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Phytochemical Screening and Potential Cytotoxic Activity of Iraqi *Corchorus olitorius* Leaves Ethyl acetate Extract against The Breast and The Esophageal Cancer Cell Lines

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Abstract

The *Corchorus olitorius* leaves, a popular 'healthier' meal in Iraq, provided cytotoxic effects against different cell lines. The main purpose of the research work was to assess the phytochemical composition (polyphenolic compounds) and the cytotoxicity of the *Corchorus olitorius* leaf ethyl acetate extract. The Iraqi *Corchorus olitorius* leaves' ethyl acetate extract was made and fractionated using the hot continuous Soxhlet device technique. Following this, a number of general and specialized tests were used to qualitatively screen for the different polyphenolic phytochemicals found within the plant extract. Then the human Iraqi breast (AMJ13) and the esophagus (SKGT-4) cancer cell lines were subjected to various ethyl acetate extract concentrations (31.2, 62.5, 125, 250, 500, and 1000 µg/ml) in order to perform the MTT assay is also known as 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, and the viability of the cell was subsequently evaluated 72 hours after the treatment. *Corchorus olitorius* leaf ethyl acetate extract exhibited cytotoxic activity against the AMJ 13 and SKGT-4 cell lines. It produced a dose-dependent action. For the AMJ13 and SKGT-4 cell lines, the values of the inhibitory concentrations at half-maximum (IC_{50}) were 139.5 µg/ml and 100.1 µg/ml, respectively. In accordance with the results of the study, the conclusion is that the *Corchorus olitorius* leaves' ethyl acetate extract exhibited cytotoxic activity and decreased the viability of Iraqi human breast and esophageal cancer cell lines due to the presence of polyphenolic compounds. To identify the polyphenolic compounds exerting this cytotoxic activity and their modes of action, more study is required.

Keywords: Breast cancer, Esophageal cancer, Flavonoids, Iraqi *Corchorus olitorius*,Phenolic compounds.

Introduction

Corchorus olitorius L. (*C. olitorius*) is an edible annual plant, locally known as Meloukia, commonly called jute mallow [(1)]. Meloukia, a member of the *Tiliaceae* family, is easy to cook and may be used in many different recipes to help people benefit from its vitamins and minerals [(2)]. *Corchorus olitorius L.*, a native of tropical and subtropical climates, has held a unique place in Southeast Asia for millennia due to its therapeutic properties. Today, it is grown and consumed in many different places, including Iraq, where it is a popular leafy soup. The leaves of *C. olitorius* contain many beneficial chemicals, such as flavonoids, phenolic compounds (like quercetin and caffeoylquinic acid), alkaloids, saponins, tannins, terpenes, and proteins. These compounds contribute to the plant's antibacterial, antidiabetic, cardioprotective, hepatoprotective, neuroprotective, antiestrogenic, and wound-healing qualities [(3, 4)]. In terms of its anticancer properties, from previous studies, the extract from *C. olitorius* has demonstrated cytotoxic effects against various cell lines, which include cells from gastric cancer, pancreatic cancer, colon adenocarcinoma, and hepatocellular carcinoma by inducing apoptosis in cancer cells and inhibiting the proliferation of various cancer cell lines. [(5, 6)].

Globally, the incidence of different cancers is on the rise, and millions of individuals are suffering from esophageal and breast cancer, causing morbidity and death [(7)]. Additionally, compared to esophageal cancer, breast cancer is more common and widespread, and different pathways cause breast cancer. The mechanisms

underlying oxidative stress are the most significant. The term "oxidative stress" refers to an imbalance between the body's oxidation and anti-oxidation processes, which causes the generation and removal

of reactive oxygen species, which in turn stimulates cell carcinogenics [8, 9]. Rarely, the esophagus becomes the site of breast cancer metastases [10, 11]. Consequently, radiation, chemotherapy, and surgery-all of which have undesirable side effects and different cancer treatment approaches use them. Because conventional anti-cancer compounds cannot differentiate between normal and malignant cells, new and novel anti-cancer compounds derived from natural plant sources are needed to increase safety, decrease recurrent resistances, and lessen harmful effects [12-14]. Therefore, novel therapeutic approaches for anticancer medicines that are both preventive and curative, have high therapeutic efficacy, and have few side effects are needed for many forms of cancer. One important source of naturally occurring substances having cytotoxic qualities is the phytochemicals obtained from different plant extracts. Consequently, it is frequently challenging to discover new plant-based anticancer medications in different parts of the world. The uses and health advantages of Meloukia leaves have been widely researched. However, data regarding the biological as well as pharmacological characteristics of special chemical compounds (polyphenolic compounds) in the leaves are still limited. The research that has been carried out only covers the effect of crude leaf extract as a cytotoxic agent, but the effect of polyphenolic leaf extract remains incompletely investigated. Furthermore, in Iraq, no studies have examined the polyphenol leaf fraction on the human breast (AMJ13) and the esophagus (SKGT-4) cancer cell line. To close this gap, this paper outlines the framework for viewing the first report on extract utilizing a sequential extraction process, identifying polyphenols through chemical testing, and assessing its cytotoxic effect on breast cancer (AMJ13) and esophageal cancer (SKGT-4) cells.

Materials and Methods

Materials

The chemicals used during the research were purchased from the following supplier n-hexane (Thomas Baker, India) ethyl acetate (Schar Lab S.L, Spain) ,ferric chloride solution (FeCl3) (Fluka, Switzerland), hydrochloric acid (HCl) (GCC, UK) ,sodium hydroxide (NaOH) (Xilong Chemical Co., China), The AMJ13 and SKGT-4 cell lines (Cellosaurus, Switzerland), trypsin-EDTA (Capricorn, Germany), The Molecular targeted therapy (MTT) stain (Bio-World, USA) and dimethyl sulfoxide (DMSO) (Santacruz Biotechnology, USA).

The collection of leaves of Corchorus olitorius

In September 2023, the leaves of the *C. olitorius* plant, which is grown in Iraq, were collected from the medicinal plants garden at the College of Pharmacy, University of Baghdad, Department of Pharmacognosy and Medicinal Plants. The studied plant sample has been identified and verified by the Department of Biology, College of Science, University of Baghdad Herbarium, which is documented under BUH No. 10908. The plant was cleaned, allowed to air out in the shade for 7 days, and then powdered into a fine powder through an electrical grinder for extraction purposes [15].

Preparation of plant sample extract

For the sequential extraction technique, 100 g of powdered *C. olitorius* leaves were placed in a thimble of a Soxhlet apparatus. The first step of the extraction process involved defatting by using 1000 ml of n-hexane for six hours, followed by filtration and allowing the plant to dry before commencing the extraction process with ethyl acetate. The dried plant is placed in a thimble, and extraction is begun using 1000 ml of ethyl acetate for 12 hours, or until the extract's color turns pale. The ethyl acetate extract was filtered and evaporated to dryness under vacuum using a rotary evaporator; the residue of ethyl acetate extract was set aside for additional examination [16- 18].

Phytochemical analysis

A preliminary qualitative phytochemical screening of polyphenolic compounds in the ethyl acetate fraction of *C. olitorius* was performed utilizing both the general and the specific testing methods, in accordance with the standard tests that are detailed in Harborne [19] and Basim S et al [20].

1-The detection of phenolic compounds

After dissolving 2 mg of ethyl acetate extract in 5 ml of distilled water, 1 ml of 1% ferric chloride solution ($FeCl_3$) was added to the filtrate. The presence of phenolic compounds is indicated by a precipitate that is either dark green or blue-black in color.

2- The detection of Flavonoid

When only a few droplets of diluted hydrochloric acid (HCl) were added to a mixture containing 1 milliliter of the ethyl acetate extract and 2 milliliters of 2% alcoholic sodium hydroxide (NaOH), the color changed gradually from yellow to colorless, indicted in the presence of flavonoids.

The maintenance of cell cultures

The AMJ13 and SKGT-4 cell lines were kept in minimal essential medium (MEM) (Capricorn, Germany) with 10% fetal bovine, 100 units /mL penicillin, and 100 µg/mL streptomycin added as supplements. Trypsin-EDTA was employed to enable the cells to pass. Additionally, the cells underwent twice-weekly culture at 37 °C and 50% confluence (Cypress Diagnostics, Belgium) [21, 22].

Cytotoxicity Assays

The impact of cytotoxicity was assessed by the test of cell viability utilizing molecular targeted treatment (MTT stain) (Bio-World, USA) 96-well plates from Santa Cruz Biotechnology. The cell lines grew at a density of one x 104 cells in each well. The cells were exposed to the trial medication for 24 hours until a consolidated monolayer formed. The vitality of the cells was assessed after a 72-hour treatment [23]. Following the removal of the media, 28 µ L of a (2 mg/mL) MTT solution was added, and they were subsequently incubated at 37 ^{0}C approximately one and a half hours. Following the MTT solution's removal from the wells, the remainders of the crystals were dissolved through the addition of 130 µL of DMSO.

This was done at 37 °C while shaking the container for 15 minutes [24]. The absorbency was determined in triplicate utilizing a microplate reader (Gennex, USA) set to the test wavelength of (492) nm. The subsequent formula [25] was used to measure the percentage of the cytotoxicity, or the rate at which cell proliferation is suppressed.

The inhibition rate (IR) equation is A-B /A * 100, where (A) indicates the control optical density and (B) represents the sample optical density.

Statistical analysis

Utilizing the GraphPad Prism 6, a statistically analyzed unpaired t-test was performed on the data in this investigation [26]. The data were shown as the triplicate measurements' mean ± SD, p values < 0.05 are considered as significant [27].

Extraction and qualitative identification of polyphenolic compounds

The use of a hot Soxhlet extraction method is preferable, as indirect heat will facilitate the penetration of plant material by the solvent by breaking the plant tissue fibers [16]. Because plant extract contains diverse classes of chemical constituents with varying polarities, sequential extraction techniques are recommended for full phytochemical profile screening for a given plant. This allows the main classes (especially polyphenolic compounds) of plant constituents to be separated from each other based on differences in polarity and solubility [17]. In addition, in this study, ethyl acetate is used as a solvent for the extraction of phenolic compounds and flavonoids due to its moderate polarity and selectivity, with a yield of 2.43 grams and a percentage of 2.43%, filling the gap for better and more efficient extraction with less destruction of chemicals yielded. Different extraction solvents were utilized for the extraction and separation of polyphenolic compounds from *C. olitorius* L leaves, but for the first time, the sequential extraction technique and ethyl acetate solvent were applied for assessing cytotoxic activity against AMJ13 and SKGT-4 cancer cell lines. The Iraqi *C. olitorius* leaves' ethyl acetate extract included significant phytochemicals, mainly flavonoids and phenolic compounds, as determined by qualitative phytochemical analysis. These results are consistent with other research [28, 29]. And is displayed in Table 1.

Results and Discussions

Table 1 . Qualitative identification of polyphenolic compounds in the ethyl acetate fraction of *C. olitorius*

Phytochemical classes	Results
Phenolic compounds	+
Flavonoids	+

Cytotoxicity assay

The percentage of the cell inhibition by treating the AMJ13 and SKGT-4 cell lines with different concentrations of the *C.olitorius* leaves' ethyl acetate extract elevated in a manner that is dose-dependent, as demonstrated in Figure 1.

The IC_{50} of the Iraqi *C. olitorius* leaves was demonstrated in Figure 2 to be 100.1 µg/ml in SKGT-4 cells , however, was shown to be 139.5 µg/ml in AMJ13.

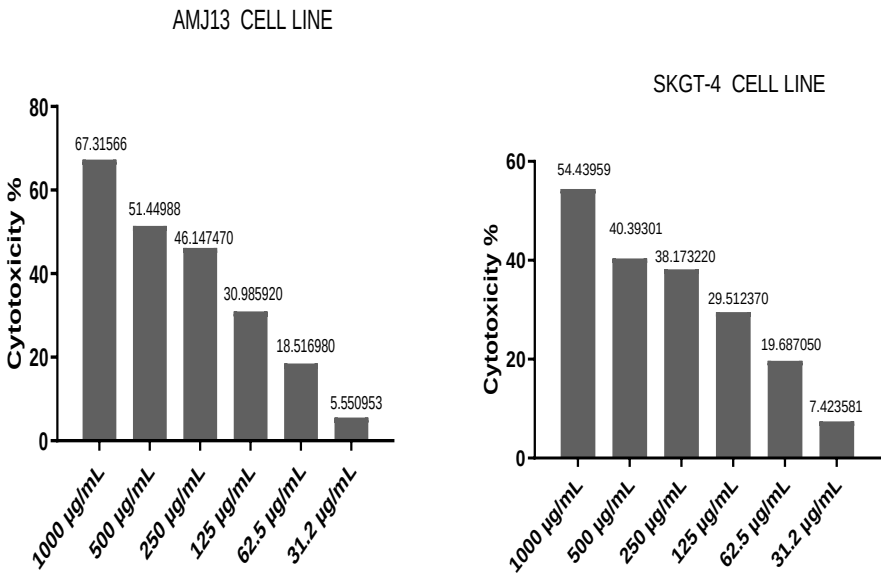


Figure 1. The percentage of cell inhibition in the A- AMJ-13 and B-SKGT-4 cell lines at different concentrations of the Iraqi *C. olitorius* ethyl acetate extract.

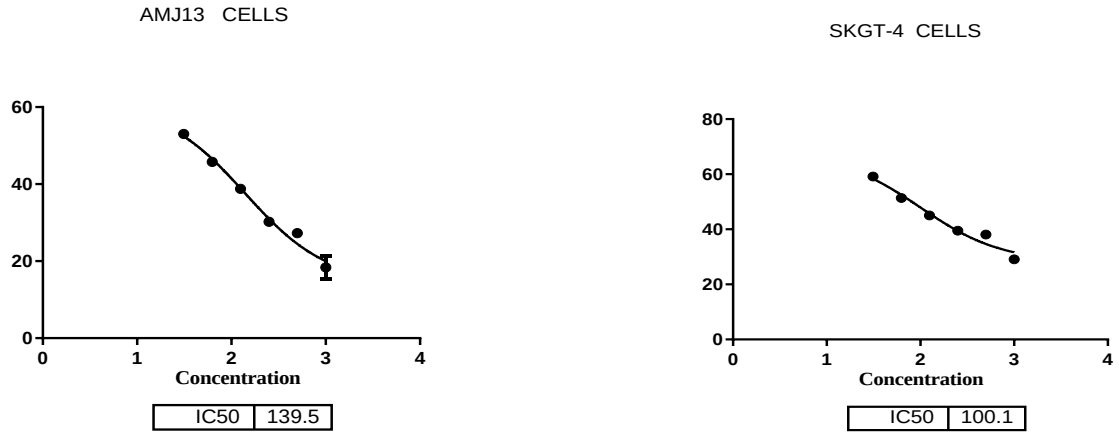


Figure 2. Iraqi *C. olitorius* leaves ethyl acetate extract lowers cell viability to various degrees, shown by the IC50 values for the A. AMJ13 and B. SKGT-4 cell lines.

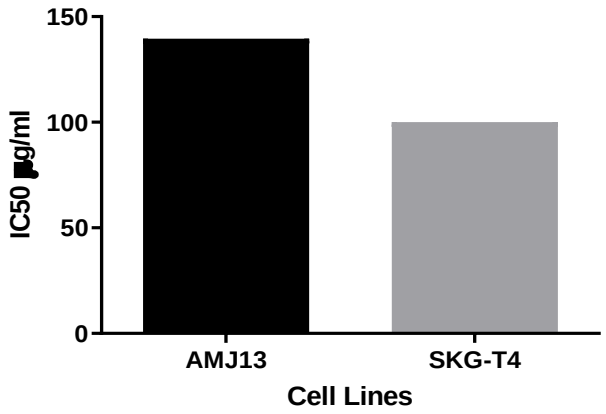
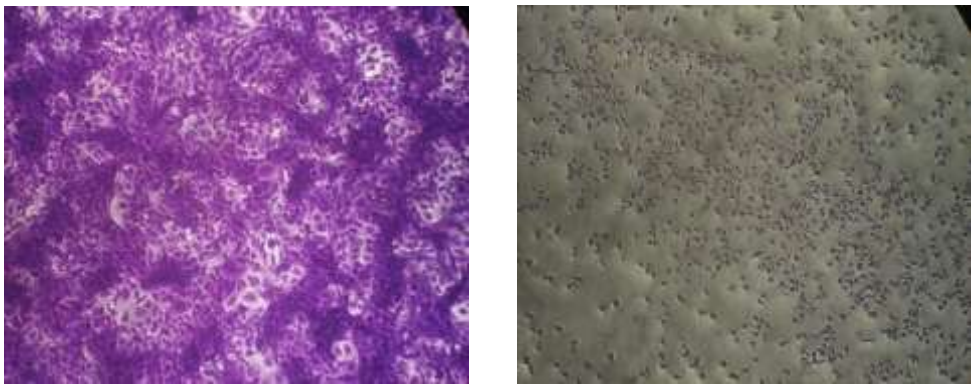


Figure 3. Comparisons between cancer cell lines according to their sensitivity to Iraqi *Corchorus olitorius* extract.

The cytotoxic activity was considered moderate, with the IC_{50} value ranging between 21 and 200 µg/mL [30, 31]. Furthermore, depending on the type of cancer cell line, *C. olitorius* leaf extracts have different IC_{50} values. The IC_{50} of the *C. olitorius* leaves was 139.5 µg/ml in AMJ13; and it was 100.1 µg/ml in SKGT-4 cells, as shown in Figure 3. Additionally, Figures 4 and 5 illustrate the cytotoxic effects of the AMJ13 and the SKGT-4 cell lines treated with an ethyl acetate extract of *C.olitorius* leaves at a dose equal to the corresponding IC_{50} when compared to untreated cells.

Control **Treated**

Figure 4 . The viability of AMJ13Cells treated with the Iraqi *corchorus olitorius* leaves' ethyl acetate fraction at IC_{50} (right) and Untreated Cells (left). The inverted microscope (Nikon, Japan) was utilized to take the microscopic images at ×10.



Control **Treated**

Figure 5. The viability of SKGT-4 cells treated with the Iraqi *corchorus olitorius* leaves' ethyl acetate fraction at IC_{50} (right) and untreated Cells (left). The inverted microscope (Nikon, Japan) was utilized to take the microscopic images at ×10.

Regarding esophageal and breast cancer, these are considered highly aggressive and metastatic types of cancer that exhibit high mortality rates and poor prognoses [32].In this research , the Iraqi *C. olitorius* leaves' ethyl acetate extract demonstrated a moderate cytotoxic impact on the two cell lines of cancer, which include the esophageal (SKGT- 4) and the breast (AMJ13) cells. This effect was seen in a dose - dependent manner, as illustrated in Figures 1 and 2. These results are consistent with the previous research [33, 34]. According to previous research, the same type of AMJ13 and SK-GT-4 cell lines were used, but the phenolic and terpene fractions from Iraqi Prunus Arabica were used instead. induced damaging impacts on these cell lines; they stimulated apoptosis and autophagy, inhibited the growth and invasiveness of cancer cells, and altered the activities of ROS-scavenging enzymes to cause cytotoxicity on cancer cells [35].Furthermore, the *C.*

olitorius inhibitory concentrations at half- maximum (IC_{50}) were lower in SKGT-4 cells compared to AMJ13 cells ,as shown in Figure 3. In addition, in this study the *C. olitorius* leaves had a cytotoxic effect by inhibiting the proliferation of two cancer cell lines, breast (AMJ13) and esophageal (SKGT-4) cells, as shown in Figures 4 and 5. Regarding these studies, the cytotoxic effects of *C. olitorius* may be attributed to their high phenolic compound and flavonoid content and their antioxidant properties, and these results are consistent with previous studies [36, 37]. Additionally, according to earlier research, flavonoids—widely distributed secondary metabolites that exist in the plants possess a range of beneficial pharmacological and biological properties. Which improve the body functions and offer the potential in the prevention and treatment of cancers by antioxidant effects in normal cells and pro-oxidant effects in cancer cells, then induce apoptotic pathways in cancer cells [38, 39].

Furthermore, the possible role of synergistic interactions between the numerous phytoconstituents of *C. olitorius* [40, 41] boosts their anticancer effects by exhibiting anti-inflammatory, antioxidant, and antiproliferative properties that make it a prospective candidate for cancer treatment. They also have anti-angiogenic activity because they can damage DNA in cancer cells and regulate various proteins and signaling pathways. [34, 42]. In this article, researchers showed for the first time how an ethyl acetate extract from *C. olitorius* leaves affects human cancer cell lines for the breast and. esophagus. Previous research either demonstrated the effect of *C. olitorius* on other cancer cell lines or the effects of ethyl acetate fraction from other Iraqi plants on human Iraqi breast and esophageal cell lines. It would therefore be necessary to do more studies on the mechanisms that govern the impact of the ethyl acetate extract from *C. olitorius* leaves on different cancer cell lines and to assess the potential cytotoxicity of Iraqi *C. olitorius* using animal models. Additionally, knowing the bioactive substances found in Iraqi *C. olitorius* can help us understand their potential as a treatment. The development of efficient and focused treatments for different forms of cancer may be assisted by this research.

Conclusion

The study's findings showed that the Iraqi *C. olitorius* leaves' ethyl acetate extract had cytotoxic action and decreased the viability of human esophagus and breast cancer cell lines and was more effective on SKGT-4 than AMJ13 cancer cell lines. The phytochemical components that have been identified during the preliminary phytochemical screening could possibly be contributing to this effect. Consequently, it is suggested that Iraqi *C. olitorius* is a palatable plant that may be used to create chemopreventive drugs for esophageal and breast cancer. So that more research will need to determine the precise component(s), the molecular mechanism(s) of Iraqi *C. olitorius* that inhibit the growth of cancer cells, and the potential candidate to be used as anticancer agents.

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Conflicts of Interest

The authors declared no conflict of interest.

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Ethics Statements

The research not required ethical statements.

Author Contribution

The authors confirm contribution to the paper as follows: study conception and design: Farah Kais Abdul-Wahab., Thukaa Z. Abdul-Jalil; data collection, analysis and interpretation of results and draft manuscript preparation : Farah Kais Abdul-Wahab., Zahraa Muhsin Hammodi, Nabaa M. Ibrahim,Thukaa Z. Abdul-Jalil. All authors reviewed the results and approved the final version of the manuscript.

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الفحص الكيميائي النباتي والنشاط السام المحتمل للخلايا لمستخلص أسيتات الإيثيل من أوراق نبات *Corchorus olitorius* العراقي ضد خطوط خلايا سرطان الثدي والمريء.

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الخلاصة

أظهرت أوراق نبات *Corchorus olitorius* العراقي ، وهي وجبة صحية شائعة في العراق، تأثيرات سامة للخلايا على سلالات خلوية مختلفة. كان الهدف الرئيسي من هذا البحث هو تقييم التركيب الكيميائي النباتي (المركبات متعددة الفينول) والسمية الخلوية لمستخلص أسيتات الإيثيل من أوراق *Corchorus olitorius* تم تحضير مستخلص أسيتات الإيثيل العراقي من أوراق *Corchorus olitorius* وتجزئته باستخدام تقنية جهاز سوكسليت الساخن المستمر. بعد ذلك، استُخدمت عدة اختبارات عامة ومتخصصة للكشف النوعي عن مختلف المركبات الكيميائية النباتية متعددة الفينول الموجودة في المستخلص النباتي. بعد ذلك، خضعت خطوط خلايا سرطان الثدي العراقي البشري (AMJ13) والمريء-(SKGT-4)لتراكيز مختلفة من مستخلص أسيتات الإيثيل (٣١,٢، ٦٢,٥، ١٢٥، ٢٥٠، ٥٠٠ و١٠٠٠ ميكروغرام/مل) لإجراء اختبارMTT ، المعروف أيضًا باسم بروميد ٣-(٤,٥ ثنائي ميثيل ثيازول-٢-يل)-٢,٥-ثنائي فيل تترازوليوم، وتم تقييم قابلية الخلية للحياة بعد ٧٢ ساعة من العلاج. أظهر مستخلص أسيتات الإيثيل لأوراق نبات *Corchorus olitorius* نشاطًا سامًا للخلايا ضد خطوط خلايا AMJ 13 و .SKGT-4وقد أحدث تأثيرًا يعتمد على الجرعة. بالنسبة لسلالات الخلايا AMJ13 وSKGT-4، بلغت قيم التراكيز المثبطة عند نصف الحد الأقصى (IC_{50}) ١٣٩,٥ميكروغرام/مل و١٠٠,١ ميكروغرام/مل، على التوالي ووفقًا لنتائج الدراسة، يُستنتج أن مستخلص أسيتات الإيثيل من أوراق نبات *Corchorus olitorius* أظهر نشاطًا سامًا للخلايا، وخفض من قابلية بقاء سلالات خلايا سرطان الثدي البشرية العراقية والمريء ، وذلك لاحتوائه على مركبات بوليفينولية. ولتحديد المركبات البوليفينولية التي تمارس هذا النشاط السام للخلايا وطريقة عملها، هناك حاجة إلى مزيد من الدراسة.

الكلمات المفتاحية: سرطان الثدي ، سرطان المريء ، الفلافونويدات ، *Corchorus olitorius* العراقي ، المركبات الفينولية.