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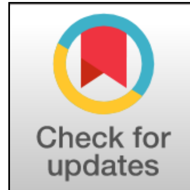

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The Effects of Crude Alkaloid Extracts of Convolutes Arvensis on Tumour Cell Lines and Their Potential For Toxicity

Efek Ekstrak Alkaloid Kasar dari Convolutes Arvensis pada Garis Sel Tumor dan Potensi Toksisitasnya

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Abstract

General Background: Cancer remains a leading cause of mortality worldwide, and the search for effective treatments continues to intensify. **Specific Background:** *Convolvulus arvensis* has been traditionally used for medicinal purposes, but its potential as an anticancer agent is underexplored. **Knowledge Gap:** The cytotoxic and apoptotic mechanisms of crude alkaloids extracted from *C. arvensis* against specific cancer cell lines have not been fully characterized. **Aims:** This study investigates the antioxidant activity and cytotoxic effects of *C. arvensis* crude alkaloids on mouse liver cancer (HC) and human breast cancer (AMJ13) cell lines, focusing on apoptosis-related gene expression. **Results:** The antioxidant activity of *C. arvensis* was comparable to that of ascorbic acid, with inhibition rates of 92.01% at 500 µg/ml. Crude alkaloids demonstrated dose-dependent cytotoxicity, with a maximum inhibition rate of 82.65% in AMJ13 cells and 79.49% in HC cells at 500 µg/ml. Gene expression analysis revealed upregulation of caspase-9, indicating apoptosis via the intrinsic mitochondrial pathway. **Novelty:** This study is among the first to provide molecular evidence of *C. arvensis*-induced apoptosis through the intrinsic pathway, offering a novel insight into its anticancer potential. **Implications:** These findings suggest that *C. arvensis* alkaloids could be developed as a therapeutic option for cancer treatment, with future studies needed to isolate specific compounds and assess their in vivo efficacy.

Highlights:

1. *C. arvensis* alkaloids show antioxidant activity similar to ascorbic acid.
2. Alkaloids inhibit liver and breast cancer cell growth dose-dependently.
3. Apoptosis triggered via caspase-9 through the mitochondrial pathway.

Keywords: *Convolvulus arvensis*, alkaloids, cancer, apoptosis, antioxidant

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Introduction

Plants are the source of more than 60 chemotherapeutic drugs now in use, and they are important components of chemotherapy (1). Research is being done to find out if these substances protect from the mutagenic and carcinogenic effects of other chemicals. Convolvulus arvensis, another name for hoarse tails, is a perennial herb that reaches a height of 10 cm (2). Numerous compounds were identified by the phytochemistry of this plant, including flavonoids (such as isoquercetin and apigenin), alkaloids, and caffeic acid. (3).

This herb has been used traditionally to cure ulcers, kidney issues, TB, and bleeding (4). This plant's cytotoxic properties offer a fresh way to employ it. Different C. arvensis extracts have different capacities to stop cell development; these variations depend on the type of cell line, the sort and method of extraction, and the conditions under which the cells are incubated. Human leukemia cell proliferation is significantly inhibited by the plant's ethyl acetate extract; this cytotoxic effect is assumed to be dose-dependent. (5). The aqueous extract of aerial portions of C. arvensis has an inhibitory impact on HIV-1-induced cytopathy. Testing several plant extracts for their capacity to suppress HIV-1 and its essential enzymes led to the discovery of this. (6) reported that the great majority of anticancer medications now used in clinical practice induce cell death using apoptosis. Because of its significance for tissue homeostasis and the development of cancer, apoptosis modulation has become an intriguing target in cancer treatment (7). apoptosis is a highly efficient and tightly regulated method of cell death that depends on several interdependent elements. It is believed that elements of the apoptotic signalling network are present in a nucleated cell, ready to be triggered by an event that results in cell death. Numerous internal and external variables, such as the lack of survival signals, cytotoxic drugs or radiation therapy, irregular cell cycle signalling, or developmental death signals, can cause cell death. Cell surface receptor ligation and DNA damage can result from insufficient DNA repair systems. Various death signals trigger a similar cell death machinery that causes the outward manifestations of cell death. (2).

Methods

The plant used

Figure 1 illustrates where the plant in these experiments originated: the Diyala. Family of Convoluluceae, C. arvensis.

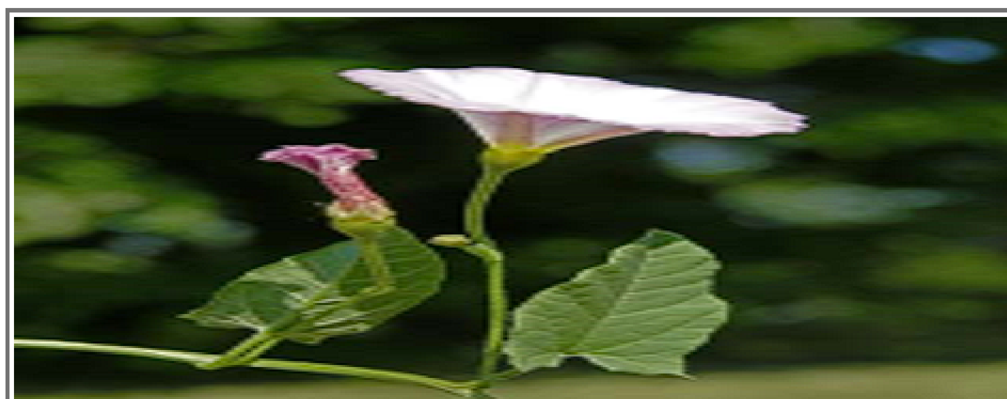

Figure 1. *Convolvulus arvensis plant*

Alkaloids Extraction

This plant's crude alkaloids were extracted following the guidelines provided by Cannel (1998) (8). Dragendroff's reagent was used to identify the alkaloids. After the extract was exposed to Dragendroff's reagent, a brown-orange colour emerged, indicating the presence of alkaloids. Concentrations of 15.15, 31.25, 62.5, 125, 250, and 400 were employed in this investigation and (500) µg\ml.

Antioxidant assay

The DPPH assay described by Zhu (2006)(9) was used to evaluate C. arvensis's antioxidant activity. Each concentration of E. arvense (15.15, 31.25, 62.5, 125, 250, 400, and 500) was mixed with DPPH (60 M) in an equal volume (0.5 ml), and the mixture was allowed to settle at 37 for 30 minutes. Measurements of the absorbance were made at 517 nm. A component of vitamin C called ascorbic acid was used as a positive control. The following formula was used to determine the alkaloid extract's % inhibition of DPPH, which was utilized to express its

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capacity to scavenge free radicals.

DPPH inhibition (%)=(AC-AS/AC)X100

where the test sample solvent and the DPPH peak intensity are represented by the letters AS and AC, respectively.

Preparation of tumour cell line

This in vitro technique was used to assess the effect of the crude alkaloid from C. arvensis on the (AMJ13 and HC cell lines). The Iraqi Center for Medical and Genetic Research's regular procedure was used to arrive at the answers. These cells were incubated at 37°C in a humid environment with 5% CO_2 and were maintained viable in RPMI-1640 media containing 15% serum.

Toxicity assay

Freshney (2000) measured the cytotoxic test using the crystal violate stain. Following the seeding of 96-well microplates with tumour cells, serum was diluted with the cells and incubated for 24 hours at 37 C at concentrations of 15.25, 31.25, 62.5, 125, 250, 400, and 500 g/ml. The plate was incubated for a full day following the substitution of a new medium for the old. SFM with different extract concentrations. 100µl crystal violet dye wells were added to the wells to treat them after the media was removed and the exposure intervals had passed. The plates were allowed to cool for 15 minutes after the wells were cleaned with phosphate-buffered saline (PBS) and incubated for 20 minutes at 37 degrees Celsius. The absorbency of the wells was then measured at 492 nanometers using an ELISA reader.

(qRT-PCR) Cell seeding

Two falcons, each with 106 cells, were seeded with the AMJ13 and HC cell lines, respectively. The cells were then allowed to adhere, multiply, and form a confluent monolayer by incubating the falcons for 24 hours at 37°C and 6% CO_2.

Exposure stage Each cell line, AMJ13 and HC, was exposed for 24 hours following the creation of the monolayer. The alkaloid extract was applied to one falcon at its IC50 (33.90 gml for AMJ13 and 10.70 gml for HC), and only SFM therapy was given to the second falcon, which was kept as a control.

Collect cells The falcons were given the medium back after the exposure period. The cells were washed with 5 millilitres of PBS. Each cell population was separated using the abm EXCellenCT Lysis Kit by scraping the bottom of the cell plate in 50 litres of cold PBS and then moving the relevant solution to a new 200-litre Eppendorf tube. The tubes were immediately placed in an ice box in order to preserve the biological material as fresh as possible. -80 degrees Celsius.

RNA Extraction: The RNA from cultivated cells was extracted using the abm EXCellenCT Lysis Kit (Abm, Canada) after frozen cells were thawed at room temperature by the manufacturer's instructions. To create cDNA from pure extracted RNA, the First Chain cDNA Synthesis Kit (TonkBio, USA) was utilized in accordance with the manufacturer's instructions. A Nano-drop spectrophotometer was used to measure the cDNA's concentration and purity.

Statistical Gene expression analysis using real-time PCR

In the cancer cell lines AMJ13 and HC, real-time PCR was utilized to analyze the BAX, P53, Caspase8, and Casp. 9, and GAPDH ' mRNA level. and they were lyophilized at -20°C following the guidelines provided by the National Center for Biotechnology Information (NCBI). Every mRNA sequence that was found was selected with care. All of the genes in this study, including those in Table 1, were produced using the NCBI's Primer-Blast program.

The PCR amplification procedure made use of the USA-based Kappa Syber Green Master Mix Kit. The reaction happened in a container holding 20µl. For every 20-litre qRT-PCR reaction, two litres of cDNA (100 ng), ten litres of KAPA SYBR Green Master Mix, six litres of RNase-free water, and one litre of each primer (Forward and Reverse) at a concentration of one million times the concentration (100 µM) were utilized. Table 2 displays the temperature profile of gene expression in our study. By replacing the calibrator with sample 2ΔCt, the expression ratio was obtained

ΔCT (test) = CT internal control - CT target gene of interest

Ultimately, the expression ratio was computed using the following formula: 2-ΔCt=ratio of normalized expression. After cleaning the wells with phosphate-buffered saline (PBS), the plates were allowed to cool for fifteen minutes. After that, the absorbency (O.D.) of the wells was measured using an ELISA reader set at 492 nanometers, and Caspase8 and Caspase9 were computed. Alongside the internal control gene GAPDH:

ΔCT(test) = CT gene of interest (target, test) - CT internal control

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CT internal control - CT = ΔCT (calibrator) The target, or calibrator gene of interest. Choosing the calibrator involved using the control samples. To calculate ΔΔCT, the following formula was used:ΔΔCT = ΔCT (calibrator) - ΔCT (test) Lastly, the following formula was used to get the expression ratio:

2-ΔΔCt= (Livak and Schmittegen, 2001) (11) Normalized expression ratio).

5´...........3´	F/R	Human/Mice	Primer
iCCG TCCi CAAi GCAi ATGi GATi Gi	F	H	P53
iGAA GATi GACi AGGi GGCi CAGi	R		
iGAT CTGi TAGi CTGi CCCi CAGi GATi	F	M	
iAGA TGAi CAGi GGGi CCAi TGGi AGTi	R		
iCCT CTCi CCCi ATCi TTC AGAi TCAi	F	H	BAX
iTCA AGTi CAAi GGTi CACi AGTi GAGi	R		
CGC AAGi AGAi GGCi CAGi AATi GAii	F	M	
iTGT GGAi GAGi AATi GTTi GGCi GTi	R		
iGAC CACi GACi CTTi TGAi AGAi GCTi	F	H	Caspase 8
iCAG CCTi CATi CCGi GGAi TATi ATCi	R		
iGCT CTGi AGTi AAGi TTTi AAGi Gi	F	M	
iGAT CTTi GGGi TTTi CCCi AGAi Cii	R		
iCTC TTGi AGCi AGTi GGCi TGGi TCi	F	H	Caspase 9
iGCT GATi CTAi TGAi GCGi ATAi CTi	R		
iGCT GTTi TCTi GCGi AAAi GGGi ACTii	F	M	
iAGG GCAi CAAi TCCi CTAi ACCi ACi	R		
iGGG TTCi TTTi GTGi CTGi AGCi GGi	F	H	GAPDH
iTGC AGAi TAGi GAAi GGGi CTTi TGi	R		
iTCT CCAi TGGi TGGi TGAi AGAi	F	M	
iTGG CCGi TATi TGGi GCGi CCTi	R		

Table 1. *The Primers used in the experiment*

Step	Temperature	Duration	Cycles
Enzyme	95ºC	30 sec	Hold
Denature	95ºC	5sec	
Anneal/extend	62 ºC	20 sec	40
Dissociation	1min /95 ºC-30 sec /55 ºC-30sec/95 ºC		

Table 2. *qRT-PCR*

Result and Discussion

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Crude alkaloid antioxidant activity extraction from plant

The antioxidant activity results indicate that the alkaloid extract is a useful scavenger of free radicals. Seven distinct concentrations were monitored, as Table (3) illustrates. There was a general con.-dependent inhibition observed, with 500 µ g/ml outperforming the other concentrations. The con. (15.15, 31.25) g/ml and con. (62.5, 125, 250, 400, and 500 g/ml and ascorbic acid) did not differ from one another, as Figure 2 illustrates.

SD ± Mean	Concentrations (µg\ml)
75.59 ± 6.29 a	15.15
87.21 ± 4.39 ab	31.25
86.60 ± 2.55 b	62.5
89.69 ± 2.30 b	125
90.10 ± 2.27 b	250
91.12 ± 2.54 b	400
92.01 ± 2.62 b	500
92.23 ± 1.46 b	Ascorbic Acid

Table 3. *Alkaloid extract from C. arvensis has antioxidant properties*

The various letters signify statistical differences at the level of P 0.05.

Figure (2): Alkaloid extract of C. arvensis has antioxidant properties.

The C. arvensis plant exhibited elevated quantities of copper and zinc, in addition to high levels of vitamins C and E. For superoxide dismutase to combat active oxygen species, these were necessary. (12)

Huh and Hun, (2015). (13) reported that at 4.0 mg/ml, C. arvensis exhibited 94.7% DPPH scavenging activity for roots and rhizomatous stems.

Extracts from C. arvensis were tested for their antioxidative potential using DPPH and reactive hydroxyl radicals. The outcomes demonstrated that the free radical scavenging activity was influenced by the kind and amount of administered extracts.The n-butanol extract produced the highest levels of DPPH (EC50 = 0.65 mg/ml) and hydroxyl radical scavenging (EC50 = 0.74 mg/ml)(14). Onitin and luteolin, which were isolated from the methanolic extract of C. arvensis, were found to have DPPH free radical scavenging activity, with IC50 values of 35.8 0.4 microM and 22.7 2.8 microM, respectively.(15)

Cytotoxicity of C. arvensis crude alkaloid extract on cell lines

Table (4) displays the percentages of the alkaloid extract that inhibited the growth of the AMJ13 cancer cell line: 33.78% at concentrations of 15.15 gml, 48.92% at concentrations of 31.25 and 60.01%, 69.90%, 70.76%, and 78.62%, and 79.49% at concentrations of 31.25 gml, 62.5 gml, 125 gml, 250 gml, and 500µ gml, respectively. There were no changes in the concentrations (62.5, 125, 250) g/ml and (400 and 500 g/ml) at P 0.05, as Figures 3 and 4 demonstrate.

The HC cell line exhibited an inhibitory effect, as shown in Table 5, that started at doses of 15.1 gml and grew to 31.2 gml, 62.5 gml, 125 gml, 250 gml, 400 gml, and 500 gml, with corresponding inhibitory effects of 60.01%, 59.60%, 75.20%, 76.91%, 79.40%, and 82.65%. As can be seen in Figures 3 and 4, there were no significant changes at the P 0.05 level between the amounts of 15.1, 32.2, 62.5, 125, 250, 400, and 500 µg/ml.

SD ± Mean	(µg\ml) Concentrations in
33.78 ± 2.8 d	15.1.5
48.92 ± 7.3 c	31.25
60.01 ± 7.6 b	62.5
69.90 ± 8.6 b	125
70 .76± 8.1 b	250
78.62 ± 3.4 a	400
79.49 ± 11.4 a	500

Figure 2. *Cytotoxicity of crude alkaloid extract on growth inhibition percentage in AMJ13 cell line after 24 hours of exposure*

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*The various letters signify statistical differences at the level of P 0.05.

SD ± Mean	(µg\ml) Concentrations in
1.1±44.65 d	15.15
0.7 ± 56.50 c	31.25
3.8± 69.60 b	62.5
3.6± 75.2 b	125
3.4± 76.91 a	250
4.2± 79.40 a	400
3.5±82.56 a	500

Figure 3. *Alkaloid extract's cytotoxicity over 24 hours as measured by the percentage of HC cell line growth inhibition*

*The various letters signify statistical differences at the level of P 0.05.

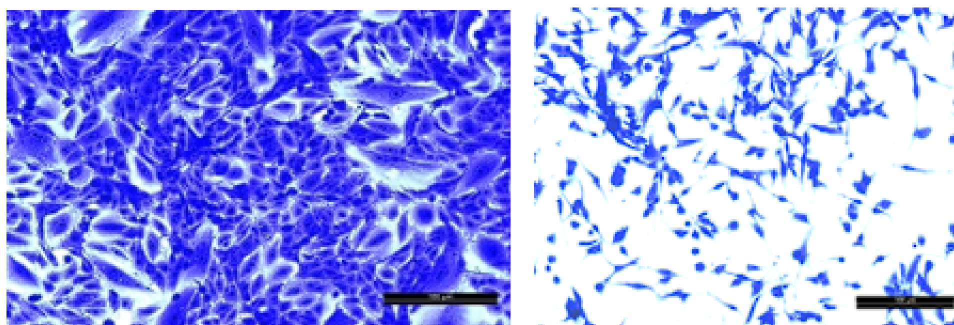

Figure 4. *Effect crude alkaloid After 24 hours, in AMJ13 cell line was exposed to C. arvensis. A. AMJ13 as control B. crude alkaloid with treatment.*

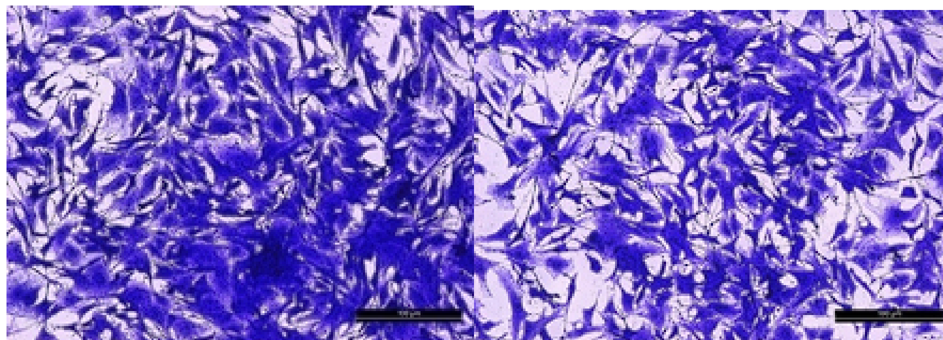

Figure 5. *Effect of crude alkaloids After being exposed for 24 hours, A. HC a control B. cell line treatment with alkaloids the HC cell line was affected by the alkaloid extract of C. arvensis.*

The cytotoxic activity of the alkaloid extract from C. arvensis was examined on the AMJ13 and HC cell lines. This cytotoxic activity was influenced by the concentration of the extract and the cell line. The aqueous extract from sterile stems of C. arvensis exhibited dose-dependent toxicity against human leukemic U937 cells. When DNA fragmentation, phosphatidylserine externalization, and the collapse of mitochondrial transmembrane potential were observed in cells cultivated for 48 hours, apoptosis was clearly visible.

Freshney, (2000) (14). Human embryonic kidney cells, breast adenocarcinoma, and lung fibroblasts were among the cancer cell lines that were subjected to a methanolic extract of the dried aerial section of C. arvensis to test its cytotoxicity. The relative percentages of live and dead cells in the cells were assessed 72 hours after treatment. All four test cell lines were destroyed by the extract; however, the most detrimental effects were seen in human embryonic kidney and breast cancer cells. However, depending on the kind of cell and the concentration of extract utilized, the level of toxicity differed. The plant extract had a significant cytotoxic impact on breast cancer cells

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compared to untreated cells.e (15).

Quantitative Real-time PCR

Real-time PCR was used to evaluate the expression levels of GAPDH and other genes. The housekeeping gene GAPDH, which was used in this investigation, has a Ct value that is displayed in Table (6). In the AMJ13 cell line, the folds of gene expression for P53, BAX, Caspase8, and Caspase9 were 2.98, 0.05, 0.65, and 0.15, respectively.

GAPDH Ct levels were 25,09 and 26,90 in the treatment and control groups, respectively. The HC cell line's P53, BAX, Caspase8, and Caspase9 gene expression folds were 3.45, 1.36, 0.12, and 0.19, respectively, as shown in Figure 7. The GAPDH Ct value is shown in Table (7). The HC cell line range for the treatment group was (21.84), whereas the range for the control group was (21.61).

Fold of Gene expression	ΔΔCT	ΔCTC	ΔCTT	CT (GAPDH)	CT(Gene)	T/C	Gene
1.04	-4.18	-	-0.35	23.02	23.56	T	BAX
1.01	0.01	-4.50	-	25.94	21.32	C	
0.64	0.65	-	7.73	25.07	31.78	T	P53
1.01	0.01	7.08	-	26.92	32.97	C	
0.14	0.95	-	4.85	23.07	28.92	T	Cas9
1.01	0.01	2.15	-	26.91	29.03	C	
2.97	-1.56	-	-3.88	25.08	23.21	T	Cas8
1.01	0.00	-2.34	-	25.99	24.60	C	

Table 4. *Gene expression fold in the AMJ13 cell line*

Fold of Gene expression	ΔΔCT	ΔCTC	ΔCTT	CT (GAPDH)	CT(Gene)	T/C	Gene
1.65	-0.45	-	8.18	22.84	30.70	T	BAX
1.32	0.00	8.64	-	22.16	29.97	C	
0.87	2.96	-	10.12	22.84	30.06	T	P53
1.11	0.00	7.09	-	22.16	28.18	C	
0.10	2.37	-	11.24	21.84	33.86	T	Cas9
1.87	0.00	8.88	-	22.16	31.00	C	
3.76	-1.79	-	7.09	21.84	28.09	T	Cas8
1.98	0.00	9.34	-	21.16	31.10	C	

Table 5. *Gene expression patterns in the HC cell line*

In contrast to necrosis, apoptosis is characterized by a precise sequence of events that leads to the disintegration of internal cell contents, including swelling and rupture of afflicted cells. The death receptor (extrinsic pathway) and the mitochondria (intrinsic pathway) are the two primary pathways by which apoptosis can occur. These two paths lead to the same death program.(16)

In contrast to necrosis, apoptosis is characterized by a precise sequence of events that leads to the disintegration of internal cell contents, including swelling and rupture of afflicted cells (16). The death receptor (extrinsic pathway) and the mitochondria (intrinsic pathway) are the two primary pathways by which apoptosis can occur. These two paths lead to the same death program. (17).

Numerous research have shown that plant extracts are harmful to cancer cells. Vetineria zizanioides root water extract proved cytotoxic to MCF-7 cells by increasing nuclear material fragmentation and chromatin intensification (18). In response to Cinnamomum zeylanicum methanolic extract, HepG2 cancer cells undergo morphologically planned apoptosis, as seen by fragmentation, nuclear material intensification, and cell shrinkage. (19). By compressing chromatin and raising the proportion of nuclear material, berberine inhibits HL-60 leukaemia cells (20). Peganum harmala's alkaloid extract is said to impede the proliferation of cancer cells by encouraging nuclear fragmentation and the release of cytochrome C cells. Plant extracts are effective at inducing programmed cell death in cancer cells, as demonstrated by their effects on several cellular markers, some of which were found in this study. These findings support the current study's discovery that alkaloid extracts have a significant effect on the induction of apoptosis in cancer cell lines.(21),

Conclusion

The present study demonstrates that crude alkaloid extracts from Convolvulus arvensis exhibit potent antioxidant

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activity and significant cytotoxic effects against both mouse liver cancer (HC) and human breast cancer (AMJ13) cell lines, with dose-dependent inhibition of tumor growth. The highest cytotoxicity was observed in the AMJ13 cell line, reaching an inhibition rate of 82.65% at 500 µg/ml. Additionally, the extract's ability to induce apoptosis through the mitochondrial (intrinsic) pathway was confirmed by increased expression of caspase-9 in both cell lines, highlighting the potential of C. arvensis as an anticancer agent. This research not only underscores the therapeutic potential of crude alkaloids for cancer treatment but also opens avenues for further investigation into their mechanisms of action, optimal dosages, and efficacy across different cancer types and clinical settings. Future studies should focus on isolating specific alkaloid compounds and evaluating their in vivo efficacy to enhance the understanding of their biomedical applications.

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