Anticancer Activity of Ferulic acid Purified from Iraqi Apple petioles

Rihab J. Mansoor[1*] , Yasir H. Zaidan[1] , Zainab Y. M. Hasan[2]

[1]Department of Biology, College of Science, Mustansiriyah University, Iraq
[2] Biotechnology Research Center / Al-Nahrain University, Iraq

*Correspondence email: rihabaldulaimi@gmail.com

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ABSTRACT

Background: Ferulic acid (F), a hydroxycinnamic acid (4-hydroxy-3-methoxycinnamic acid), is one of the primary phenolic acids found in apples. F has potential biological activity, such as antioxidant, anti-inflammatory, and anticancer properties. **Objective:** The present study aimed to purify F from Iraqi Apple petioles crude extract and investigate the effect of F on cell viability in (human colon cancer cell/ SW480, human hepatocyte carcinoma/ Hep G2, adenocarcinoma of the stomach/ AGS, and human lung adenocarcinoma/ A549), beside the normal cell line (Human Embryonic Kidney/HEK 293). **Methodology:** F was determined quantitatively and qualitatively in different aerial parts for Iraqi apple *(Malus domestica*), cv." Ibrahimi" included leaves, petioles, and stems extracts. Petioles represented a rich source of F, which was isolated and purified by preparative HPLC. Various analytical characterization methods confirmed the chemical structure of the isolated Ferulic acid (F2): Melting point, High-performance Liquid Chromatography (HPLC), Carbon Hydrogen and Nitrogen elemental analyses (CHN), and Identification by Fourier-transform infrared spectroscopy (FTIR). Cytotoxicity was evaluated using MTT assay after exposure to escalating doses (500, 250, 125, 62.5, 31.25, and 15.625) µg/ml of F and Cisplatin (standard chemotherapy) against cancer cell lines and normal cell line. **Results:** About 0.952 g F2 was obtained from 14.086 g apple petioles (6.8%). The MTT test results showed that the purified F2 possessed significant anticancer activity on all cancer cell lines. **Conclusion:** Results suggested that purified F2 from Iraqi apple petioles has potential cytotoxicity, which may benefit human health.

Keywords: *Malus domestica*, Petiole, Ferulic acid, Anticancer.

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INTRODUCTION

Cancer is the second leading cause of death worldwide, after cardiovascular diseases (1). Cancer, a multifaceted and heterogeneous disease, is a substantial health obstacle for humans (2). Despite notable progress in medical science, pharmaceutical chemistry, and cell biology, the prevalence and death rates of cancer persistently increase worldwide, frequently considered untreatable (3). It is projected that the global cancer rate will experience a substantial rise from 2020 to 2040, with approximately 28.4 million additional cancer cases anticipated in 2040 (4). Crucial lifestyle factors that lead to the onset of cancer include, but are not limited to, the consumption of carcinogens through foods, exposure to solar radiation, smoking, alcohol consumption, and lack of physical exercise. Many of these factors can be altered, suggesting that cancer can be avoided by adopting an appropriate lifestyle (5). Around half a century ago, Michael B. Sporn first proposed the concept of cancer prevention and introduced the word "chemoprevention." Tumorigenesis inhibition is the utilization of non-toxic substances to prevent or reverse the progression of tumor formation (6).

Pharmacotherapy (Chemotherapy) is a widely used global treatment for cancer. It involves using several substances, including platinum-containing compounds like carboplatin, oxaliplatin, and cisplatin (7). Approximately half of cancer patients undergo cisplatin treatment (8). Unfortunately, cisplatin has many negative aspects, such as inducing harmful effects on the patient's healthy tissues (neurotoxicity, nephrotoxicity, hepatotoxicity, ototoxicity, haematological toxicity, gastrointestinal toxicity, and cardiotoxicity) and the emergence of cisplatin resistance in cancer cells during treatment (9). Consequently, it significantly reduces the patient's quality of life. It requires a reduction in the amount of this medication or maybe stopping it completely, which lowers the effectiveness of anticancer treatment (10). In addition, the diverse composition of cancer poses a significant challenge in developing efficient anticancer treatments. This frequently leads to chemotherapy ineffectiveness or increased treatment resistance, ultimately culminating in cancer resurgence (11).

Plant-based therapies are commonly utilized in traditional medicines (12). Specific botanical remedies exhibit significant efficacy in treating and preventing extremely lethal cancers (13, 14). Drugs containing plant secondary metabolites as the main active components have been employed to treat human diseases (15).

F is the predominant phenolic acid found in plant cell walls and is abundant in vegetables, fruits, and grains (16). F is present in a free form or bound to hydroxy acids in vegetables and fruits or hemicellulosic polysaccharides in grains; it is classified as a hydrophobic phenolic compound because of its poor solubility in water(17, 18).

It has been characterized as a powerful antioxidant by effectively eliminating free radicals and improving the cellular stress response by activating Cytoprotective mechanisms (19). Previous studies have shown that F has the potential to be used as a treatment for various disorders, including skin disease (20), Alzheimer's disease (21), diabetes mellitus (22), cardiovascular diseases (23), anti-angiogenic (24), antimicrobial (25), anti-inflammatory (26), wound healing (27), cholesterol-lowering(17), radioprotective properties (20) and anticancer properties (28). For these benefits, it is widely used in the cosmetic, food, and pharmaceutical industries (29).

The extraction of F from natural plant sources is crucial for industrial production. Having plant material that contains the necessary components can lower the overall cost of processing. There has been a new upsurge in scientific curiosity about F. The increasing interest in this compound can be ascribed to its indispensable anticancer efficacy, rendering it a very interesting field of investigation for future research. Apple parts, specifically apple peel, pomace, and other aerial parts, are a cost-effective and plentiful source of polyphenols (30, 31) . This study presents the process of isolating, characterizing the structure, and identifying F from the petioles of *M. domestica* in Iraq.Consequently; we aim to investigate the antioxidant activity and cytotoxicity of Ferulic acid on four cancer cell lines. Additionally, we will compare F with cisplatin (the traditional chemotherapy anticancer drug). This study aims to gain greater knowledge of the bioactivity of Ferulic acid derived from Iraqi *M. domestica* and provide supporting evidence. So far, no prior documentation exists regarding the isolation of Ferulic acid from apples cultivated in Iraq.

METHODOLOGY

Plant material Collection and Preparation of Samples

In November 2022, during the early winter season, the aerial parts (leaves, petioles, and stems) of Iraqi *M. domestica* were collected from a privately owned orchard in the Ala'bara region of Diyala Governorate, Iraq. The specific cultivar was named "Ibrahimi". Dr Sukina Abbas, a senior Taxonomist from the University of Baghdad Herbarium, identified the plant. The aerial parts of the plant, which were in good health and fresh, were cleaned and then dried in the air for various durations: 15 days for the leaves, 20 days for the petioles, and 25 days for the stems. The drying process occurred at room temperature, with sufficient airflow, and in a shaded area. The dried materials were ground to a fine powder by a two-step procedure involving (manual) grinding followed by (electrical) grinding and employed for extraction.

Extraction Procedure

Each dried aerial part, including leaves, petioles, and stems, was separately treated with 500ml of 70% ethanol through a process called triple cold maceration. This process included immersing them for 24 hours while sometimes stirring and filtering the mixture. The marc underwent two more macerations in 70% ethanol, each

lasting 24 hours. The maceration process was repeated up to three times, and the resulting filtrate was combined. The filtrate underwent a final desiccation process utilizing a rotary evaporator at 40°C. As a result, highly concentrated dry extracts were formed, which were then weighed and stored at 4°C until they were available for use. Marc was ejected(32, 33).

Identification and Quantification of Ferulic acid by HPLC Method

HPLC analysis was conducted to identify Ferulic acid in Ethanolic extracts (Leaves, stems, and petioles) of Iraqi *M. domestica*. Quantitative estimation was carried out using liquid chromatography (equipment SHIMADZU, Japan), the column was ODS-C18 (250 mm× 4.6 mm I.d.), particle size 5µm, the flow rate was 0.8 ml/min, room temperature, column pressure was 8.9, the Injection Volume was 20 µl, and the mobile phase was 0.1% Acetic acid 20% (A) and Methanol 80% (B), and detection wavelength was UV-Vis at λ 254nm, at the Ministry of Science and Technology, the Department of Environmental and Water Research, Iraq, Baghdad.

Isolation and purification of proposed Ferulic acid from the crude leaves extract by preparative HPLC (PHPLC)

A Ferulic acid was isolated from the ethanolic extract of the petioles performed according to conditions (34) by HPLC equipment(SHYKAM, Germany), the column was ODS-C18 (250 mm× 4.6 mm I.d.), particle size 5µm, the flow rate was 1.0 ml/min, column temperature(25 ± 1°C), the Injection Volume was 0.1 ml, and the mobile phase was = Methanol 70% (A), water 25%(B), and formic acid 5% (C), filtered and degassed before use, and detection wavelength was UV-Vis at λ 280 nm, at the Ministry of Science and Technology, the Department of Environmental and Water Research, Iraq, Baghdad. A fraction collector collects the target peak from the time of its appearance until it's near or almost end. The proposed Ferulic acid was subjected to a final drying process using a rotary evaporator at a temperature of 40°C. This resulted in the formation of powder concentrate dried proposed Ferulic acid, which was subsequently weighed and kept at a temperature of 4°C until ready for use. The identification and purity of the target compound (proposed Ferulic acid) were checked using chromatographic (HPLC), spectroscopic (FT-IR), Melting point, CHN analysis, ^{1}HNMR and ^{13}C NMR spectra. The isolated Ferulic acid is symbolized as F2.

Structural Identification and characterization of the proposed Ferulic acid (F2)

F2 was identified by using different identification methods as follows:

Identification by HPLC

The purity of F2 was checked using HPLC and the same conditions previously discussed in previous above step.

Melting point

The melting point of F2 was evaluated using electrothermal melting point equipment within the Environmental and Water Research Department of the Ministry of Science and Technology, Iraq, Baghdad.

Identification by Fourier Transforms Infrared (FT-IR) Spectroscopy

The functional groups in F2 were detected using Fourier transform infrared spectroscopy (FTIR) with apparatus (BRUKER, USA). The KBr method was utilized to transform the sample into pellets for examination. FTIR analysis is a spectroscopic method employed to determine the absorption spectrum of a material inside the infrared region, especially within the wavelength band of 400-4000 cm^{-1}. The samples were analyzed to determine the structure and molecular composition of the compounds by measuring the absorbance of infrared light energy at various wavelengths(35). The analysis was carried out within the Environmental and Water Research Department of the Ministry of Science and Technology, Iraq, Baghdad.

CHN Elemental Analysis

The CHN analysis approach precisely measures the carbon, hydrogen, and nitrogen elemental composition of F2 sample. Analyzed with the PERKIN-ELMER 2400 CHN analyzer. The oxygen percentage is calculated by subtracting the combined percentage of carbon (C) and hydrogen (H) from the total percentage (36).

Cytotoxicity assay of isolated Ferulic acid from Iraqi *M.domestica* petioles by MTT test

For evaluating the cytotoxicity of F2 obtained from the Iraqi *M. domestica* petioles. The MTT test is widely used to assess the cells viability exposed to compounds that might have cytotoxic effects. The MTT test is quick, efficient, and economical in vitro technique employed to quantify the number of viable cells by evaluating their capacity to transform a yellow (tetrazolium salt) into a purple (formazan molecule) (37).

Cell culture

The cancer cell lines utilized in this study were human colon cancer cell (SW480), human hepatocyte carcinoma (Hep G2), adenocarcinoma of the stomach (AGS), and human lung adenocarcinoma (A549). The human embryonic kidney cell line is the normal Human Embryonic Kidney (HEK 293). These cell lines were incubated in a humid environment at 37 °C in CO_2 incubator. The RPMI-1640 medium was supplemented with 100 U/mL of streptomycin, 100 µg/mL of penicillin and fetal bovine serum(10%) (38).

MTT assay

The SW480, Hep G2, AGS, A549, and HEK 293 cell lines were initially seeded at a density of 1×10^4 cells per well. Subsequently, the plates were carefully transferred to a CO_2 incubator and adhered overnight. The following day, the medium was replaced with a fresh medium 100 µl that contained varying concentrations (500, 250, 125, 62.5, 31.25, and 15.625) µg/ml of F 2, as well as control samples. In addition, Cisplatin (Accord, Ireland) was used for the positive control. The cells were subsequently placed in an incubator for 24 hours. Afterwards, the medium was removed and replaced with 100 µl of an MTT solution containing 0.5 mg/ml. The cells were subsequently cultured for 4 hours at 37 °C. Afterwards, the MTT solution was extracted and substituted with 100 µl of dimethyl sulfoxide (DMSO) in every well. The plate was placed in an incubator at 37°C and subjected to continuous agitation for 15 minutes. Ultimately, absorbance was measured using a microplate reader configured to a specific wavelength of 570 nm(39). The experiment was performed three times. To determine the fraction of live cells, we compared the absorbance values of cells treated with F2 and Cisplatin to those of untreated control cells. Cell viability percentage was calculated using the equations outlined by Al-Shammari (40).

$$Percent\ cell\ viabilty = \frac{Average\ absorbance\ of\ treated\ cells}{Average\ absorbance\ of\ untreated\ cells} \times 100$$

$$Percent\ cell\ inhibition = 100 - Percent\ cell\ viabilty$$

Statistical Analysis

The Statistical Analysis System- SAS (41) program was used to detect the effect of difference factors in study parameters. Least significant difference-LSD was used to significant compare between means in this study ($P \leq 0.05$).

RESULTS

Ferulic acid concentration in different aerial parts of the apple

The concentration of F quantified by HPLC in the apple aerial parts is shown in (Table 1). Highly varied concentration ranges of F were found in the results; the highest concentration of F detected was in the petioles at 0.248mg/g DW and the lowest in the stems at 0.014mg /g DW. The retention times of standard and extracts are shown in (Figure 1).

Table (1): The retention time and Concentration of Ferulic acid identified by the HPLC analysis in the different aerial parts.

Ferulic acid	Ret. Time for Standard	Ret. Time for extract	Concentration (mg /g DW)
leaves	11.563	11.546	0.015
stems	11.563	11.547	0.014
petioles	11.563	11.475	0.248

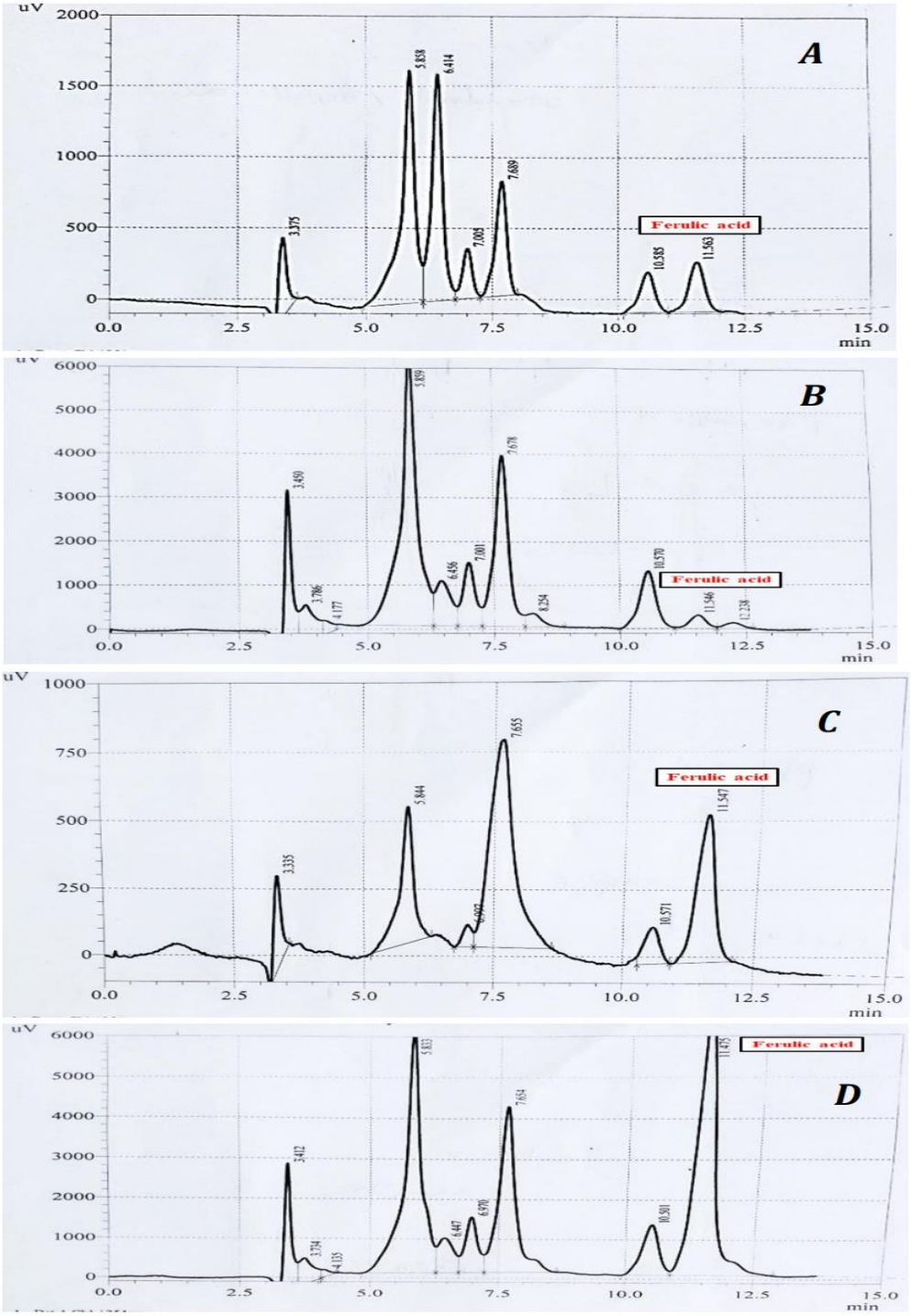
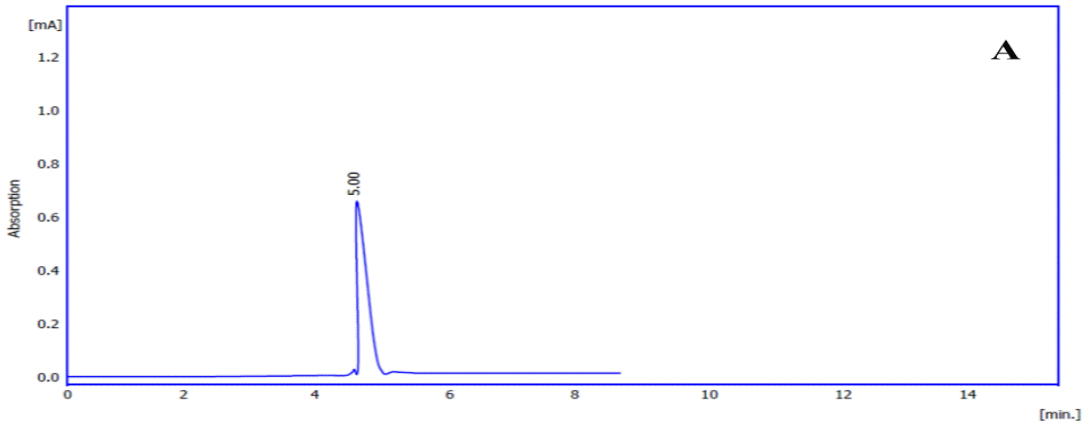


Figure (1): HPLC chromatogram of mixed standard solution, (A) ferulic acid Standard, (B) leaves, (C) stems, and (D) petioles.

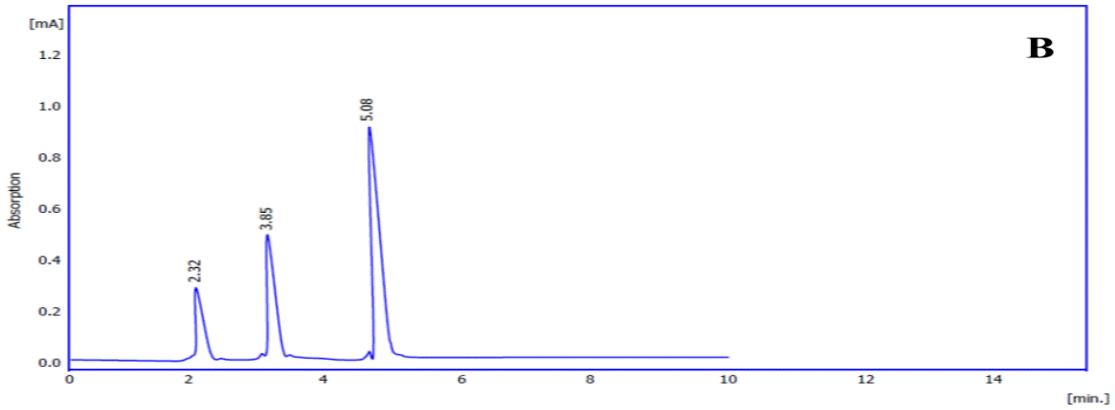
Isolation and purification of ferulic acid from the crude petioles extract by PHPLC

According to HPLC results, the presence of F was documented in the extracts of *M. domestica* by comparison with Rt values of the Ferulic acid standard. (F) In the extract of petioles isolated by PHPLC, the PHPLC chromatogram of the petioles extract exhibited several peaks corresponding to various compounds based on their retention times. One of these peaks, F, had a retention time of 5.08 min, which was identified by comparison with the F standard with a retention time of 5.00 min. The target peak was collected by a fractions collector from the time of its appearance until its descendants, as shown in (Figure 2).



Result chromatography Table (Uncal - F:\ Ferulic acid (10 PPM)

No	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W 05 [min]	Compound Name
1	5.00	8547.59	600.02	100.00	100.00	0.25	
	Total	8547.59	600.02	100.00	100.00		



Result chromatography Table (Uncal - F:\ extracted

No	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W 05 [min]	Compound Name
1	2.32	2451.59	300.12	20.00	20.00	0.15	
2	3.85	4587.49	501.26	30.00	30.00	0.20	
3	5.08	98542.22	904.58	50.00	50.00	0.30	
	Total	105581.31	1705.96	100.00	100.00		

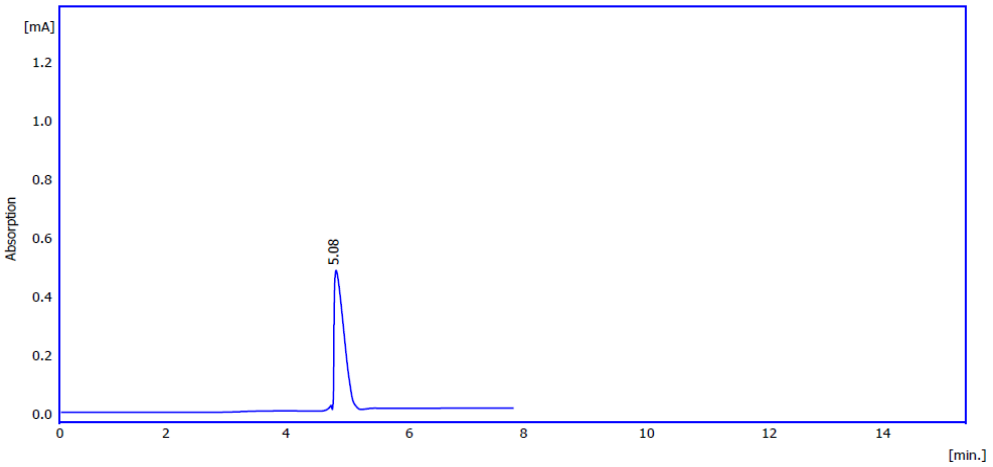
Figure (2): PHPLC chromatogram for Ferulic acid standard (A) matched with petioles extract (B).

Identification and characterization of the proposed Ferulic acid

Additional investigation was performed utilizing the following procedures to verify the identity of F2.

HPLC

The identification of F2 was carried out by HPLC, comparing its HPLC retention time with that of the standard. Peak F2 had a retention time of 5.08 min, compared with the Ferulic acid standard with a retention time of 5.00 min, as shown in (Figure 3).



Result chromatography Table (Uncal - F:\ fraction Ferulic

No	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W 05 [min]	Compound Name
1	5.08	1204.59	530.22	100.00	100.00	0.25	
	Total	1204.59	530.22	100.00	100.00		

Figure (3): HPLC chromatogram for proposed Ferulic acid (F2).

Melting point

Table (2) compares the melting point of F2 and the reference melting point.

Table (2): Melting point of isolated proposed Ferulic acid compared to reference melting point

Isolated compound	**Tested Melting Point °C**	**Reference Melting Point°C**	**Ref.**
Ferulic acid	170 – 173 °C	168 - 172 °C	(29)

FTIR

The FT-IR spectrum of the sample indicates the presence of the main functional groups in the structure of ferulic acid, as shown in Figure (4). The strong and broad band observed at 3,300 cm^{-1} is characteristic of the OH group in phenolic compounds, while the C-H stretching of the aromatic ring is observed at 3062 cm^{-1}. The bands at 2956, 2927, and 2864 cm^{-1} are assigned to the stretching vibration of C-H aliphatic groups. The band observed at 1735 cm^{-1} corresponds to the carbonyl group (C=O). The bands at 1654 and 1550 cm^{-1} are attributed to (aromatic C=C), thus confirming the skeleton of F. The stretching band observed at 1328 cm^{-1} is characteristic of the C-H vibration of the methyl group, and the stretching of the C-O carboxylic acid is visible at 1207 cm^{-1}. Moreover, the vibration for C-O on the aromatic ring appears at 663 cm^{-1}(42, 43).

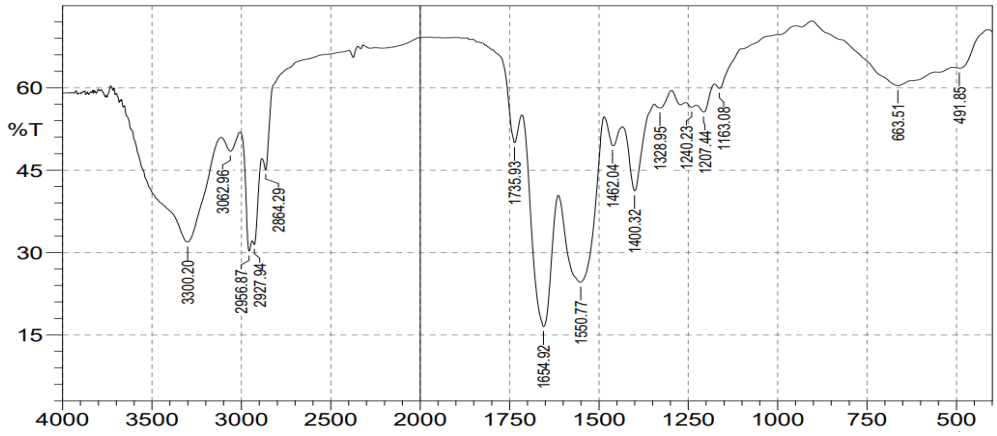


Figure (4): FT-IR spectra of isolated Ferulic acid (F2).

CHN analysis

The CHN analysis approach can accurately quantify the elemental composition of carbon, hydrogen, and nitrogen in F2 sample. The analysis was conducted using the PERKIN-ELMER 2400 CHN analyzer. Table 3 displays the percentages of (C, H, N) constituents within the examined sample. The findings demonstrate a significant agreement between the calculated theoretical data and the found experimental data for F2.

Table (3): CHN/O analysis results of proposed Ferulic acid (F2).

Compound	**C %**	**H %**	**N %**	**O % ***
Proposed ferulic acid (F2)/Found	61.11	4.99	0.00	33.90
ferulic acid standard / Calculated	61.80	5.15	—	32.96
*O% was determined by C % and H % from the total composition./ -- = absent				

Based on all these results, the target compound (F2) structure was identified as Ferulic acid (F).

Cytotoxic Effects of Ferulic acid (F2) Four Cancer Cell Lines

In vitro, the cytotoxicity potential of F2 was evaluated in SW480, Hep G2, AGS, A549, and HEK 293 cell lines (Table 4). The effect of cytotoxicity on cell growth was assessed at various concentrations (15.625 – 500 µg/ml), and the results measured the ability of F2-induced inhibition. The results show no cytotoxic effects towards the F2 treated Noncancerous cell line of HEK-293. In SW480, Hep G2, AGS, and A549 cells, the viability percentage of cancerous cells decreased with the increasing concentrations of F2. It shows that the cytotoxic effect of the F2 against cancer cell lines was stronger than the Noncancerous cell line. In vitro, the cytotoxicity potential of Cisplatin was evaluated in cancer and non-cancer cell lines (Table 5). Our findings indicate that various cells exhibited varying degrees of sensitivity to the inhibitory action of F2.

Table (4): The percentage of inhibition per different doses of the isolated Ferulic acid of *M. domestica* Petioles in different cell lines

Conc. (µg/ml)	Means ±SE					LSD value
	Hep G2	A549	AGS	SW480	Hek 293	
500	29.17 ±1.74 A d	74.50 ±3.68 A b	84.67 ±4.68 A a	90.38 ±4.54 A a	49.24 ±2.51 A c	9.52 *
250	16.07 ±0.82 B d	70.83 ±2.96 AB b	80.34 ±4.03 A a	69.69 ±3.19 B b	33.11 ±1.87 B c	9.05 *
125	15.01 ±0.78 B d	69.28 ±3.05 AB a	76.15 ±3.19 AB a	43.17 ±2.74 C b	30.59 ±2.04 B c	8.61 *
62.5	12.88 ±0.83 BC d	64.73 ±2.57 B a	69.57 ±3.62 B a	33.07 ±1.93 D b	22.95 ±1.37 B c	8.26 *
31.25	7.83 ±0.52 CD c	44.15 ±2.09 C a	32.75 ±1.72 C b	33.93 ±1.26 D b	14.45 ±0.92 C c	7.55 *
15.625	2.37 ±0.13 DE d	7.98 ±0.54 D cd	11.97 ±0.69 D bc	28.79 ±2.08 D a	12.92 ±0.76 C bc	6.79 *
0	0.00 ±0.00 E a	0.00 ±0.00 E a	0.00 ±0.00 E a	0.00 ±0.00 E a	0.00 ±0.00 D a	0.00 NS
LSD value	6.92 *	7.75 *	8.91 *	9.34 *	7.86 *	---
Means having with the different big letters in same column and small letters in same row differed significantly. * (P≤0.05).						

Table (5): The percentage of inhibition per different doses of the standard chemotherapy (Cisplatin) in different cell lines

Conc. (µg/ml)	Means ±SE					LSD value
	Hep G2	A549	AGS	SW480	Hek 293	
500	48.02 ±2.36 A b	65.44 ±3.07 A a	60.35 ±2.95 A a	65.19 ±3.17 A a	19.15 ±0.86 A c	8.96 *
250	51.71 ±2.85 A b	54.76 ±2.77 B ab	56.44 ±2.68 AB ab	62.71 ±2.94 AB a	17.02 ±0.79 A	8.52 *
125	51.72 ±2.69 A a	49.28 ±2.63 B a	49.41 ±2.71 B a	54.87 ±2.36 B a	16.28 ±0.62 A b	8.47 *
62.5	34.94 ±1.86 B b	16.13 ±0.95 C c	36.26 ±2.04 C ab	41.78 ±2.83 C a	16.37 ±0.75 A c	7.92 *
31.25	30.99 ±1.57 B b	13.99 ±0.68 C c	27.82 ±1.57 D b	40.39 ±2.16 C a	11.09 ±0.62 A c	7.39 *
15.625	24.10 ±1.42 C b	11.76 ±0.74 C c	21.42 ±1.06 D b	31.18 ±1.09 D a	9.91 ±0.58 A c	6.88 *
0	0.00 ±0.00 D a	0.00 ±0.00 D a	0.00 ±0.00 E a	0.00 ±0.00 E a	0.00 ±0.00 B a	0.00 NS
LSD value	8.34 *	9.51 *	8.73 *	8.07 *	7.18 *	---
Means having with the different big letters in same column and small letters in same row differed significantly. * (P≤0.05).						

DISCUSSION

The variation in phenolic compounds concentrations and yields in apples can be attributed to factors including variety, plant parts, environment conditions, maturity, time, and storage conditions and is additionally affected by the extraction conditions, extraction method (44).In our study, this variation was observed in the difference in F concentration between different aerial parts) leaves, petioles, and stems).

Extracting important phenolic compounds from plant residues is crucial for creating valuable products from renewable by-products. F is a functional bioactive compound that can be isolated from agricultural biomass (45, 46, 47). Phenolic compounds, including phenolic acid, are purified through traditional methods such as Extraction by using precipitation, organic solvents, chromatography, and crystallization, which can increase the concentration of extracted phenolic compounds by removing unwanted compounds and residual solid particles(48). Chromatography is a highly effective purification method that yields high purity (49). HPLC is commonly employed to efficiently separate bioactive compounds and achieve a high recovery rate(50); the principle of separation depends on the bonds between the chromatographic phases (stationary and mobile phases) and each sample component. The choice of phases depends on the sample's characteristics (structure, solubility, and polarity) and the target compound for isolation(51, 52).

F is an efficient scavenger of free radicals of both reactive nitrogen species and reactive oxygen species (RNS and ROS, respectively. The antioxidant activity of F is attributed to its chemical structure (unsaturated side chain and phenolic nucleus), which contributes to forming resonance compounds (17, 23). Previous research has demonstrated that F is a potent antioxidant, protecting DNA against oxidative damage and inhibiting lipid peroxidation by decreasing oxidative stress (53).In our study, the activity of F in the cell line conditions was also assessed. The biocompatibility of F was tested in vitro using the SW480, Hep G2, AGS, A549, and Hek-293 as model cells in a cytotoxicity study.

F, a type of natural antioxidant, has shown cytotoxic effects in several types of cancers, either when used alone or in combination with other bioactive chemicals and can inhibit the expression and function of cytotoxic enzymes, such as inducible nitric oxide synthase, caspases, and cyclooxygenase-2 (54, 55). F considerably induces apoptosis by enhancing the activity caspase-3 (activation of caspase-3 in the apoptosis pathway) in cancer cells, inhibits cell migration, inhibiting and preventing metastasis, and suppresses cancer cell proliferation(23). The possible adjuvant effect of F in cancer therapy can be attributed to its ability to activate cytoprotective enzymes and suppress cytotoxic mechanisms(28).

Our data is Similar to a previous study conducted by Choi et al. (56), who showed that F(500 μg mL−1) significantly decreased the cancer cells' viability HepG2 but did not induce any toxicity on 3T3-L1 and NIH-3T3 normal cells. (26) Proposed that F exhibits toxicity that varies depending on the cell type and should be utilized carefully. Two separate studies have demonstrated that the antitumor properties of F against cervical carcinoma cells(human Caski and Hela cervical cancer cells) (28) and osteosarcoma cells (57) were associated with induction of apoptosis and G0/G1 cell-cycle arrest (F performs its antitumor activity by interference in the cell cycle). In addition, studies have demonstrated that F suppresses cellular functions and increases oxidative DNA damage in HeLa and ME-180 human cervical cancer cells(58). Another study performed on intestinal cells (Caco2) showed a significant effect of F at a concentration of 10 and 20 mg L−1(59). There is much evidence about the anticancer activity of F, and it has been shown to promote cytotoxicity against various tumour cell lines, including human glioblastoma (U87MG) (60), prostate cancer(61), colon cancer (62), and breast cancer(23).

In the present study, HepG2 cells are resistant to Cisplatin, It has been proven that cancer cells develop several mechanisms that protect them from the cytotoxic effect of cisplatin, such as changes in DNA damage repair mechanisms or changes in the function of membrane transporters (7, 63). This problem resulted in intense searches to increase the effectiveness of cisplatin and overcome the mechanisms of cell resistance. Recent studies indicate that such methods may include combined therapy of cisplatin and radiotherapy, as well as a combination of cisplatin with compounds of plant origin and other drugs (64). F significantly inhibits SW480, Hep G2, AGS, and A549 cancer cells. This study provides a preclinical theoretical basis for utilizing F as a potential therapy for cancer cells. Finally, Ferulic acid's various sources and pharmacological activities have led to new drug development derived from apples.

CONCLUSION

Apples are among the most widely grown and consumed fruits worldwide. Ferulic acid is a vital compound found in apple trees. This compound is crucial for enhancing human health because of its strong antioxidant, anti-inflammatory, and anticancer activities. In this study, we successfully purified F from apple petioles by PHPLC. The structural and physicochemical characterization using Melting point, HPLC, FTIR, CHN elemental analyses, and NMR revealed that the purified compound was ferulic acid. The MTT assay demonstrated increased antiproliferative activity of F compared to Cisplatin (standard chemotherapy) against SW480, Hep G2, AGS, and A549 cancer cell lines. Our results confirmed the F, which could increase cytotoxicity and anticancer potential. In conclusion, petioles are a potent source of ferulic acid that can be extracted, isolated, and used as an antioxidant and anticancer. Experimental findings and pre-clinical data serve as a basis for developing potential natural drugs.

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REFERENCES

1. Alansari WS, Eskandrani AA. The anticarcinogenic effect of the apple polyphenol phloretin in an experimental rat model of hepatocellular carcinoma. Arabian Journal for Science and Engineering. (2020); 45(6): 4589-4597.
2. Jarallah SA, Al-Fartusie FS, Zageer DS. Assessment of Insulin and Cortisol Levels in Iraqi Women with Breast Cancer. Al-Mustansiriyah Journal of Science. (2023); 34(1): 32-36.
3. Tuli HS, Rath P, Chauhan A, Ramniwas S, Vashishth K, Varol M, Jaswal VS, Haque S, Sak K. Phloretin, as a potent anticancer compound: from chemistry to cellular interactions. Molecules. (2022); 27(24): 8819.
4. Sung, H, Ferlay, J, Siegel, R L, Laversanne, M, Soerjomataram, I, Jemal, A, Bray, F. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: a cancer journal for clinicians. (2021); 71(3): 209-249.
5. Sepand, M R, Bigdelou, B, Maghsoudi, A S, Sanadgol, N, Ho, J Q, Chauhan, P, Zanganeh, S. Ferroptosis: Environmental causes, biological redox signaling responses, cancer and other health consequences. Coordination Chemistry Reviews. (2023); 480: 215024.
6. Choi BY. Biochemical basis of anti-cancer-effects of phloretin—A natural dihydrochalcone. Molecules. (2019); 24(2): 278.
7. Zoń A, Bednarek I. Cisplatin in ovarian cancer treatment—known limitations in therapy force new solutions. International Journal of Molecular Sciences. (2023); 24(8): 7585.
8. Ghosh S. Cisplatin: The first metal based anticancer drug. Bioorganic chemistry. (2019); 88: 102925.
9. Qi L, Luo Q, Zhang Y, Jia F, Zhao Y, Wang F. Advances in toxicological research of the anticancer drug cisplatin. Chemical research in toxicology. (2019); 32(8): 1469-1486.
10. Amable L. Cisplatin resistance and opportunities for precision medicine. Pharmacological research. (2016); 106: 27-36.
11. Lecker, L S, Berlato, C, Maniati, E, Delaine-Smith, R, Pearce, O M, Heath, O, Balkwill, F R. TGFBI production by macrophages contributes to an immunosuppressive microenvironment in ovarian cancer. Cancer research. (2021); 81(22): 5706-5719.
12. Mormone E, George J, Nieto N. Molecular pathogenesis of hepatic fibrosis and current therapeutic approaches. Chemico-biological interactions. (2011); 193(3): 225-231.
13. Kamil AM, Hussain M, Kadoori YT, Ahmad SJ. Eruca sativa and Raphanus sativus Oils Enhance Hepatic and Renal Tissues Regeneration in White Mice. Al-Mustansiriyah Journal of Science. (2018); 29(4).
14. Srinivasan K. Antimutagenic and cancer preventive potential of culinary spices and their bioactive compounds. PharmaNutrition. (2017); 5(3): 89-102.
15. Negrean O-R, Farcas AC, Pop OL, Socaci SA. Blackthorn—A Valuable Source of Phenolic Antioxidants with Potential Health Benefits. Molecules. (2023); 28(8): 3456.

16. Tang PL, Hassan O. Bioconversion of ferulic acid attained from pineapple peels and pineapple crown leaves into vanillic acid and vanillin by Aspergillus niger I-1472. BMC chemistry. (2020); 14: 1-11.
17. Rezaei A, Varshosaz J, Fesharaki M, Farhang A, Jafari SM. Improving the solubility and in vitro cytotoxicity (anticancer activity) of ferulic acid by loading it into cyclodextrin nanosponges. International journal of nanomedicine. (2019): 4589-4599.
18. AL-JURAISY YH. A review on the potential effects of plant metabolites. International Journal of Pharmacy Research & Technology (IJPRT). (2023); 13(1): 43-59.
19. Sun X, Ma L, Li X, Wang J, Li Y, Huang Z. Ferulic acid alleviates retinal neovascularization by modulating microglia/macrophage polarization through the ROS/NF-κB axis. Frontiers in Immunology. (2022); 13: 976729.
20. Maya-Cano DA, Arango-Varela S, Santa-Gonzalez GA. Phenolic compounds of blueberries (Vaccinium spp) as a protective strategy against skin cell damage induced by ROS: A review of antioxidant potential and antiproliferative capacity. Heliyon. (2021); 7(2).
21. Turkez H, Arslan ME, Barboza JN, Kahraman CY, de Sousa DP, Mardinoğlu A. Therapeutic potential of ferulic acid in Alzheimer's disease. Current Drug Delivery. (2022); 19(8): 860-873.
22. Janus, E, Pinheiro, L R, Nowak, A, Kucharska, E, Świątek, E, Podolak, N, Klimowicz, A. New ferulic acid and amino acid derivatives with increased cosmeceutical and pharmaceutical potential. Pharmaceutics. (2022); 15(1): 117.
23. Zhang X, Lin D, Jiang R, Li H, Wan J, Li H. Ferulic acid exerts antitumor activity and inhibits metastasis in breast cancer cells by regulating epithelial to mesenchymal transition. Oncology Reports. (2016); 36(1): 271-278.
24. Yang GW, Jiang JS, Lu WQ. Ferulic acid exerts anti-angiogenic and anti-tumor activity by targeting fibroblast growth factor receptor 1-mediated angiogenesis. International journal of molecular sciences. (2015); 16(10): 24011-24031.
25. Nowak, A, Cybulska, K, Makuch, E, Kucharski, Ł, Różewicka-Czabańska, M, Prowans, P, Klimowicz, A. In vitro human skin penetration, antioxidant and antimicrobial activity of ethanol-water extract of fireweed (Epilobium angustifolium L.). Molecules. (2021); 26(2): 329.
26. Li D, Rui YX, Guo S D, Luan F, Liu R, Zeng N. Ferulic acid: A review of its pharmacology, pharmacokinetics and derivatives. Life sciences. (2021); 284: 119921.
27. Ali, A, Garg, P, Goyal, R, Kaur, G, Li, X, Negi, P, Kulshrestha, S. A novel herbal hydrogel formulation of moringa oleifera for wound healing. Plants. (2020); 10(1): 25.
28. Gao, J, Yu, H, Guo, W, Kong, Y, Gu, L, Li, Q, Wang, Y. The anticancer effects of ferulic acid is associated with induction of cell cycle arrest and autophagy in cervical cancer cells. Cancer Cell International. (2018); 18: 1-9.
29. Zhai Y, Wang T, Fu Y, Yu T, Ding Y, Nie H. Ferulic acid: a review of pharmacology, toxicology, and therapeutic effects on pulmonary diseases. International Journal of Molecular Sciences. (2023); 24(9): 8011.
30. Alara OR, Abdurahman NH, Ukaegbu CI. Extraction of phenolic compounds: A review. Current research in food science. (2021); 4: 200-214.
31. Ben-Othman S, Kaldmäe H, Rätsep R, Bleive U, Aluvee A, Rinken T. Optimization of ultrasound-assisted extraction of phloretin and other phenolic compounds from apple tree leaves (Malus domestica Borkh.) and comparison of different cultivars from Estonia. Antioxidants. (2021); 10(2): 189.
32. Marcato DC, Spagnol CM, Salgado HRN, Isaac VLB, Corrêa MA. New and potential properties, characteristics, and analytical methods of ferulic acid: A review. Brazilian Journal of Pharmaceutical Sciences. (2022); 58: e18747.
33. Pandey, J, Bastola, T, Tripathi, J, Tripathi, M, Rokaya, R K, Dhakal, B, Poudel, A. Estimation of total quercetin and rutin content in Malus domestica of Nepalese origin by HPLC method and determination of their antioxidative activity. Journal of Food Quality. (2020); 2020: 1-13.
34. Mradu G, Saumyakanti S, Sohini M, Arup M. HPLC profiles of standard phenolic compounds present in medicinal plants. International Journal of Pharmacognosy and Phytochemical Research. (2012); 4(3): 162-167.
35. Khadam AA, Salman JAS, Hijri M. Determination the Optimum Conditions for β-glucan Production Extracted from Saccharomyces cerevisiae. Al-Mustansiriyah Journal of Science. (2023); 34(2): 32-43.
36. Van Eldik R. Advances in Inorganic Chemistry: Recent Highlights II: Academic Press; (2022).
37. Tolosa L, Donato MT, Gómez-Lechón MJ. General cytotoxicity assessment by means of the MTT assay. Protocols in in vitro hepatocyte research. (2015): 333-348.
38. Suleiman, M, Alali, A, Aljayyousi, N, Alkanad, K, El-khatatneh, N, Almaqashah, M, Abu-Rayyan, A. Sulfur Nanoparticle as an Effective HEK-293 Anticancer Agent. Moroccan Journal of Chemistry. (2023); 11(2): J. Chem. 11 N° 2 (2023): 434-443.

39. Mariadoss, A V A, Ramachandran, V, Shalini, V, Agilan, B, Franklin, J H, Sanjay, K, Ernest, D. Green synthesis, characterization and antibacterial activity of silver nanoparticles by Malus domestica and its cytotoxic effect on (MCF-7) cell line. Microbial pathogenesis. (2019); 135: 103609.
40. Al-Shammari AM, Abdullah AH, Allami ZM, Yaseen NY. 2-Deoxyglucose and Newcastle disease virus synergize to kill breast cancer cells by inhibition of glycolysis pathway through glyceraldehyde3-phosphate downregulation. Frontiers in molecular biosciences. (2019); 6: 90.
41. Cary N. Statistical analysis system, User's guide. Statistical. Version 9. SAS Inst Inc USA. (2012).
42. Robert MS, Francis XW, David JK, David L. Spectrometric identification of organic compounds. John wiley & Sons, Inc, Hoboken, edn. (2005); 7: 106.
43. Aarabi A, Honarvar M, Mizani M, Faghihian H, Gerami A. Extraction and purification of ferulic acid as an antioxidant from sugar beet pulp by alkaline hydrolysis. Italian Journal of Food Science. (2016); 28(3): 362.
44. Sajjadi SE, Shokoohinia Y, Moayedi NS. Isolation and Identification of Ferulic Acid From Aerial Parts of Kelussia odoratissima Mozaff. Jundishapur Journal of Natural Pharmaceutical Products. (2012); 7(4): 159.
45. Bedan DS. Extraction, precipitation and characterization of urease from Vicia faba L. Al-Mustansiriyah Journal of Science. (2020); 31(1): 9.
46. Dheeb BI, Hashim SS, Hameed RK, Hasan SW. Phytochemistry and biological activity of Prosopis fracta. Journal of Biotechnology Research Center. (2020); 14(1): 25-33.
47. Zhang, Y, Zeng, M, Zhang, X, Yu, Q, Zeng, W, Yu, B, Jiang, X. Does an apple a day keep away diseases? Evidence and mechanism of action. Food Science & Nutrition. (2023); 11(9): 4926-4947.
48. Hassanein EH, Abdel-Wahab BA, Ali FE, Abd El-Ghafar OA, Kozman MR, Sharkawi SM. Trans-ferulic acid ameliorates cisplatin-induced testicular damage via suppression of TLR4, P38-MAPK, and ERK1/2 signaling pathways. Environmental Science and Pollution Research. (2021); 28: 41948-41964.
49. Al-Khuzaay H, Al-Juraisy Y, Alwan A. PURIFICATION, CHARACTERIZATION, AND IN VITRO ANTITUMOR ACTIVITY OF A NOVEL GLUCAN FROM PHOENIX DACTYLIFERA L. FRUITS. Georgian Medical News. (2023); (340-341): 71-75.
50. Wang L, Wang H, Liu D, Han Z, Fan J. A review of the polyphenols purification from apple products. Critical Reviews in Food Science and Nutrition. (2023): 1-11.
51. Jiang T, Ghosh R, Charcosset C. Extraction, purification and applications of curcumin from plant materials-A comprehensive review. Trends in food science & technology. (2021); 112: 419-430.
52. Tenório CJL, Ferreira MRA, Soares LAL. Recent advances on preparative LC approaches for polyphenol separation and purification: Their sources and main activities. Trends in Food Science & Technology. (2022); 128: 129-146.
53. Bahedh SB, Al-Habib AAS. Evaluation the activity of Petroselinum crispum aqueous extract as promoter rooting for stem cuttings of some plants. Al-Mustansiriyah Journal of Science. (2020); 31(1): 22-30.
54. Fang L, Chen M, Liu Z, Fang X, Gou S, Chen L. Ferulic acid–carbazole hybrid compounds: combination of cholinesterase inhibition, antioxidant and neuroprotection as multifunctional anti-Alzheimer agents. Bioorganic & Medicinal Chemistry. (2016); 24(4): 886-893.
55. Zduńska-Pęciak K, Kołodziejczak A, Rotsztejn H. Two superior antioxidants: Ferulic acid and ascorbic acid in reducing signs of photoaging—A split-face comparative study. Dermatologic Therapy. (2022); 35(2): e15254.
56. Choi JH, Park JK, Kim KM, Lee HJ, Kim S. In vitro and in vivo antithrombotic and cytotoxicity effects of ferulic acid. Journal of biochemical and molecular toxicology. (2018); 32(1): e22004.
57. Wang T, Gong X, Jiang R, Li H, Du W, Kuang G. Ferulic acid inhibits proliferation and promotes apoptosis via blockage of PI3K/Akt pathway in osteosarcoma cell. American journal of translational research. (2016); 8(2): 968.
58. Karthikeyan S, Kanimozhi G, Prasad NR, Mahalakshmi R. Radiosensitizing effect of ferulic acid on human cervical carcinoma cells in vitro. Toxicology in Vitro. (2011); 25(7): 1366-1375.
59. Truzzi, F, Valerii, M C, Tibaldi, C, Zhang, Y, Abduazizova, V, Spisni, E, Dinelli, G. Are supplements safe? Effects of gallic and ferulic acids on in vitro cell models. Nutrients. (2020); 12(6): 1591.
60. Markiewicz-Zukowska, R, Naliwajko, S K, Bartosiuk, E, Moskwa, J, Isidorov, V, Soroczynska, J, Borawska, M H. Chemical composition and antioxidant activity of beebread, and its influence on the glioblastoma cell line (U87MG). Journal of Apicultural Science. (2013); 57(2): 147-157.
61. Eroğlu C, Seçme M, Bağcı G, Dodurga Y. Assessment of the anticancer mechanism of ferulic acid via cell cycle and apoptotic pathways in human prostate cancer cell lines. Tumor Biology. (2015); 36: 9437-9446.
62. Janicke, B, Hegardt, C, Krogh, M, Önning, G, Åkesson, B, Cirenajwis, H M, Oredsson, S M. The antiproliferative effect of dietary fiber phenolic compounds ferulic acid and p-coumaric acid on the cell cycle of Caco-2 cells. Nutrition and cancer. (2011); 63(4): 611-622.

63. Schoeberl, A, Gutmann, M, Theiner, S, Corte-Rodríguez, M, Braun, G, Vician, P, Koellensperger, G. The copper transporter CTR1 and cisplatin accumulation at the single-cell level by LA-ICP-TOFMS. Frontiers in Molecular Biosciences. (2022); 9: 1055356.
64. Song M, Cui M, Liu K. Therapeutic strategies to overcome cisplatin resistance in ovarian cancer. European Journal of Medicinal Chemistry. (2022); 232: 114205.

الفعالية المضادة للسرطان لحمض الفريوليك المنقى من سويقات التفاح العراقي

رحاب جمعة منصور [1]* ، ياسر حسين زيدان [1] ، زينب ياسين محمد حسن [2]

[1] قسم علوم الحياة ، كلية العلوم ، الجامعة المستنصرية ، العراق
[2] مركز بحوث التقنيات الاحيائية ، جامعة النهرين ، العراق

الخلاصة

خلفية عن الموضوع: حمض الفيروليك (F)، وهو حمض هيدروكسينّامك (حمض 4-هيدروكسي-3-ميثوكسيسينناميك)، هو أحد الأحماض الفينولية الأساسية الموجودة في التفاح و يمتلك نشاطًا بيولوجيًا محتملاً، مثل خصائص مضادة للأكسدة ومضادة للالتهابات ومضادة للسرطان. **الهدف من الدراسة**: تهدف الدراسة الحالية إلى تنقية حمض الفيروليك من المستخلص الخام لسويقات اوراق التفاح العراقي ودراسة تأثيره على حيوية الخلايا في (خلية سرطان القولون البشري/ SW480، سرطان الكبد البشري/ Hep G2، سرطان المعدة الغدي/ AGS، وسرطان الرئة البشري الغدي/ A549). بجانب خط الخلية الطبيعي (الكلى الجنينية البشرية/HEK 293). **المواد و طرائق العمل:** تم تحديد حمض الفريوليك نوعياً وكمياً في مستخلصات الأجزاء الهوائية المختلفة من التفاح العراقي *Malus domestica* صنف "الإبراهيمي" شملت الأوراق، سويقات الاوراق و السيقان. تم عزل حمض الفريوليك وتنقيته بواسطة جهاز الكروماتوغرافيا السائلة عالية الأداء التحضيريPHPLC. تم تأكيد التركيب الكيميائي لحمض الفريوليك المعزول باستخدام تقنيات التوصيف التحليلي المختلفة وهي الكروماتوغرافيا السائلة عالية الأداء HPLC، مطيافية الأشعة تحت الحمراء FTIR، نقطة الانصهار و تحليل عناصر الكاربون-الهيدروجين-النتروجين CHN. تم تقييم التأثير السمي للخلايا بواسطة اختبار MTT بعد تعريضها لجرعات متصاعدة (500، 250، 125، 62.5، 31.25، 15.625) مايكروغرام / مل من حمض الفريوليك و سيسبلاتين (العلاج الكيميائي القياسي) ضد خطوط الخلايا السرطانية و خط الخلايا غير السرطانية الطبيعية. **النتائج:** تم الحصول على حوالي 0.952 غم من حمض الفريوليك المنقى من 14.086 غم من مستخلص أوراق التفاح (6.8%) وأظهرت نتائج اختبار MTT أن حمض الفريوليك المنقى يمتلك تأثيرات كبيرة مضادة للسرطان على جميع خطوط الخلايا السرطانية. **الاستنتاج:** تشير النتائج إلى أن حمض الفريوليك المنقى من سويقات أوراق التفاح العراقي يحتوي على مضادات أكسدة محتملة وسمية للخلايا، والتي قد تفيد صحة الإنسان.

الكلمات المفتاحية: التفاح ، سويق ، حمض الفريوليك ، مضاد للسرطان.