Kirkuk University Journal for Agricultural Sciences
ISSN:2958-6585
https://kujas.uokirkuk.edu.iq

RESEARCH ARTICLE https://doi.org. 10.58928/ku25.16215

Phytochemical profiling, antioxidant and anticancer properties of wild *Artemisia haussknechtii* in Sulaymaniyah-Iraq.

Zainab Sabah Lazim

Department of Horticulture, College of Agricultural Engineering Science, University of Sulaimani, Sulaymaniyah, IRAQ.

*Corresponding Author: zaynab.lazim@univsul.edu.iq.

Received: 15/03/2025 Revised: 13/04/2025 Accepted: 07/05/2025 Published: 01/06/2025

ABSTRACT

This study was conducted on the wild wormwood plant collected from Mount Piramagroon with coordinates 35°46'21.6" N, 45°13'46.7" E and an elevation of 2611 m, Sulaymaniyah, Iraqi Kurdistan, aiming to focus on the phytochemical profiling, antioxidant, and anticancer activities of this plant. The results showed that the methanolic extract of this plant contained total phenols at 61.45 mg GA/g, while total flavonoids reached 32.51 mg rutin/g. The plant extract also contained total glycosides (18.9 mg securidaside/g), total saponins (25.5 mg/g), and total alkaloids (23.5 mg/g). By using advanced HPLC technology, ferulic acid, p-coumaric acid, apigenin, catechin, gallic acid, and kaempferol at concentrations (30.7, 491.3, 307.1, 663.1, 256.5, and 384.1 µg/g), respectively, were detected, with a dominance of catechin, followed by p-coumaric acid. The volatile oil extracted by hydro-distillation, Clevenger apparatus, and GC-MS technology was found to contain 16 active compounds, with the highest concentration being the α-pinene compound at a concentration of 12.33%, followed by myrcene (9.15%). Furthermore, strong antioxidant effects were observed. The IC50 value of the extract was 62 µg/mL compared to 250 µg/mL for ascorbic acid. The results of cytotoxicity testing using the Cell Line technique depicted that the methanolic extract showed a highly significant anticancer activity against MDA-MB231 cells at a concentration of 10000 µg/mL after 72 h of incubation. Based on these comprehensive results, we conclude that there were very high concentrations of active compounds present in the extract of this plant, in addition to its high antioxidant and anti-breast cancer activities, making the plant a promising natural source containing powerful antioxidants and anti-breast cancer agents.

Keywords: *Artemisia haussknechtii*; Antioxidant; Anticancer; Phenols; Volatile oils.

INTRODUCTION

Research on *Artemisia* species' chemistry and biological activity has gained more attention recently. This is certainly related to the 2015 Nobel Prize in Medicine, which was given for discovering artemisinin. This sesquiterpenoid lactone effectively treats malaria and is present in *Artemisia annua* (annual mugwort) [1].

The greatest genus of aromatic plants in the Asteraceae family, which is the most prominent flowering plant family, is *Artemisia*. More than 500 different plant species were included in this genus, including grasses, shrubs, and occasionally trees [2]. The *Artemisia* species are found in temperate regions of North America, the Mediterranean, Asia, Africa, and Australia. Some species are widely dispersed, while others have a limited range. The species have grown from sea to high mountains, from arid regions to marshes [3]. Wormwood, mugwort, and sagebrush are common names for *Artemisia* species [1]. In the Kurdistan region of Iraq, this plant is known locally in Kurdish as "Gyaband".

The Flora of Iraq [4] documented 8 species distributed across all physiographic regions and districts of Iraq, which are *Artemisia vulgaris, A. splendens, A. haussknechtii, A. campestris, A. absinthium, A. scoparia, A. sieberi,* and *A. jordanica.* A study carried out by Rechinger [5] in Flora of Iranica mentioned only one species, which is *Artemisia maderaspatana*. While the Flora of Turkey [6] revealed 22 species belonging to the genus *Artemisia*.

In many nations, the plants in the genus *Artemisia* are extensively utilized in supplementary medicine systems and folklore. Furthermore, this genus has many plants used in contemporary medicine to treat various conditions, including anxiety, depression, epilepsy, fever, gastrointestinal disorders, hepatitis, inflammatory illnesses, insomnia, irritability, malaria, psychoneurosis, and stress [7]. Numerous biological actions, such as antioxidant, antiseptic, anti-spasmodic, anti-rheumatic, anti-cancer, anti-nociceptive, anti-obesity, anti-fungal, antibacterial, antiviral, anti-coagulation, hepatoprotective, and COVID-19 [8] are possessed by the *Artemisia* species.

Their volatile oil content, which is found in use in medicine, cosmetics, spices, drinks, and food preparation, is partly responsible for the activities of the plants in this genus. Alongside, there are edible *Artemisia* species. Apart from additional secondary metabolites such as lignin, tannins, alkaloids, coumarins, carotenoids, flavonoids, phenolic acids, glycosides, and saponins [9].
Treatment plans are based on the kind and rate of progression of breast cancer, which is still one of the most common and serious cancers in women. Standard treatments like immunotherapy, radiation, chemotherapy, and surgery are frequently employed, but they all have drawbacks [10]. Medicinal plants have long been used to treat various illnesses, especially those that contain cytotoxic secondary metabolites. The use of these plant-based chemicals in cancer treatment has gained attention in recent years. Additionally, the genus *Artemisia* is notable among therapeutic plants because of its many bioactive chemicals, which have antiviral, antibacterial, antifungal, antimalarial, anti-inflammatory, antioxidant, and anticancer effects. Notably, several investigations have shown that artemisinin and its derivatives have anticancer potential [11]. Monoterpenes, terpenes, and phenolic compounds are the main anticancer chemicals found in *Artemisia*. These compounds are known to cause cancer cells to undergo apoptosis utilizing caspase activation, depolarization of the mitochondrial membrane potential, and downregulation of BCL-2 expression [12]. Research has demonstrated that exposure to extracts from *Artemisia* results in common apoptotic alterations across a range of cell lines, such as cell shrinkage and chromatin condensation. *Artemisia* species are still being investigated for their medicinal potential, especially in cancer treatment, because they contain strong bioactive chemicals [13].
Due to the high concentrations of active compounds in the *Artemisia* plant, its multiple medicinal uses, and its widespread use in Kurdistan, Iraq, this study aimed to collect the plant, analyze its contents of active compounds, study its antioxidant activity and its effectiveness against breast cancer using the MDA-MB231 cell line.

Material and Methods

Plant Collection and Identification

Aerial parts of *Artemisia haussknechtii* were collected from a wild population grown in the Piramagroon mountain at an altitude of 2611 m in June 2023 in Sulaymaniyah city, Iraq. The latitude of the sampling place is 35°46'21.6" N, and the longitude is 45°13'46.7" E. The plant was identified at the Iraqi National Herbarium by the Taxonomist Ali Haloob Kadhim.

Extraction

The aerial plant parts were thoroughly cleaned and air-dried at room temperature (25 °C), then ground using an electric blender before being stored at 4 °C. Specifically, 100 g of the powdered plant material was soaked in 1000 mL of 80% methanol. The mixture was placed in a water bath at 38 °C for approximately two hours, shaking every 15 minutes. Afterwards, it was left to stand at room temperature for 22 h. Finally, the extract solution was filtered through the Whatman No. 1 filter paper. The methanol extract was concentrated by evaporating the solvent at 38 °C until it reached a constant weight, and the resulting extract was stored at 4 °C [14].

Estimation of Total Phenolic Compounds

Total phenolic content was determined in the methanolic extract with the Folin-Ciocalteu reagent described by [15] with minor modifications. 100 µL of extract (1 mg/mL methanol) was mixed with 4 mL of 10% Folin-Ciocalteu reagent and allowed to react for 5 min. at room temperature (RT). After that, 2 mL of 20% sodium carbonate solution was added and left for 60 min at RT. Concerning the blank, the same steps were repeated except for the extract. The measurement was done by using a spectrophotometer at 765 nm. The total amount of phenolic compounds was represented in milligrams of gallic acid equivalent (GAE) (Sigma-Aldrich, Germany) per gram of dried extract.

Determination of Total Flavonoids

The total flavonoid content of the crude extract was determined by the aluminum chloride colorimetric method [16]. In brief, 0.5 mL of crude extract (1 mg/mL methanol) was made up to 1 mL with methanol, mixed with 4 mL of distilled water, then 0.3 mL of 5% $NaNO_2$ solution was added followed by 0.3 mL of 10% $AlCl_3$ solution which was added after 5 min of incubation. After standing for 6 min., 2 mL of 4% NaOH solution was added, and the mixture's final volume was adjusted to 10 mL with double-distilled water. Concerning the blank, the same above steps were repeated except for the extract. After a 15-minute incubation at room temperature, absorbance was measured at 510 nm. The total flavonoid content was calculated from a calibration curve, and the result was expressed as mg rutin equivalent per g dried extract.

Determination of Total Glycosides

To assess the glycoside content, 1 mL of extract (1 mg/mL methanol) was mixed with 1 mL of freshly prepared Baljeet's reagent, which consists of (95 mL of 1% picric acid and 5 mL of 10% NaOH). After allowing the mixture to react for one hour, 20 mL of distilled water was added to dilute the solution. The absorbance was then measured at 495 nm using a spectrophotometer. The total glycoside content was determined from a standard curve and expressed as milligrams of securidaside per gram of dried extract [17].

Estimation of Total Saponins

The total saponin content in the plant powder was assessed using the method described in [18]. Five grams of the powder were combined with 50 mL of 20% ethanol and then heated at 55 °C for 90 min. in a water bath. The solution was filtered through Whatman filter paper No. 42, and the remaining residue was extracted again with another 50 mL of 20% ethanol. The two extracts

were mixed, reduced to about 40 mL at 90 °C, and combined with 40 mL of diethyl ether in a separating funnel. This process was repeated until the aqueous layer became clear. 60 mL of normal butanol was used to extract the saponins. The combined extracts were washed with a 5% sodium chloride (NaCl) solution and evaporated to dryness in a pre-weighed dish. After drying at 60 °C in an oven, the sample was weighed again after cooling in a desiccator. This process was repeated two additional times to get an average. The saponin content was calculated as a percentage of the original sample using the following formula:

Percentage of saponins = [(W2 - W1) / Weight of sample] x 100

W1 = weight of the empty evaporating dish

W2 = weight of the evaporating dish with the dried sample

Estimation of Total Alkaloids

To assess the alkaloid content, 1mL of the extract (1mg/1mL methanol) mixed with 5 mL of 2N hydrochloric acid, filtered, and 1 mL of the resulting solution was transferred to a separation funnel. To this, 10 mL of chloroform was added, and the mixture was shaken thoroughly. The solvent was removed, and the pH of the solution was adjusted to neutral using 0.1 N sodium hydroxide. Following this, 5 mL of Bromocresol Green (BCG) solution and 5 mL of a buffered phosphate solution were added, and the absorbance was measured at a wavelength of 470 nm. The total alkaloid content was calculated from a calibration curve, and the result was expressed as mg atropine equivalent per g dried extract [19].

HPLC Analysis of Plant Extract

Extraction and Isolation of Phenolic Compounds by HPLC

For the HPLC analysis, 100 mL of chloroform was added to 20 grams of powdered plant material and the mixture was vibrated electrically for 3 h to remove fats. The chloroform layer was separated using a funnel, and the sample was dried at 38 °C to ensure no chloroform residue remained. Subsequently, 10 grams of the dried sample were extracted with 100 mL of 70% methanol using an ultrasonic bath at room temperature for 1 h. After extraction, the solvent was removed under vacuum using a rotary evaporator, and the residue was dried again at 38 °C. The phenolic compounds were quantified using reversed-phase HPLC, which involved a chromatographic system with a UV detector, chemstation software, binary pump, autosampler, online vacuum degasser, and a Zorbax Eclipse Plus-C18-ODS column. A gradient elution was performed with methanol (eluent A) and 1% formic acid in water (eluent B), where the composition started at 40% B (0-4 minutes) and shifted to 50% B (4-10 minutes). The analysis was carried out at 30 °C with a flow rate of 0.7 mL/min, and a 100 µL sample or standard was injected automatically by the autosampler. Spectral data were recorded at 280 nm [20].

Extraction and Analysis of Volatile Oils by GC-MS

For oil extraction, 20 g of the dry weight of the aerial plant part was placed in a 2-litre flask containing distilled water (1000 mL). The Clevenger set was placed under optimal operating conditions for 3 h. The essential oil was collected, and then 20 mL of hexane was added to separate the oil from the water droplets collected with the oil. The oil was collected and stored in the refrigerator until the analysis process [21].

The essential oil was analyzed using a GC-MS (Agilent 5977A Series) equipped with an auto-sampler and a mass spectrometer. The analysis was performed under the following conditions: A 30 m × 0.25 mm internal diameter Elite-1 fused silica capillary column (HP-5MS) was used, operating in electron impact mode at 70 eV. Helium (99.999%) served as the carrier gas at a constant flow rate of 1 mL/min, and a 0.5 µL injection volume was applied with a split ratio of 10:1. The injector temperature was set at 250 °C. In contrast, the ion-source temperature was maintained at 280 °C. The oven temperature was programmed as follows: starting at 60 °C (held isothermally for 2 min.), then increasing at 10 °C/min. to 270 °C, followed by a 5 °C/min. increase to 290 °C, and concluding with a 9-min. Isothermal hold at 310 °C. Mass spectra were recorded at 70 eV with a scan interval of 0.5 seconds and a mass range of 45 to 450 Da. The total running time for the GC analysis was 60 min. [22].

Antioxidant Activity

The DPPH assay was performed based on a modified version of the method described by [23]. To evaluate the antioxidant activities of ascorbic acid and the extract, they were dissolved in 95% methanol at various concentrations (30, 60, 120, 250, 500 µg/ml). Then, 50 µL of each solution was mixed with 1950 µL of a DPPH solution (6×10^5 M). The mixture was shaken thoroughly and incubated at room temperature in the dark for 30 min. A control was prepared by combining 50 µL of 95% methanol with 1950 µL of methanolic DPPH. The absorbance of the solution was measured at 515 nm. The percentage of antioxidant activity was calculated using the following formula:

Antioxidant activity (%) = (Absorbance of control - Absorbance of the sample) / (Absorbance of control) × 100.

The IC50 value, which indicates the concentration required to inhibit 50% of the DPPH free radicals, was determined based on the relationship between antioxidant concentration and its inhibition capacity.

Cell Line Cytotoxicity Assays

The crystal violet cell viability assay was employed to evaluate the cytotoxic effects of the plant extract. Human breast cancer cells (MDA-MB-231) were seeded at a density of 7,000 cells per well in 96-well plates. After 24 hours of incubation, or once a confluent monolayer was formed, the cells were treated with the plant extract at two-fold dilutions (78.125, 156.25, 312.5, 625, 1250, 2500,

5000, and 10000 µg/mL) in culture media. The assay was performed in triplicate. Cell viability was assessed at 48 and 72 h post-treatment by staining the cells with 50 µL of crystal violet and incubating at 37 °C for two hours. The stain was then removed, and the wells were washed with PBS. Absorbance was measured at 492 nm using a microplate reader. The results were expressed as the percentage of cell proliferation relative to the control group (the control group was not treated with plant extract) [24].

The inhibitor rate was calculated following the method described by Ghaidan *et al*. [25] using the formula:

Inhibitor rate (%) = (Abs. Control−Abs. test**)** / (Abs. control) ×100

Statistical Data Analysis

The statistical software package. XLSTAT (Version: 2019.2.2), was used to perform statistical analysis. For the cell line experiment, a Complete Randomized Design (CRD) was conducted on one factor with 5 replications. Means were compared by Duncan's new Multiple Range Test ($P \leq 0.01$).

Results and Discussion

Figure 1 shows the concentrations of total phenols, flavonoids, alkaloids, glycosides, and saponins in the dry extract of the wild *Artemisia haussknechtii*. Total phenols are expressed as milligrams of gallic acid equivalent per gram of dry extract (mg GAE/g). The results indicate a moderate phenolic content in the dry plant extract, measuring 61.45 mg GAE/g. This finding contrasts with another study, which reported lower phenolic levels of 35.81 mg GAE/g in ethyl acetate extracts of the *A. ifranensis* [26]. Conversely, a different investigation indicated a much higher total phenolic content of 125.04 mg GAE/g in ethanolic extracts of *A. vulgaris* and 68.22 mg GAE/g in ethanolic extracts of *A. absinthium* [27].

The flavonoid content was measured in milligrams of rutin equivalent per gram of dry extract (mg RE/g), with the analysis revealing a total flavonoid content of 32.51 mg RE/g. Saunoriūtė *et al.* [28] found that the total flavonoid content in *A. abrotanum* and *A. absinthium* during vegetative stages ranged from 3.37 to 8.61 mg RE/g DW. Trifan *et al.* [29] reported that flavonoid levels in the roots and aerial parts of *A. absinthium* ranged from 0.37 to 28.74 mg RE/g. In comparison with our study, there were different flavonoid concentrations in *Artemisia* species, possibly due to factors like species, plant part used, extraction method, environmental conditions, and geographical locations.

Regarding the concentration of total glycosides, the glycoside content is expressed as milligrams of securidaside per gram of dry extract (mg securidaside/g). The methanolic extract showed a total glycoside content of 18.9 mg Securidaside/g. There are many factors influencing glycoside content, like the extraction method; the choice of extraction method significantly affects the yield of glycosides. For instance, classical extraction techniques have been shown to yield higher glycoside content compared to methods like Soxhlet, microwave, and ultrasonic-assisted extraction [30] the solvent sed. used; The polarity of the solvent influences the extraction efficiency of glycosides. Acetone and methanol are commonly used solvents that can effectively extract glycosides from *Artemisia* species [31].

The saponin content in the plant powder extract was found to be 25.5 mg/g. These results are similar to the study by Kumar *et al.* [32] conducted on *A. vulgaris*, where they found that the total saponin content was 2.8%**,** indicating that the saponin content in the samples is comparable to the results obtained in our study.

Additionally, the concentration of total alkaloids is measured in milligrams of atropine per gram of dry extract (mg atropine/g). The methanolic extract revealed a total alkaloid content of 23.5 mg atropine/g DW. In a study conducted by Kumar *et al.* [32] on *A. vulgaris* plant, it was found that the total alkaloid concentration was 1.5%.

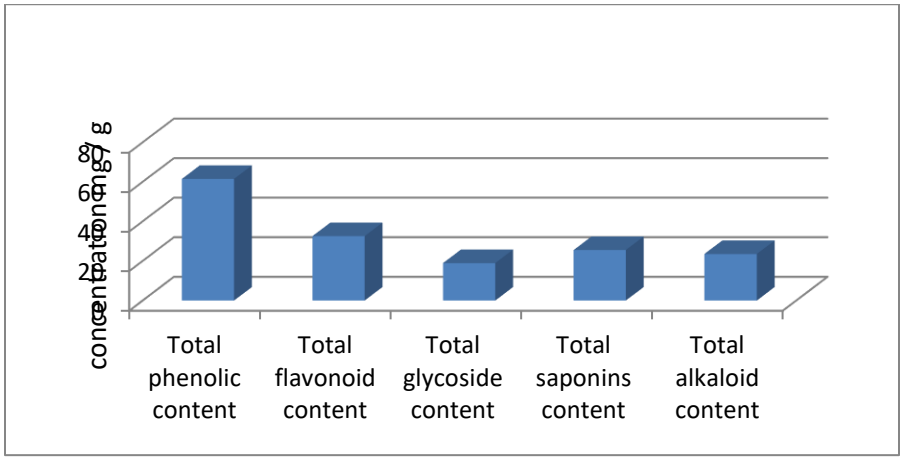


Figure 1. The total content of various components in *Artemisia haussknechtii* extract

HPLCAnalysis

The quantitative analysis of *Artemisia haussknechtii* by HPLC identified several phenolic compounds, including ferulic acid, p-coumaric acid, apigenin, catechin, gallic acid and kaempferol (Fig. 2). The predominant compounds were catechin (663.1 µg/g) and p-coumaric acid (491.3 µg/g), followed by kaempferol (384.1 µg/g) and apigenin (307.1 µg/g), then gallic acid (256.5 µg/g) and ferulic acid was present in the lowest amount at 30.7 µg/g.

These results are consistent with the findings of Hussain *et al.* [33] in their study on *Artemisia absinthium*, as it contained numerous flavonoids, including quercetin, kaempferol, apigenin, artemisinin, and rutoside and numerous phenolic acids such as chlorogenic, ferulic, gallic, caffeic, syringic, and vanillic, and derivatives of caffeoylquinic acid. Other compounds found in smaller amounts are the chalcone—cardamonin, coumarins (herniarin, coumarin, fatty acids, tannins, carotenoids, and lignan).

When determining the concentrations of phenolic compounds in plants using (HPLC), several key factors influence the accuracy and reliability of the results: environmental and physiological factors (plant species, growth stage, environmental conditions), extraction method (solvent type, pH level, temperature) and HPLC analysis conditions (column type, mobile phase composition, flow rate and column temperature). To achieve accurate measurements of phenolic compounds in plants using HPLC, it is essential to meticulously control these factors during sample collection, extraction processes, and analytical procedures [34].

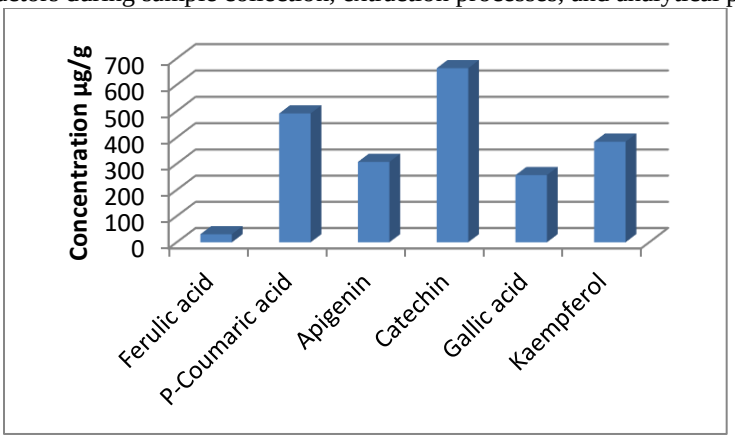


Figure 2. HPLC analysis for phenolic contents of *Artemisia haussknechtii* methanolic extract

GC-Ms Analysis

GC-MS analysis was conducted to identify the most effective compounds in the volatile oils of *Artemisia haussknechtii*. The results revealed a total of 16 compounds (Table 1) among these; α-pinene was the most abundant compound with 12.33% followed by myrcene at 9.15%, linalool at 6.25%, then camphene 5,49%, g-terpinene at 5.49%, nerol 5.12%, limonene 4.89%, eucalyptol 4.11%, camphor 3.25%, p-cymene 2.56%, β-caryophyllene 2.14%, caryophyllene 1.15%, α-thujone 0.59% and the least value was thymol 0.11%.

Table 1. Chemical compounds in Artemisia haussknechtii essential oils were identified by GC-MS analysis.

Peak#	Ret.Time	Area	Con %	Name
1	1.793	4164883388		
2	5.513	107376	5.49	g-Terpinene
3	6.240	2679885	12.33	α-Pinene
4	8.668	457911	9.15	Myrcene
5	10.736	848008	5.49	Camphene
6	12.552	577463	4.11	Eucalyptol
7	14.186	1145725	3.25	Camphor
8	14.978	533828	2.14	β-caryophyllene
9	15.685	976828	1.15	Caryophyllene
10	16.347	1176502	0.59	α-Thujone
11	17.036	327764	1.44	Eugenyl acetate
12	18.164	3151660	4.89	Limonene
13	18.601	2250767	5.12	Nerol
14	19.229	6488713	6.25	Linalool
15	20.581	1498772	2.56	P-cymene
16	20.923	6129838	0.11	Thymol

Total		4193234428			

Based on GC-MS analysis of *A. absinthium*, the results indicated that a total of 34 compounds representing 99.98% of the essential oil of the plant were identified; among them, cis-davanone was found at the highest concentration (52.51%) compared to the other constituents. In addition, α-gurjunene (7.15%), chamazulene (3.38%), camphene (3.27), γ-eudesmol (2.49%), pinocarvone (2.18%), and ocimenone (2.03%) were also identified as major constituents of the plant's essential oils. The total percentage of cis-davanones (53%) was the highest in the plant species growing elsewhere globally [35].

Antioxidant Activity

The methanolic extract demonstrated a significant capacity to neutralize DPPH radicals (Fig. 3). Antioxidant activity increased with higher concentrations of *Artemisia haussknechtii* methanolic extract and ascorbic acid. The maximum antioxidant activity was observed at a concentration of 500 µg/mL, with the plant extract achieving 91.2% and ascorbic acid reaching 71.59%. The lowest concentration of *Artemisia haussknechtii* extract inhibited 50% of the DPPH radicals was 62 µg/mL, while the IC50 for ascorbic acid was 250 µg/mL.

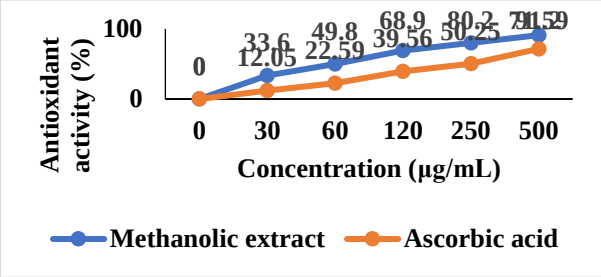


Figure 3. Antioxidant activity (%) of *Artemisia haussknechtii* methanolic extract

Since IC50 is the measure of inhibitory concentrations, a lower IC50 value would reflect the greater antioxidant activity of the sample. Hence, the ethanolic extract of *A. haussknechtii* displayed higher antioxidants with lower IC50 values of 0.15 mg/mL [36]. In another study, Elazzouzi *et al.* [26] found that the IC50 of *A. ifranensis* was 0.656 mg/mL.

Reactive oxygen species (ROS) are modulated uniquely by phytochemicals and their derivatives, extracts, and essential oils from the *Artemisia* plant. They have potent antioxidants and radical scavenging properties against hydrogen peroxide and hydroxyl ions, offering superior protection by boosting the antioxidant defence system and lowering the production of ROS. The process entails attaching to ferrous iron (heme, for example) and producing ROS, which can have cytotoxic or cytostatic consequences. Additionally, the generation of ROS can cause cellular damage by lipid peroxidation, pro-apoptotic pathway activation, or instability of mitochondrial and genomic DNA. These characteristics demonstrate the potential therapeutic use of *Artemisia* chemicals in oxidative stress-related diseases [37].

Effect of *Artemisia haussknechtii* on Human Breast Cancer Cell Line MDA-MB231 *In vitro*

Figure 4 shows the cytotoxic effect of different concentrations of *Artemisia haussknechtii* on the cancer cell line MDA-MB231 in vitro after an incubation period of 48 and 72 h. The results showed significant differences in cell growth inhibition among the concentrations used. The alcoholic extract recorded the highest percentage of cell growth inhibition at the concentrations of 5000 µg/mL and 10000 µg/mL. At the same time, it did not show a significant effect on cell viability ($P \leq 0.01$) at the low concentrations of 78.125 and 156.25 µg/mL. The results denote a significant effect of the incubation period on cell vitality after 48 h, giving the inhibition rates of 44.7% and 62.8%, respectively, while the highest effect of the *Artemisia haussknechtii* on the cells was after 72 h of incubation, reaching 53.2 and 78.9%, respectively, for the same concentrations (Fig. 4). The anti-proliferation was time dependent. The secondary metabolites, such as terpenoids, phenolic compounds, coumarins, alkaloids, and flavonoids, are types of phytochemical compounds that can be responsible for cytotoxic effects on cancer cell lines in the genus *Artemisia* [38]. Through their antioxidant properties, which include causing cell death, stopping the cell cycle, preventing angiogenesis, and neutralizing free radicals, medicinal herbs and their bioactive components exhibit encouraging anti-cancer benefits. Because of these characteristics, plant extracts and chemicals are useful for creating novel cancer therapies [39]. Given their effectiveness and safety, researchers are currently concentrating on ARTs that seem broad-spectrum antitumor agents [40].

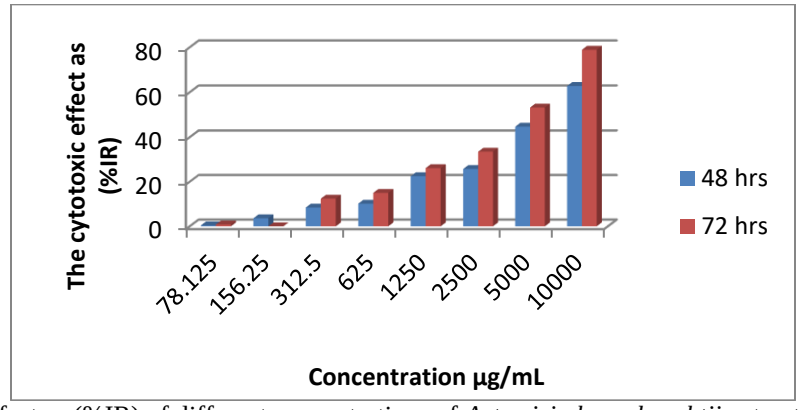
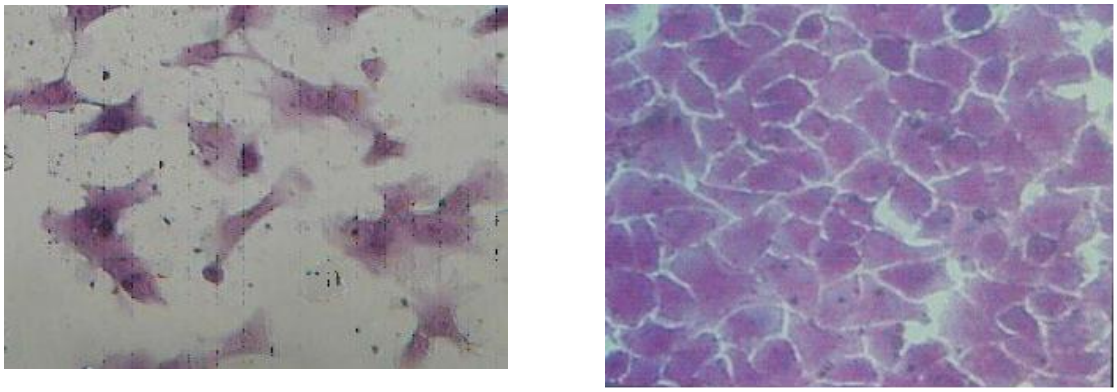


Figure 4. The cytotoxic effect as (%IR) of different concentrations of *Artemisia haussknechtii* extract on MDA-MB231 cell line at 48 and 72 h



A **B**

Figure 5. Comparison between cells in the control sample with another sample treated with *Artemisia haussknechtii* extract at a concentration of 10000 µg/mL for 72 h (Crystal violet, 100x). A- Control (without treatment), B- Cell treatment with *Artemisia haussknechtii* extract after 72 h

Conclusions

Based on the results obtained, it was concluded that *A. haussknechtii* represents a promising low-cost, phytochemical-rich, antioxidant, and might be a potential source of new compounds limiting the development of breast cancer.

References:

[1]. Szopa, A., Pajor, J., Klin, P., Rzepiela, A., Elansary, H.O., Al-Mana, F.A., Mattar, M.A. and Ekiert, H., 2020. *Artemisia absinthium* L. importance in the history of medicine, the latest advances in phytochemistry and therapeutical, cosmetological and culinary uses. Plants, 9(9), p.1063. https://doi.org/10.3390/plants9091063.

[2]. Mohammed, H.A., Eldeeb, H.M., Khan, R.A., Al-Omar, M.S., Mohammed, S.A.A., Sajid, M.S.M., Aly, M.S.A., Ahmad, A.M., Abdellatif, A.A.H. and Eid, S.Y., 2021. Sage, *Salvia officinalis* L., constituents, hepatoprotective activity, and cytotoxicity evaluations of the essential oils obtained from fresh and differently timed dried herbs: A comparative analysis. Molecules, 26(19), p. 5757. https://doi.org/10.3390/molecules26195757.

[3]. Koul, B. Taak, P., Kumar, A., Khatri, T. and Sanyal, I., 2017. The *Artemisia* genus: A review on traditional uses, phytochemical constituents, pharmacological properties, and germplasm conservation. Journal of Glycomics and Lipidomics, 7(1), p. 1000142. https://doi.org/10.4172/2153-0637.1000142.

[4]. Ghaznfar, S.A., Edmondson, J.R. and Hind, J.N., 2019. Flora of Iraq. Vol. 6. Ministry of Agriculture and Agrarian Reform of Iraq.

[5]. Rechinger, K. H., 1963. Flora of the Iranian highlands and surrounding mountains: Persia, Afghanistan, parts of western Pakistan, Northern Iraq, Azerbaijan, Turkmenistan. Akad. Druck- und Verl.-Anst, Graz.

[6]. Davis, P. H., 1970. Flora of Türkiye and the East Aegean Islands. Vol. 3. 645 pp.

[7]. Mohmmed, H.A., Qureshi, K.A., Ali, H.M., Al-Omar, M.S., Khan, O. and Mohammed, S.A.A., 2022. Bio-evaluation

of the wound healing activity of *Artemisia judaica* L. as part of the plant's use in traditional medicine; phytochemical, antioxidant, anti-inflammatory, and antibiofilm properties of the plant's essential oils. Antioxidants, 11(2), p. 332. https://doi.org/10.3390/antiox11020332.

[8]. Qiao, Y., Wu, L., Yang, S., Wang, Q., Gu, H., Wei, L., Liu, G., Zhou, S., Wang, P. and Song, M., 2023. Metabolomic and transcriptomic analyses provide insights into variations in flavonoids contents between two *Artemisia* cultivars. BMC Plant Biology, 23(1), p. 288. https://doi.org/10.1186/s12870-023-04295-8.

[9]. Bisht, D., Kumar, D., Dua, K. and Chellappan, D.K., 2021. Phytochemistry and pharmacological activity of the genus *Artemisia*. Archives of Pharmacal Research, 44(5), p.439. https://doi.org/10.1007/s12272-021-01328-4.

[10]. Sung, H., Ferlay, J., Siegel, R.L., Laversanne, M., Soerjomataram, I., Jemal, A. and Bray, F., 2021. Global Cancer Statistics. 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: A Cancer Journal for Clinicians, 71(3), p.209. https://doi.org/10.3322/caac.21660.

[11]. Wen, L., Chan, B.C.L., Qiu, M.H., Leung, P.C. and Wong, C.K., 2024. Artemisinin and its derivatives as potential anticancer agents. Molecules, 29(16), p. 3886.

[12]. Anibogwu, R., Jesus, K.D., Pradhan, S., Pashikanti, S., Mateen, S. and Sharma, K., 2021. Extraction, isolation, and characterization of bioactive compounds from *Artemisia* and their biological significance: A review. Molecules, 26(22), p. 6995. https://doi.org/10.3390/molecules26226995.

[13]. Kamarya, Y., Lijie, X. and Jinyao, L., 2022. Chemical constituents and antitumor mechanisms of *Artemisia*. Anti-Cancer Agents in Medicinal Chemistry, 22(10), p.1838. https://doi.org/10.2174/1871520621666210708125230.

[14]. Yousif, A.A. and Hassan, W.A., 2023. HPLC analysis and antifungal activity of some plant extracts against decay apple fruits. Iraqi Journal of Agricultural Sciences, 54(1), p.291.

[15]. Ahmad, Z.M., Hamzah, H.M. and Lazim, Z.S., 2023. Analysis of chemical profiles of different *Pistacia atlantica* parts at Sulaymaniyah and Halabja region in Iraq. Applied Ecology and Environmental Research, 21(1), p.561. DOI: 10.15666/aeer/2101_561574

[16]. Lammam, S.N. and Mahmood, Z.F., 2023. Activity of *Marticaria chamomilla* crude and total flavonoid extracts as anti-virulence factor for clinically isolated *Pseudomonas aeruginosa*. Iraqi Journal of Agricultural Sciences, 54(1), p.59.

[17]. Hassan, A.Y., 2023. The effect of spraying with the hormone brassinolide on the production of oil and glycosides of goat pea (*Securigera securidaca*). In IOP Conference Series: Earth and Environmental Science, IOP Publishing, 1214(1), p. 012046. https://doi.org/10.1088/1755-1315/1214/1/012046.

[18]. Lazim, Z.S., Ahmad, Z. and Faizy, H., 2024. Estimating phytochemical content, antioxidant, and antibacterial efficacy of wild *Adiantum capillus-veneris*. Anbar Journal of Agricultural Sciences, 22(2), p. 981. https://doi.org/10.32649/ajas.2024.184464.

[19]. Ajanal, M., Gundkalle, M.B. and Nayak, S.U., 2012. Estimation of total alkaloid in Chitrakadivati by UV Spectrophotometer. Ancient Science of Life, 31(4), p.198.

[20]. Al-Zubaidy, A.M.A., Hassan, K.I. and Jabbari, B.S., 2016. Analysis of naturally occurring phenolic compounds of the genera *Clinopodium* L., Hymen crater Fish, C. A. Mey. and Melissa L. Iraqi Journal of Agricultural Sciences, 47(1), p.379.

[21]. Lazim, Z.S., Jasim, S.N. and Mustafa, H.A., 2022. Effect of bat guano fertilizer and drying methods on growth and volatile oil yield of geranium plant (*Pelargonium graveolens* L.). Indian Journal of Ecology, 49(20), p.52.

[22]. Eswaran, R., Anandan, A., Doss, A., Sangeetha, G. and Anand, S.P., 2012. Analysis of chemical composition of *Cissus quadrangularis* Linn. by GC-MS. Asian Journal of Pharmaceutical and Clinical Research, 5(2), p. 139.

[23]. Alsaloom, A.N., 2023. Testing and evaluation of bioactive compounds in soybean. Iraqi Journal of Agricultural Sciences, 54(1), p. 85-92. https://doi.org/10.36103/ijas.v54i1.1678.

[24]. Almzaien, A.K., Mohammad, M.H. and Al-Shammari, A. M., 2022. Cytotoxicity of ethanolic extract of *Petroselinum sativum* against esophageal cancer cell lines. International Journal of Cancer Management and Genetics, 15 (1), p. 15.

[25]. Ghaidan, H., Stenlo, M., Niroomand, A., Mittendorfer, M., Hirdman, G., Gvazava, N., Edström, D., Silva, I.A.N., Broberg, E., Hallgren, O., Olm, F., Wagner, D.E., Pierre, L., Hyllén, S. and Lindstedt S., 2022. Reduction of primary graft dysfunction using cytokine adsorption during organ preservation and after lung transplantation. Nature Communications, 13(1), p. 4173. https://doi.org/10.1038/s41467-022-31811-5.

[26]. Elazzouzi, H., Zekri, N., Zair, T. and El Belghiti, M.A., 2022. Total phenolic and flavonoid contents of *Artemisia iranensis* J. Didier plant extracts and their antioxidant activity. Journal of Medical Pharmaceutical and Allied Sciences, 11(1), p. 4419. https://doi.org/10.22270/jmpas.V11I1.2642.

[27]. Teterovska, R., Sile, I., Paulausks, A., Kovalcuka, L., Koka, R., Maurin, B. and Bandere, D., 2023. The antioxidant activity of wild-growing plants containing phenolic compounds in Latvia. Plants, 12(24), p. 4108. https://doi.org/10.3390/plants12244108.

[28]. Saunoriūtė, S., Ragažinskienė, O., Ivanauskas, L., Marksa, M., Laužikė, K. and Raudonė, L., 2023. Phenolic diversity and antioxidant activity of *Artemisia abrotanum* L. and *Artemisia absinthium* L. during vegetation stages. Separations, 10(10), p. 545. https://doi.org/10.3390/separations10100545.

[29]. Trifan, A., Zengin, G., Sinan, K.I., Sieniawska, E., Sawicki, R., Maciejewska-Turska, M., Skalikca-Woźniak, K. and Luca, S.V., 2022. Unveiling the phytochemical profile and biological potential of five *Artemisia* Species. Antioxidants, 11(5), p. 1017. https://doi.org/10.3390/antiox11051017.
[30]. Khan, F.A., Khan, N. M., Ahmad, S., Nasruddin, Aziz, R., Ullah, I., Almehmadi, M., Allahyani, M., Alsaiari, A.A. and Aljuaid, A., 2022. Phytochemical profiling, antioxidant, antimicrobial and cholinesterase inhibitory effects of essential oils isolated from the leaves of *Artemisia scoparia* and *Artemisia absinthium*. Pharmaceuticals, 15(10), p. 1221. https://doi.org/10.3390/ph15101221.
[31]. Salih, A.M., Qahtan, A.A. and Al-Qurainy, F., 2023. Phytochemicals identification and bioactive compounds estimation of *Artemisia* species grown in Saudi Arabia. Metabolites, 13(3), p. 443. https://doi.org/10.3390/metabo13030443.
[32]. Kumar, G.S., Venkataramaiah, C. and Rajendra, W., 2018. Qualitative and quantitative phytochemical profile and in vitro antioxidant activity of methanolic extract of *Artemisia vulgaris*. The Pharma Innovation Journal, 7(9), p. 348.
[33]. Hussain, M., Raja, N.I., Akram, A., Iftikhar, A., Ashfaq, D., Yasmeen, F., Mazhar, R., Imran, M. and Iqbal, M., 2017. A status review on the pharmacological implications of *Artemisia absinthium*: A critically endangered plant. Asian Pacific Journal of Tropical Disease,7(3), p. 185. https://doi.org/10.12980/apjtd.7.2017D6-385.
[34]. Abdul Karim, S., Al-Hilifi, S.A.H. and George, S.S., 2024. Phenolic compounds in plants: Extraction, analysis, and biological activity. Humanities and Natural Sciences Journal, 5(8), p. 261. https://doi.org/10.53796/hnsj58/17.
[35]. Mohammed, H.A., 2022. Phytochemical analysis, antioxidant potential, and cytotoxicity evaluation of traditionally used *Artemisia absinthium* L. (wormwood) growing in the Central Region of Saudi Arabia. Plants, 11(8), p. 1028. https://doi.org/10.3390/plants11081028.
[36]. Khanahmadi, M., Rezazadeh, S., Shahrezaei, F. and Taran, M., 2009. Study on chemical composition of essential oil and antioxidant and anti-microbial properties of *Artemisia haussknechtii.* Journal of Medicinal Plants, 8(31), p. 132.
[37] Kshirsagar, S.G. and Rao, R.V., 2021. Antiviral and immunomodulation effects of *Artemisia*. Medicina, 57(3), p. 217. https://doi.org/10.3390/medicina57030217.
[37]. Nasseri, S., Delnavazi, M.-R., Shirazi, F.H. and Mojab, F., 2022. Cytotoxic activity and phytochemical analysis of *Artemisia haussknechtii* Boiss. Iranian Journal of Pharmaceutical Research, 21(1), p. e126917. https://doi.org/10.5812/ijpr-126917.
[38]. Forouhandeh, H., Tarhriz, V., Zadehkamand, M. and Asgharian, P., 2023. Anti-proliferative activity of *Artemisia marschalliana* on cancerous cell lines. BMC Complementary Medicine and Therapies, 23(1), p. 119. https://doi.org/10.1186/s12906-023-03887-z.
[39]. Pulito, C., Strano, S. and Blandino, G., 2020. Dihydroartemisinic: From malaria to the treatment of relapsing head and neck cancers. Annals of Translational Medicine, 8(10), p. 612.

التوصيف الكيميائي النباتي والخصائص المضادة للأكسدة والسرطان لنبات الشيح البري *Artemisia haussknechtii* في السليمانية-العراق.

زينب صباح لازم
قسم البستنة -كلية علوم الهندسة الزراعية -جامعة السليمانية -السليمانية، العراق

الخلاصة

أجريت هذه الدراسة على نبات الشيح البري الذي تم جمعه من جبل بيرەمگرون على خط عرض 35°46'21.6" شمالاً وخط طول 45°13'46.7" شرقاً وعلى ارتفاع 2611 متراً فوق سطح البحر في السليمانية، كردستان العراق، بهدف التركيز على الملف الكيميائي النباتي والأنشطة المضادة للأكسدة والسرطان. أظهرت النتائج أن المستخلص الميثانولي لهذا النبات يحتوي على إجمالي الفينولات بنسبة 61.45 ملغم حمض الجبريليك/غرام. في حين بلغ إجمالي الفلافونويدات 32.51 ملغم روتين/غرام. يحتوي المستخلص النباتي على إجمالي الغليكوسيدات (18.9 ملغم سيكيورياسيد/غرام) وإجمالي الصابونين (25.5 ملغم/غرام) وإجمالي القلويدات (23.5 ملغم/غرام). وباستخدام تقنية *HPLC* تم الكشف عن حمض الفيروليك وحمض الباراكوماريك والأبيجينين والكاتشين وحمض الغاليك والكامبفيرول بتركيزات (30.7 و491.3 و307.1 و663.1 و256.5 و384.1 ميكروجرام/جرام) على التوالي مع هيمنة الكاتشين يليه حمض الباراكوماريك. أما الزيت المتطاير المستخرج بالتقطير المائي وجهاز كلافنجر وتقنية كروماتوغرافيا الغاز-مطياف الكتلة فقد احتوى على 16 مركب نشط وكان أعلى تركيز لمركب ألفا بينين بتركيز 12.33% يليه الميرسين (9.15%). كما لوحظت تأثيرات قوية مضادة للأكسدة. وكانت قيمة *IC50* للمستخلص 62 ميكروغرام/مل مقارنة بـ 250 ميكروغرام/مل لحمض الأسكوربيك. أظهرت نتائج السمية الخلوية باستخدام تقنية *Cell Line* أن المستخلص الميثانولي أظهر نشاطًا مضادًا للسرطان عالي الأهمية ضد خلايا *MDA-MB231* بتركيز 10000 ميكروغرام / مل بعد 72 ساعة من الحضانة. بناءً على هذه النتائج، نستنتج أن هناك تركيزات عالية جدًا من المركبات النشطة في مستخلص هذا النبات، بالإضافة إلى نشاطه المضاد للأكسدة ومضاد لسرطان الثدي، مما يجعل النبات مصدرًا طبيعيًا واعدًا يحتوي على مضادات الأكسدة وعوامل مضادة لسرطان الثدي.

الكلمات المفتاحية: الشيح البري، مضاد للأكسدة ، مضاد السرطان ،الفينولات، ، الزيوت الطيارة.