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Preliminary Tests, Phytochemical Investigation (GC/MS, HPLC), and Cytotoxic Activity of *Pyrus calleryana* Fruits Cultivated in Iraq

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Abstract

Worldwide neither information is available regarding the chemical constituents of *Pyrus calleryana* fruits nor its pharmacological effects. Previous studies demonstrated that Pyrus is a rich source of phenolics and has various pharmacological actions. Therefore, this study aimed to identify the secondary metabolites, especially phenolic compounds, and isolate phenolic acids in addition to investigating the fruits' cytotoxic effect.

powdered fruits were defatted with hexane and hexane extract was subjected to GC/MS. The defatted fruits were extracted through reflex (80% ethanol), phytochemical tests, and then acidic hydrolysis was done for the extract. The hydrolyzed extract was subjected to sequential extraction using chloroform, ethyl acetate, and n.butanol. TLC was done for these fractions to identify phenolic acids and flavonoids. Phenolic acids were identified and isolated from ethyl acetate fraction using HPLC. MTT test was used to determine the cytotoxic effect of ethyl acetate fraction on the A549 cell line. GC/MS analysis revealed that fatty acids esters and fatty acids were the most predominant compounds in hexane extract. For the first time, two known phenolic acids: p.coumaric and caffeic acid were obtained and identified from the ethyl acetate fraction of this plant. This fraction demonstrated no cytotoxic on the A549 cell line (IC_{50} of ethyl acetate fraction was 245319 µg/ml). This is the first study demonstrating the phenolic profile of *Pyrus calleryana* fruits, qualitatively Ethyl acetate fraction was a rich source of phenolic compounds, and Pyrus fruits exert no cytotoxic effect, further studies are required to evaluate the cytotoxic effect of this plant using other assays.

Keywords: *Pyrus calleryana,* IC50, caffeic acid, methyl ester, non-cytotoxic.

Introduction

Nowadays, there is an increasing interest in the discovery of unstudied plants for finding new chemical entities that serve directly as drugs or as templates for synthesizing new drugs[(1)].The Pyrus genus belongs to the subtribe Pyrinae, family Rosaecea. More than eighty species are specified in this genus. Pears in addition to apples are included in this genus. This genus demonstrates a remarkable therapeutic and economic importance [(2, 3)]. *Pyrus calleryana* Dcne is commonly known as the gallery pear. It is a deciduous, conical to rounded crown tree, that has gorgeous white flowers blossom early at three years of age[(4)]. Callery pear is cultivated in urbanized residential and commercial zones as an ornamental tree. This tree is native to Taiwan, Korea, eastern and southern China, and Japan[(5, 6)]. In the United States, the callery pear is an invasive plant[(7)]. Phytochemical investigation of stem bark revealed the presence of ursolic acid, acetyl ursolic acid, hydroxy ursolic acid, friedelinol, epifriedelinol, and euscaphic acid. Phenolic acid, phenolic acid ester, and glycosides such as chlorogenic acid, caffeic acid, p-hydroxybenzoic acid, methyl gallate, arbutin, lanceoloside A, protocatechuic acid-3-glucoside, calleryanin, protocatechuoyl calleryanin were isolated from hydroalcoholic leaves extract. Regarding pharmacological effects, till now only the antioxidant activity of the plant has been studied, and proven[(8)].Over the world, lung cancer is one of the most prevalent cancers. Radiotherapy and chemotherapy are effective therapeutic measures for its treatment but these measures are accompanied by intense side effects, undesired complications, and increased resistance[(9)]. As a result, there is a growing interest in the identification and isolation of cytotoxic agents from natural sources such as plants. Pyrus was among the plants that were studied for its cytotoxic effect. El-Hawary in a previous study revealed the cytotoxic effect of *Pyrus communis* fruit volatile oil on the A549 cell line with IC50 30.9 µg/ml[(10)].

This work aimed to investigate the phytochemicals in *Pyrus calleryana* Dcne fruits, detect phenolic acids and flavonoids using TLC and detect phenolic acids, and flavonoids using TLC and HPLC, and also evaluate its cytotoxic effect on the A549 human lung cancer cell line since no previous work has been done.

Materials and Method

Collection of plant materials

Fruits of *Pyrus calleryana* were collected from the gardens of the College of Pharmacy / University of Baghdad in June 2021. The plant was authenticated by Assist. Prof. Dr. Khansaa Ghazi Rasheed at the National History Museum and Research Centre. The fruits were washed with water, air dried for 2 weeks, and milled in an electrical miller to powder.

Preparation of the hexane extract

Dried fruit powder (75 gm) was defatted in 400 ml hexane for one week twice with occasional shaking. The solvent was distilled off in a rotary evaporator yielding yellow to brown sticky extract which was subjected to GC/MS analysis.

GC/MS analysis

GC/MS analysis was performed for hexane extract to identify the extract constituents. The analysis was done using an Agilent (7820A) USA GC Mass Spectrometer. The injection volume was 1 without derivatization, Injector temperature: 250 °C, Injection type: Splitless Column; Agilent HP-5ms Ultra Inlet (30 m length x 250µm diameter x 0.25 µm inside diameter, Carrier gas: helium 99.99%, pressure 11.933 psi. Scan range: m/z 50-500. GC inlet line temp. :250 °C Aux heaters temp. 310 °C. Temperature Ramp 1 60 °C hold to 2°C/min, Ramp 2 55°C to 180 °C hold to 7 °C/min, Ram 3 180 °C to 280°C hold to 1°C/min, and Ramp 4 280 °C hold to 1°C/min. the time amounted to approximately 33 minutes.

Preparation of the hydroalcoholic extract

The hydroalcoholic extract was prepared as follows: 75 gm of the defatted sample was reflexed with 400 ml 80% ethanol for 3 hours. The marc was removed, and the extract was dried in a rotary evaporator.

Phytochemical investigation of hydroalcoholic extract

Qualitative preliminary phytochemical analysis was performed on hydroalcoholic extract as follows:

1. Alkaloids were analyzed by Mayers and Dragendroff test.
2. Carbohydrates by Benedict's test.
3. Tannins by ferric chloride test.
4. Phlobataninns by reaction with hydrochloric acid.
5. Flavonoids by alkaline reagent test.
6. Anthraquinone glycosides by Borentrager's test.
7. Saponins by foam test.
8. Steroids by Lieberman Burchard test [11, 12]

Acidic hydrolysis of hydroalcoholic extract

The extract was subjected to acidic hydrolysis (reflex using 100 ml 5% HCl for 2 hours), filtration, and evaporation under reduced pressure to dryness.

Fractionation of extract

The fractionation of the acidic hydrolyzed extract was done by suspending it in water (100 ml) and shaking it in a water bath till solubilization. Into a separatory funnel, the aqueous solution was extracted sequentially and separately with chloroform, ethyl acetate, and n.butanol respectively. Using the rotary evaporator these fractions were concentrated under reduced pressure to obtain the dry extract[13].

TLC for chloroform, ethyl acetate, and n.butanol fractions

Preliminary TLC analyses were done for phenolic acids and flavonoids aglycone.

Silica gel TLC plate GF $_{254}$ was the stationary phase, three different solvent systems were used as eluent for phenolic acids identification; S_1 (chloroform: ethyl acetate: formic acid 25:20:5)[14], S_2 (toluene: ethyl acetate: acetic acid: formic acid 45:30:7.5:7.5), and S_3 (ethyl acetate: hexane: formic acid 20:19:1)[15].

For flavonoid aglycones, the used eluents were S_4 (toluene: ethyl acetate: acetic acid: formic acid 23:13:4:4), S_5 (toluene: methanol: glacial acetic acid 20:3:2[16], S_6 (toluene: ethyl acetate: formic acid 10:9:1[17].

The standard solutions of compounds: are flavonoids (quercetin, kaempferol, myricetin, apigenin, and luteolin). phenolic acids (caffeic acid, gallic acid, and p.coumaric acid) in addition to cinnamic acid and resorcinol were prepared (1 mg of each was reconstituted in 1 ml methanol). For samples (chloroform, ethyl acetate, and n.butanol), 3 mg for each was reconstituted in 1 ml of methanol.

The separated spots on the chromatogram were identified by UV light at 254nm, and by spraying with 2% alcoholic $FeCl_3$, and 5% alcoholic KOH for phenolic acid and flavonoids respectively [18]. Determinations of R_f values were done only for spots observed after spraying.

Identification and isolation of caffeic acid and p. coumaric acid by semi-preparative HPLC

A reverse semi-preparative HPLC technique was used for the detection and the isolation of the phenolic acids, using a SYKAMN HPLC chromatographic system equipped with a UV of solvent A (methanol) and solvent B (1% formic acid in water (v/v)). The initial composition of the eluent was maintained at 40% B for 0-4 minutes, and 50% B for 4-10 minutes. The flow rate was 0.7 ml/min, injection volume was 100 µl for ethyl acetate, p.coumaric, caffeic acids standards, and for the isolated phenolic acids. Spectra were obtained at 280 nm, and the analysis was performed at room temperature.

The standards and the sample were filtered through a 0.45 µm filter before HPLC injection [19]. The separated constituents from the ethyl acetate fraction were collected in different flasks at respective retention times. The isolated compounds were subjected to HPLC for identification and to check their purity.

Determination of the cytotoxicity of ethyl acetate fraction

A549 cell line A549 cells were used as models for the evaluation of the cytotoxic effect of ethyl acetate fraction against lung cancer[20, 21]. A549 cells are adenocarcinoma human alveolar basal epithelial cells, this cell line was first developed by D. J. Giard, et al. in 1972. The designated cells were obtained through the exclusion and culturing of cancerous lung tissue in the explanted tumor in a Caucasian male of a 58-year-old [22].

Maintenance of cell cultures

A549 cells were maintained in MEM (Minimum Essential medium) appended with 10% Fetal bovine, 100 µg/ml streptomycin, and 100 units/ml penicillin. Cells were passage through trypsin-EDTA, reseeded at 50% confluence two times a week, and then incubated at 37 °C[23].

Combination Cytotoxicity Assays

96-well plates were used to conduct an MTT cell viability assay for cytotoxic effect determination[24]. Cell lines were seeded at 1×10^4 cells/well. After 24 hours or once a confluent monolayer was attained, the cells were treated with the tested compound (ethyl acetate fraction). Cell viability was determined 72 hours post-treatment by medium eradication, the addition of 28 µL of 2 mg/ml MTT solution, then incubated at 37 °C for 1.5 h. MTT solution was removed, and the remaining crystals in the wells were solubilized by the addition of 130 µL of dimethyl sulphoxide, then incubated at 37 °C for 15 min with shaking [25]. The absorbency was measured on a microplate reader at 492 nm (test wavelength); the test was done in triplicate. The cell growth inhibitory rate (the percentage of cytotoxicity) was determined according to the following equation[26]:

detector, Chemstation, a Zorbax Eclipse Plus-C18-OSD .25cm, 4.6mm column. The column temperature was 30 ºC.

The gradient elution method was used for the analysis of the phenolic acid. The eluent consisted

% Cell viability = $(A_1 / A_2) \times 100$

% Cytotoxicity = 100 – cell viability

A_1: Absorbance of treated cell

A_2: Absorbance of non-treated cell

Prism 6 [27] was used. The values were displaced as the mean ± SD of triplicate measurements [28].

Result and Discussion

Plant samples regularly hold various bioactive moieties whose isolation, identification, and characterization are crucial for the discovery and development of a new therapeutic approach.

According to GC-MS analysis, 12 compounds were detected in the n.hexane extract, the retention time ranges from 15-27 minutes. From these compounds, only eight had been identified. These compounds were categorized into three classes; fatty acids, fatty acid esters, and alkanes. The compounds, their molecular formula, molecular weight, peak area, and the nature of chemical compounds are presented in Table 1 and their gas chromatogram is displayed in Figure1.

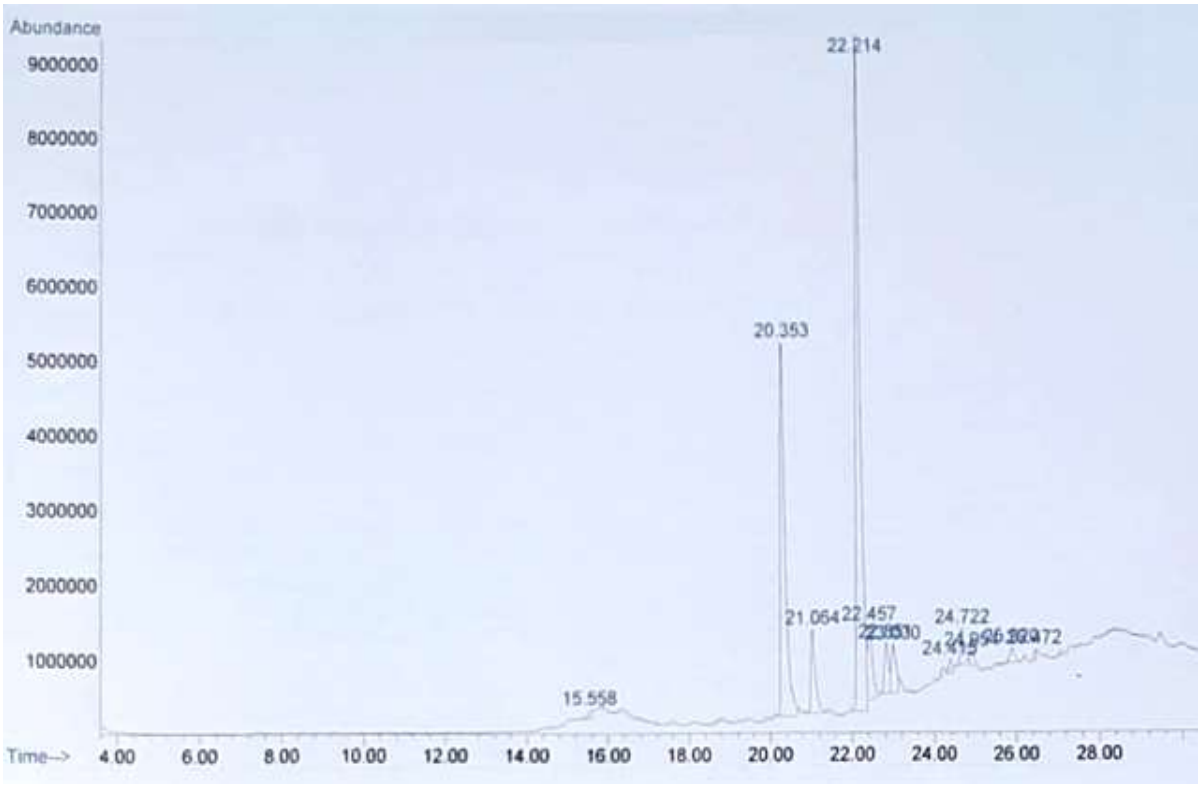


Figure 1. GC / MS Chromatogram of hexane extract of *Pyrus calleryana* fruit.

Table 1. Phyto-components identified in hexane extract *Pyrus calleryana* fruit

Peak Number	Retention time	Compound name	Molecular formula	Molecular weight	%Peak area	Nature of the compound
1	15.558	Heptacosane, 1-chloro	$C_{27}H_{55}Cl$	415.18	**0.55**	Aliphatic
2	20.353	Hexadecanoic acid, methyl ester (Palmitic acid, methyl ester	$C_{17}H_{34}O_2$	270.450	27.79	Aliphatic
3	21.06	Hexadecanoic acid, ethyl ester (Palmitic acid, methyl ester)	$C_{18}H_{36}O_2$	284.477	5.961	Aliphatic
4	22.214	Cis-13-Octadecenioc, methyl ester	$C_{19}H_{36}O_2$	296.487	48.312	Aliphatic
5	22.457	Methyl stearate	$C_{19}H_{38}O_2$	298.505	5.064	Aliphatic
6	22.851	Not identified			3.573	
7	23.030	Not identified			3.454	
8	24.415	Not identified			0.717	
9	24.722	Cis-vaccenic acid	$C_{18}H_{34}O_2$	282.461	2.542	Aliphatic
10	24.953	Erucic acid	$C_{22}H_{42}O_2$	338.6	0.717	Aliphatic
11	25.92	Oleic acid	$C_{18}H_{34}O_2$	282.47	1.061	Aliphatic
12	26.472	Not identified			0.501	

Based on the displayed data the majority of the identified compounds were fatty acid esters; cis-13-octadecenioc acid methyl ester and palmitic acid, methyl ester, while cis-vaccenic acid was the main identified fatty acid (Figure 2). Cis-vaccenic acid was detected in the hexane extract of *Pyrus pashina* [29].

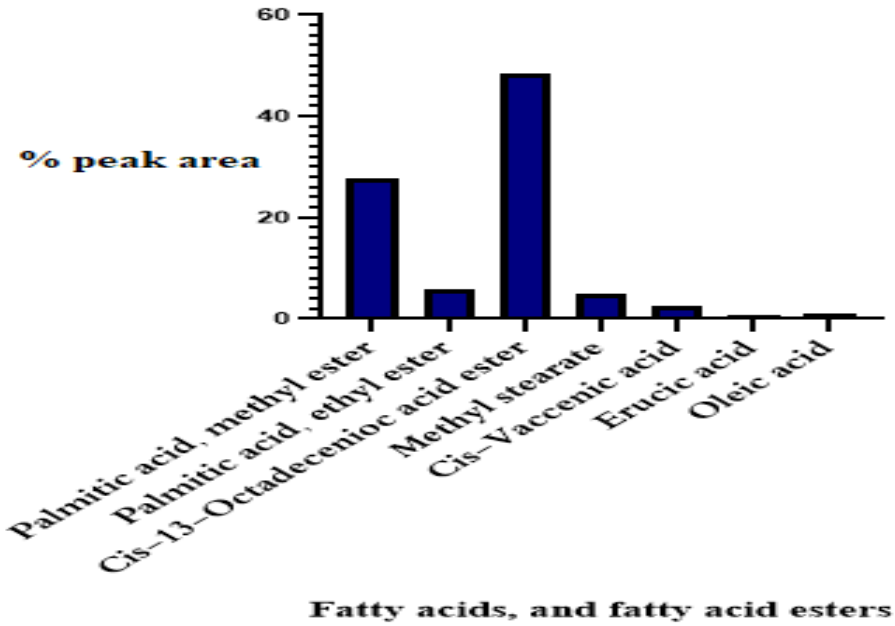


Figure 2. Quantitative analysis of fatty acids and fatty acid esters of *Pyrus calleryana* fruit hexane extract.

The phytochemical analysis of crude hydroalcoholic extract (Table 2) revealed the presence of flavonoids, tannins (pyrogallol and catechol type)[30], anthraquinone glycosides[31], saponins, and sugar[32], meanwhile, alkaloids and steroids are absent.

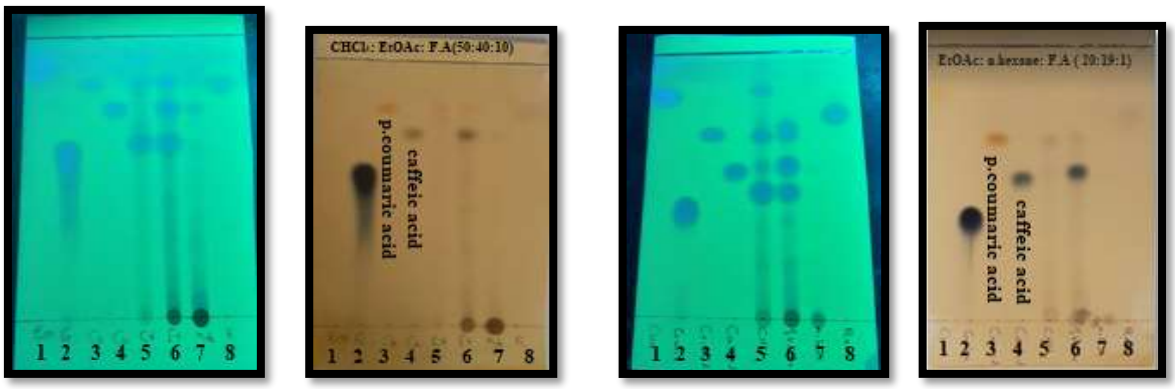
Table 2. Phytochemical analysis of crude hydroalcoholic extract

Chemical test	Result
Benedict's test (Reducing sugar)	+
Mayer's test (Alkaloids)	-
Borntrager's test (Anthraquinone glycosides)	+
Foam test (Saponins)	+
Alkaline reagent test (Flavonoids)	+
Ferric chloride test (Tannins)	+
Phlobaphin test (Condensed tannins)	+
Liberman-Burchard test (sterols)	-

Preliminary TLC analysis were done to provide basic information regarding the number and the type of phenolics present in chloroform, ethyl acetate, and n.butanol fractions. Under UV light at 254 nm, multiple dark spots were observed in chloroform, and ethyl acetate fractions, while in the n.butanol fraction, faint spots were observed. Based on R_f values (Table 3), and the color of the reference standards spots and separated spots on the sprayed chromatogram (Figure 3); p.coumaric acid was identified in both chloroform and ethyl acetate fraction as orange spot (R_f value 0.8, 0.69, 0.66 in S_1, S_2, and S_3 respectively). Caffeic acid was detected in the ethyl acetate fraction in the three used solvent systems (S_1, S_2, and S_3) as a grey spot (R_f value 0.72, 0.5, 0.53) and in the n.butanol fraction as a very faint spot only in S_1 solvent system. Two different solvent systems in which the separated unknown compound and standard have the same R_f values on the same TLC plate in the desired solvent system are required to prove the compound identity[33]. In a previous study, alcoholic esters of caffeic acid were detected in the *Pyrus calleryana* hydroalcoholic leaves extract[8].Chlorogenic acid was identified as one of the major components in the pear also caffeic acid and p.coumaric acid were detected in a previous study in *Pyrus communis* fruits [34]. Caffeic and quinic acid are the hydrolyzed products of chlorogenic acid[13, 35]. Phenolic acids are the main type of phenolic compounds present in the pear fruit[36].

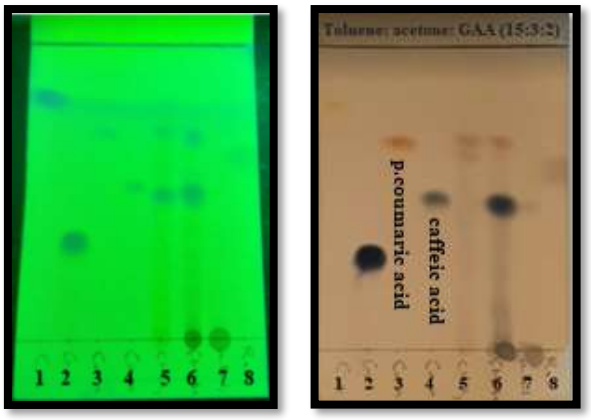
Table 3. Solvent systems for phenolic acids, retardation factor values, and the color of standard compounds and separated components.

S1	**Chloroform: ethyl acetate: formic acid (50:40:10)**		
	Compound	**R_f value**	**Color of the spot after spraying**
	Cinnamic acid	0.89	Yellow
	Gallic acid	0.57	dark blue
	p.coumaric acid	0.8	faint orange
	Caffeic acid	0.72	Grey
	Chloroform fraction	0.8	faint orange
	Ethyl acetate fraction	0.72 0.8	Grey faint orange
	n. butanol	0.72	very faint
	Resorcinol	0.89	faint grey
S_2	**Toluene: acetone: glacial acetic acid (15:3:2)**		
	Cinnamic acid	0.81	Yellow
	Gallic acid	0.3	dark blue
	p.coumaric acid	0.69	faint orange
	Caffeic acid	0.5	Grey
	Chloroform fraction	0.64 0.69	Faint orange
	Ethyl acetate fraction	0.51 0.69	Grey faint orange
	n. butanol	No spots were observed	
	Resorcinol	0.58	faint grey
S_3	**Ethyl acetate: hexane: formic acid (20:19:1)**		
	Cinnamic acid	0.83	yellow spot
	Gallic acid	0.35	dark blue
	p.coumaric acid	0.66	faint orange
	Caffeic acid	0.53	Grey
	Chloroform fraction	0.66	Faint orange
	Ethyl acetate fraction	0.54 0.66	Grey faint orange
	n.butanol	No spots were observed	
	Resorcinol	0.73	Faint grey



(a) **(b)** **(a)** **(b)**

(A) **(B)**



(a) (b)

(C)

Figure 3. TLC chromatogram of standard reference materials and tested fractions. A: developed in the S1 system, B: developed in the S_2 system, C: developed in the S_3 system. 1: Cinnamic acid, 2: Gallic acid, 3: p. coumaric acid, 4: caffeic acid, 5: Chloroform fraction, 6: ethyl acetate fraction, 7: n. butanol fraction, and 8: Resorcinol. Detection (a) under UV light 254nm, (b): after spraying with 2% alcoholic $FeCl_3$ solution and observation at daylight.

Concerning flavonoids, in the S_3 solvent system kaempferol was detected in ethyl acetate fraction (R_f value 0.71). In S_4, S_5, and S_6 systems, quercetin was identified (R_f value for quercetin was 0.74, 0.3, 0.6, in ethyl acetate fraction the separated spot had R_f= 0.74, 0.28, 0.58 respectively). Luteolin was identified in S_6 (R_f value for luteolin standard was 0.52, in EtOAc fraction it was 0.53). A previous study showed the presence of quercetin as a major flavonol in pear Korean fruits[37]. Table 4, and Figure 4 demonstrate the solvent systems, R_f values, and the color of the identified spots.

Table 4. Solvent systems for flavonoids, Retardation factor values, and the color of standard compounds and separated components.

	Compound	**R_f value**	**Color of the spot after spraying**
S_3	**Ethyl acetate: n.hexane: formic acid (20:19:1)**		
	Quercetin	0.6	yellow spot
	Kaempferol	0.71	yellow spot
	Myricetin	0.44	Grey
	Apigenin	0.63	yellow spot
	Chloroform fraction	No spots were observed	
	Ethyl acetate fraction	0.55, 0.71	faint yellow
	n.butanol	No spots were observed	
	Luteolin	0.49	Very faint yellow
S4	**Toluene: ethyl acetate: acetic acid: formic acid (23:13:4:4)**		
	Quercetin	0.74	Very light brown
	Kaempferol	0.79	Very light brown
	Myricetin	0.65	Very light brown
	Apigenin	0.77	Very light brown
	Chloroform fraction	No spots were observed	
	Ethyl acetate fraction	0.72	Very light brown
	n. butanol	0.72	
	Luteolin	0.69	Light brown
S_5	**Toluene: methanol: glacial acetic acid (20:3:2)**		
	Quercetin	0.3	Light brown
	Kaempferol	0.42	Light brown
	Myricetin	0.2	Light brown
	Apigenin	0.41	Light brown
	Chloroform fraction	No spots were observed	
	Ethyl acetate fraction	0.28	Light brown

	n. butanol	No spots were observed	
	Luteolin	0.31	Light brown
S6	**Toluene: ethyl acetate: formic acid (10:9:1)**		
	Quercetin	0.6	Light yellow
	Kaempferol	0.68	Light yellow
	Myricetin	0.48	Light grey
	Apigenin	0.63	Light brown
	Chloroform fraction	No spots were observed	
	Ethyl acetate fraction	0.53 0.58, 0.65	Light brown Very light brown
	n.butanol	No spots were observed	
	Luteolin	0.52	Light brown

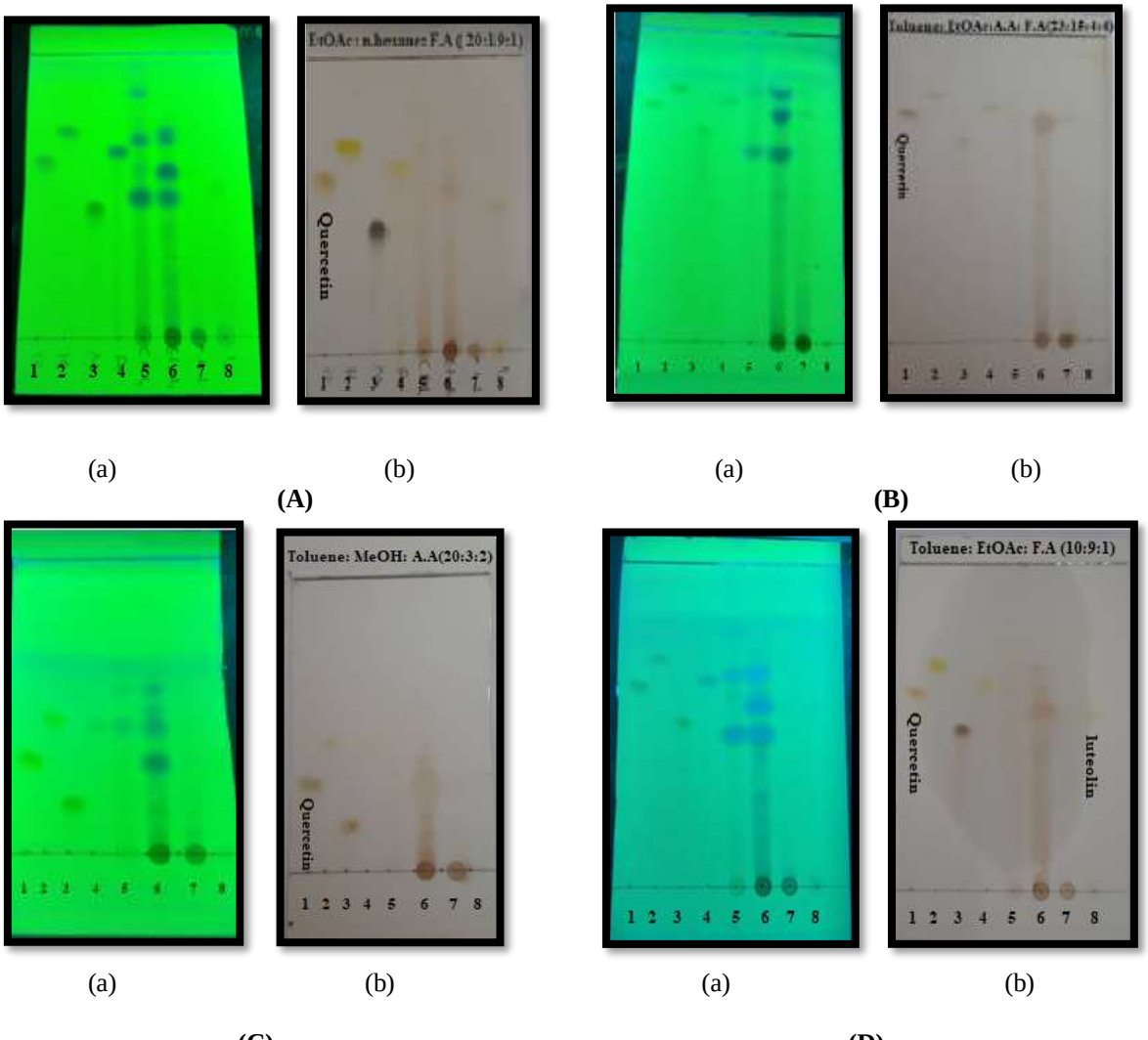


Figure 4. TLC chromatogram of standard reference materials and tested fractions. A developed in the S1 system, B developed in the S_2 system, C developed in the S_3 system and D developed in the S_4 system. 1: Quercetin, 2: kaempferol, 3: Myricetin, 4: Apigenin, 5: Chloroform fraction, 6: Ethyl acetate fraction, 8: n.butanol. Detection (a) under UV light 254nm, (b): after spraying with 5% alcoholic KOH solution and observation at day light.

Identification and isolation of phenolic acid by semi-preparative HPLC

Qualitative and quantitative estimation of phenolic compounds, including phenolic acids, can be accomplished by HPLC [26]. As phenolic compounds have a wide range of polarity, a gradient-type elution was developed [27].

After initial TLC identification of p.coumaric and caffeic acids in all the tested solvent systems, this analysis was performed to confirm their existence. The HPLC chromatogram (Figure 5) showed three major peaks, with Rt of 1.85 min, 2.24 min, and 3.5

min respectively. Two of these compounds were separated by semi-preparative HPLC, and their purity was tested by HPLC, each of the isolated compounds showed a single peak (Figure 6) Based on results demonstrated in Table 5 and by comparing the retention times for the standards, the separated and isolated compounds on HPLC chromatogram, p. coumaric and caffeic acid presence were confirmed. Compounds with Rt 3.47 min and 2.59 min represent the isolated p.coumaric acid, and caffeic acid respectively. Hydroxycinnamic acids (caffeic, ferulic, p-coumaric, and sinapic acid) were reported as pear major constituents [28]. Besides, previous studies on *Pyrus communis* fruits reported the presence of these acids [23, 29].

Table 5. Retention time for standards, ethyl acetate fraction, and isolated phenolic acid

Compounds	Retention time for standards	Retention time for the separated compounds in ethyl acetate fraction	Retention time for isolated phenolic acids
p. coumaric acid	**3.6**	3.5	3.47
Caffeic acid	**2.46**	2.24	2.59
Unknown		1.85	

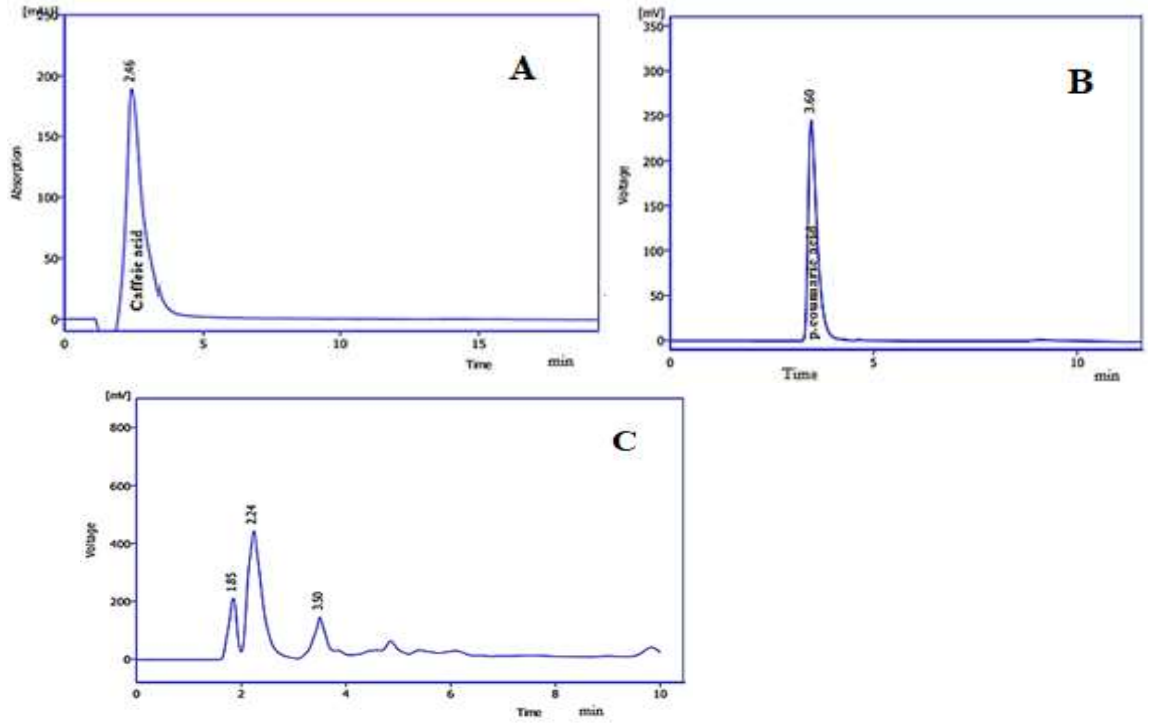


Figure 5. HPLC chromatogram, (A): p.coumaric acid standard, (B): Caffeic acid standard, (C): Ethyl acetate fraction

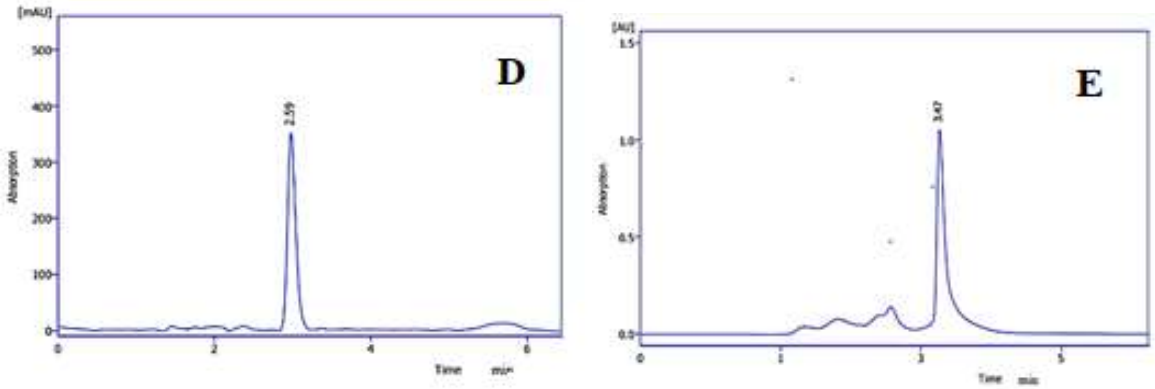


Figure 6. HPLC chromatogram for D: Isolated caffeic acid, and E: Isolated p. coumaric acid.

Cytotoxic effect of ethyl acetate fraction

The cytotoxic effect was studied against the A549 lung cancer cell line using the (MTT) test; 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide this colorimetric test is sensitive, credible, and quantitative (quantify cell viability) [38]. Ethyl acetate fraction was chosen to evaluate the cytotoxic effect against the selected cell line based on preliminary TLC and HPLC which revealed the presence of phenolic compounds mainly phenolic acids. According to the result tabulated in Table 6, figure 7, and 8 after 72 hours of incubation, a concentration of 3.125 µg/ml, and 100 µg/ml produce a cytotoxic effect of 15.4% and 64.8% respectively so the effect is dose-dependent.

Table 6. Extract concentrations and percent cytotoxicity

Concentration µg/ml	3.125	6.25	12.5	25	50	100
%Cytotoxicity	15.4	18.5	20.7	23.8	40	64.8

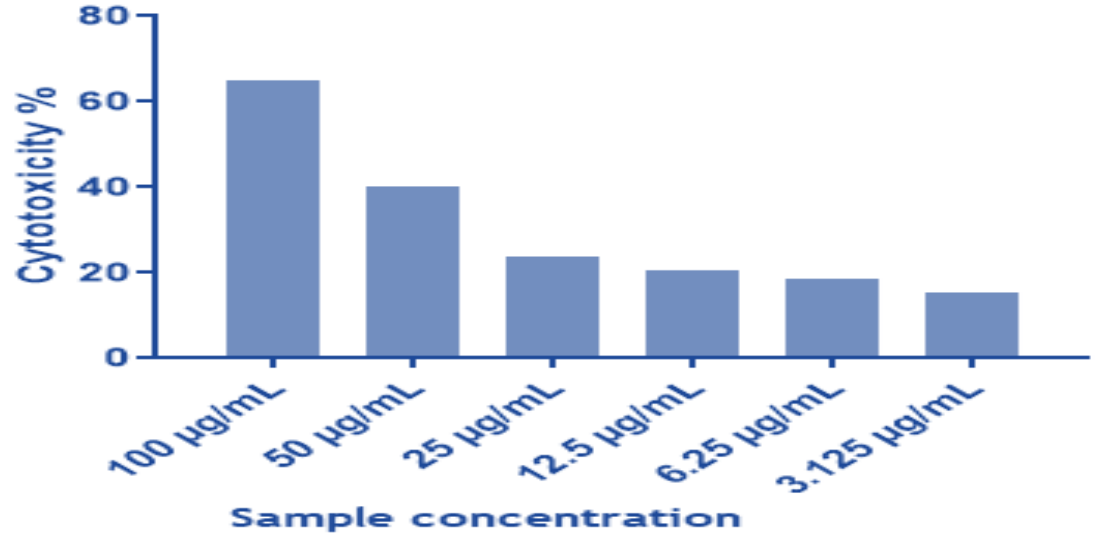
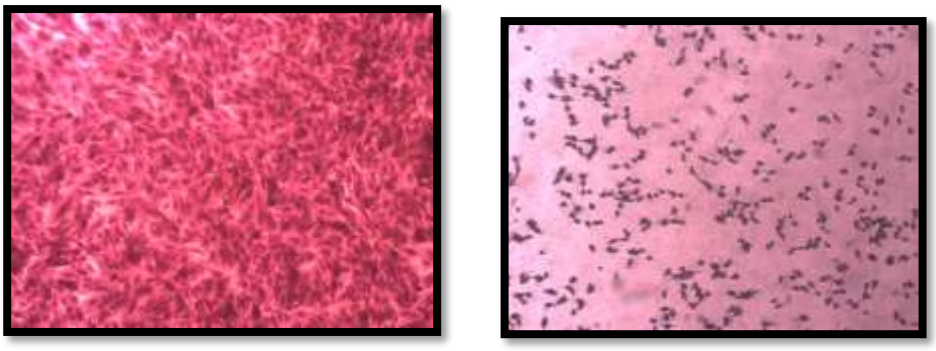


Figure 7. Cytotoxicity percent for examined ethyl acetate fraction concentrations



(a) (b)

Figure 8. Morphological picture for A549 lung cancer cell line in vitro (a) Control cells (b) After treatment with ethyl acetate fraction under an inverted microscope, 10x.

Extract cytotoxicity is evaluated by measuring the inhibitory concentration (IC50)

IC50 is the concentration of the test compound that reduced the cell viability by 50%. IC50 values are predictive of the cytotoxic effect, the smaller the value the more cytotoxic effect, meanwhile the higher value the non-cytotoxic compound [32]. According to the American National Cancer Institute, the product is considered to be cytotoxic when the IC50 value is less than 30 µg/ml. Atjanasuppat *et al* categorized the extract cytotoxicity-based IC50 into four groups; IC50 ≤ 20 µg/ml (very active), IC50 > 20–100 µg/ml (moderately active), IC50 > 100–1000 µg/ml (weakly active), IC50 > 1000 µg/ml (inactive)[39].

The MTT assay was performed and the result demonstrated that the IC50 of ethyl acetate fraction was 245319 µg/ml (figure 8), thus this fraction exhibits no-cytotoxic effect against the A549 lung cancer cell line because IC50 is greater than 1000 µg/ml[39, 40].

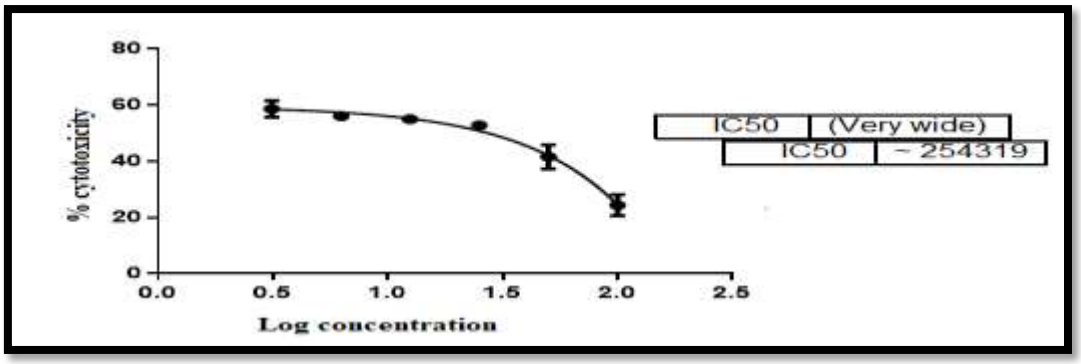


Figure 8. % cytotoxicity versus log concentration (µg/ml) of ethyl acetate fraction of *Pyrus calleryana* fruits.

This result was in accordance with a previous study that demonstrated that the leaves and bark methanolic extracts of *Pyrus spinosa* did not show cytotoxic effects against the Fem-x and normal MRC-5 cell lines [41].

Conclusion

Internationally, this is the first study displaying the phytochemical constituents of *Pyrus calleryana*, particularly phoenolic compounds. p, coumaric, and caffeic acids were the most important identified and isolated compounds from ethyl acetate fraction that demonstrate no cytotoxic effect on the A549 lung cancer cell line.

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Declaration of Interest

None.

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الاختبارات الأولية والتحقيق الكيميائي النباتي (GC/MS، HPLC) والنشاط السمي للخلايا لثمار *Pyrus calleryana* المزروعة في العراق

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الخلاصة

لا توجد معلومات متاحة في جميع أنحاء العالم فيما يتعلق بالمكونات الكيميائية لفاكهة *Pyrus calleryana* ولا آثارها الدوائية. أظهرت الدراسات السابقة أن البيروس مصدر غني للفينولات وله تأثيرات دوائية مختلفة. ولذلك هدفت هذه الدراسة إلى التعرف على المركبات الثانوية وخاصة المركبات الفينولية وعزل الأحماض الفينولية بالإضافة إلى التحقق من تأثير السمية الخلوية للثمار.

تمت إزالة دهن الثمار المسحوقة باستخدام الهكسان وتم تعريض مستخلص الهكسان إلى GC/MS تم استخلاص الثمار منزوعة الدهن بطريقة المنعكس (٨٠٪ إيثانول) وأجريت الاختبارات الكيميائية النباتية ثم إجراء التحليل المائي الحمضي للمستخلص. تم تعريض المستخلص المتحلل مائيًا للاستخلاص المتسلسل باستخدام الكلوروفورم، وأسيتات الإيثيل، والبوتانول. تم إجراء TLC لهذه الأجزاء للتعرف على الأحماض الفينولية والفلافونويدات. تم التعرف على الأحماض الفينولية وعزلها من جزء خلات الإيثيل باستخدام HPLC. تم استخدام اختبار MTT لتحديد التأثير السام للخلايا لجزء خلات الإيثيل على خط الخلية A549. كشف تحليل GC/MS أن استرات الأحماض الدهنية والأحماض الدهنية كانت أكثر المركبات السائدة في مستخلص الهكسان. لأول مرة، تم الحصول على اثنين من الأحماض الفينولية المعروفة: حمض الكوماريك وحمض الكافيك وتم التعرف عليهما من جزء أسيتات الإيثيل في هذا النبات. لم يظهر هذا الجزء أي سمية للخلايا على خط الخلايا A549 (IC50) لجزء أسيتات الإيثيل كان ٢٤٥٣١٩ ميكروغرام/مل .(هذه هي الدراسة الأولى التي توضح المظهر الفينولي لثمار Pyrus calleryana ، حيث كان جزء أسيتات الإيثيل نوعيًا مصدرًا غنيًا للمركبات الفينولية، ولا تمارس ثمار Pyrus أي تأثير سام للخلايا، وهناك حاجة إلى مزيد من الدراسات لتقييم التأثير السام للخلايا لهذا النبات باستخدام فحوصات أخرى.

الكلمات المفتاحية: الكمثرى المزهرة ، IC50، حمض الكافيين، ، ميثيل استر، غير سام للخلايا.