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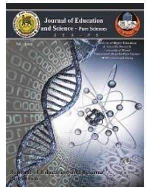

Separation and Identification of Bioactive Compounds from the Seeds of Iraqi *physalis angulata* L. Using Chromatographic Techniques

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Abstract

This study aimed to investigate the phytochemical composition of *Physalis angulata* L collected from the Rabia region, west of Mosul, Nineveh Governorate/ Iraq. The active compounds of selected *P. angulata* seeds were identificated sued chromatographic techniques. The extraction of those active compounds was performed employing the Soxhlet apparatus. The results showed the identification of six phenolic compounds in the 70% alcoholic extract by High-Performance Liquid Chromatography (HPLC) analysis following acid hydrolysis (Quercetin, Apigenin, Ferulic acid, P-coumaric acid, Gallic acid, and Chlorogenic acid). And the Chlorogenic acid was exhibited the highest concentration in the extract reaching 0.0904 mg\ml. Fourteen compounds were identified in the same crude extract used Gas Chromatography-Mass Spectrometry (GC- MS)analysis, among which the most prominent were (17-Octadecynoic acid, 3-Hexanol, 1,2-Benzenedicarboxylic acid, Heptanoic acid, 2-ethyl, and Lauric aacid) Gas-liquid chromatography (GLC) analysis of the saponified petroleum ether extract revealed presence of six fatty acids (Oleic, Stearic, Linoleic, Palmatic, Arachidonic, Linolenic). Among these, Linoleic acid was the most abundant, constituting 19.80% of the extract. These results indicate that the seeds of *P. angulata* contain compounds of medical importance, which opens the door to its potential use in future therapeutic pharmaceutical applications.

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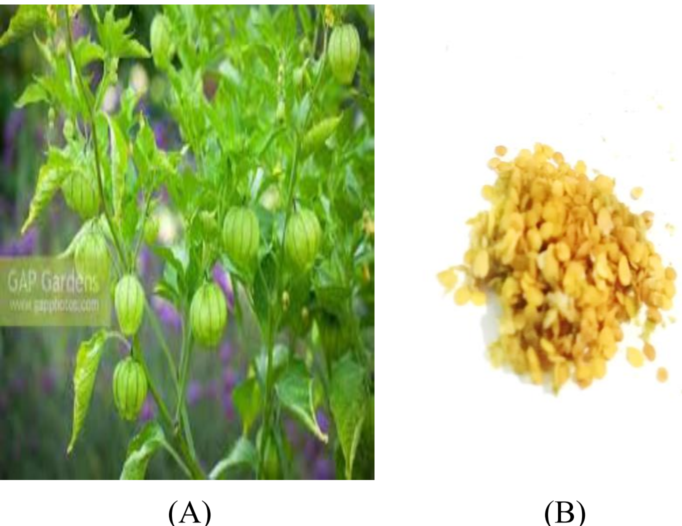
1. Introduction

Physalis angulata L. belongs to the Solanaceae family, which is characterized by its annual and perennial herbaceous species. The genus *Physalis* is the fifth largest genus of nightshades, comprising approximately 70 to 90 species [1], [2]. It is an annual herb native to tropical regions and also found in Asia, Africa and the Americas. The plant grows to a height of one meter, is erect, strong and has smooth, glossy parts [3]. The plant has ribbed hollow stems which may be hairless or with short hairs. The leaves are alternate, simple, ovate to lanceolate. The flowers are bisexual, bearing five-lobed, yellow petals with spot. The calyx leaves are light green with toothed margins and pointed apices and are covered with villous and glandular hairs, and it is characterized by the fruit calyx which develops into a covering enclosing the fruit, which are round, juicy, green or light before ripening, and become yellow when fully ripe. They contain many small seeds, which are disc-shaped or flat with surface decorations of a reticulate or wavy type, and their color is yellowish-off white [3], [4], [5], [6]. It was first recorded in the Encyclopedia of Botany in Iraq by Al-Alaq in 2012, as the study showed its spread in the alluvial plains areas in Baghdad and Baqubah, as well as in the Governophrates of Kirkuk and Tuz Khurmatu. The species is not native to Iraq: rather, it was introduced through the importation of tomatoes, eggplant, tobacco and peppers. In the current study, it was noted that it has a wide spread in the agricultural fields of the Rabia region in Nineveh Governorate. Previous studies have shown that *P. angulata*.

contains many active compounds, including terpenes, saponins, flavonoids, alkaloids, tannins, and phenolics[7].It also contains palmitic, acetic, malic, chlorogenic, and citric acids [7],[8]Currently, there is a global trend towards shifting from synthetic medicines to herbal medicine (complementary medicine), or what is called "back to nature," as the use of plant extracts as active compounds is a modern, environmentally friendly approach [9], [10].Researchers in the field of natural products have shown enclosing growing interest in extracting active compound from plant extracts. This interest has expanded our understanding of physiological activity of the body and how these compounds affect the production of effective drugs against many diseases caused by fungi, bacteria, and viruses [11] Medicinal plants and their active compounds produce approximately 78% of medicines [12]*P.angulata*. plant has many medicinal benefits and uses, as it is used as an antibacterial, antiparasitic, anti-inflammatory, analgesic, and a treatment for diabetes. In addition, it is an antioxidant due to its active properties [13], [14].In addition, it is used to treat many diseases such as dermatitis, intestinal worms, abdominal pain, wounds, hepatitis, anemia, urinary tract infections, and tumors. It has been used in folk medicine in many countries of the world [15][16].Chromatography techniques, including GLC, HPLC, and GC-MS, are essential technologies in the pharmaceutical, food, and chemical industries. Due to their efficiency and important role in analyzing, separating, and identifying compounds from a mixture quantitatively and qualitatively, and their ability to handle large and small samples, they have become widely used in chemical and biological research in examining plant extracts, pharmaceutical preparations, and many other important applications.[17]

This study aims to isolate and identify the active compounds from the seeds of the *P. angulata*. using chromatographic techniques in order to provide preliminary data for evaluating their biological significsnce.



(A) (B)

Figure 1; showing the plant *P. angulate. A- P. angulata* in the field [18]. B- The dried seeds of *P, angulata*

2. Research Method

2.1 Preparation of Plant Extracts:

Physalis angulata plants were collected from agricultural fields in the Rabia region, west of Mosul. The seeds were air-dried in shaded, moisture-free conditions with daily stirring. Plant extracts were prepared used the method described by [19], [20]. Extraction was performed used a Soxhlet continuous extraction apparatus, depending on the boiling point of each solvent. Two types of solvents were employing: petroleum ether 60-80°C (supplied by Scharlau) and ethanol 70% (supplied by Chemstok Chemicals). 27 g of seed dried powdered were placed in a batch in the Soxhlet apparatus for extraction, and 400 ml of the first solvent, petroleum ether, was added to it. The batch was macerated for 48 h. at a 25 ± 2°C, and the extraction was completed until the solvent used became colorless. Then 400 ml of 70% ethanol was added, and the extracts were concentrated used a rotary evaporator (RVE) at a 20°C up to the boiling point of each solvents.The crude extracts were transferred to tightly sealed amber glass bottle and stored in the refrigerator until futher analysis [19], [21]

2.2 Separation and purification of fatty acids by saponification

10 ml of the crude petroleum ether extract were mixed with (100 ml) of 7.5 M potassium hydroxide (KOH) solution (prepared by Scharlau). The solution was heated at 100°C for 90 minutes, then allowed to cool to room temperature. subsequently, (50 ml) of distilled water was added, and the mixture was transferred to a separating funnel, Then, (50 ml) of diethyl ether (prepared by Scharlau) was added to it in two portions to remove the unsaponified fats. The saponified layer (lower

layer) was collected and acidified with 10% sulfuric acid H_2SO_4 (prepared by Sigma-Aldrich) until the solution became clear at pH = 2. After that, the mixture was transferred to separating funnel, followed by the addition of (50 ml) of diethyl ether in two portions with a well shaking. This resulted in the formation of two layers: the upper layer is the fatty acids, while the lower layer is the aqueous layer. The upper layer shown in (Figure 2) was collected and (3 g) of anhydrous magnesium sulfate $MgSO_4$ was added to remove residual moisture. Then, the solution was filtered and the samples were kept in the refrigerator in tightly sealed opaque glass bottles.[19] [22]

Figure2;the fatty acid layer resulting from the soaping process of the petroleum ether extract of the seeds of the plant *P.angulata*

2.3 Identification of fatty acids using gas-liquid chromatography (GLC)

Identification of fatty acids was carried out by GLC Analyses were performed at the laboratories of the Ministry of Science and Technology (Environment and Water Directorate), Baghdad, Iraq, using a Shimadzu GLC device(model 2010, Japan). A flame ionization detector (FID) was used with an SE-30 separation column with wavelengths of 30m x 0.25mm, at a temperature of 310°C in the detector zone and 280°C in the injection zone. The separation column temperature ranged from 120-290°C (10°C-min) to a gas flow rate of 100kPa.

2.4 Separation and purification of phenolic compounds by acid hydrolysis

Since phenolic compounds naturally occur in plants predominantly as glycosides rather than in their free forms, an acid hydrolysis process was performed to cleave the glycosidic bonds and obtain the free, pure compounds A (10 ml) of the ethanolic plant extract was placed in a (500 ml) glass beaker and (100 ml) of hydrochloric acid (HCl) 1N (provided by Sigma-Aldrich) was added to the beaker. The mixture was heated at 100°C for 60 minutes, and then allowed to cool to room temperature. It was subsequently transferred into a separating funnel, followed by addition (50 ml) of ethyl acetate (provided by Scharlau) was added in two portions, with well shaking, until two layers formation. The lower sugar layer was discarded, and the upper layer, consisting of ethyl acetate (as shown in Figure 3), was collected. Then tree gram of anhydrous magnesium sulfate (MgSO4) was added to remove residual moisture., then the solution was filtered using filter paper. Samples were stored in sealed, opaque glass bottles in a refrigerator at 4°C until further use with HPLC [23]

2.5- Identification of phenolic compounds using high-performance liquid chromatography (HPLC)

After acid hydrolysis of phenolic compounds, were identified by HPLC. Analyses were performed at the laboratories of the Ministry of Science and Technology (Environment and Water Directorate), Baghdad, Iraq. using a German-made SYKAM HPLC using a C18-ODS column measuring 25 cm x 4.6 mm, using a mobile phase (methanol:D.W.:formic acid) (70:25:5) at a wavelength of 280 nm, at a flow rate of 1 ml/min.

2.6-. Identification of Active Compounds Using Gas Chromatography-Mass Spectrometry (GC-MS)
Analyses were performed at the laboratories of the Ministry of Science and Technology (Environment and Water Directorate), Baghdad, Iraq, the analysis was performed using an Agilent 5977A gas chromatograph equipped with an autosampler and connected to a mass spectrometer. The following conditions were used: the column was a HP-5MS fused silica capillary column (30 mm × 0.25 mm internal diameter), helium (99.999%) was used as the carrier gas at a constant flow rate of 1 mL/min, and the injection volume was 0.5 µL (split ratio 10:1). The injector temperature was 250°C; the oven temperature was set at 60°C (isothermal for 2 minutes), and the analysis was completed at 310°C. The total run time for the gas chromatograph was 60 minutes. The analysis was then transferred to the mass spectrometer with an ion source at 280°C and an ionization energy of 70 eV. With a scan interval of 0.5 seconds, and fragments of 45 to 450 Daltons.

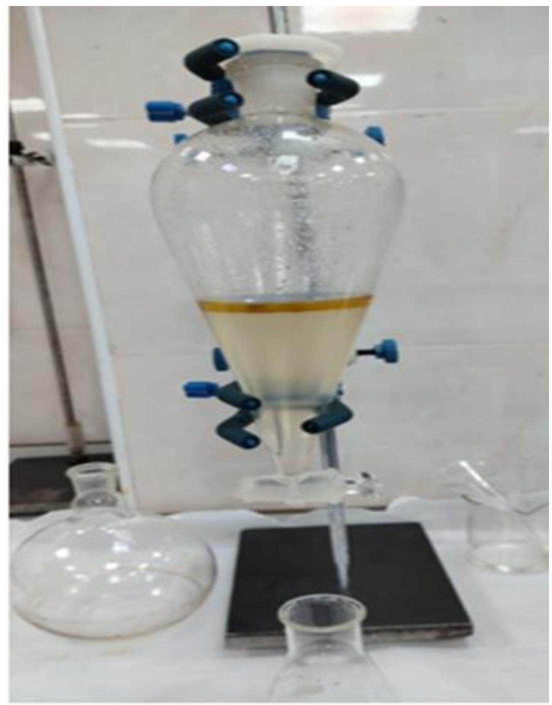


Figure3; the phenolic layer resulting from the acid hydrolysis of the ethanolic extract of the seeds of the plant *P.angulata*

3. Results and Discussion

3.1 Identification of fatty acids saparated by GLC chromatography

The results are presented in Table (1) six fatty acids from the saponified petroleum ether extract of *P. angulata* seeds using GLC, depending on the retention time of each compound. Variations in the concentrations of the identified fatty acids were observed. Linolic acid exhibited the highest concentration of 19.80% in the extract, while arachidonic acid recorded the lowest concentration in the extract, at 0.88%.

Table 1; fatty acids were saparated and identified by GLC technique and their Retention time and concentrations were determined**.**

Fatty acids	R. Time of standard samples (min)	The petroleum ether extract of seeds		
		Concentration (%)	R. Time (min)	Peak area
Oleic	4.22	14.58	4.241	0.2434
Stearic	4.951	3.21	4.907	0.1085
Lenoleic	5.999	**19.80**	5.965	1.4176
Palmatic	8.11	5.99	8.090	0.2319

Arachidonic	8.510	**0.88**	8.535	0.0612
Linolenic	12.691	2.04	12.727	0.0678

The chromatographic curves presented (4) confirm the presence of the fatty acids identified by the GLC, as listed in Table (1), with each appearance at its characteristic retention time as illustrated in the chromatographic profile; oleic at 4.241 minutes, Stearic at 4.907 minutes, Lenoleic at 5.965 minutes, Palmatic at 8.090 minutes, arachidonic at 8.535 minutes and linolenic at a retention time of 12.727 minutes, and comparing them with retention time of standard samples.

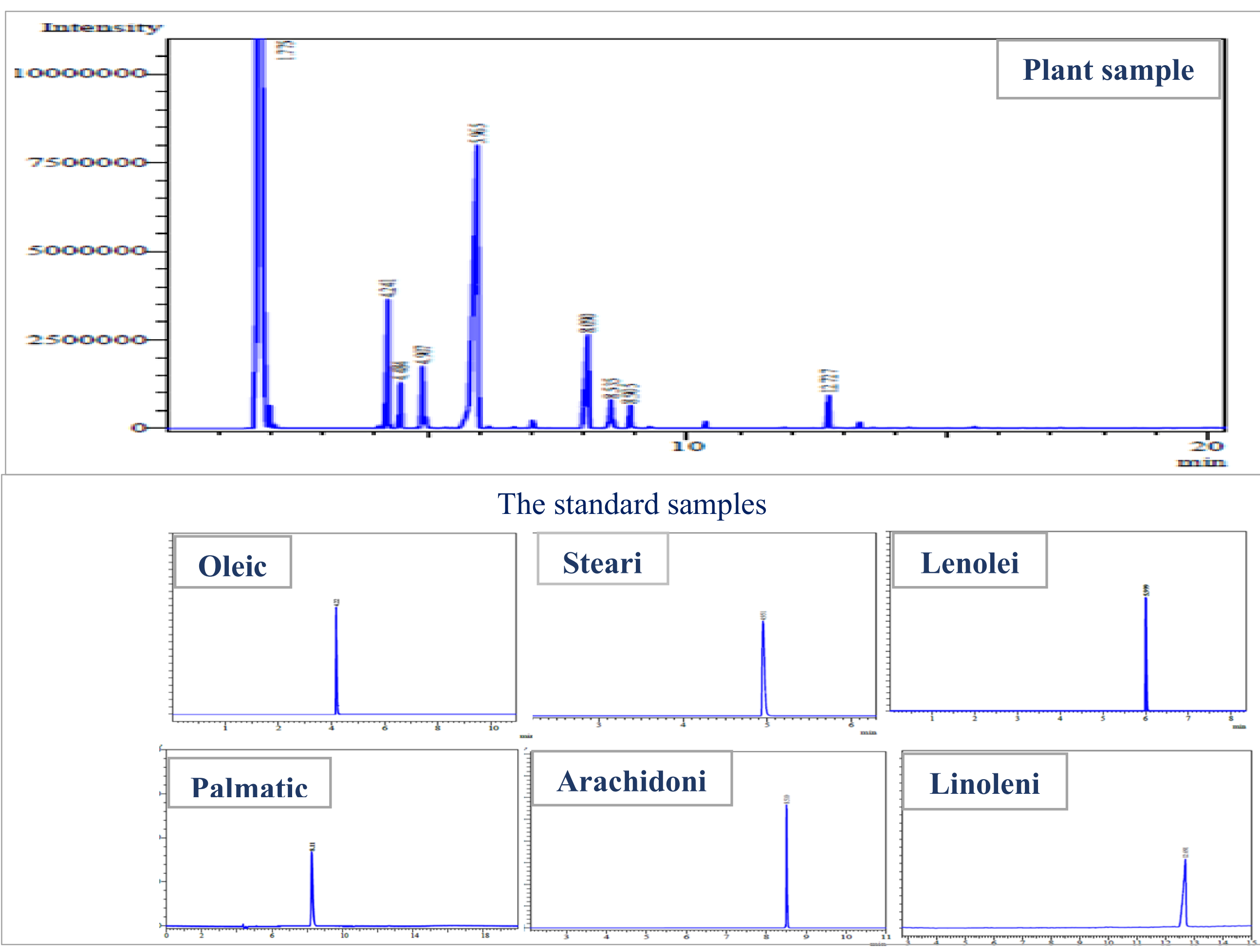


Figure 4; GLC chromatogram of the seed extract of *P. angulata* showing fatty acids identified in sequence with the standard samples

3.2 Identification of phenolic compounds saparated by HPLC

The results in Table (2) indicate that six phenolic compounds were identified by HPLC in the ethanolic extract of *P. angulata* seeds following acid hydrolysis. Based on the retention time of each compound and comparison with standard samples, a variation in the concentrations of phenolic compounds observed. The chlorogenic compound exhibited the highest concentration (0.0904 mg/ml) in the extract, while the apigenin compound recorded the lowest concentration (0.0526 mg/ml) in the extract. And this low content of phenolic compounds in the seeds is consistent with the findings when comparison with the other vegetative parts of plant.[24]

The chromatographic curves presented in Figure (5) confirm the presence of all the phenolic compounds identified by HPLC and listed in (Table 2), which were identified in the ethanolic extract of plant, with each appearance at its characteristic retention time as illustrated in the chromatographic profile (Qurcetine at 3.74 minutes, Apigenin at 4.18 minutes, Ferulic acid at 5.95

minutes, P-coumaric acid at 7.08 minutes, Galic acid at 7.93 minutes and Chlorogenic compound at a retention time of 11.88 minutes).

Table 2;The phenolic compounds that were separated and identified from the seed extracts of *P. angulata* by HPLC.

Phenolic compounds	R.Time of standard samples (min)	The 70% alcoholic extract of seeds		
		Concentration	R.Time (min)	Peak area
Qurcetine	3.79	0.0714	3.74	20.00
Apigenin	4.15	**0.0526**	4.18	10.00
Ferulic acid	5.98	0.0665	5.95	15.00
P-coumaric acid	7.00	0.0621	7.08	15.00
Galic acid	7.90	0.0740	7.93	13.00
Chlorogenic	11.80	**0.0904**	11.88	27.00

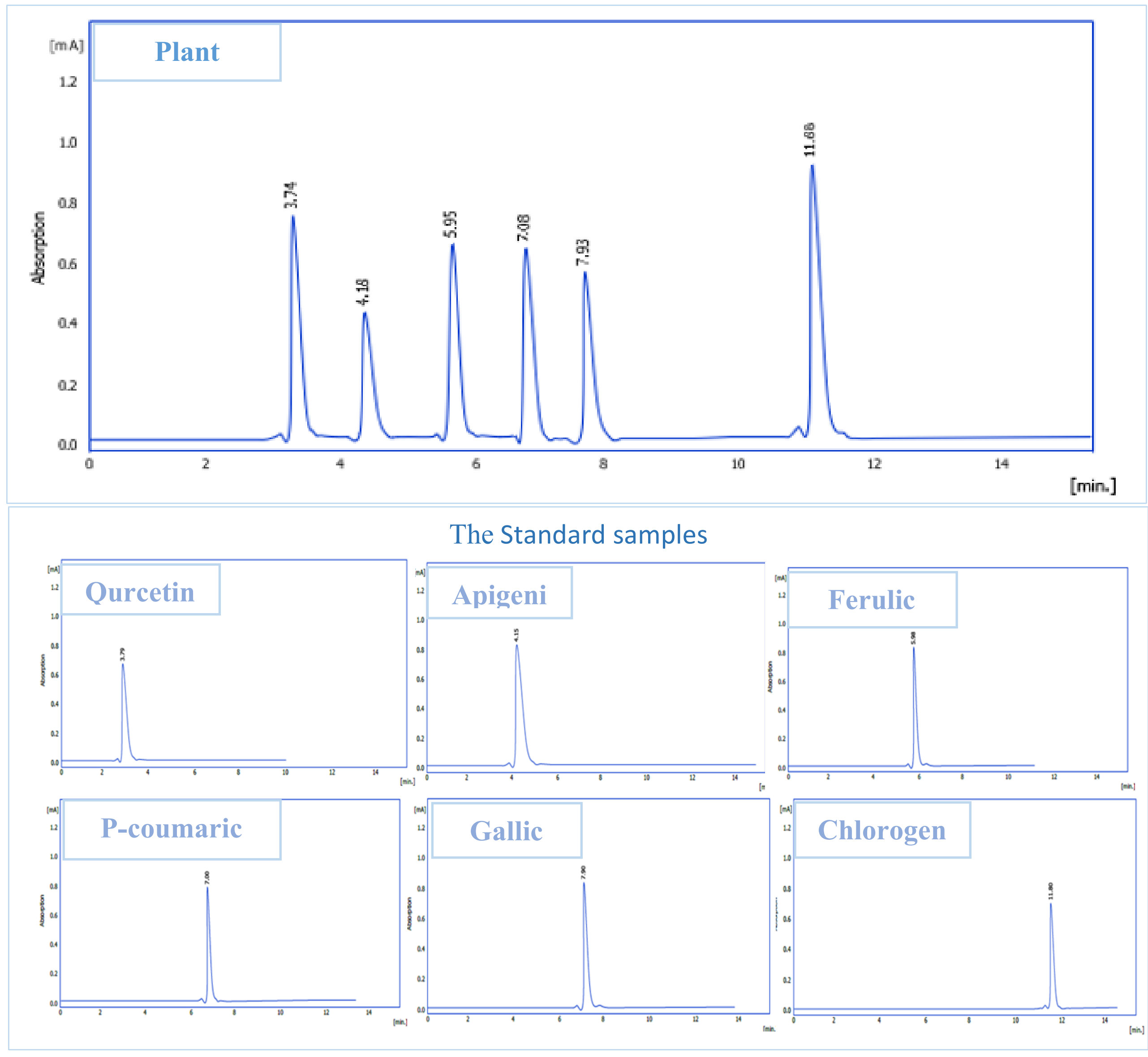


Figure 5; HPLC chromatogram of the seed extract of *P. angulata* showing phenolic compounds identified in sequence with the standard samples

3.3 Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

The active compounds were identified by comparing the mass spectra of the compounds with the NIST scientific library in the device's database. The analysis revealed the presence of a number of diverse active compounds, such as fatty acids and their derivatives, alcohols, and esters, as shown in Table (3), which is reinforced by the curve in Figure (6).

Table 3; the Compounds were identified by GC-MS based on their retention time, structural composition and molecular weight and further confirmed through compared with the NIST database.

Compound name	Paek Area%	R.Time (min)	Molecular formula	Molecular weight (g\mol)	Structure
Methanamine, N-butylidene	0.42	10.475	$C_5H_{11}N$	85	N
2-Amino-1,3-propanediol	0.45	10.700	$C_3H_9NO_2$	91	HO OH NH2
1-Bromo-3-butene-2-ol	0.50	13.100	C_4H_7BrO	150	Br OH
Butanoic acid, (tetrahydro-2-furanyl)methyl ester	0.40	15.458	$C_9H_{16}O_3$	172	
2-Imidazolidinone	1.53	16.817	$C_3H_6N_2O$	86	HN NH O
Lauric acid	18.97	17.383	$C_{12}H_{24}O_2$	200	OH
Heptanoic acid, 2-ethyl	0.87	17.517	$C_9H_{18}O_2$	158	HO
17-Octadecynoic acid	61.79	19.192	$C_{18}H_{32}O_2$	280	
Octadecanoic acid	8.19	19.358	$C_{18}H_{36}O_2$	284	OH
L-Serine, methyl ester	0.57	19.525	$C_4H_9NO_3$	119	HO NH2
3-Hexanol	4.14	21.133	$C_6H_{14}O$	102	OH
1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	0.70	22.600	$C_{16}H_{22}O_4$	278	
Cyclopentane, 1,2,4-trimethyl	0.73	24.183	C_8H_{16}	112	

Compound name	Paek Area%	R.Time (min)	Molecular formula	Molecular weight (g\mol)	Structure
2,3−Dibromo−6−chlorophenanthrene−9−carboxylic acid	0.74	27.042	$C_{15}H_7Br_2ClO_2$	412	

The results of the GC-MS analysis revealed the presence of fourteen compounds in the alcoholic seeds extract, as presented in Table (3), these compounds differed in their chemical formulas, retention times, and relative abundance percentage. Identification was achieved based on the comparison of the obtained mass spectra with those stored in the instrument database belonging of the NIST scientific library. Among the identified compounds, 17-Octadecynoic acid ($C_{18}H_{32}O_2$) was detected at a retention time of 19.192 minutes and exhibited the highest abundance percentage accounting for 61.79% of the total compounds, In contrast Methanamine, N-butylidene ($C_5H_{11}N$), recorded the lowest percentage at 0.42% and detected at a retention time of 10.475 minutes. Other notable compounds identified in the extract include lauric acid ($C_{12}H_{24}O_2$),at a retention time of 17.383 minutes, heptanoic acid 2-ethyl ($C_9H_{18}O_2$) at a retention time of 17.517 minutes, octadecanoic acid (also known as stearic acid),at a retention time of 19.358 minutes; 3-hexanol ($C_6H_{14}O$) at a retention time of 21.133 minutes; and 1,2-benzenedicarboxylic acid ($C_{16}H_{22}O_4$), at a retention time of 22.600 minutes. These results corresponds to the chromatographic profile illustrated in Figure (6)

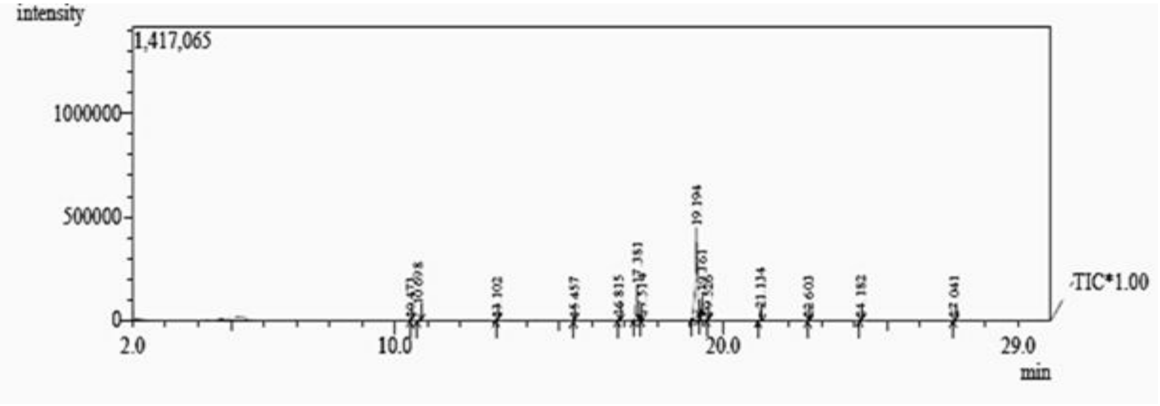


Figure6; GC-MS chromatogram of the crude ethanolic extract of *P. angulata* seeds, showing the compounds separated at different retention times**.**

from the results presented above, and in order to obtain a comprehensive chemical profile of the active compounds in the seeds of the *P. angulata* L. multiple methods and analytical approaches were employed. A several of fatty acids were identified using the GLC technique after their liberation through the saponification of the petroleum ether extract, resulting in the detection of six fatty acids (Oleic, Stearic, Linoleic, Palmatic, Arachidonic, Linolenic). In addition, a several phenolic compounds were identified from the ethanolic extract following acid hydrolysis. Six phenolic compounds were subsequently characterized using the HPLC technique (Quercetin, Apigenin, Ferulic acid, P-coumaric acid, Gallic acid, and Chlorogenic). Furthermore, the same crude extract was analyzed using GC-MS technique to detect more additional volatile and semi-volatile compounds. This analysis led to the identification of fourteen compounds, the most prominent of which were (17-Octadecynoic acid, 3-Hexanol, 1,2-Benzenedicarboxylic acid, Lauric acid and Heptanoic acid, 2-ethyl).

Previous studies have shown that several of the compounds identified in the current study possess significant medicinal importance and health-promoting properties. Notably, the identified fatty acids include members of the well-known Omega family. Linolenic acid, known as Omega-3, is associated with enganced cognitive function which and improved concentration in infants when taken by the mother. It also supporting lung health and reduces the risk of cardiovasuclar diseases. Linoleic acid, known as Omega-6, is essential for the proper functioning of all body tissues. Meanwhile, Stearic and Oleic acid together

constitute Omega-9, which has been reported to lower levels of (LDL) cholesterol while increases (HDL) cholesterol, thereby contributing to reduce risk of stroke and cardiovascular diseases [25].

In addition, the Phenolic compounds, such as gallic acid, have antioxidant, anti-inflammatory, and antitumor properties. They are also beneficial in treating neurological, psychological, and gastrointestinal disorders [26].Qurcetin and chlorogenic acid are antioxidant, anti-inflammatory, antibacterial, and antiviral compounds with immunomodulatory activities [20] previous Studies have confirmed that ferulic acid is used in the cosmetics and food industries, and has anticancer, diabetes, and neurodegenerative effects [27], [28]. 17-Octadecynoic acid, 3-Hexanol, 1,2-Benzenedicarboxylic acid, and Lauric acid also have antioxidant, antiparasitic, antibacterial, and antifungal properties, and act as a vasoconstrictor. [29], [30], [31], [32]

4. Conclusion

Based on the results obtained, the seeds of *P. angulata* contain many important active compounds (fatty acids, phenolic compounds, alcohols, and esters) which are associated with multiple health benefits. This indicates the potential for using the seeds as a natural source for treating various diseases in the future. It is also clear that the application of multiple chromatography techniques (GLC, HPLC, GC-MS) provided a more comprehensive profiling, allowing the identification a greater number of active compounds in these seeds.

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6. Declarations

6.1 Ethics approval and consent to participate

Not applicable.

6.2 Consent for publication

Not applicable.

6.3 Availability of Data and Materials

Data will be provided upon receiving a valid request.

6.4 Conflicts of interest

The authors declare that there is no conflict of interest

6.5 Funding

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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فصل وتشخيص بعض المركبات الفعالة من بذور نبات الحرنكش العراقي ***Physalis angulata* L.** باستخدام تقنيات الكروماتوغرافيا

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الخلاصة:

هدفت هذه الدراسة الى دراسة التركيب الكيميائي النباتي لنوع *Physalis angulata* L. الذي تم جمعه من ناحية ربيعة غربي مدينة الموصل شمال العراق وتم اختيار البذور لتشخيص ماتحتويه من مركبات فعالة باستخدام تقنيات الكروماتوغرافية من خلال استخلاص المركبات الفعالة باستخدام جهاز Soxhlet، اذ أظهرت النتائج تشخيص ستة مركبات فينولية من المستخلص الكحولي 70% بواسطة HPLC بعد التحلل الحامضي وهي: (Quercetin، Apigenin، Ferulic acid، P-coumaric acid، Gallic acid، وChlorogenic). وسجل اعلى تركيزللمركبChlorogenic في المستخلص بلغ 0.0904 mg\ml. و أربعة عشر مركب باستخدام تقنية GC-MS لنفس المستخلص الخام وكان ابرزها (17-Octadecynoic acid و3-Hexanol و 1,2-Benzenedicarboxylic acid و Heptanoic acid, 2-ethyl وLauric acid). كما وشخصت ستة أنواع من الاحماض الدهنية (Oleic، Stearic، Linoleic، Palmatic، Arachidonic، Linolenic) من مستخلص الايثر بترولي المتصوبن بوساطة تقنية GLC، وسجل الحامض الدهني Linoleic في المستخلص النباتي اعلى تركيز بلغ 19.80 %. تشير هذه النتائج احتواء بذور نبات الحرنكش *P. angulata* L. على مركبات ذات أهمية طبية تفتح المجال الى إمكانية استخدامه في تطبيقات دوائية علاجية مستقبلا.