DOI: https://doi.org/10.31351/vol35iss3pp68-69

From Invasive to Investigation: Polyphenolic Diversity and Cytotoxicity of Ethyl Acetate Extract of Iraqi *Eichhornia crassipes*

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Received 30/3/2025, Accepted 21/2/2026, Published 28/9/2026

Abstract

Eichhornia crassipes (Mart.) is a free-floating macrophyte that exerts a substantial influence on aquatic ecosystems. Often called water hyacinth, it also possesses notable industrial and therapeutic applications that are related to an existence of various secondary phytochemicals in the *Eichhornia crassipes*. This Iraqi research article widens previous studies carried out and issued by others, demonstrating for the first time the separation of polyphenolic compounds from the entire plant of *Eichhornia crassipes* using a Soxhlet Sequential Hot Continuous Extraction method with High-Performance Liquid Chromatography (HPLC) qualification and quantification estimations, as well as an assessment of cytotoxic activity against the human Iraqi breast cancer (AMJ13) cell line. The method of the Soxhlet apparatus was used to prepare the *Eichhornia crassipes* ethyl acetate extract. The next step is HPLC screening for various polyphenolic compounds, and in order to perform the MTT cytotoxic assay test that also known as 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, the breast (AMJ13) cancer Iraqi human cell line was then exposed to six concentrations of ethyl acetate extract [31.2, 62.5, 125, 250, 500, and 1000 µg/ml]. The viability of the cell was then assessed 72 hours after the treatment. Eight polyphenolic compounds were found via a qualitative phytochemical study designated as diverse phenolic acids, such as tri-hydroxy-benzoic acid [gallic acid] and hydroxy-cinnamic acid [caffeic acid and p-coumaric acid], polyphenol esters [chlorogenic acid], and flavonoids such as flavone [luteolin and apigenin], flavanone [naringenin], and flavonol glycoside [quercitrin]; this is the first investigation on quercitrin in *Eichhornia crassipes* conducted in Iraq, and potentially globally. Quantitative examination revealed that chlorogenic acid was present in greater quantities than the other components in the ethyl acetate extract, followed by gallic acid and p-coumaric acid, with naringenin exhibiting the lowest concentration. The data indicate that phenolic acid concentrations exceed those of flavonoids. The present investigation demonstrated that *Eichhornia crassipes* can be utilized therapeutically as an oncolytic agent, evidenced by an IC_{50} value of 127.5 µg/ml. These findings help people understand that, although invasive plants are detrimental to the environment and human livelihoods, they may also be used to make medicinal compounds that treat cancer, the most prevalent and deadly disease.

Keywords: Breast cancer (AMJ13) cell line, *Eichhornia crassipes*, high-performance liquid chromatography, polyphenolic chemicals, water hyacinth.

Introduction

Mammary neoplasm has overtaken lung cancer as the most common neoplasm diagnosed in women globally. It was projected that there would be at least 2.3 million new cases of mammary neoplasm in the last five years, accounting for 11.7% of all new malignancies and 684,996 deaths [(1)]. There are several different subgroups within this extremely varied neoplasm. These subtypes are generally classified according to the immunohistochemical expression of hormone receptors into four groups: G1: Triple-Negative Breast Cancer (TNBC), G2: Human Epidermal Growth Factor Receptor Positive (HER2+), G3: Progesterone Receptor Positive (PR+), and G4: Estrogen Receptor-Positive (ER+) [(2)]. Evaluation of tumor burden and genetic factors, as well as interdisciplinary teamwork, are essential for the management of mammary neoplasm. The conventional treatment of early-stage mammary neoplasm is either mastectomy alone or breast-conserving surgery in conjunction with radiation therapy. The state of the nodal metastasis, hormone receptors, and human epidermal growth factor receptor-2 determines whether adjuvant systemic therapy is used [(3)]. Less than 10% of the more than 250,000 plant species in the kingdom have been

studied or recognized for possible medicinal application, according to current data [4,5]. The water hyacinth (Mart.) with botanical name; *Eichhornia crassipes* (*E. crassipes*); is a freely floating aquatic monocot plant that originated in Brazil as well as Amazon and prevalence to tropical and subtropical regions. It is a member of the Pontederiaceae family [6]. The plant's exceptional growth, quick and widespread reproduction, and strong resistance to changes in pH, nutrients, and temperature are some of its distinctive characteristics. It is one of the top ten most harmful weed plants in the world and ranked among the hundred most aggressive species, according to the International Union for Conservation of Nature [7]. While considered the most harmful aquatic weed in the world, water hyacinth is used in some regions and unknown in others. Phenolic acids, flavonoids, tannins, alkaloids, steroids, glycosides, volatile and fixed oils, resins, and a number of other metabolites are among the phytochemicals found in water hyacinth. These substances have biological properties including antiviral, antifungal, anticancer, and antibacterial activities, and are necessary for nourishment. Increased oxidative enzyme levels, non-enzymatic antioxidant activities, tissue repair capacity, and cytotoxic qualities are all present in water hyacinth [8]. Water hyacinth was used as a phytoremediation agent for wastewater treatment due to the capacity to grow in contaminated water and absorb heavy metals [9]. Moreover, it has been regarded as a possible source of bioenergy [10] and bio-fertilizers [11]. For a long time, the herb has been used to cure a variety of digestive issues, including flatulence, diarrhea, and intestinal worms. The seeds were used to keep the spleen functioning at its best [12]. The uses and health advantages of water hyacinth have been widely researched. However, data regarding the biological as well as pharmacological characteristics of special chemical compounds (polyphenolic compounds) in the entire plant are still limited. The research that has been carried out only covers the effect of crude extract as a cytotoxic agent, but the effect of polyphenolic extract remains incompletely investigated. Furthermore, in Iraq, no studies have examined the polyphenol fraction on the human breast (AMJ13) cancer cell line. To close this gap, this research outlines the framework for viewing the first study on extraction using a sequential extraction process, identifying polyphenols qualitatively and quantitatively using HPLC, and assessing its cytotoxic effect on breast cancer (AMJ13) cells.

Materials and Methods

Botanical substances

In April 2024, the whole plant parts (leaves, stems, flowers, and roots) of the *E. Crassipes*, which is wild grown in Iraq, were collected from the Euphrates River in the Karbala Governorate. The studied plant sample has been identified and verified by the Department of Biology in the College of Science, at University of Baghdad Herbarium, which is documented under BUH No. 89019. The plant was cleaned to eliminate any extraneous contaminants, allowed to air out in the shade for 10 days. An electric grinder was subsequently employed to pulverize the desiccated plant into a powder. Subsequently, the powder was prepared for extraction and stored in a securely sealed glass container.

Preparation of E. crassipes for extraction:

One hundred grams of powdered *Eichhornia crassipes* whole plant were put in a thimble of a Soxhlet device for the sequential extraction method. Defatting with 1000 ml of n-hexane for six hours at 50°C was the initial step in the extraction procedure, followed by filtration and allowing the plant to dry before commencing the extraction process with ethyl acetate. After the plant has dried, it is put in a thimble and extracted for 12 hours at 50°C, using 1000 milliliters of ethyl acetate. The ethyl acetate extract has been filtered and vacuum-evaporated by rotary evaporator [13,14], as seen in Figure 1. The extraction yield was 8.2%, and the dried ethyl acetate extract was set aside for further examination.

Qualitative and quantitative evaluation of polyphenolic substances with High-Performance Liquid Chromatography (HPLC)

With the help of high performance liquid chromatography (HPLC), the predicted polyphenolic components contained in the entire *Eichhornia crassipes* ethyl acetate extract were analyzed for both quality and quantity in the Ministry of Science and Technology at Department of Water and Environmental Research. Comparing the retention times of the extracted ethyl acetate components to those of the legitimate standards, which were run under the identical chromatographic conditions, allowed for the determination of the qualitative identifications [15]. In order to proceed quantification measurements, the calibration curve was created by graphing the area under the curve (AUC), represented by the y-axis, against the four (5, 10, 15, and 20 µg/ml) concentration levels of the standards, represented by the x-axis. The concentration of each analyst sample was calculated using this equation (Y = AX + B), in which; A is the slope gradient and B is the y-axis intercept. [16].

HPLC parameters for the ethyl acetate extract

The ethyl acetate extract was undergoing to a High-Performance Liquid Chromatography (HPLC) study utilizing four Flavonoids [luteolin, naringenin, quercitrin, and apigenin] and four Phenolic acids [chlorogenic, gallic, caffeic as well

as p-coumaric acids] as authenticated standards for qualitative and quantitative evaluation [17]. The chromatographic parameters employed are detailed in Table (1).

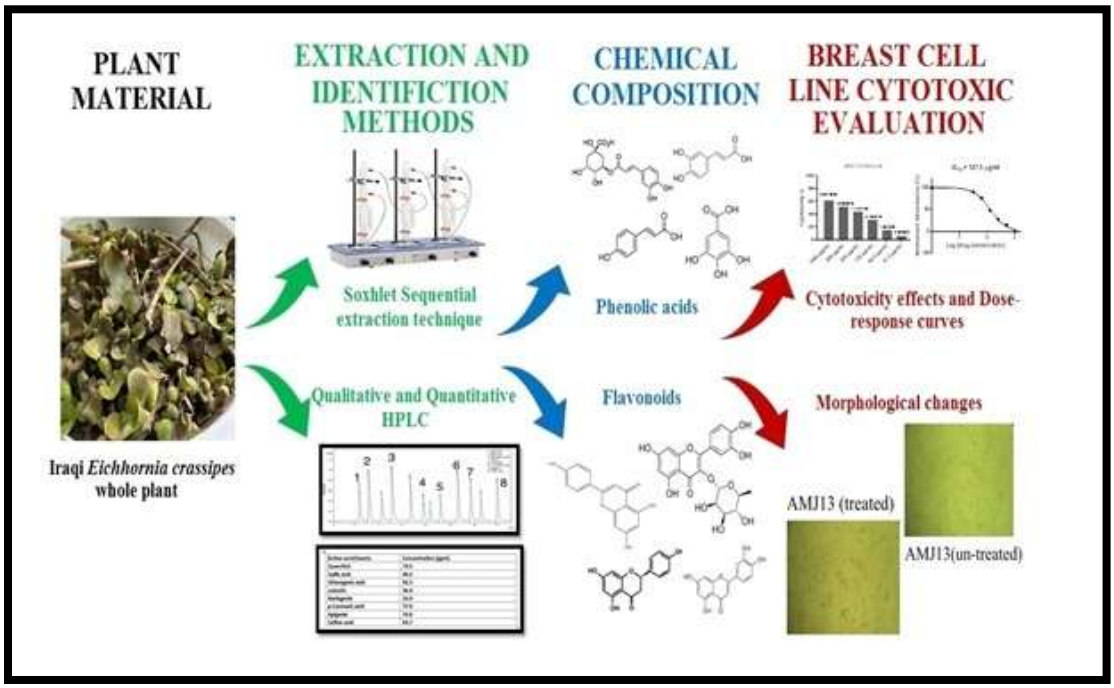

Figure 1. Overview of the phytochemical and cytotoxic investigation of *Iraqi Eichhornia crassipes*.

Table 1. Chromatographic parameters employed for the ethyl acetate fraction [17]

Mobile phase	Elution: Gradient mode Solvent (A): 1% Aqueous Formic acid Solvent (B): Acetonitrile 0 - 5 min: (A) :90% while (B):10% 5 - 9 min: (A) :80% while (B):20% 9- 15 min: (A) :70% while (B):30% 15 - 25 min: (A) :60% while (B):40%
Column	LC SYKAM C18 (250 mm * 4.6 mm; 5 µm particle size).
Sample	Whole plant ethyl acetate extract at concentration 1mg/ml
Standards	four Phenolic acids [chlorogenic, gallic, caffeic as well as p-coumaric acids] and four Flavonoids [luteolin, naringenin, quercitrin and apigenin] by using four concentrations (5, 10, 15, 20 µg/ml) for each one.
Flow rate	[1 ml/min]
Injected volume	[50 µL]
Injected concentration	[1 mg /ml]
UV detector	Detection at λ 254 nm

Cytotoxicity assessment of ethyl acetate extract from Eichhornia crassipes

This methodology investigates the impact of *Eichhornia crassipes* ethyl acetate extract on the viability of the breast cancer Iraqi cell line [Ahmed Murtada Jabria 2013 [(AMJ13)], which was provided by Gen Target Inc. (United States). The experiment was carried out at the lab of Tech Cell Technologies in Iraq by utilizing the MTT [3-(4,5-Dimethyl, thiazol-2-yl)-2,5-Diphenyl-tetrazolium Bromide] colorimetric assay, which evaluates metabolic cellular activity as well as cellular oxido-reductase NADPH-dependent enzymes may, in certain instances, serve as indicators of viable cell count. These enzymes can transform the MTT dye; tetrazolium; into a purple, insoluble formazan.

Cell Cultivation

The cell culture was grown in Minimum Essential Medium [US Biological, USA] supplemented with Fetal Bovine Serum [FBS] at concentration 10% (v/v), Penicillin [100 IU], and Streptomycin [100 µg] (Capricorn-Scientific, Germany), and incubated at 37°C in a moisturized

atmosphere. Exponentially expanding cells were utilized in the tests [18].

Cell Viability Assessment

At a particular wavelength, 570 nm, to assess the concentration of formazan. The count of live cells is directly proportional to the quantity of formazan produced. Consequently, treatment with the ethyl acetate extract results in a reduction of formazan production, indicating cytotoxicity through a decrease in absorbance. The dose-response curve facilitates the determination of the half-maximal inhibitory concentration [IC_{50}], which indicates the quantity of the ethyl acetate extract that require inhibiting 50% of the cells. A widely utilized method for evaluating cell viability and cytotoxicity is the MTT [3-(4,5-Dimethyl, thiazol-2-yl)-2,5-Diphenyl-tetrazolium Bromide] cytotoxicity assay. This chromatic assay relies on the ability of living cells to utilize mitochondrial dehydrogenases to transform yellow MTT into purple formazan crystals. Cells are typically installed in a ninety-six well plate and subjected to diverse concentrations of the plant extract [ethyl acetate] to conduct the MTT assay. After the incubation time, MTT is inserted to each well and incubated again. Viable cells transform tetrazolium MTT into insoluble formazan. A spectrophotometer measures the absorbance of a substance that causes a 50% decrease in cell viability [19].

MTT protocol

Ten thousand cell lines were inoculated in a microplate / 96-well (NEST Biotech, China). Subsequently, they were cultured for 72 hr. at 37°C until single-layer confluence was attained. Cytotoxic influence was evaluated with the [3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyl-tetrazolium bromide] (MTT) test (Elabscience, China). The cells have been subjected to diverse concentrations of ethyl acetate extract (31.2, 62.5, 125, 250, 500, and 1000 µg/ml). After a 72-hour exposed period, 28 µL of MTT dye solution (2 mg/ml) was introduced to all evenly. The incubation phase persisted for three hours. Following the addition of 100µl of dimethyl sulfoxide (DMSO) to each well, the wells were incubated for 15 minutes. The optical density at 570 nm was assessed utilizing a microplate reader. The cytotoxicity percentage was determined using the formula provided below:

The percentage of cytotoxicity is calculated as **(OD Control – OD Sample) / OD Control × 100**.

OD Sample denotes the optical density of treated wells, while OD Control signifies the average optical density of untreated wells [20].

Investigation of structural alterations

The inverted microscope's forty-power magnification (Optika, Italy) was utilized to examine the morphology of (AMJ13) cells to observe and document the morphological alteration of apoptotic cells. Apoptotic features were observed by the presence of cellular membrane-bound bodies and/or cellular shrinkage after a 72-hour incubation period.

Quantitative examination

Tukey's ANOVA multiple differentiation test was employed to statistically analyze the collected data utilizing GraphPad Prism 8 [21]. The mean ± standard deviation of three distinct measurements was utilized to denote the values [20].

Results and Discussion

Soxhlet sequential extraction methodology

The extraction process used depends on a number of variables; including the particular phytochemical to be separated, the consistency and moisture ratio of the plant parts being extracted, and other factors. As a result, choosing the best extraction method is crucial and, in certain cases, depends on how the extract will be used. By applying various solvents in succession, sequential extraction selectively separates phytochemicals according to their polarity and solubility, resulting in high extraction efficiency and reduced solvent consumption. Due to its remarkable resistance to high temperatures, the entire *E. crassipes* plant was eliminated in this manner [22].

Qualitative and quantitative analysis of polyphenolic substances via HPLC

The polyphenols [phenolic acids & flavonoids] in the *E. crassipes* ethyl acetate extract were qualitatively analyzed using RP-HPLC, comparing the retention times of eight standards [caffeic acid, chlorogenic acid, gallic acid, p-coumaric acid, luteolin, naringenin, quercitrin & apigenin] with the plant extract under the same chromatographic conditions. The RP-HPLC analysis indicated the presence of eight distinct polyphenolic compounds in the plant, comprising tri-hydroxy-benzoic acid [gallic acid], hydroxycinnamic acids [caffeic acid & p-coumaric acid], polyphenol esters [chlorogenic acid], and flavonoids [flavone (luteolin and apigenin), flavanone (naringenin), and flavonol glycoside (quercitrin)]. These are illustrated in figures (2–10) and Table (2).

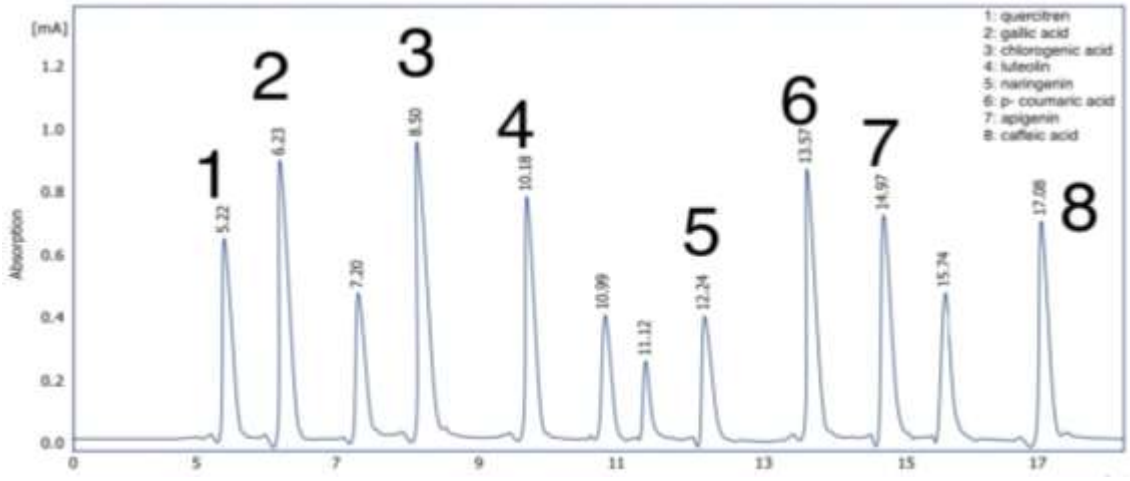

Figure 2. HPLC chromatogram of the entire *Eichhornia crassipes* ethyl acetate extracted plant

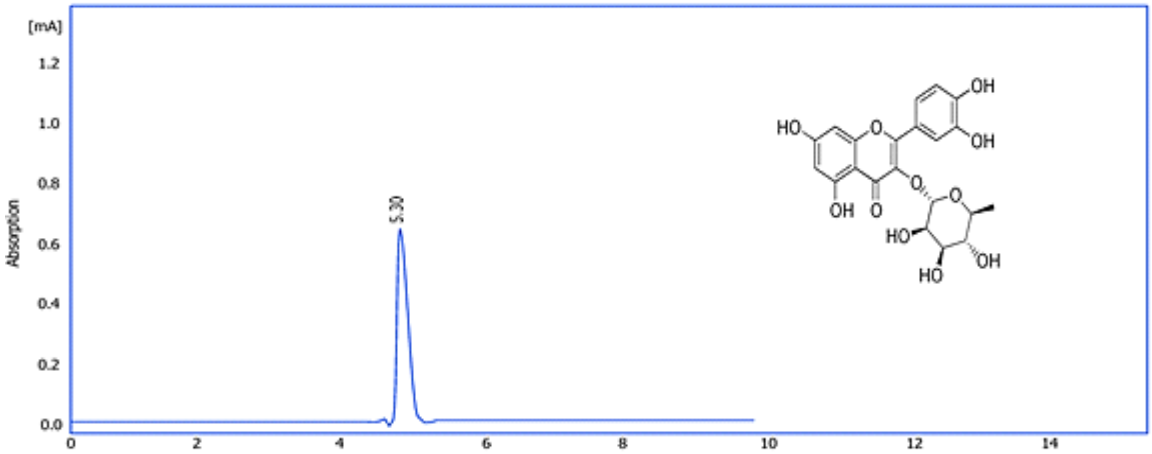

Figure 3. Chromatogram of HPLC for the standard quercitrin

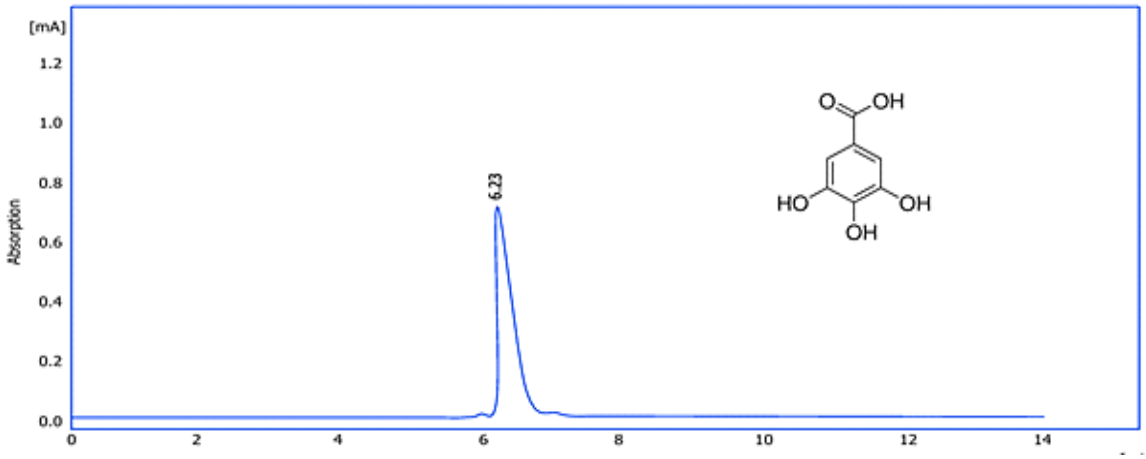

Figure 4. Chromatogram of HPLC for the standard gallic acid

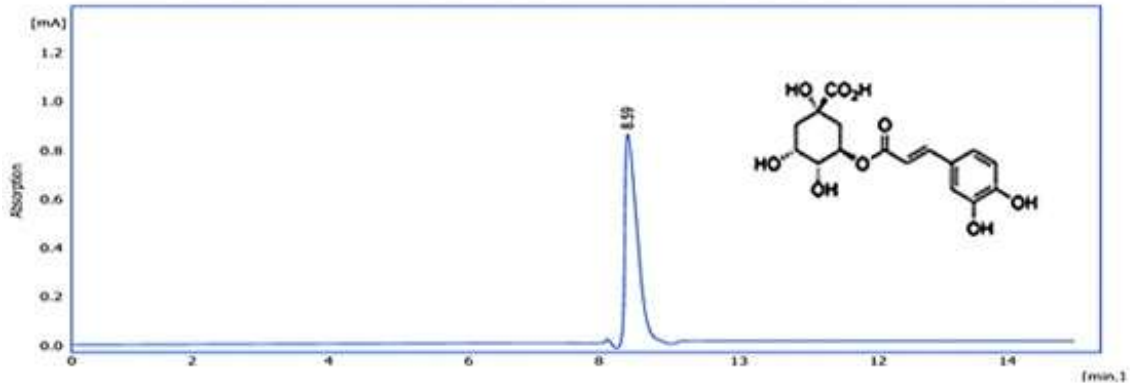

Figure 5. Chromatogram of HPLC for the standard chlorogenic acid

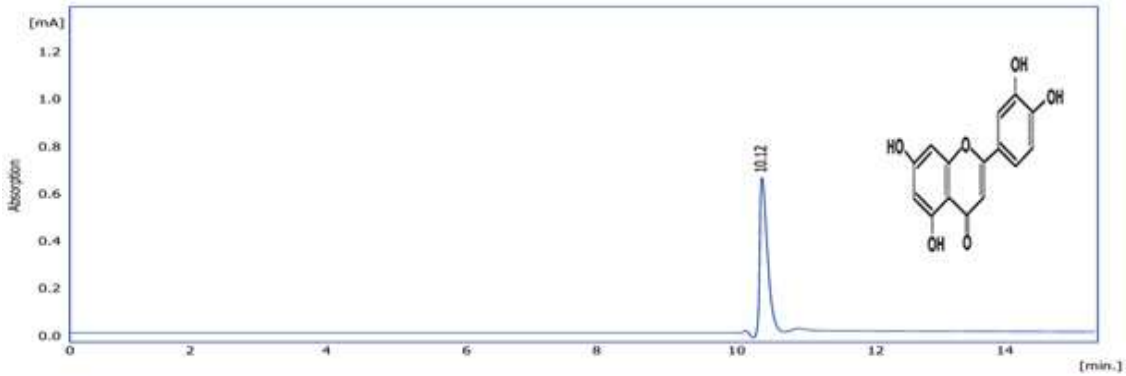

Figure 6. Chromatogram of HPLC for the standard luteolin

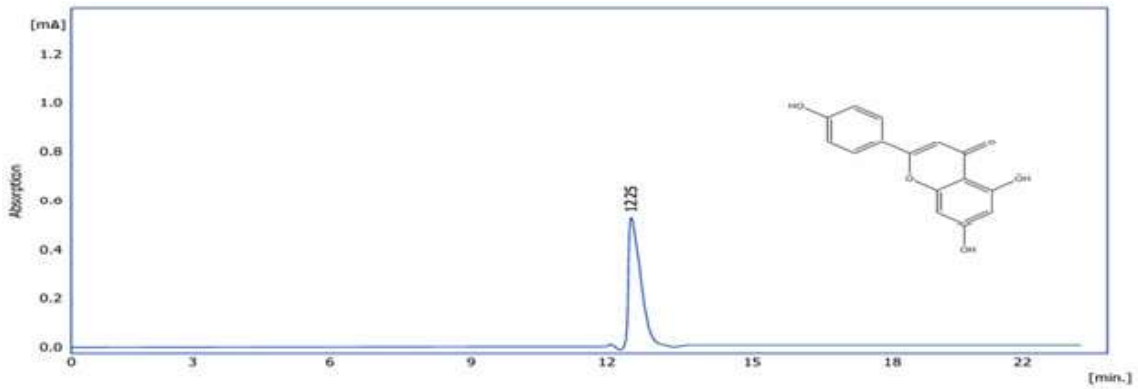

Figure 7. Chromatogram of HPLC for the standard naringenin

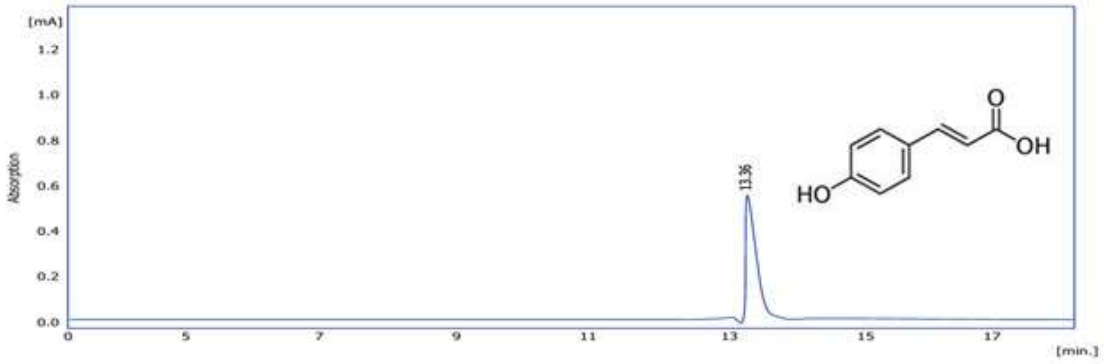

Figure 8. Chromatogram of HPLC for the standard p-coumaric acid

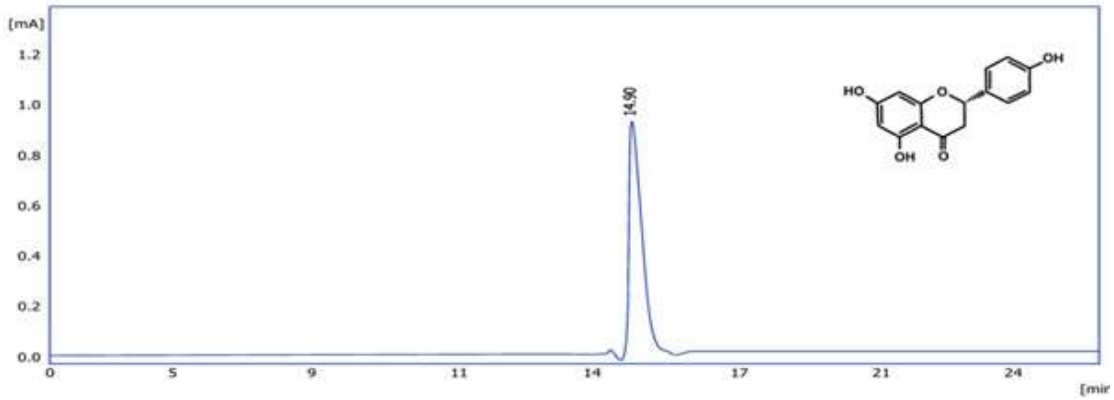

Figure 9. Chromatogram of HPLC for the standard apigenin

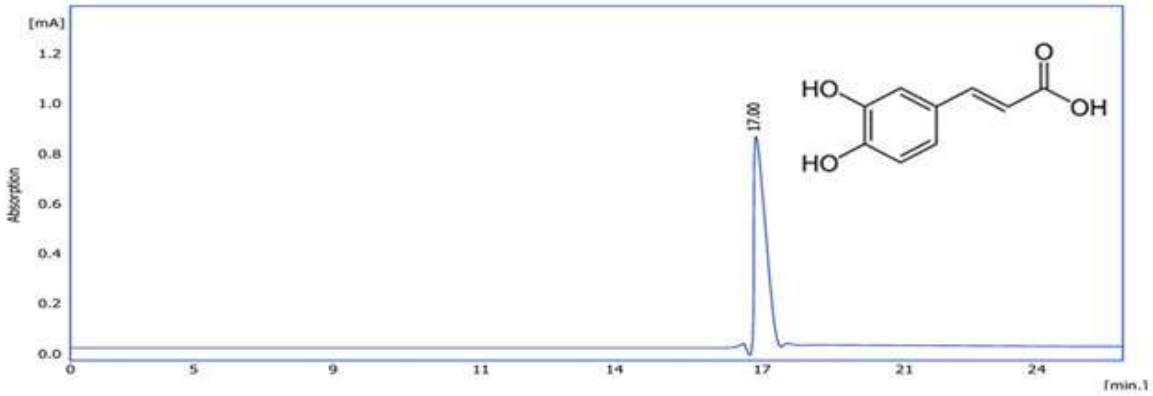

Figure 10. Chromatogram of HPLC for the standard caffeic acid

Analysis –quantitative of the supposed concentration of polyphenols in the sample-ethyl acetate extract was calculated via a mathematic equation (straight-line) by curve-calibration using AUV [area under the curve] against four standard concentrations for each of the following: caffeic acid, chlorogenic acid, gallic acid, p-coumaric acid, luteolin, naringenin, quercetin & apigenin]; as revealed in Figure (11) and Table (2).

Table 2. Retention time of standards, sample, and concentration in plant extract (ppm) of polyphenolics in ethyl acetate extract of *E. crassipes*

Active constituents	R_t of standard	R_t of sample constituents	Concentration in plant extract (ppm or µg/gm)
Quercitrin	**5.30**	**5.22**	**74.5**
Gallic acid	**6.23**	**6.23**	**90.2**
Chlorogenic acid	**8.59**	**8.50**	**92.3**
Luteolin	**10.12**	**10.18**	**38.4**
Naringenin	**12.25**	**12.24**	**16.9**
p-Coumaric acid	**13.36**	**13.57**	**77.9**
Apigenin	**14.90**	**14.97**	**74.8**
Caffeic acid	**17.00**	**17.08**	**43.7**

Table (2) demonstrates the concentrations of phenolic acids in *E. crassipes* exceed those of flavonoids, while the concentration of chlorogenic acid surpassed those of other elements in the sample-ethyl acetate extract.

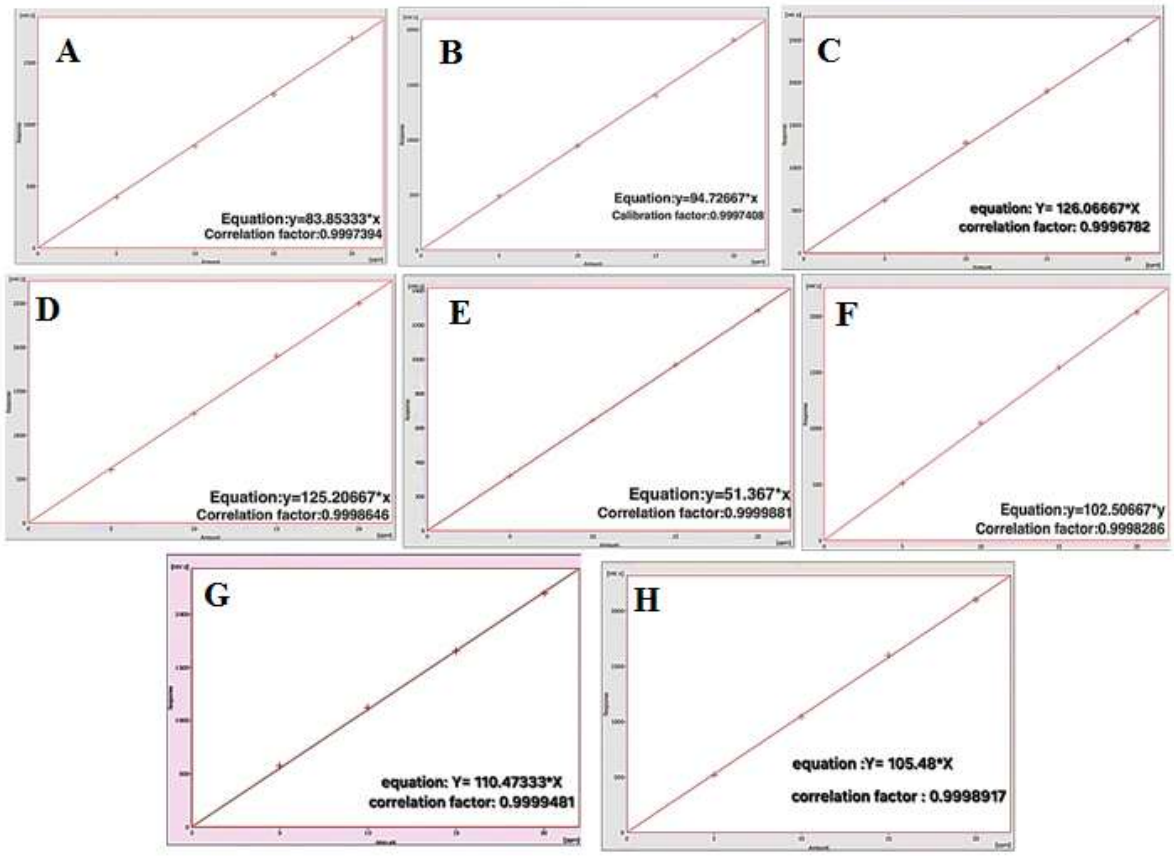

Figure 11. Calibration curve of standards A = quercitrin, B = gallic acid, C = chlorogenic acid, D = luteolin, E = naringenin, F = p-coumaric acid, G = apigenin, H = caffeic acid.

The results indicate that chlorogenic acid, gallic acid, and p-coumaric acid had the highest concentrations, respectively, in contrast to Rufchaei et al. [23], which illustrated that chlorogenic acid, quercetin, and catechin had the highest concentrations. This could be explained by the fact that the chemical composition of water hyacinth varies depending on the location, as well as by the availability of biochemical factors that are thought to be necessary for the formation of the building block of these secondary metabolites [6]. The concentration measure in ppm because some phytochemicals have concentration lower than 1000µg/ml so to unify the concentration of all compounds used this unit. This study is the inaugural investigation conducted in Iraq, and may be globally, on the presence of quercitrin in *Eichhornia crassipes*.

Cytotoxicity assay

The cytotoxic assay and the MTT test were applied for the evaluation of the cytotoxic affection of *E. crassipes*-ethyl acetate extract on the distinctive human/DC tumor cell line [breast ductal carcinoma AMJ-13] [24]. The *E. crassipes* exhibit a major cytotoxicity at 1000 µg/mL and a minor cytotoxicity at 31.2 µg/ml. The ethyl-acetate extract of *E. crassipes* had provoked cytotoxicity at the AMJ 13 cell line; as illustrated in Figure 12 and Table 3.

Table 3. Percentage of cytotoxicity (%) of *E. Crassipes* Ethyl acetate extract at various concentrations

Concentrations	1000 µg/mL	500 µg/mL	250 µg/mL	125 µg/mL	62.5 µg/mL	31.2 µg/mL
% Cytotoxicity	60.8103	52.0939	44.1947	31.5287	14.5046	6.0265

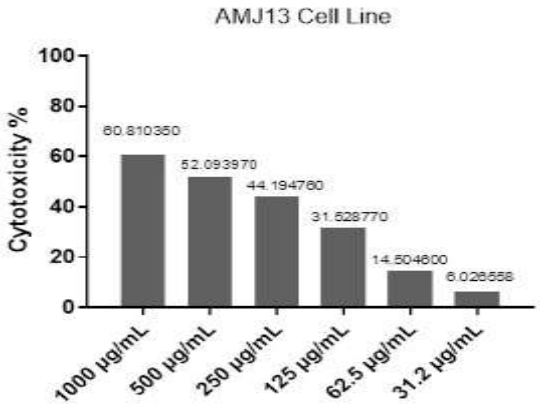

Figure 12. Cytotoxicity of ethyl-acetate *E. Crassipes* extract on AMJ-13 in a concentration-dependent manner. The Y-axis reveals the cell death percentage, and the X-axis reveals the influencing concentrations of ethyl-acetate.

The IC_{50} value ranged from 21 to 200 µg/mL, indicating moderate cytotoxic action [24]. The ethyl acetate extract demonstrated substantial activity ($p<0.0001$) versus human AMJ13breast cell line at all five- concentrations tested (62.5, 125, 250, 500, and 1000 µg/mL); when compared to the control (untreated cells), but the strongest effect had been noted at the highest concentration (1000 µg/mL), as illustrated in Figure 13.

As shown in Figure 14, the tested extract's IC_{50} value was 127.5 µg/ml, which resulted in a 50% reduction in cell proliferation. Additionally, cytotoxicity rose in tandem with concentration. Depending on the concentration, the cytotoxicity, optical density, and IC_{50} of the *E. crassipes* ethyl acetate extract vary.

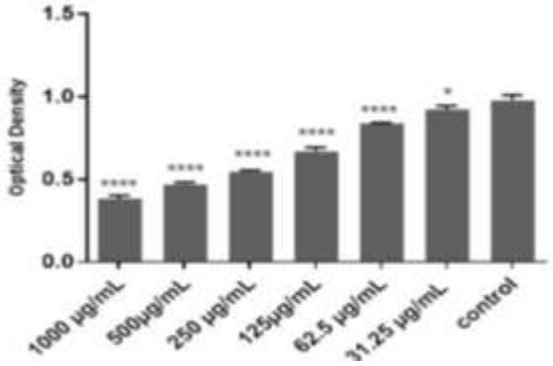

Figure 13. Depicts the optical density (OD) measurement for AMJ13 subjected to treatment with *E. crassipes* Ethyl acetate extract. The X-axis denotes concentrations in micrograms per milliliter, while the Y-axis indicates the optical density value at 570 nanometers. Each observation signifies the mean ± standard deviation of triple measurements.

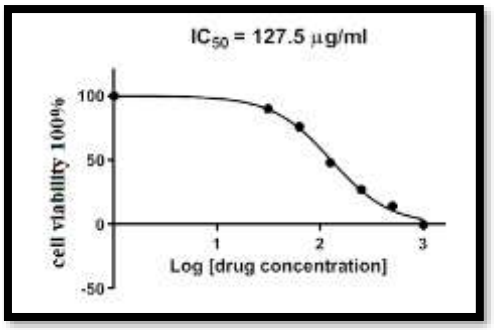

Figure 14. Dose-response curve utilized to ascertain the IC50 value of *E. crassipes* ethyl-acetate extract on cells. The IC50 value is denoted by the logarithm of the drug concentration of the test *E. crassipes* ethyl acetate extract that reduces cell viability by 50%.

The effect of ethyl-acetate extract at a dose corresponding to the relevant IC50 is illustrated in Figures (12-15). It exhibits remarkable cytotoxicity against AMJ13 cancer cells, as indicated by Tukey's ANOVA multiple comparisons test and morphological alterations, resulting in a significant reduction in viable cancer cell count and morphological changes, including cell separation and detachment. The phytochemical components (polyphenols) that have been identified during qualitative and quantitative estimation by HPLC could be contributing to these effects.

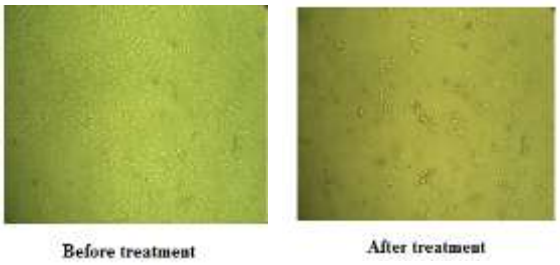

Figure 15. The morphological profile of AMJ13 cells before and after treatment with ethyl acetate extract

According to the outcomes of the phytochemical analysis, the ethyl-acetate extract of *E. crassipes* had polyphenolic constituents. Phenolic compounds exhibit properties related to free radical scavenging and antioxidant protection. These chemicals have been linked to anticancer effects on several critical components, including angiogenesis, cell proliferation, metastasis, and apoptosis [25,26].

The ability of the extracts from various parts of *E. crassipes* to inhibit the growth of multiple forms of cancer has been demonstrated in numerous *in-vitro* experiments [27-30]. Researchers presented the effects of an ethyl-acetate extract

from the entire plant of *E. crassipes* on human AMJ13 cancer cell lines for the first time in this study. They also evaluated the quantitative levels of flavonoids and phenolic acids, especially chlorogenic acid, which exceeded the concentrations of other constituents in the ethyl-acetate extract, as well as gallic acid, p-coumaric acid, quercitrin and apigenin. This suggests that the polyphenolic compounds identified in the extract of *E. crassipes* implement an obvious role in the reported cytotoxic activities.

Moreover, the quantities of phenolic acids contained in the extract are higher than those of flavonoids produced with ethyl acetate. These results are consistent with the previous research done by Rufchaei R and colleagues, which demonstrated that the total phenolic content of the hydro-methanolic and aqueous extracts of *E. crassipes* was markedly higher than the total flavonoid levels. [23].

Current information indicates that chlorogenic acid may possess anticancer properties by interrupting the cell-cycle, inducing apoptosis, and obstructing the cancer cells proliferation. The SRY-box transcription factor 2 gene and the B cell-specific Moloney murine leukemia virus integration site 1 protein are downregulated, whilst the nuclear factor of activated T cells 2 (NFATC2) and NFATC3 genes relevant to the immunological system are elevated. This is executed to facilitate the eradication of malignant cells. This component facilitates intracellular DNA damage and the formation of topoisomerase I- and topoisomerase II-DNA complexes, both essential for apoptosis [25,29].

Gallic acid (naturally occurring) polyphenolic compound that exists in several forms, including both polymeric and free forms. Gallic acid exhibits anti-cancer properties that are incongruent with numerous biological systems, including the initiation of apoptosis, arresting the cell cycle, inhibition of vascular and neoplasm migration, and alteration of inflammatory reactions. Gallic acid has additional synergism with other commercially available chemotherapeutic agents, also possessing anti-cancer properties with a promising candidate for future therapeutic development [31].

A multitude of flavonoids has demonstrated anticancer effects, and data substantiates this assertion. Flavonoids inhibit cell proliferation in human breast cancer cells. The flavonoids comprise luteolin, apigenin, and naringenin. Furthermore, these flavonoids induce cell cycle arrest, autophagy, or ultimately apoptosis. Adjuvant flavonoids may reduce the incidence of breast cancer and systemic side effects, hence improving the quality of life for individuals who have successfully battled breast cancer [32].

Quercitrin collaborates with other factors to modulate the production of IFNγ-R, p-JAK2, p-STAT1, and PD-L1 in the JAK/STAT1 signaling pathway, hence inducing apoptosis and eradicating breast cancer cells. Furthermore, it promotes the control of γδ T cells. Quercitrin may be employed in the adjuvant therapy of the mammary gland tumors and in the prevention of its provoking conditions [33].

Apigenin can suppress the activity of the MCF-7/Akt clone, which facilitates cell proliferation. Apigenin induces arresting cell cycle with the apoptosis during the G2/M phase, hence inhibiting E2's role as a proliferative factor. Apigenin is a crucial molecule as it inhibits FOXM1 expression; a transcription factor integral to the cell cycle. Consequently, it inhibits the Akt/FOXM1 signaling pathway. Apigenin additionally modifies the FOXM1 controlled gene expression; including those related to the cell cycle, particularly in the MCF-7/Akt clone [34].

Conclusion

The study discovered a new finding that the ethyl-acetate extracts of *E. crassipes* concelate a cytotoxic effect upon AMJ13 cell lines with a 127.5 μg/ml IC_{50} value, when varying the concentrations from 31.2 μg/ml to 1000 μg/ml. It is further deduced that the cytotoxic characteristic of the MTT assay increased with increasing concentrations of active ingredients. The polyphenolic components (flavonoids and phenolic acids) that have been identified during qualitative and quantitative estimation by HPLC could be contributing to this effect. Accordingly, Iraqi *E. crassipes* is a fast-growing, widely distributed plant that could be utilized to make chemopreventive medications for breast cancer. Therefore, the additional research is required to identify the specific component(s), the molecular mechanism(s), and the possible candidate for use as anticancer medicines. As a result, the current work alerts us to the problem and provides useful information for future polyphenolic extraction from *E. crassipes* optimization.

Acknowledgment

Great gratitude to the College of Pharmacy-University of Baghdad for supporting this work.

Conflicts of Interest

The authors had nothing to declare.

Funding

Self-fund supply

Ethics Statements

None.

Author Contribution

The contributants in this work issued an A-Z plan with meticulous observational considerations for each step in this harmonic work-ladder starting by preparations of samples to statistical analysis and completing it with writing the manuscript.

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من الاجتياح إلى البحث: التنوع متعدد الفينول والفعالية السامة للخلايا في مستخلص أسيتات الإيثيل من نبات عكر الماء البري العراقي الكامل

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الخلاصة

نبات عكر الماء (ورد النيل) هو نباتٌ كبيرٌ عائمٌ حرّ الحركة، يُمارس تأثيرًا كبيرًا على النظم البيئية المائية. يُسمى غالبًا ياقوتية الماء، وله أيضًا تطبيقات صناعية وعلاجية بارزة مرتبطة بوجود مركبات ثانوية مُتنوعة فيه. تُوسّعت هذه المقالة البحثية العراقية نطاق الدراسات السابقة التي أجراها وأصدرها آخرون، مُظهرةً لأول مرة فصل المركبات البوليفينولية من نبات عكر الماء بالكامل باستخدام طريقة استخلاص سوكسليت المُتسلسلة الساخنة المُستمرة مع تقدير النوعي والكمية باستخدام كروماتوغرافيا السائل عالية الأداء، بالإضافة إلى تقييم النشاط السام للخلايا ضد سلالة خلايا سرطان الثدي البشري العراقية .استُخدمت طريقة جهاز سوكسليت لتحضير مُستخلص أسيتات الإيثيل من عكر الماء. الخطوة التالية هي فحص كروماتوغرافيا السائل عالي الأداء للكشف عن مركبات بوليفينولية مختلفة، ولإجراء اختبارMTT ، المعروف أيضًا باسم بروميد ٣-(٤،٥-ثنائي ميثيل ثيازول-٢-يل)-٢،٥-ثنائي فينيل تترازوليوم، عُرضت بعد ذلك سلالة خلايا سرطان الثدي العراقية البشرية (AMJ13) لتركيزات مختلفة من مستخلص أسيتات الإيثيل (٣١,٢، ٦٢,٥، ١٢٥، ٢٥٠، ٥٠٠، و١٠٠٠ ميكروغرام/مل). ثم قُيّمت قابلية الخلية للبقاء على قيد الحياة بعد ٧٢ ساعة من العلاج. عُثر على ثمانية مركبات بوليفينولية من خلال دراسة كيميائية نباتية نوعية، عُرفت بأحماض فينولية متنوعة، مثل حمض ثلاثي هيدروكسي بنزويك (حمض الغالك) وحمض هيدروكسي سينامك (حمض الكافيين وحمض البارا-كوماريك)، وإسترات البوليفينول (حمض الكلوروجينيك)، والفلافونويدات مثل الفلافون (اللوتولين والأبيجينين)، والفلافانون (النارينجنين)، وجليكوسيد الفلافونول (الكيرسيترين)؛ وهذا أول بحث يُجرى على الكيرسيترين في نبات عكر الماء في العراق، وربما عالميًا. كشف الفحص الكمي عن وجود حمض الكلوروجينيك بكميات أكبر من المكونات الأخرى في مستخلص أسيتات الإيثيل، يليه حمض الغالك وحمض البارا-كوماريك، مع وجود أقل تركيز للنارينجنين. تشير البيانات إلى أن تركيزات حمض الفينول تفوق تركيزات الفلافينويدات. أظهرت هذه الدراسة إمكانية استخدام نبات عكر الماء علاجيًا كعامل مُذيب للأورام، ويتجلى ذلك في قيمة IC50 البالغة ١٢٧,٥ ميكروغرام/مل. تُساعد هذه النتائج الناس على فهم أنه على الرغم من أن النباتات الغازية تُضر بالبيئة وسبل عيش الإنسان، إلا أنه يُمكن استخدامها أيضًا في صنع مُركّبات طبية تُعالج السرطان، وهو أكثر الأمراض شيوعًا وفتكًا.

. الكلمات المفتاحية: سلالة خلايا سرطان الثدي (AMJ13)، عكر الماء (ورد النيل)، كروماتوغرافيا سائلة عالية الأداء، مواد كيميائية متعددة الفينول، زهرة ياقوتية الماء.