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Flavonoid Glycosides from *Convolvulus arvensis* Induce Selective Cytotoxicity and Caspases-Mediated Apoptosis in MCF-7 Cells

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ABSTRACT

Objective: *This research was an attempt to isolate, characterize and investigate the cytotoxicity of flavonoid glycosides derived from its leaves against MCF-7 breast cancer cell line.* ***Method:*** *Plant compounds were extracted using 70% ethanol and subsequently fractionated by silica gel column. TLC, UV-Vis and FTIR spectroscopic methods were employed for phytochemical characterization. The MTT assay was used to evaluate the cytotoxic activity against MCF-7 and Vero cell lines.* ***Results:*** *Chromatographic separation resulted in obtaining two glycosides (F2, F1). The UV spectra showed characteristic absorption bands at 270-292 nm and 330 nm, that corresponding for flavonoids structure, whilst FTIR revealed presence of hydroxyl, aromatic and glycosidic functional groups. MCF-7 was sensitive to both fractions in a dose-dependent manner, with F2 exhibiting greater potency (IC50= 33 µg/mL) than F1 (IC50= 42µg/mL). selective anticancer activity indicated by lower toxicity in Vero cells. AO/EB staining demonstrated morphological characteristics of apoptosis, such as chromatin condensation and membrane damage. Moreover, ELISA analyses revealed a significant activation of both caspase-8 and caspase-9 with a pronounced activation of the extrinsic apoptotic pathway.* ***Novelty:*** *Convolvulus arvensis can potentially provide natural sources of flavonoid glycosides that may be useful in the treatment of cancer. These results recommend also means that future studies are needed to isolate these active compounds.*

INTRODUCTION

Cancer is among the leading causes of death across the globe and one of the most dangerous problems any health care system faces anywhere. Breast cancer is the most diagnosed malignancy in women and the main cause of mortality and senescence from cancer among female patients with all types of malignancies. Clinical application of these novel treatment strategies; chemotherapy, radiotherapy and targeted therapies is severely hampered when their clinical efficacy is strongly limited by mechanisms of drug resistance, toxicity or disease recurrence. Recently, one of the most promising areas of interest has been the pursuit for new, safe and effective agents in cancer prevention and treatment; specifically, the natural product compounds [1,2].

In the field of drug discovery and development, samples of natural products are taken as soon as possible after they are extracted from their source, however there may be resources related to the information capabilities provided by large-scale sorting operations in these areas. Many useable bioactive secondary metabolites are obtained from diverse plant tissues, including alkaloids, terpenoids, phenolics and flavonoids, that employ various pharmacological effects. Flavonoids are the largest group of this phytonutrients, which have been studied due to antioxidant, anti-inflammatory and anticancer activities. Different functions of flavonoids had been shown on cancer

prevention, based upon multiple mechanisms such as inhibition of cell proliferation, apoptosis induction and modification of pathways involved in tumorigenesis [3,4]. Flavonoid glycosides fall into one of the two categories of flavonoids, in which sugar moieties are bonded to the flavonoid skeleton. that are found throughout the plant kingdom. They can thus, from the point of view of natural products chemistry, be regarded as phytochemicals in food. Their bioactivity is therefore further enriched and their bioavailability augmented. On its potential anti-cancer effects information indicate that they affect programmed cell death, suppress cell cycle progression, and prevent metastasis and angiogenesis. Especially, some specific aglycone flavonoid glycosides have been shown to modulate the NF-κB, PI3K/Akt and MAPK signaling pathways, which are indispensable for the survival and proliferation of cancer cells [4-6].

Convolvulus arvensis (Field bindweed) is used as herbal medicine over a wide distributed in many regions. A traditionally used for many therapeutic purpose, it's have a great number of bioactive compounds such as flavonoids, phenolic acids and their glycosides have been detected by phytochemical investigations carried out on this plant and these compounds may account for some of the biological activities exhibited by this plant. Nonetheless, few studies have concentrated on isolation and characterization of anticancer active flavonoid glycosides in breast cancer model systems [7]. In addition to cytotoxic effects, the induction of apoptosis is considered one of the most important through which anticancer agents exert their activity. Apoptosis is the regulated process of inherently programmed cellular death occurs through caspases activation that involves caspase-8 (the extrinsic pathway) and caspase-9 (the intrinsic pathway). Many flavonoid compounds have been shown the ability to selectively the activation of these pathways and induce apoptotic cell death in cancer cells, leading to elimination of malignant cells whereas minimizing damage of normal cells [8-10]. Thus, the objectives of the present study were to isolate and characterize flavonoid glycoside compounds (using chromatographic and spectroscopic methods) from leaves of *C. arvensis* as well as evaluate of cytotoxicity and apoptotic effects in an MCF-7 breast cancer cell line. This study will help to provide further evidence that these plant products can be used as potential anticancer agent.

RESEARCH METHOD

Plant Material Collection

A complete plant of *Convolvulus arvensis* was collected from the field in University of Basrah, Iraq, during April to May 2023 (**Fig. 1**). A plant was taxonomically identified by Pro. Dr. Abdul Redha Akbar Al-Mayah (plant taxonomist in the Department of Environment, College of Science, University of Basrah). After identification, the leaves of plant were collected in large quantities and transport to the extraction laboratory at the department of Biology, College of Education for pure science, University of Basrah. The leaves were washed thoroughly, then dried at room temperature away from direct sunlight in order to preserve their active compounds.

The dried leaves were ground into a fine powder by electric grinder, then the powdered was stored in a sealed dark glass container -20 ºC until the time of extraction.

Figure 1. The plant of *Convolvulus arvensis*.

Extraction of Active Compounds with 70% Ethanol

The extraction and isolation of glycosides were performed according to the method described by Al-Asady et al., with slight modification [11]. Approximately 400 g of powdered leaves were soaked in 2 L of 70% ethanol in conical flask. The flask tightly closed and wrapped with aluminum foil and the mixture was stirred using a magnetic stirrer at 50 ºC for 48 h. After the extract was cool to room temperature, the mixture was filtered through Whatman No.1 filter paper using a Buchner funnel under vacuum. These process was repeated twice with the plant residue to ensure complete extraction of the active compounds. The combined filtrates were concentrated by a rotary evaporator at 60 ºC to remove the ethanol and obtain a concentrated crude extract, then transferred into glass petri dishes and complete drying at room temperature. The derided extract was carefully scraped from petri dishes and stored in a sterile tube at -20 ºC until further use.

Isolation of Glycosides by Column Chromatography

The separation of glycosides was performed using silica gel column chromatography according to Harborne [12], with slight modifications [11]. The mobile phase BAW (n-Butanol: Acetic acid: Water) was prepared in a ratio of 4: 1: 5 by mixing 800 mL n-butanol, 200 mL Acetic acid and 1000 mL distilled water in a separating funnel. The mixture was shaken vigorously for 15 min. The Column was packed by first placing a cotton plug at the bottom of the column. A slurry of silica gel (60-200 mesh) was prepared by mixing 100 g silica gel with 250 mL BAW solvent. The slurry was carefully poured into the column while gently tapping the column walls to eliminate air bubbles, additional BAW solvent was added until the solvent level was above the

surface of silica gel. Finally, layer of sand (1 cm) was placed on top of silica bed to protect the column surface. Approximately 500 mg of crude glycoside extract was dissolved in 10 mL of BAW solvent and mixed thoroughly, a sample solution was carefully loaded onto column by dropper, the follow rate maintained at 1-1.5 mL/min, eluted fractions were collected in sterile glass tube approximately 5 mL per fraction.

Thin Layer Chromatography (TLC)

The TLC plate (Silica gel 60 GF 254) was performed to monitor the separation and determine the purity of the collected fractions, the mobile phase solvent used was BAW (4:1:5). The developed plates were examined under UV lamp at 254 nm, and spots were visualized using iodine vapor in a closed chamber. Fractions showing similar values of RF were combined and dried at room temperature. Two major spots were observed and named as fraction1 (F1) and fraction 2 (F2).

Spectroscopic Analysis

UV–Visible Spectroscopic Analysis

Isolated glycosidic fractions were recorded by using UV–Visible spectrophotometer at wavelengths ranging from 200 to 600 nm. The fractions were diluted with the corresponding solvent (methanol) and transferred to a quartz cuvette at 1 cm path length. Absorbance spectra were recorded relative to methanol as blank. These absorption maxima (λmax) provide preliminary insights into the structural attributes of these isolated compounds.

Fourier Transform Infrared Spectroscopy (FT-IR)

The isolated fractions were analyzed using FT-IR spectrophotometer (BRUKER, FTIR ALPHA II) 400–3800 cm^{-1} spectral range. Analysis of the samples was performed following proper preparation and recorded spectra to identify functional group existing in collected product. FTIR spectra were analyzed by comparing observed wave numbers with those of different functional groups.

The Cell Lines in Current Study

The cell lines used in the current study were Veroslem (Vero as a model for normal cells) and cancer cell lines MCF-7, they were brought from the Cell Bank Unit, Cell Culture Laboratory, Department of Biology, College of Science, University of Basrah.

Cytotoxicity Assay

The cytotoxic activity of the isolated glycoside fractions (F1 and F2) was evaluated on MCF-7 and Vero cell lines using the MTT assay according to the method that described by Freshney [13], and Bahuguna et al. [14]. The cells were seeded in 96-well plates approximately 100 µL of cell suspension (1×10^4 cells per well) and incubating for 24h, to allow cell attachment (70-80% confluence), the culture medium was removed and replaced with 100 µL of glycosides solutions at different concentrations (15,30,60,120, and 240 µg/mL) that prepared in serum – free DMEM medium, each concentration was tested in four replicates for both normal (Vero) and cancer (MCF-7) cell lines. The treated cells were incubated for 48 h at 37 ºC in a humidified incubator (5% CO_2). After the treatment period, 10 µL of MTT solution (5 mg/mL) and 90 µL serum–free medium was added to each well and the plate was incubated for 3 h at 37

^{o}C. During this period, viable cells reduce the yellow tetrazolium salt (MTT) into purple formazan crystals by mitochondrial dehydrogenase enzymes. Following incubation, the culture medium was removed and 50 µL of DMSO was added to each well to dissolve the formed formazan crystals. The absorbance was then measured using the microplate reader at 570 nm, and the optical density values were used to determine cell viability%.

Determination of the IC_{50} value

The half maximal inhibitory concentration (IC_{50}) values of glycoside fractions (F1 and F2) were determine from the dose- response curves obtained by plotting the percentage of cell viability against the logarithm of compound concentration.

Morphological Assessment of Apoptosis

Acridine orange/ethidium bromide (AO/EB) double dye was used to differentiate viable, early apoptotic, late apoptotic, and necrotic cells. The cells were seeding into 8-wells chamber slide one day before and left obtaining 70-80% confluence, then treated with IC_{50} concentrations of glycosides F1and F2 for 48 h and observed under fluorescence microscopy [15,16].

Quantification of Caspase-8 and Caspase-9 by Sandwich ELISA

After treatment at IC_{50} concentration, cells were lysed using cold lysis buffer (EDTA Anticoagulant 1X) supplemented with protease inhibitors (PMSF 100X). Lysates were cooling centrifuge, and the supernatants were collected and stored at −80 °C. Caspase-8 and Caspase-9 levels were quantified using a commercial human ELISA kit (sandwich method), its procedure was done according to the manufacturer's instructions. Treated cell lysates were added to antibody-precoated microplates, followed by biotinylated detection antibody and HRP conjugate. The level of Caspase-8 and Caspase-9 was measured using the MultiScan SKANLT device at 540 nm followed by 4 PL equation.

Statistical analysis

The data was statistically analyzed using SPSS V. 26., The T-test and One-way analysis of variance (ANOVA) were used. Graph Pad Prism version 6 was used to implement IC_{50} values, curves, and graphs. The results of the analysis and statistics were submitted to the purpose of understanding the differences between normal and cancerous cell lines, and all data were presented as an average±standard deviation, and ($p<0.05$) was considered statistically significant.

RESULTS AND DISCUSSION

Results

Properties, yields of *C. arvensis* extracts and qualitative chemical Screening

The yield weight (g) with the color and nature of each obtained extract of the *C. arvensis* leaves were illustrated in (**Table 1**). The phytochemical qualitative of the 70 % ethanol crude extract and isolated glycoside fractions (F1 and F2) revealed a presence of several classes of compounds. The crude extract displayed positive reactions of alkaloids, flavonoids, polyphenolic compounds, saponins, terpenids and glycosides. Whereas, the glycoside fractions (F1 and F2) showed a more limited of phytochemical

compounds, that positive tested for polyphenolic, flavonoids and glycosides, but alkaloids and terpenoids were not detected. These findings indicate the fractionation process enriched the glycosidic fractions mainly with phenolic and flavonoid compounds.

Table 1. The yield weight (gm) with color and nature of the extracts

Type of leaves extract		Yield weight (g)	Color and nature of extract
70% Ethanol crude extract		55.9	dark brown solid
Glycosides	F1	6.8	dark green crystalline
	F2	8.5	dark brown viscous

Structural analysis of the glycoside Fractions

The structural characteristics of the isolated glycoside fractions were determined using optical spectroscopic techniques. The characterization was carried out using thin layer chromatography (TLC), UV–Vis and FT-IR spectroscopy. This was done to identify any functional groups and confirm electronic transitions. The details of this process are described below.

Thin Layer Chromatography (TLC)

The TLC plate showed two different spots when visualized under UV light at 245 nm. These two spots demonstrate that the isolate emerged with two glycoside fractions. The RF values obtained for these fractions differed in a rate flow (RF) on TLC plate F1= 0.64 and F2= 0.28, signifying that the compounds have different polarities and molecular structures. Fractions with similar RF values combined to each other were pooled and dried at room temperature. The resulting fractions were named F1 and F2 and were subsequently subjected to further spectroscopic analyses for structural characterization.

Ultra Violet-Visible Light (U.V.) Spectra

Ultra violet spectra of the glycosidic fractions F1 and F2 revealed the characteristic absorption peaks of flavonoid glycosides presence in the extracts of *Convolvulus arvensis* . F1 showed three maximum absorption limits at 271, 292, and 329 nm, typically Band II and Band I transitions of flavonoid structures (**Fig. 2, A**). Whereas, F2 showed maximum absorption at 275nm and 330 nm, this is also in line with the presence of flavonoid glycosides (**Fig. 2, B**).

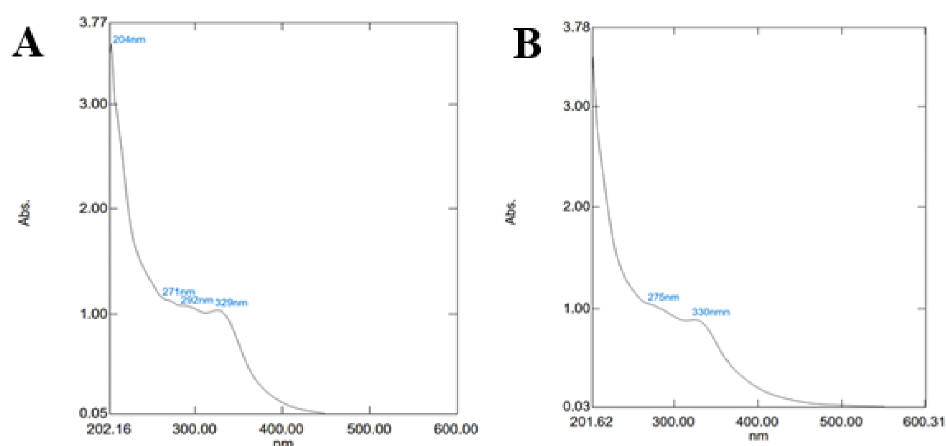

Figure 2. UV–Visible Spectrum of Glycoside Fractions Isolated from *Convolvulus arvensis*, A) F1 and B) F2.

Fourier Transform Infrared (FT-IR) Spectroscopy

The FT-IR spectra showed the main functional groups in the isolated glycoside fractions (F1 and F2) including characteristic absorption bands of the hydroxyl group (O–H), the carbonyl group (C=O), the aromatic stretch C=C and the glycosidic C–O–C bond. These functional groups are common in plant glycosides and flavonoid derivatives. Both fractions showed a presence of hydroxyl groups involved broad absorption band at 3330 cm^{-1} stretching of phenolic groups, the peak at 1706 cm^{-1} was related to carbonyl group (C=O) distinctive in flavonoid backbone and the presence of aromatic ring observed at 1630 - 1660 cm^{-1} associated with flavonoid structures. In other regions, peak in the range of 1157 cm^{-1} (C–O–C) are related to glycosidic bonds vibrations. Additionally, strong bands of stretching (C–O) around 1040- 1059 cm^{-1} correspond to sugar moieties. These results indicate that both fractions contain a flavonoid glycoside compounds, with fraction F2 possessing a higher glycosidic character (**Fig. 3, A & B**).

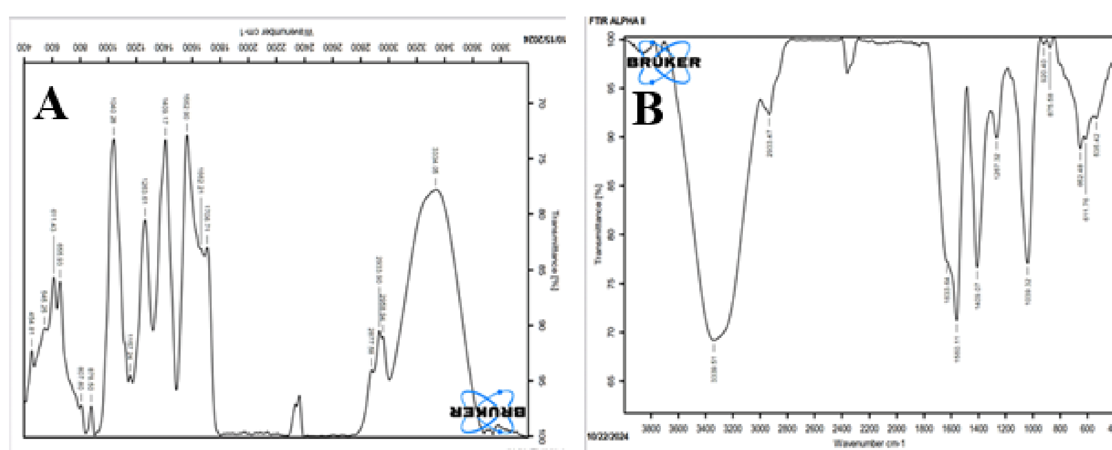

Figure 3. FT-IR spectrum of glycoside fractions (A- F1; B- F2) isolated from *C. arvensis* showing characteristic groups of glycosidic compounds.

Cytotoxicity of glycoside fractions (F1 and F2)

The cytotoxicity of F1 and F2 glycosides on MCF-7 cells was confirmed after treated time 48 h, both fractions showed highly cytotoxic effects on cell viability, significant statistical($p<0.05$) differences were observed between the treated groups and the control groups. At low concentrations (15 µg/mL) the viability was decreased to 83.06%±9.08% and 85.65%±1.38% for F1 and F2, respectively. At the intermediate concentrations (30 and 60 µg/mL) viability reduced markedly to 63.41%±4.52 and 50.03%±2.63 for F1, and 64.82%±2.32 and 48.25%±4.05 for F2 compared with the controls. In compare, at the higher concentrations (120 and 240 µg/mL) both fractions revealed a strong cytotoxic action, with F1 decreasing cell viability to 31.72%±5.73 and 22.54%±2.58 respectively, whereas F2 reduced this parameter to values of 29.24%±4.32 and 20.51%±1.94 (**Fig. 4, A**). From the comparative analysis, the results showed a slightly higher cytotoxicity in F2 compared to F1, especially at higher doses, as shown by the lower percentages of viability and the results show that both glycosides fractions have a dose-dependent anti-proliferative activity on the MCF-7 cell lines.

The cytotoxic effect of glycoside fractions on Vero normal cell after 48 h are presented in (**Fig. 4, B**). F1 showed higher efficacy at longer treated with decreasing of viability. Although the concentration of 15 µg/mL did not show high toxicity, the high concentrations (120 and 240 µg/mL) cause decreasing in viability to 29.88%±4.91 and 25.98%±4.22 respectively. This behavior reveals that the glycosides F1 has a sequential effect which means that cells become more sensitive over time, perhaps due to the accumulation of the compound within cells or the activation of cellular response pathways that lead direct stimulation of cell death.

The effectiveness of glycosides F2 decreased significantly ($p<0.05$) with 48 h of treated time, and the results indicated a greater decrease in viability, mainly at high concentrations. Survival lowered to 82.11%±4.69 in concentration 15 µg/mL, whereas viability reaching 31.3% and 27.8% to 120 µg/mL and 240 µg/mL, respectively. The interpretation of these relatively cytotoxicity results conclude a slight higher cytotoxicity in F2 compared to F1, particularly at high doses, given the lower viability percentages and the results indicate a dose-dependent anti-proliferative activity of both glycosides fractions on the MCF-7 cell lines.

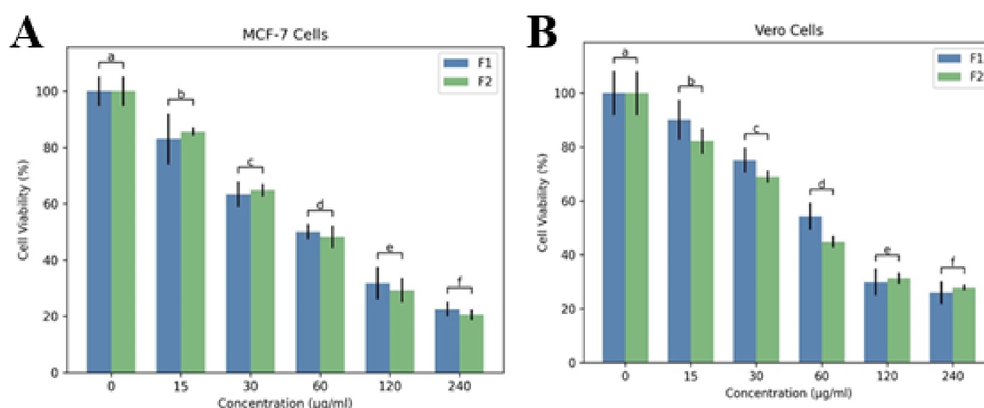

Figure 4. Comparative cytotoxic activity of glycoside fractions (F1and F2) on: (A) MCF-7 breast cancer and (B) Vero normal cells after 48 h.

Evaluate the half maximal inhibitory concentration (IC_{50}) of extracts

The data obtained from the IC_{50} show the difference in treated of the two cell lines when exposed to the compounds tested for toxicity. The glycoside fraction F1 exhibited a strong toxic activity on the cell lines with the lowest IC_{50} values (42 µg/mL within 48 h) registered by MCF-7 cells compared to the Vero cells the highest IC_{50} values indicated for the compound F1 of 75 µg/mL within 48 h indicating a relatively higher resistance compared to the cancer cells. The toxic potency of glycoside fraction F2 was also observed to be higher compared to F1 in most cases, especially on MCF-7 cell lines responded well to the potency of the F2, with an IC_{50} value of 33 µg/mL at 48 h The IC_{50} value of Vero cell lines, however, elevated high at 85 µg/mL at 48 h, thus clearly showing that the less toxic potential of the compound compared to cancerous cell lines lay in its effects on normal cell lines.

Induction of programmed cell death (Apoptosis)

Cytomorphological observation of apoptosis

The morphological characteristics of cell death were further investigated using the technique of dual fluorescent staining with orange acridine and ethidium bromide (AO/EB), which allowed clear discrimination between viable, early apoptotic, late apoptotic and necrotic cells. In this assay, two cell lines (MCF-7 and Vero) were exposed to concentrations of IC_{50} of two glycosidic fractions (F1 and F2) for 48 hours. This method of staining relies on the differential permeability of cell membranes and color dependence on nucleic acids, allowing for an accurate assessment of the progression of apoptosis.

In MCF-7 cells, control culture showed healthy with normal cell morphology, uniform green fluorescence, and intact cell membranes, thus indicating that the cells were viable with complete cell nuclei (**Fig. 5, A**). In contrast, the exposed cells with IC_{50} of two glycosidic fractions (F1 and F2), for 48 hours (42 µg/mL and 33 µg/mL) respectively, displayed prominent features of apoptosis. The cells retained their green fluorescence during the early stages of the apoptosis; however, their nuclei showed bright green punctate dots. The presence of nuclear fragmentation and chromatin condensation, which are signs of programmed cell death, take on the observed nuclear characteristics. The unequal and contracted cell shape along with the accumulated yellow-green fluorescence represented cells that were characterized by nuclear degradation as well as increased membrane permeability to the late stage of apoptosis (**Fig. 5, B & C**). Comparing it to Vero cells, the cells treated with IC_{50} concentrations of F1 and F2 for 48 hours (75 µg/mL and 85 µg/mL) respectively, resulted in relatively milder morphological changes. A large number of treated cells exhibited green fluorescence, along with bright green or yellow-green nuclear dots showing a minimal induction of apoptosis. These observations clearly reveal a selective cytotoxicity of the tested compounds on cancer cells with a relatively greater preservation of normal cells (**Fig. 5, D, E & F**).

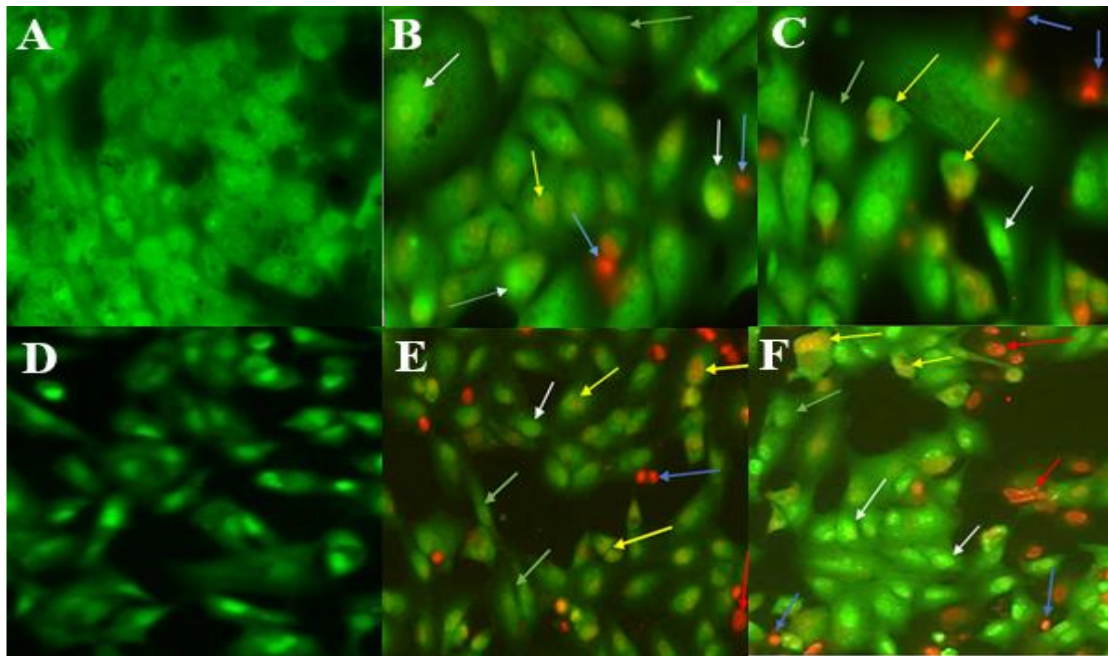

Figure 5. Fluorescent microscope images at 48 h of MCF-7 Cell, (A) control cells, appear as green with uniform color, (B) cells treated with IC_{50} of glycoside F1, (C) cells treated with IC_{50} of glycoside F2. Vero Cell, (D) control cells, appear as green with uniform color, (E) cells treated with IC_{50} of glycoside F1, (F) cells treated with IC_{50} of glycoside F2. Morphological features of MCF-7 and Vero cells are: early apoptotic cells appeared as bright green (white arrow), late apoptotic cells stain dense yellow-green (yellow arrow), apoptotic body (blue arrow), necrotic cells (red arrow), healthy cells (green arrow). AO/EB stain, (400x).

Biochemical analysis of Apoptosis

The results showed that the compounds; glycoside fractions 1 and fractions 2 activation of apoptosis by induced of death receptor-mediated apoptotic signaling in treated cell lines with IC_{50} dose for 48h (MCF-7 and Vero) occurred through both extrinsic and intrinsic pathways through initiation of caspases (**Table 2**) and (**Fig. 6**). In MCF-7 cells, the difference between caspase-8 and caspase-9 concentrations differed more significantly among treatment groups ($p<0.05$). In the control groups, caspase-9 levels (0.122±0.010 ng/mL) were significantly lower ($p<0.05$) than caspase-8 levels (0.133±0.005 ng/mL), which indicate that a majority signals for cell death were extrinsic apoptosis. After treatment with glycoside F1, the level of caspase-8 markedly increased (2.430±0.035 ng/mL) while the level of caspase-9 remained low (1.381±0.021 ng/mL). This treatment led to a statistically significant difference for both proteins, and a greater activation of the extrinsic cell death pathway.

Likewise, in glycoside F2 treated samples, the highest levels of both caspases were detected, with caspase-8 reaching 3.178±0.038 ng/mL and caspase-9 at 2.529±0.000 ng/mL. This difference remained strongly statistically significant ($p<0.05$) while switching dominant activation of caspase-8-mediated signaling, and concurrent to strong activity of the intrinsic pathway (**Fig. 6**). In Vero cells, caspase-8 (0.131±0.002 ng/mL) and caspase-9 (0.113±0.060 ng/mL) did not differ significantly in control

groups. We showed that treatment with glycoside F1 led to a statistically significant increase ($p<0.05$) of caspase-8 (1.645±0.073 ng/mL) as compared to caspase-9 (0.920±0.009 ng/mL) and therefore activated the extrinsic pathway of programmed cell death. Glycoside fraction 2 activation of caspase-8 (2.646±0.246 ng/mL) and also higher than caspase-9 (0.523±0.034 ng/mL) which persisted statically significant and indicating dominance of the extrinsic apoptotic pathway.

Table 2. Caspase-8 and Caspase-9 protein concentration (ng/mL) in MCF-7 and Vero cell lines after 48 h of treatment with IC_{50} dose of glycoside F1 and F2

Treatment	MCF-7 (Mean±SD ng/mL)		Vero (Mean±SD ng/mL)	
	Caspase-8	**Caspase-9**	**Caspase-8**	**Caspase-9**
Control	0.112±0.017[a]	0.118±0.089[a]	0.131±0.002[a]	0.113±0.060[a]
Glycoside F 1	2.182±0.028[b]	2.438±0.060[b]	1.645±0.073[b]	0.920±0.009[b]
Glycoside F 2	2.701±0.023[c]	1.646±0.036[c]	2.646±0.246[c]	0.523±0.034[b]

Values are expressed as mean±SD (n=3). Different letters within the same column indicate statistically significant differences compared with the control group ($p<0.05$) as determined by one-way ANOVA followed by Tukey's post hoc test.

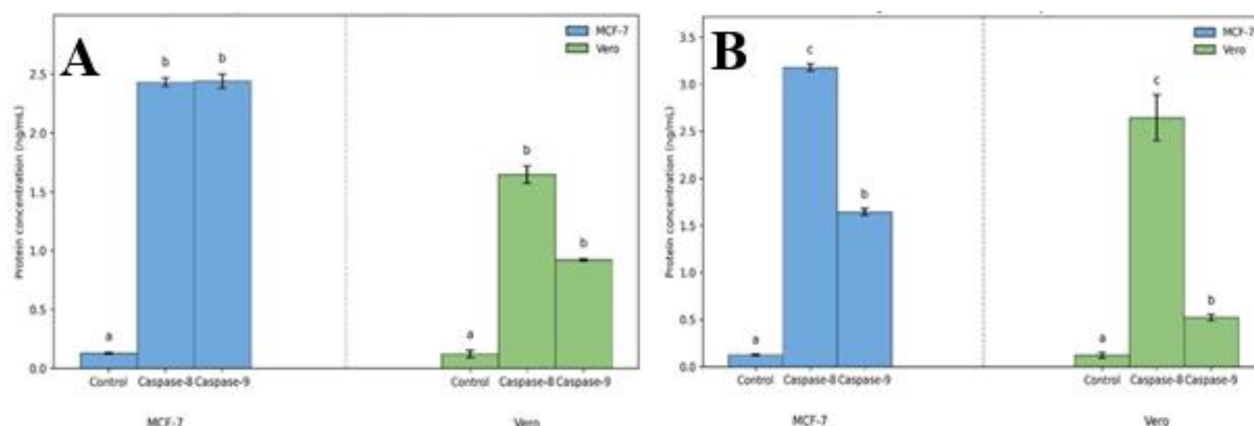

Figure 6. Effect of glycoside fractions on Caspase-8 and Caspase-9 protein levels in MCF-7 and Vero cell lines. **(A)** Glycoside F1 and **(B)** Glycoside F2.

Discussion

The obtained spectroscopic results in this study support the successful enrichment of flavonoid glycosides in the isolated fractions of *convolvulus arvensis*. The UV spectra indicated characteristic absorption within the ranges of 270-390 nm and 329-332 nm, which are consistent with Band II and Band I transitions of the typical flavonoid derivatives. This interpretation is in agreement with recent studies showing that combined of UV and FTIR profiling is effective for the characterization of flavonoid rich plant extracts, particularly when flavonoid glycosides are presented rather than free aglycones, related conclusions were reported for the leaves of *Spiraea japonica* , that

collectively of UV , FTIR and LC-MS confirmed of occurrence the phenolic and flavonoid constituents, in addition , in a recent chromatographic review that highlighted UV, FTIR and LC-MS support as one of the preferred approaches for flavonoid profiling in complex plant grounds [17-19].

The FTIR spectra of both fractions further supported this interpretation through the presence of broad O–H stretching absorption band around 3330 cm^{-1}, the aromatic stretch C=C vibrations at 1630-1660 cm^{-1}, and strong bands (C–O and C–O–C) in the region of 1000-1260 cm^{-1}, which are commonly related to phenolic hydroxyl groups and glycosidic linkage. The stronger carbohydrate-related absorptions that observed in F2 fraction suggest a higher glycosidic relative to F1 fraction, this indicating F2 is likely richer in flavonoid glycosides, this pattern is comparable to recent studies for plant profiling in which FTIR bands in the hydroxyl and fingerprint reign that used together with UV data for verify glycosidic and phenolic structures in extract of leaves. Therefore, the combined TLC, UV and FTIR data in the present study reasonable suggest that both fractions (F1 and F2) contain flavonoid glycosides with F2 appearing of possess a higher glycosidic compound than F1 [17,20-22].

The cytotoxicity results reveal that both fractions inhibited MCF-7 cell growth in a dose-dependent manner, with fraction F2 being more potent than F1. The increased activity can possibly be associated with higher glycosidic flavonoids contents of F2 based on the relative strong glycosidic signals in FT-IR spectra. Similar results are reported by other studies showing that flavonoids glycosides potently suppress the proliferation of cancer cells via several mechanisms, including modulation of oxidative stress and cell cycle arrest [4,6]. Notably, the cytotoxicity of both fractions toward Vero normal cells was lower, demonstrating the selective cytotoxicity. The aforementioned selectivity is an advantageous quality for anti-cancer agents as this property has been previously reported for several plant-derived flavonoids which displayed selective action towards cancer cells owing to the distinctive metabolic activity and redox status of cancer cells [4,10].

Apoptosis induction was confirmed using AO/EB staining that characterized the morphological changes as evidenced by chromatin condensation and membrane damage observed in treated MCF-7 cells. This was further supported by ELISA results reflecting significant activation of apoptotic protein levels, particularly, caspase-8 and caspase-9 (extrinsic and intrinsic pathways, respectively). In MCF-7 breast cancer cells following treatment with glycoside fractions. This change was significantly greater than that measured in the normal Vero cells, indicating selective stimulation of cancer cells [10,23].

Caspase-8 is a major upstream activator of the extrinsic pathway upon death receptor signals and due to its increased activity, it could help contribute to anticancer activity. In the latter case, the major upregulation in caspase-8 levels found for MCF-7 cells treated with the F2 glycoside moiety suggests a more dominant contribution of the extrinsic pathway in induction of apoptotic programmed cell death. Comparable pathways have been discovered in both flavonoid glycosides-linked studies in which

flavonoid glycosides activated death receptor-mediated apoptosis via caspase-8 activation [4,24,25].

On the other hand, caspase-9 is a central regulator of the intrinsic (mitochondrial) apoptotic pathway. The high levels of caspase-9 in MCF-7 cells indicate the involvement of mitochondrial- mediated apoptosis. The simultaneous activation of caspase-8 and caspase-9 indicate that the glycoside fractions induce apoptosis through double signaling pathways and thereby enhancing the efficiency of cancer cells eliminations [10,26]. Furthermore, the higher expression of apoptotic proteins in MCF-7 cells compared with Vero cells highlights the selective cytotoxicity of the flavonoid glycoside fractions. The cancer cells are more vulnerable to apoptosis due to their altered metabolic activity, increasing of oxidation stress and dysregulated signaling pathways. In contrast, normal cells exhibit better resistance to apoptotic stimuli. Similar selectivity has been reported in several studies involving flavonoid compounds as anticancer thereby [27,28].

The difference in activity between the F1 and F2 glycoside fractions highlights the importance of chemical composition in regulating biological activity. These differences between the F1 and F2 activity in induce of caspases (the F2 exhibits relatively higher caspase-8 activity, indicating greater activation of the extrinsic pathway, whilst the F1 fraction exhibits higher caspase-9 activity, suggesting a greater contribution from the intrinsic pathway) with the results of previous studies that have demonstrated the role of both pathways in inducing programmed cell death. These differences may be due to variations in the structure of flavonoid glycosides, which can be influenced by their interaction with cellular targets and the signaling pathways involved in programmed cell death [25,29].

Furthermore, flavonoids not only directly activate the caspase enzymes involved in apoptosis but they also inhibit and modulate associated signaling pathways involved in the proliferation and survival of cancer cells such as the NF-κB, PI3K/Akt and MAPK pathways. The inhibition of these pathways decreases the levels of anti-apoptotic proteins and induces apoptotic mechanisms, which in turn results in an increase in apoptosis [4,30].These findings of the present study provide strong evidence for the potent pro-apoptotic effect of glycoside fractions, especially regarding their anti-cancer effect. The selective caspase pathways and the strong differences between F1 and F2 confirm their potential for development as anti-cancer drugs. Nonetheless, this will require more studies to isolate pure active compounds and determine their specific molecular mechanisms using advanced analytical and in vivo techniques on separated tissues.

CONCLUSION

Fundamental Finding : The present study demonstrated that glycoside fractions isolated from leaves of *C. arvensis* possess significant phytochemical and biological activities, with spectroscopic analyses (TLC, UV-Vis, and FT-IR) confirming flavonoid glycosides, and fraction F2 showing higher glycosidic character and greater cytotoxic

potency against MCF-7 cells with lower IC_{50} values. **Implication :**Both fractions exhibited dose-dependent cytotoxic effects with reduced toxicity toward Vero normal cells, suggesting selective anticancer activity mediated through apoptosis via activation of caspase-8 and caspase-9, indicating that *Convolvulus arvensis* may serve as a natural source of anticancer flavonoid glycosides. **Limitation :** The study is limited to fraction-level analysis without isolating specific active compounds or fully elucidating their detailed molecular mechanisms of action. **Future Research :** Future studies are needed to isolate the active compounds and conduct further assays to investigate their precise molecular mechanisms and therapeutic potential.

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