38,39].

The highest value (23.453 %) of the yield was for 80% Ethanol because it has the highest polarity among the solvents, then it was decreased to 20.716 % Ethyl Acetate. This is due to the fact that Ethyl Acetate has a lower polarity than 80% Ethanol. Finally, it was found that the yield decreased significantly when using the non-polar Petroleum Ether. The yield decreased to a very small values that are not sufficient to be used in the experiments. Therefore, it was compensated by increasing the weight of the plant sample and the volume of solvent (Petroleum Ether) used in the extraction process. The yield results of this study relatively agree with [40] as illustrated in table (1).

From the phytochemical analysis results (Table 2), it was observed that the Petroleum Ether extract did not contain any glycosides, nor did it contain any phenols in either the 80% Ethanol or Petroleum Ether extract. Additionally, all extracts lacked alkaloids, proteins, resins, steroids, and terpenes, but all of them contained carbohydrates, coumarins, flavonoids, saponins, and tannins. Solvents with different polarity can solubilize distinct phenolic compounds depending on their structural variations. As a result, the kind of solvent used and the extraction technique can significantly affect the amount of polyphenols that are recovered from plant materials [41,42].

Cytotoxic effect of Quercus infectoria galls Ethanol crude extract on MCF-7 Cell Line In vitro:

The results show an increase cell proliferation in all the concentrations after 24 hours treatment, in the concentrations of 6.25 µg/ml, 50 µg/ml, and 100 µg/ml after 48 hours treatment, and in the concentrations of 25 µg/ml, 50 µg/ml, and 100 µg/ml after 72 hours treatment. This could be as a result of the crude extract's numerous phytochemicals, which may promote the growth of cancer cells. The ethanol extract from oak galls was shown to have anticancer properties on mice mammary carcinoma cell line 2003 AMN3 cancer cell line [11], a finding that is in conflict with the current study results.

Cytotoxic effect of Quercus infectoria galls Ethyl Acetate crude extract on MCF-7 Cell Line In vitro:

The current results showed a clear increase in cells after 24 hours for all concentrations tested, ranging from 0.78 µg/ml to 50 µg/ml, in the concentrations of 12.5 µg/ml, 50 µg/ml, and 100 µg/ml after 48 hours treatment and in the concentrations of 25 µg/ml, 50 µg/ml, and 100 µg/ml after 72 hours treatment. This could be because the crude extract contains a variety of phytochemicals that promote the growth of cancer cells. These findings conflict with those of [43], who discovered that the ethyl acetate extract of oak galls exhibited cytotoxic action against the MCF-7 cell line.

Cytotoxic effect of Quercus infectoria galls Petroleum Ether crude extract on MCF-7 Cell Line In vitro:

Statistical analysis of the results, after 24 hours treatment revealed obvious increase in proliferation of tumor cells when treated with concentrations 3.125 µg/ml and 6.25 µg/ml, the same applies on the doses ranging from 0.78 to 25 µg/ml after 72 hours. While the concentrations of 25 and 100 µg/ml were the only doses and the most successful in suppressing the proliferation of the MCF-7 cell line after 48 hours treatment.

This could potentially be attributed to the existence of quercetin, a flavonoid group antioxidant that has strong anticancer properties [44]. On the other hand, Sangweni NF. (2021) [45] found that DMSO doses higher than 0.001% but not more than 0.5% stimulate MCF- 7 Cells proliferation activity.

CONCLUSIONS

It can be concluded from the results of the current study that the best inhibitory activity of the Oak Galls Petroleum Ether extract on MCF-7 cell line was found at 25 µg/ml concentration after 48 hours of treatment, while the effect of all other concentrations of the three extracts for 24, 48 and 72 hrs. exposure periods varied between inducing proliferation activity and being non-significantly effective on MCF-7 cell line.

Conflict of Interests

The authors did not declare any conflict of interest.

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