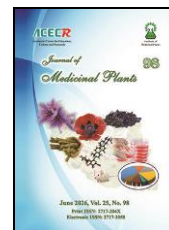




Institute of
Medicinal Plants

Journal of Medicinal Plants

Journal homepage: www.jmp.ir



Research Article

Phytochemical profiling and *in vitro* cytotoxic evaluation of alkaloids isolated from *Equisetum arvense* L. native to northern Iraq against human cancer cell lines

Hadeel M. Habeeb¹, Sumaya F. Hamad¹, Shaymaa Al-Majmaie^{2,*}, Ghussun S. Salih¹, Ismail A. Murtadeh¹, Zainab Anwar¹, Ibraheem Hadi³

¹Department of Biology, College of Science for Women, University of Baghdad, Baghdad, Iraq

²Department of Biotechnology, College of Science, University of Diyala, Diyala, Iraq

³Department of Biology, College of Science, University of Diyala, Diyala, Iraq

ARTICLE INFO

Keywords:

Equisetum arvense

Alkaloids

Cytotoxicity

Cancer cell lines

Iraqi flora

Phytochemical profiling

ABSTRACT

Background: *Equisetum arvense* L. (Horsetail) is a medicinal perennial herb widely distributed across the Northern Hemisphere. Traditionally utilized for gastrointestinal, renal, and musculoskeletal disorders, its specific alkaloid profile and associated anticancer properties (particularly regarding regional chemotypes) remain understudied. **Objective:** This study evaluated the phytochemical composition of alkaloids isolated from *E. arvense* growing in northern Iraq and evaluated their cytotoxic potential against three human cancer cell lines (HePG2, WRL68, and SK-GT2) alongside a normal cell line (REF). **Methods:** Purified alkaloids were extracted from *E. arvense* and subjected to qualitative and quantitative chemical profiling. Cytotoxicity was evaluated across a concentration range of 25 to 1600 µg/ml at multiple exposure intervals using standard cell viability assays. **Results:** Chromatographic analysis identified five distinct alkaloids: hordenine (426.4 µg/g), caulerpin (324.1 µg/g), martensine (139.3 µg/g), isoquinoline (139.1 µg/g), and almazolone (77.6 µg/g). The total alkaloid extract demonstrated concentration- and time-dependent cytotoxicity across all cancerous cell lines ($P < 0.05$). Following 24 hours of exposure at 1600 µg/ml, maximum growth inhibition was observed in HePG2 (78.90 %) and SK-GT2 (77.40 %) cells, with moderate inhibition in WRL68 cells (39.90 %). Notably, no significant cytotoxic effect was observed against the normal REF cell line. **Conclusion:** The alkaloid fraction of *E. arvense* native to northern Iraq exhibits selective cytotoxic activity against human cancer cell lines while sparing normal cells, supporting its potential as a source for natural lead compounds in oncology drug discovery.

Abbreviations: HePG2, WRL68 and SK-GT2, cancerous cell lines; RFF, Normal cells line; BHA, [butylated hydroxyl anisole; BHT, butylated hydroxyl toluene; REF, Rat Embryo Fibroblast; ICCMGR, Iraqi Center for Cancer and Genetic Research; FVS, Fetal Bovine Serum

*Corresponding author: shaymaa@uodiyala.edu.iq

doi:

Received 4 April 2024; Received in revised form 3 July 2026; Accepted 26 July 2026

© 2023. Open access. This article is distributed under the terms of the Creative Commons Attribution-NonCommercial 4.0 International License (<https://creativecommons.org/licenses/by-nc/4.0/>)

1. Introduction

Ferns have many species which have been used in dietary consumption as a vegetable or as folk medicine. In recent years, considerable research has focused on the identification and characterization of secondary metabolites present in ferns. They have also investigated the potential medical applications of these compounds [1]. *Equisetum arvense* is a member of the fern family. It is a vascular plant that belongs to the Equisetaceae family [2] distributed in Asia and Africa. The plant spreads in northern Iraq [3]. Traditionally, *Equisetum arvense* has been used to manage gastric bleeding, promote wound healing, relieve menstrual disorders, and treat rheumatic conditions. [4]. It is also used to treat various conditions such as oral infections, alcohol addiction, and thiamine deficiency [5]. Additionally, *E. arvense* is used to manage diarrhoea, urethritis, epidemic conjunctivitis, joint pain, kidney stones, and urinary tract infections. [6].

The plant has antioxidant, antibacterial, antifungal, and anticancer properties [4]. It also helps with inflammation, dizziness, headaches, tremors, and paralysis. Moreover, it is used for hyperglycemia, cerebral ischemia, memory disorders, and hepatitis [7]. The plant contains many secondary metabolites. These include flavonoids, phenols, cinnamic acid, and caffeic acid [8]. It also has essential oils, tannins, triterpenoids, and steroids. In addition, it contains various types of alkaloids. [9, 10].

Alkaloids are bioactive compounds found in plants. They are widely used as anticancer agents. The *E. arvense* plant contains several types of alkaloids. These include soquinoline, isoquinoline, hordenine, caulerpin, almazolone, and martensine. These compounds can produce strong anticancer effects. They work by

stopping the cell cycle. They also trigger apoptosis. This process leads to the death of cancer cells [11].

Reactive oxygen species (ROS) are chemically reactive molecules. That are naturally produced during cellular metabolism. When the balance between free radical production and antioxidant defenses is disturbed, oxidative stress occurs [4, 12]. Synthetic antioxidants, including butylated hydroxyl anisole (BHA), butylated hydroxyl toluene (BHT), and α -tocopherol are widely used to control ROS levels in the food and pharmaceutical industries. However, concerns regarding the potential toxicity of synthetic antioxidants have stimulated increasing interest in natural products with antioxidant activity.

Phenolic compounds can neutralize free radicals. This ability depends on their chemical structure, molecular weight, and functional groups such as hydroxyl or methoxy [12]. The biosynthesis of these secondary metabolites is a dynamic process. It largely depends on environmental conditions. It also depends on the specific traits of the plant. One important factor is the type of plant parts or organs used. Extraction is an important first step in isolating, quantifying, and identifying secondary metabolites from plants. The choice of solvent system is critical. Factors such as solvent type, purity, and polarity affect how well polyphenols and their antioxidant activities are extracted [13, 14]. Phenolic compounds have different levels of stability and solubility. These can vary depending on the solvent used. This makes selecting the right solvent a complex task. It requires careful thought and cannot be taken lightly. Therefore, it is necessary to investigate which solvents work best for extracting polyphenols from each plant species.

In this study, the aim was threefold. First, to evaluate how well different solvents recover phenolic compounds from two parts of *S. tebesana*. Second, to measure antioxidant properties and the content of phenolic subgroups using these solvents. Third, to analyze and quantify individual flavonoids using HPLC for the first time.

2. Materials and methods

2.1. Plant collection

Plant materials were collected from northern Iraq, dried and ground before use. The samples were confirmed and identified by Prof. Dr. Bahram Khidhr Maulood, a botanist in the College of Science at the University of Salahaddin. Voucher specimens (No. 4, 9, 5, E.ar.) were deposited in the Kurdistan Herbarium, Howler Botanical Garden, Erbil Governorate.

2.2. Plant extraction

The extraction was performed according to the method described by [15]. Plant powder was macerated in absolute ethanol at a ratio (1:10) (w/v) for 3 days. Subsequently, the extract was filtered and concentrated to 50 ml. The concentrate was acidified with 1 ml of 0.1 N HCl and extracted with 10 ml of ethyl acetate. Ammonia (3 ml) was then added and the mixture was re-extracted with 20 ml of ethyl acetate. Finally, the ethyl acetate layer collected and evaporated to dryness.

2.3. Qualitative analysis of alkaloids

Alkaloids were detected in the extract using two different reagents:

Mayer's reagent: A few drops of Mayer's reagent were added to 1 ml of the extract in a watch glass. The formation of a white precipitate indicated the presence of alkaloids.

Wagner's reagent: Several drops of Wagner's reagent were added to 1 ml of the extract in a watch glass. The appearance of an orange precipitate suggested the presence of alkaloids [9].

2.4. Quantitative analysis of alkaloids

Quantitative HPLC analysis was performed according to the method described by [10]. Purified alkaloids were analyzed using a sykam (Germany) HPLC system equipped with a phenomenex C-8 (50 x 4.6 mm I.D). The mobile phase consisted of acetonitrile: Methanol: Ortho phosphoric acid (55:45:1 v/v), with UV detection at 280 nm and a flow rate of 1.0 ml/min. A 100 µm of sample was injected.

2.5. Cell lines cytotoxicity analysis

Four cell lines were used in the study. These three malignant cell lines (HePG2, WRL68, and SK-GT2) and one normal cell line, Rat Embryo Fibroblast (REF). The cell lines were obtained from the Iraqi Center for Cancer and Genetic Research (ICCMGR). They were cultured in RPMI1640 medium supplemented with 10 % Fetal Bovine Serum (FBS) and 1 % penicillin-streptomycin. The cells were incubated at 37 °C with 5 % CO₂ [16]. Upon reaching approximately 80-90 % confluence, the cells were treated with *Equisetum arvense* extract at concentrations 25, 50, 100, 200, 400, 800, and 1600 µg/ml. Cytotoxicity was evaluated after 24 hours of treatment. The cells were subsequently stained with 5 % crystal violet. The percentage of growth inhibition rate was calculated using following equation:

$$\text{Inhibition ratio} = \frac{A-B}{A} \times 100$$

Where, **A** represents the optical density of the untreated control cells, and **B** is the optical density of the treated cells [11].

2.6. Statistical analysis

Statistical analysis was performed using t-test to compare treated and untreated (control) cells. A $P \leq 0.05$ was considered statistically significant [17].

3. Results

3.1. Phytochemical screening for alkaloids

The results of the alkaloids extracted from the *Equisetum arvense* plant are presented in Table 1.

3.2. The quantitative analysis of *equisetum arvense* alkaloids

The results presented in Table 2 demonstrate the presence of several alkaloids in the *Equisetum arvense* extract. The identified alkaloids included isoquinoline, a heterocyclic alkaloid; hordenine, a non-heterocyclic alkaloid; and caulerpin, almazolone, and martensine, which are classified as indole alkaloids. The HPLC chromatogram of the identified alkaloids

is shown in Fig. 1, and the corresponding standard chromatogram is presented in Fig. 2.

3.3. Cytotoxicity analysis of *Equisetum arvense* alkaloids on cell lines

The cytotoxic effects of alkaloids isolated from *Equisetum arvense* were evaluated against three cell lines (HepG2, WRL68, and SK-GT2) and the normal REF cells. The inhibitory effects of the plant extract are presented in Tables 3, 4, 5, and 6, respectively.

The results demonstrated a concentration-dependent inhibitory effect, with the percentage of growth inhibition increasing from 19.97 % at 25 $\mu\text{g/ml}$ to 70.90 % at 1600 $\mu\text{g/ml}$. All concentrations tested in the experiment showed significant inhibition compared to the control, which exhibited an inhibition rate of only 3.23 % for the HepG2 cell line (as shown in Fig. 3). Notably, the SK-GT2 cell line demonstrated the highest inhibition rate, reaching 77.40 % at a concentration of 1600 $\mu\text{g/ml}$ of *Equisetum* alkaloids.

Table 1. Alkaloids phytochemical screening in *Equisetum arvense* extract.)

Phytochemical	Mayer's reagent	Wagner reagent
Alkaloids	+ / white precipitate	+ / brown precipitate

Table 2. Types and quantity of *Equisetum arvense* alkaloids

Alkaloids compounds	Quantity of alkaloids ($\mu\text{g/ml}$)
Isoquornoline	139.1
Hordinie	426.4
Caulerpin	324.1
Almazolone	77.6
Martensine	139.3

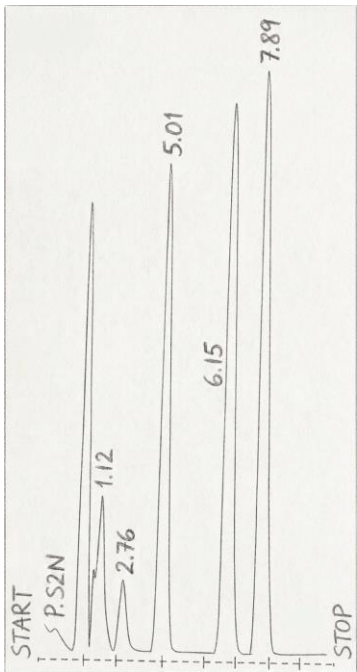


Fig. 1. HPLC chromatography of *Equisetum arvense* purified alkaloids

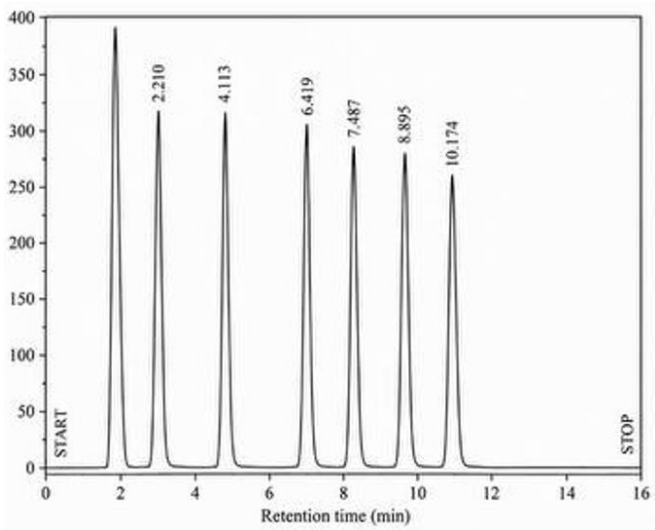


Fig. 2. HPLC chromatography of standards alkaloids

Table 3. Inhibition rate in HepG2 cell line influenced by different concentrations of extract of *E. arvense* plant after exposure for 24 h

Alkaloids Concentration $\mu\text{g/ml}$	Inhibition rate % $\pm$ Standard error
Control	3.23 ± 0.00^j
25	$19.97 \pm 0.81^{g*}$
50	$22.12 \pm 0.30^{f*}$
100	44.65 ± 0.23^e
200	$46.89 \pm 0.33^{d*}$
400	$60.97 \pm 1.12^{c*}$
800	$75.46 \pm 0.37^{b*}$
1600	$78.90 \pm 0.60^{a*}$

Values are expressed as Mean $\pm$ Standard Error. Asterisks (*) indicate a statistically significant difference compared to the untreated control group ($P \leq 0.05$, Student's t-test). Different superscript letters (s, f, e) indicate statistically significant differences between the treated groups ($P \leq 0.05$, Student's t-test)

Table 4. Inhibition rate of *E. arvense* alkaloids plant on SK-GT2 cell line influenced at 24h. exposure

Concentration $\mu\text{g/ml}$	Inhibition rate % $\pm$ Standard error
Control	1.15 ± 0.00^j
25	$15.90 \pm 0.36^{h*}$
50	$17.41 \pm 0.31^{g*}$
100	$17.09 \pm 0.79^{f*}$
200	23.16 ± 0.63^e
400	38.11 ± 1.36^d
800	$61.87 \pm 1.20^{c*}$
1600	$77.40 \pm 0.59^{b*}$

The different letters indicate that there are statistical differences at the level of $P \leq 0.05$
Values are expressed as Mean $\pm$ Standard Error. Asterisks (*) indicate a statistically significant difference compared to the untreated control group ($P \leq 0.05$, Student's t-test). Different superscript letters (s, f, e) indicate statistically significant differences between the treated groups ($P \leq 0.05$, Student's t-test)

Table 5. Inhibition rate of *E. arvense* alkaloids plant on WRL68 cell line influenced at 24h exposure

Concentration (µg/ml)	Inhibition rate % % ± Standard error
Control	0.00 ± 16.24 ^c
25	6.09 ± 2.25 ^{bc}
50	8.40 ± 6.99 ^c
100	25.13 ± 1.19 ^{ab*}
200	36.01 ± 7.55 ^{ab}
400	31.64 ± 9.52 ^{a*}
800	37.58 ± 6.61 ^{a*}
1600	39.90 ± 5.51 ^{a*}

Values are expressed as Mean ± Standard Error. Asterisks (*) indicate a statistically significant difference compared to the untreated control group ($P \leq 0.05$, Student's t-test). Different superscript letters (s, f, e) indicate statistically significant differences between the treated groups ($P \leq 0.05$, Student's t-test)

Table 6. Inhibition rate of *E. arvense* alkaloids plant on REF cell line influenced at 24h exposure

Concentration µg/ml	Inhibition rate % % ± Standard error
Control	0.0 ± 0.0 ^c
25	0.0 ± 0.0 ^c
50	0.0 ± 0.0 ^c
100	0.0 ± 0.0 ^{a*}
200	1.15 ± 14.50 ^{ab*}
400	4.0 ± 6.77 ^a
800	7.2 ± 8.33 ^{a*}
1600	10.11 ± 9.44 ^{a*}

Values are expressed as Mean ± Standard Error. Asterisks (*) indicate a statistically significant difference compared to the untreated control group ($P \leq 0.05$, Student's t-test). Different superscript letters (s, f, e) indicate statistically significant differences between the treated groups ($P \leq 0.05$, Student's t-test)

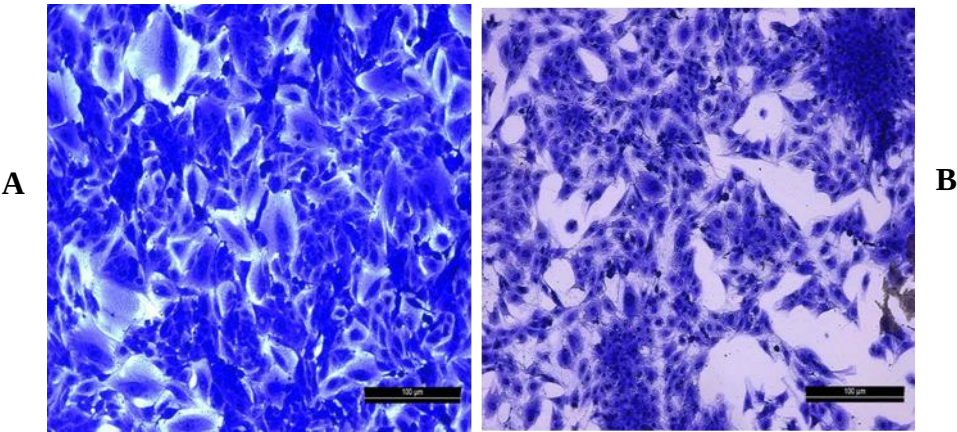


Fig. 3. Effect of alkaloid *E. arvense* on HepG2 cancer cell line after 24h. exposure (crystal violet, 100X). A: HepG2 as control showed monolayer confluent. B: HepG2 treated with extract at 1600 µg/ml, showed loss of confluent and dead of cells.

All the remaining concentrations show a significant difference from the control group (Fig. 4). A similar concentration-dependent inhibitory effect was observed in the WRL68 cell line, where growth inhibition increased with increasing alkaloid concentration and differed significantly from the control group (Fig. 5). Furthermore the

alkaloid extract exhibited the greatest cytotoxic activity against the HepG2 cell line, followed by the SK-GT2 and WRL68 cell lines. In contrast, the REF cell line showed minimal sensitivity to the treatment, with a maximum growth inhibition of only 10 % at the highest tested concentration (1600 µg/ml) (Fig. 6).

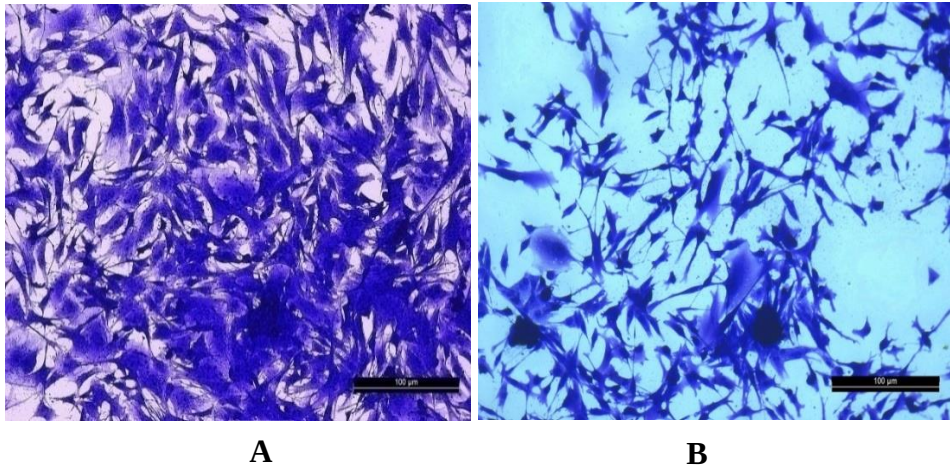


Fig. 4. Effect of alkaloid *E. arvense* on SK-GT 2 cancer cell line after 24h. exposure (crystal violet, 100X). A: SK-GT2 as control showed monolayer confluent. B: SK-GT 2 treated with extract at 1600 µg/ml, showed loss of confluent and dead of cells.

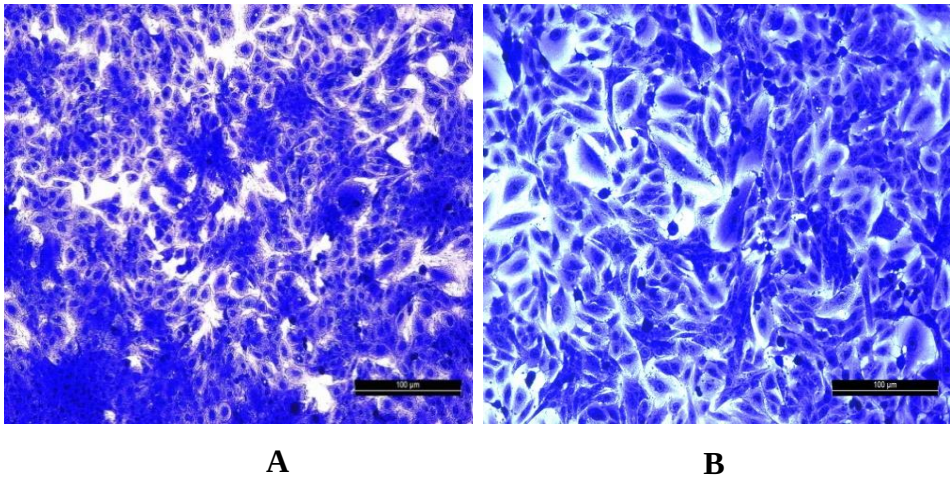


Fig. 5. Effect of alkaloid *E. arvense* on WRL68 cancer cell line after 24h. exposure (crystal violet, 100X). A: WRL68 as control showed monolayer confluent. B: WRL68 treated with extract at 1600 µg/ml, showed loss of confluent and dead of cells.

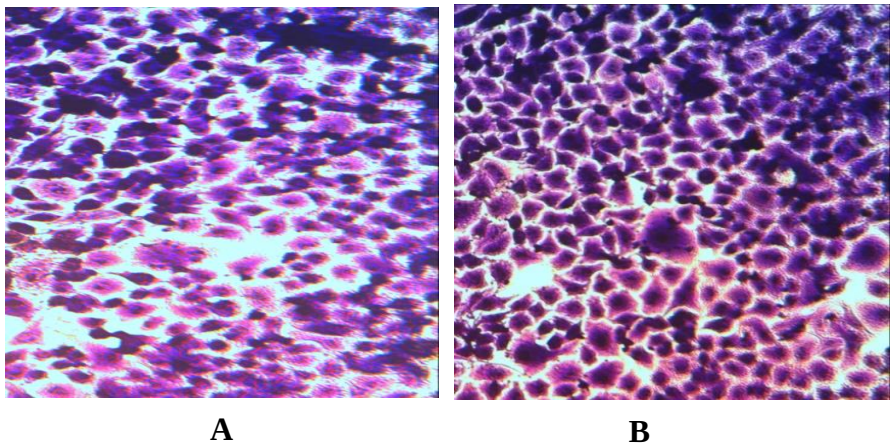


Fig. 6. Effect of alkaloid *E. arvense* on REFcell line after 24h. exposure (crystal violet, 100X). A: REF as control showed monolayer confluent. B: REF treated with extract at 1600 µg/ml, showed all cells preserved with shape and number.

4. Discussion

Alkaloids are widely distributed in higher and lower plants, algae, and other marine plants [18]. Their content varies according to several factors. These include the plant's age, geographical location, and seasonal variation. Understanding these factors is essential for evaluating the biological properties and potential applications of medicinal plants [19].

The present findings demonstrated that the cytotoxic activity of *E. arvense* alkaloids increased in a concentration- and time-dependent manner, with higher extract concentrations producing greater growth inhibition. Similar findings have been reported in previous studies [20, 21], which showed that *Equisetum* alkaloid extracts inhibit the growth of several cancer cell lines, including pancreatic carcinoma (AsPC-1), human colon carcinoma, MCF-7, and HeLa cells. The observed cytotoxic activity is likely attributable to the selective targeting of cancer cells by the bioactive alkaloid constituents.

Certain active compounds inhibit cancer cells by binding strongly to their DNA. Cancer cell DNA is more relaxed and unstable because it has many broken hydrogen bonds. This allows active compounds to attach more easily to damaged DNA regions [22]. Some researchers suggest another mechanism. Secondary metabolites selectively adhere to cancer cells because these cells have higher iron levels. Cancer cells need more iron for rapid DNA multiplication during frequent cell division. This higher iron content attracts active secondary compounds [23].

Beljanski [18] explains that alkaloids reduce the metabolic activity of cancer cells. They affect dehydrogenase enzymes and lower NAD and phosphate levels. Other researchers [24] emphasize the antioxidant and antiproliferative effects of *Equisetum* extract. The extract is

effective against HeLa, HT-29, and MCF cell lines. Alkaloids can stop cancer cell proliferation by arresting the cell cycle in G1 or G2/M phases. This arrest promotes apoptosis. Alkaloids also inhibit microtubule formation. Alkaloids from *Equisetum* significantly affect MCF7 cell growth but have little impact on REF cell lines [30]. Al-Marzook and Omran [25] highlight that *Equisetum* extract can inhibit A549 cell proliferation. This shows the extract's potential for therapeutic use.

The aqueous extract of *Equisetum* significantly inhibits metabolic activity in WM-2266-4 melanoma cells. It reduces activity by 70 % compared to controls. The aqueous extract also shows cytotoxic effects against the U937 cell line [3, 26]. Reference [19] reports that ethanolic *Equisetum* extract reduces ASPC-1 cell proliferation.

5. Conclusion

The extraction of active compounds from *Equisetum arvense*, also known as horsetail, holds great potential for medicine development. These compounds are rich in bioactive substances. They have many health benefits, such as anti-inflammatory, diuretic, and antioxidant effects. More scientific research is needed to understand how these compounds work and how effective they are. Thorough studies will help researchers use the plant's potential to create effective treatments. These treatments could improve patient outcomes. This research offers a valuable opportunity for the pharmaceutical industry. It can help connect traditional herbal remedies with modern healthcare. By combining the benefits of *E. arvense* with current medication formulations, the industry can develop innovative and scientifically supported therapies.

Authors contributions

The laboratory experiments were conducted by H. M. H., S. F. H., and S. Al-Majmaie. The statistical analysis was performed and the draft was edited by Gh. S. S. and Z. A. I. A. M. and I. H. provided invaluable advisory support and reviewed the project.

Acknowledgments

The research for this paper was supported by College of Science for Women, University of Baghdad.

Conflict of interest

The authors declare that there is no conflict of interest.

References

1. Tan ST, Ong HC, Chai TT and Wong FC. Identification of potential anticancer protein targets in cytotoxicity mediated by tropical medicinal fern extracts. *Pharmacognosy Magazine* 2018; 14(54); 227-230. doi: 10.4103/pm.pm_282_17.
2. Dao-Lan XU, Jian-Guo CAO, Quan-Xi WANG and Xi-Ling DAI. Cloning and characterization of DEAD box RNA Helicases gene from the Fern *Equisetum arvense*. *Plant Diversity* 2014; 36(06); 715-722. doi: 10.7677/ynzwyj201414036.
3. Ismail AM, Ouaid T, Al-Amery M, Maulood B and Serson W. A preliminary study of phytochemicals in *Equisetum arvense* and *E. ramosissimum* (Equisetaceae) extracts from Northern Iraq. *Fern Gaz* 2020; 21(3); 115-121.
4. Asgarpanah J and Roohi E. Phytochemistry and pharmacological properties of *Equisetum arvense* L. *J. Med. Plants Res.* 2012; 6(21): 3689-3693. doi: 10.5897/JMPR12.234.
5. Dragos D, Gilca M, Gaman L, Vlad A, Iosif L, Stoian I and Lupescu O. Phytomedicine in joint disorders. *Nutrients* 2017; 9(1): 70. doi: 10.3390/nu9010070.
6. Kunwar RM, Shrestha KP and Bussmann RW. Traditional herbal medicine in Far-west Nepal: A pharmacological appraisal. *J. Ethnobiol. Ethnomed.* 2010; 6(35): 1-18. doi: 10.1186/1746-4269-6-35.
7. Abdulrazzaq IH. The comparison effect of Nickel (II) and Cadmium (II) Complexes with aqueous extract of *Tecrium polium*. L (Ja,adah) plant on Hepatocellular Carcinoma cell line HeP2. *Baghdad Sci. J.* 2017; 14(2): 371-378. doi: 10.21123/bsj.2017.14.2.037.
8. Mimica-Dukic N, Simin N, Cvejic J, Jovin E, Orcic D. and Bozin, B. Phenolic compounds in field horsetail (*Equisetum arvense* L.) as natural antioxidants. *Molecules* 2008; 13(7): 1455-1464. doi: 10.3390/molecules13071455.
9. Cramer L, Ernst L, Lubienski M, Papke U, Schiebel HM, Jerz G and Beuerle T. Structural and quantitative analysis of *Equisetum* alkaloids. *Phytochem.* 2015; 116; 269-282. doi: 10.1016/j.phytochem.2015.03.003.
10. Alallaq SA, Murtadeh IA and Al-Majmaie SH. Determination of Alkaloids constituents in some Ferns by using high Performance Liquid Chromatography technique – Iraq. *Iraqi J. Sci.* 2023; 64(9): 4340-4346. doi: 10.24996/ij.s.2023.64.9.3.
11. Yun D, Yoon SY, Park SJ and Park YJ. The anticancer effect of natural plant Alkaloid Isoquinolines. 2021; *IJMS.* 22(4): 1653. doi: 10.3390/ijms22041653.
12. Eghbaliferiz S, Soheili V, Tayarani-Najaran Z and Asili J. Antimicrobial and cytotoxic activity of extracts from *Salvia tebesana* Bunge and *Salvia sclareopsis* Bornm cultivated in Iran. *Physiol. Mol. Biol. Plants.* 2019; 25(4): 1083-1089. doi: 10.1007/s12298-

019-00652-w.

13. Andayani DGS, Anggraeni SR, Liviawaty E, Chrisentia RM and Srikandace Y. Isolation, identification of alkaloid from *Rhizophora mucronata* and the activity of its methanol extract against barnacles. In IOP Conference Series: Earth and Environmental Science. 2018; 160: 012005). IOP Publishing. doi: 10.1088/1755-1315/160/1/012005.

14. Beljanski, M. The anticancer agent PB-100, selectively active on malignant cells, inhibits multiplication of sixteen malignant cell lines, even multidrug resistant. *Genet. Mol. Biol.* 2000; 23(1): 29-33. doi: 10.1590/S1415-47572000000100005.

15. Bhat AA, Ahamad B, Rehman MU and Ahmad P. Impact of ethanolic extract of *Equisetum arvense* (EA1) on pancreatic carcinoma AsPC-1 cells. *Saudi J. Biological Sci.* 2020; 27(5): 1260-1264. doi: 10.1016/j.sjbs.2020.01.029.

16. AL-Momen HM, Gali MA and Alwash BM. Isolation of Jasmimin from Jasmine (*Jasminum sambac*). *Iraqi J. Biotechnol.* 2015; 14(2): 113-121.

17. Syam S, Abdul AB, Sukari MA, Mohan S, Abdelwahab SI and Wah TS. The growth suppressing effects of girinimbine on HepG2 involve induction of apoptosis and cell cycle arrest. *Molecules* 2011; 16(8): 7155-7170. doi: 10.3390/molecules16087155.

18. Hussain J, Hashim I, Ibrahim mohammed F, Salman A and Saad N. Comparison of the effect of aqueous extracts of two plants, *Origanum vulgare* L. and fenugreek seeds with anticancer drug cis-platin on the growth of cancer cell lines. *Baghdad Science Journal* 2013; 10(2): 368-376. doi: 10.21123/bsj.2013.10.2.368-376.

19. Pec MK, Aguirre A, Moser-Thier K, Fernández JJ, Souto ML, Dorta J, Díaz-

González F and Villar J. Induction of apoptosis in estrogen dependent and independent breast cancer cells by the marine terpenoid dehydrothysiferol. *Biochem. Pharmacol.* 2003; 65(9): 1451-1461. doi: 10.1016/S0006-2952(03)00123-0.

20. Lunagariya J, Bhadja P, Zhong S, Vekariya R and Xu S. Marine natural product Bis-indole Alkaloid Caulerpin: Chemistry and biology. *Mini. Rev. Med. Chem.* 2019; 19(9): 751-761. doi: 10.2174/1389557517666170927154231.

21. Müller J, Puttich PM and Beuerle T. Variation of the main Alkaloid content in *Equisetum palustre* L. in the light of its ontogeny. *Toxins* 2020; 12(11): 710. doi: 10.3390/toxins12110710.

22. Wang L, Zhang L, Zheng G, Luo H, El-Kott AF and El-Kenawy AE. *Equisetum arvense* L aqueous extract: A novel chemotherapeutic supplement for treatment of human colon carcinoma. *Arch. Med. Sci.* 2023; 19(5): 1472-1478. doi: 10.5114/aoms/138146.

23. Al-Ali AA, Alsalami KA and Athbi AM. Cytotoxic effects of CeO₂ NPs and β -Carotene and their ability to induce apoptosis in human breast normal and cancer cell lines. *Iraqi Journal of Science* 2022; 63(3): 923-937. doi: 10.24996/ijs.2022.63.3.2.

24. Khalaf AN and Abed IJ. Evaluating the in vitro cytotoxicity of *Thymus vulgaris* essential oil on MCF-7 and HeLa cancer cell lines. *Iraqi Journal of Science* 2021; 62(9): 2862-2871. doi: 10.24996/ijs.2021.62.9.3.

25. Singh NP and Lai HC. Artemisinin induces apoptosis in human cancer cells. *Anticancer Res.* 2004; 24(4): 2277-2280.

26. Četojević-Simin DD, Čanadanović-Brunet JM, Bogdanović GM, Djilas SM, Četković GS, Tumbas VT and Stojiljković BT. Antioxidative and antiproliferative activities of different horsetail (*Equisetum arvense* L.) extracts.

Journal of Medicinal Food 2010; 13(2): 452-459. doi: 10.1089/jmf.2008.0159.

27. Al-Marzook FA and Omran R. Cytotoxic activity of alkaloids extracted from three Iraqi plants against breast cancer cell line. *Asian J. Pharmaceutical and Clinical Research* 2017; 10(9): doi: 10.22159/ajpcr.2017.v10i9.19134.

28. Glück J, Waizenegger J, Braeuning A and Hessel-Pras S. Pyrrolizidine alkaloids induce cell death in human heparg cells in a structure-dependent manner. *IJMS*. 2020; 22(1): 202. doi: 10.3390/ijms22010202.

How to cite this article: Habeeb HM, Hamad SF, Al-Majmaie Sh, Salih GhS, Murtadeh IA, Zainab Anwar¹, Ibraheem Hadi. Phytochemical profiling and *in vitro* cytotoxic evaluation of alkaloids isolated from *Equisetum arvense* L. native to northern Iraq against human cancer cell lines. *Journal of Medicinal Plants* 2026; 25(98): 54-64.

doi: ...