Extraction and Quantification of Vitexin and p-Coumaric Acid from Basella alba L. Cultivated in Iraq

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Abstract

The objective of this work is to isolate, characterize, and quantify two bioactive substances from *Basella alba* (Malabar spinach), a plant of nutritional and therapeutic value. Vitexin and p-coumaric acid were isolated and quantified using high-performance liquid chromatography (HPLC), and Their characterization was carried out using Fourier transform infrared spectroscopy (FT-IR), liquid chromatography-tandem mass spectrometry (LC-MS/MS), and the spiking method, where the isolated compound was compared with the standard for confirmation. confirmationThe concentrations of p-coumaric acid and vitexin in Basella alba were found to be 144 µg/g and 220 µg/g, respectively, which are higher than those identified in previous studies. This suggests that environmental factors, particularly light exposure and geographical conditions, may play a significant role in enhancing the production of these secondary metabolites. Soxhlet extraction was employed to isolate and quantify the compounds, demonstrating high recovery rates consistent with previous findings.. These results highlight the potential of Basella alba as a valuable plant for further research into its health benefits. Future studies should explore its applications in nutraceuticals and pharmaceuticals.

Keywords: Basella alba, Vitexin,p-coumaric acid, HPLC,LC-MS/MS,FT-IR

Introduction

Currently, more than 100,000 natural compounds have been isolated and identified from plants classified as terpenes, phenols and alkaloids, as these compounds such as flavonoids are part of a large group of phenolic compounds and these compounds are an essential component of plant cells [(1)]. Medicinal plant use, however, is not limited to developing countries; in fact, demand for herbal medicine is increasing in many developed countries[(2)] .Phenolic compounds are defined chemically as a substance consisting of one or more aromatic rings with hydroxyl substituents, including their functional derivatives. The plant produces them as secondary metabolites that it needs for the purposes of growth and defending itself against diseases.The antioxidant capacity of natural phenols is important for foods, especially functional foods, medicinal foods, and nutritional supplements, as multiple phenols are associated with a wide range of physiological properties, including anti-inflammatory, antimicrobial, antioxidant, and powerful radical scavengers [(3)]. The main groups of phenolic compounds are flavonoids, phenolic acids, tannins, stilbenes and lignans Flavonoid rich plant extracts from different species have been reported to possess anti activity against a wide array of

microorganisms.Thus,theirmodeof antimicrobial action may be related to their ability to inactivate microbial adhesions, enzymes, cell envelope transport proteins, and so forth.Lipophilic flavonoids may also disrupt microbial membranes [4]. .Because they are antioxidants and provide protection against heart disease, some types of cancer, and agerelated cell component deterioration, flavonids are very advantageous. Superoxide and hydroxyl radicals are among the harmful free radicals that they can scavenge because to their polyphenolic nature [5].People's interest in using wild plants as food during times of starvation is a significant aspect, but consuming natural products is also growing more popular in our contemporary culture.Basella alba L., a perennial vine that grows quickly and is tolerant to high temperatures, is a member of the Basellaceae family[6,7]. Vine spinach, Malabar spinach, Indian spinach, and Ceylon spinach are some of its common names[8]. It has long been used widely in Chinese and Indian medicine because of its therapeutic properties, which include its use as a diuretic, cleaning agent, and anti-inflammatory therapy[9]. It is also said to have several pharmacological advantages, such as antiviral, anticancer, antioxidant, anti-inflammatory, anti-cholesterol, anti-ulcer, antibacterial, hypoglycemic, wound-healing, and androgenic properties[10]. Environmental variables and cultivar type are important determinants of Basella alba's nutritional and phytochemical content. Research has indicated notable differences in antioxidant activity, phytochemical concentration, and proximate composition based on geographic location[11,12]. The influence of environmental conditions on the chemical profile of plants is highlighted by the variations in chlorophyll, carotene, flavonoid, and phenolic contents between samples from different regions[13]. It is generally known that Basella alba contains bioactive substances such phenolic compounds, peptides, and saponins. Carotenoids, organic acids, water-soluble polysaccharides, betacyanin, bioflavonoids, and vitamin K are abundant in the leaves. The plant has also yielded a number of triterpene oligoglycosides, such as momordins IIb and IIc, betavulgaroside I, and basella saponins A, B, C, and D[14]. The antioxidant activity of Basella alba is closely linked to flavonoids and other polyphenols, which shield cells from oxidative damage[15].Despite Basella alba's well-established advantages, little is known about the precise nature and levels of two of the plant's main bioactive substances, vitexin and p-coumaric acid. The flavone glycoside vitexin is well-known for its neuroprotective[16], anti-inflammatory, and antioxidant qualities[17], while hydroxycinnamic acids p-coumaric acid is an important component of plant defense and has potent antibacterial and antioxidant capabilities[18]. Using infrared (IR) spectroscopy, high-performance liquid chromatography (HPLC), and liquid chromatography- tandem mass spectrometry (LC-MS/MS), this work attempts to describe and quantify vitexin and p-coumaric acid in Basella alba. These advanced methods of analysis will offer valuable insights on Basella alba's chemical composition .

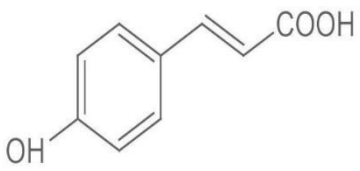

Figure1. chemical structure of p-coumaric acid

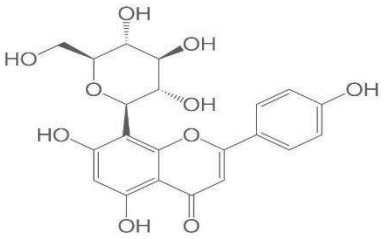

Figure2. chemical structure of Vitexin.

Materials and Methods

Preparation of plant materials

The whole Basella laba plant was acquired in September 2024 from a farm in the city of Baghdad. The University of Baghdad's Biology Department's Assistant Professor Dr. Israa Abdulrazaq Majeed has discovered the authenticity of the plant. A mechanical grinder was used to grind the plant into a coarse powder after it had been washed and allowed to dry in the shade.

Extraction and fractionation

The remaining plant material was extracted using a Soxhlet device for 12 hours with 1 liter of aqueous ethanol (ethanol-water 85:15 v/v). After filtration, the alcoholic extract was concentrated using a rotary evaporator to obtain the crude extract. The crude extract was suspended in 250 milliliters of distilled water and subjected to fractionation using a separatory funnel. It was first partitioned three times with 250 milliliters of petroleum ether to remove non-polar components. Afterward, the aqueous layer was further partitioned three times with 250 milliliters of ethyl acetate. Both petroleum ether and ethyl acetate fractions were collected separately, filtered, dried over anhydrous sodium sulfate, evaporated under reduced pressure using a rotary evaporator, and then weighed.

Preliminary phytochemical screening

Flavonoids test Shinoda test: A small piece of magnesium ribbon was added to the alcoholic solution of the crude extract, followed by the dropwise addition of concentrated HCL, which reveals the presence of flavonoids by the formation of a color ranging from orange to red within one to two minutes [(19)].

Test for phenols

Ferric chloride test: Two milliliters of 10% aqueous ferric chloride were added. The appearance of a bluish color indicated the presence of flavonoids in the extracts [19].

Thin layer chromatography (TLC):

The identification of the bioactive compounds p-coumaric acid and vitexin in the ethyl acetate fraction was performed using Thin Layer Chromatography (TLC). Silica gel-coated plates (20 × 20 cm) were used, and the ethyl acetate fraction was spotted onto the plates. The mobile phase used for the separation of p-coumaric acid was ethyl acetate:toluene:formic acid (5:5:2, v/v/v), while for vitexin it was toluene:ethyl acetate:methanol (T:E.A:M) in a ratio of 10:6:4 (v/v/v). After development, the plates were dried and visualized using ferric chloride ($FeCl_3$) spray reagent. The appearance of an orange-colored spot indicated the presence of p-coumaric acid, while a brown-colored spot confirmed the presence of vitexin in the fraction(20).

P-coumaric acid and Vitexin identification using high performance liquid chromatography (HPLC)

At the Department of Environmental and Water Research/Ministry of Sciences and Technology, the HPLC analysis was carried out using the instrument model SYKMAN (Germany) to select the p-coumaric acid and vitexin contained in the fraction of ethyl acetate. The retention times of the investigated samples under identical circumstances were compared to those of standard materials. A gradient mobile phase comprising 95% acetonitrile + 0.01% Triflouroacetic acid as solvent A and 5% acetonitrile + 0.01% Triflouroacetic acid as solvent B was used to prepare the HPLC analysis using a C18-ODS column (250 × 4.6 mm, 5 µm). The injection volume was 100 µL, and the flow rate was set at 1 mL/min. The gradient program was as follows: 10% A from 0 to 5 minutes; 25% A from 5 to 7 minutes; 40% A from 7 to 12 minutes; and then going back to the initial circumstances. The chemicals were found using a UV visible detector at 278 nm(21).

Equipement for characterization of the isolated compounds

The extracted p-coumaric acid and vitexin were characterized using several chromatographic and spectroscopic techniques. FTIR analysis was performed using a Shimadzu IRAffinity-1 spectrometer (Japan). The ^{1}H-NMR (400 MHz) spectra was obtained using a Bruker Avance III spectrometer in d_6-DMSO. LC-MS/MS analysis was carried out using a Shimadzu Triple Quad 4500 system (Japan) equipped with a GL-Science C18 column (100 mm × 4.6 mm, 5 µm), operated at 35°C, with a 5 µL injection volume, 1 mL/min flow rate, and a 25-minute run time. The isocratic mobile phase consisted of water with 0.1% formic acid and a mixture of acetonitrile:methanol (50:50, v/v) with 0.1% formic acid. Data acquisition was performed in positive ESI mode over an m/z range of 50–800(22,23).

Results and Discussion

Quantity and percentage yield of fractions

Different percentages were obtained from the extracts of Basella alba based on extraction and fractionation using ethyl acetate solvent. Table 1 displays the quantities and percentage yields of extracts in ethyl acetate and aqueous ethanol.

Table 1. The quantities and percentage yield of the extracts with aqueous ethanol, and ethyl acetate.

Fraction of plant extract	Quantity in gram	Percentage yield
Aqueous ethanol	5.85	24%
Ethyl acetate	0.5	0.5%

It was determined by the initial phytochemical analysis that flavonoids and phenols were present.

Table2. Preliminary screening of flavonoids and phenols in ethyl acetate fraction.

Test	Result

Flavonoids	+ pink to reddish color
Phenols	+ (Deep green color

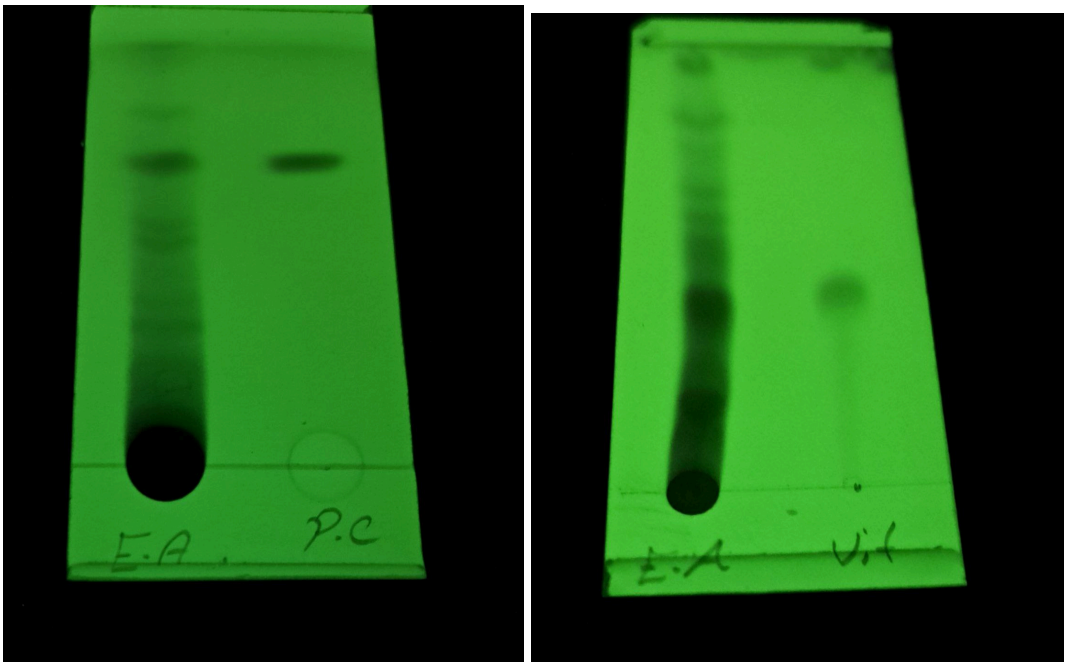

Figure 3. TLC chromatogram of p-coumaric acid and vitexin under UV light at 256 nm, showing the characteristic spots of the compounds in the ethyl acetate fraction.

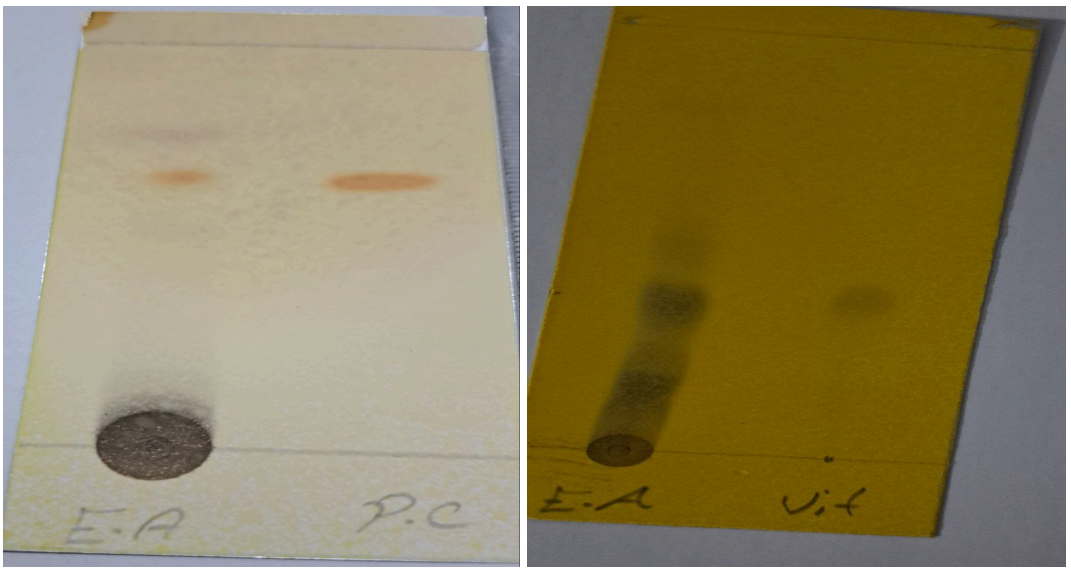

Figure 4. TLC chromatogram showing p-coumaric acid with Rf = 0.66 in the sample and Rf = 0.65 in the standard, and vitexin with Rf = 0.42 in the sample and Rf = 0.49 in the standard after spraying with $FeCl_3$ reagent.

Table 3. Rf values of the sample and standard compound using Thin Layer Chromatography (TLC).

compounds	Rf of identified compounds	Rf of standards
p-coumaric acid	0.66	0.65
vitexin	0.42	0.49

High-performance liquid chromatography (HPLC) for the identification of the two compounds

comparing to the retention times of p-coumaric acid and vitexin standards, which are displayed in Figures 4 and 5 below, the ethyl acetate fraction analysis results based on retention times, as indicated in Table 3 and the chromatogram in Figure 3, proved the presence of p-coumaric acid and vitexin compounds.

Table 4. Standards' retention time(measured in minutes) and the associated components of Iraqi Basella alba

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compounds	Rf of identified compounds	Rf of standards
p-coumaric acid	5.85	5.97
vitexin	6.30	6.36

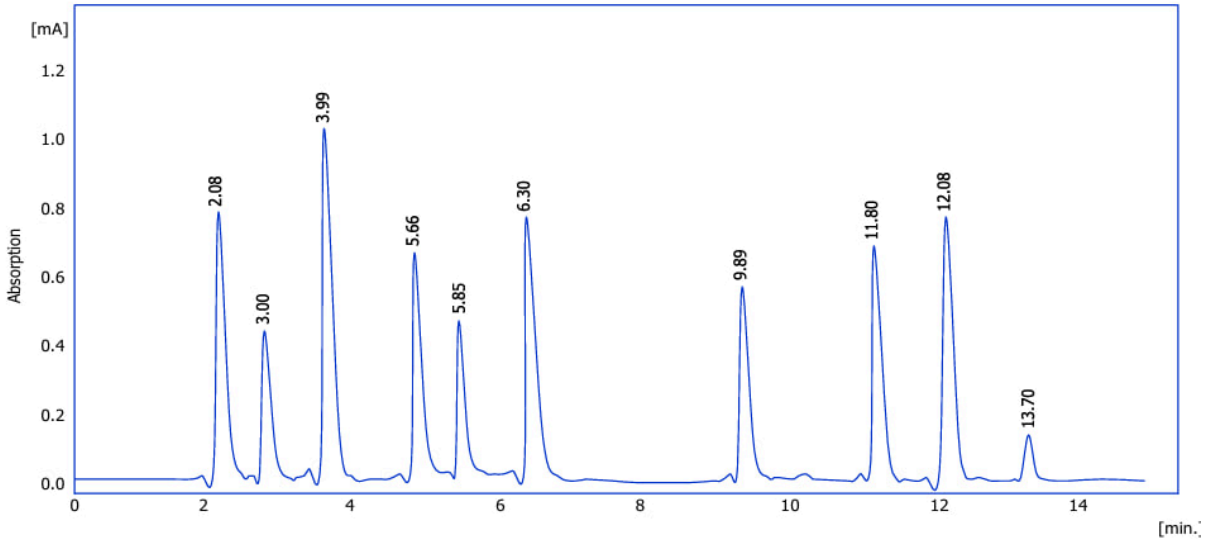

Figure 5. HPLC chromatogram for the fraction of ethyl acetate.

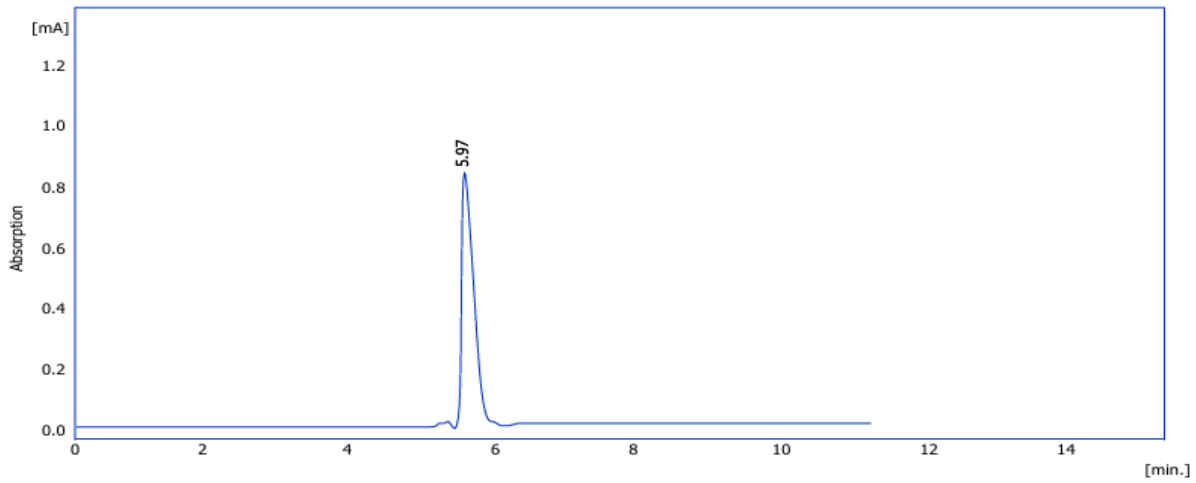

Figure 6. HPLC chromatogram for the standard p-coumaric acid.

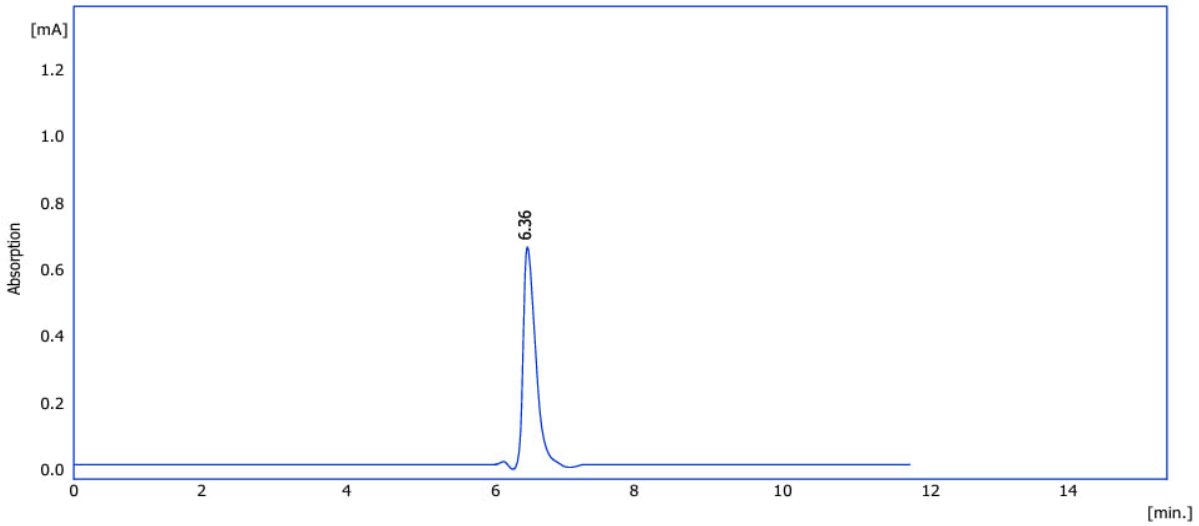

Figure 7. HPLC chromatogram for the vitexin standard.

Spiking analysis by HPLC

P-coumaric acid and vitexin retention times in Iraqi Basella alba extract were identified and confirmed using High-Performance Liquid Chromatography (HPLC) analysis. To confirm the existence of the discovered compounds, their retention times (Rt) were compared to those of the matching standards.

There was a close match between the standard retention time of 5.97 minutes and the actual retention time of 5.85 minutes for p-coumaric acid in the sample. Likewise, the sample's vitexin retention time was 6.30 minutes, whereas the standard's was 6.36 minutes.

Given that their retention times closely match those of the corresponding standards, our findings validate the existence of p-coumaric acid and vitexin in the Iraqi Basella alba extract

High performance liquid chromatography (HPLC) to isolate Vitexin and p-coumaric acid

Table 5- Retention Time and Peak Area of the Isolated Compounds

Standard used	Area of Standard	Area of isolated	Rt of isolated spiked with standard	Area of spiking
P-coumaric acid	415.08	985.07	5.98	1389.11
Vitexin	520.11	1421.00	6.38	1932.44

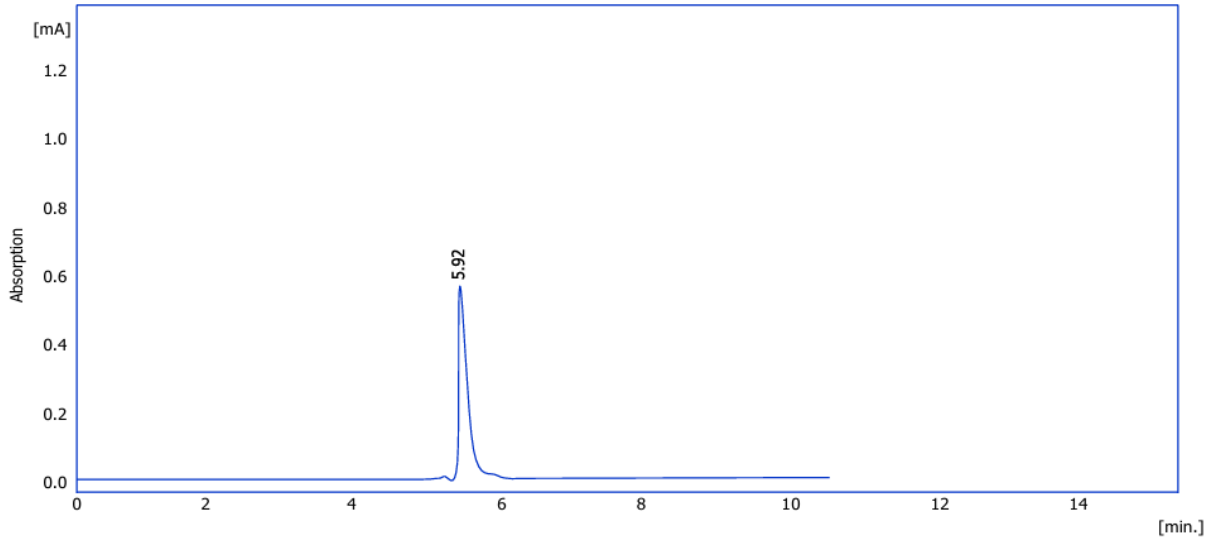

Figure8- Chromatogram of HPLC for isolated p-coumaric acid

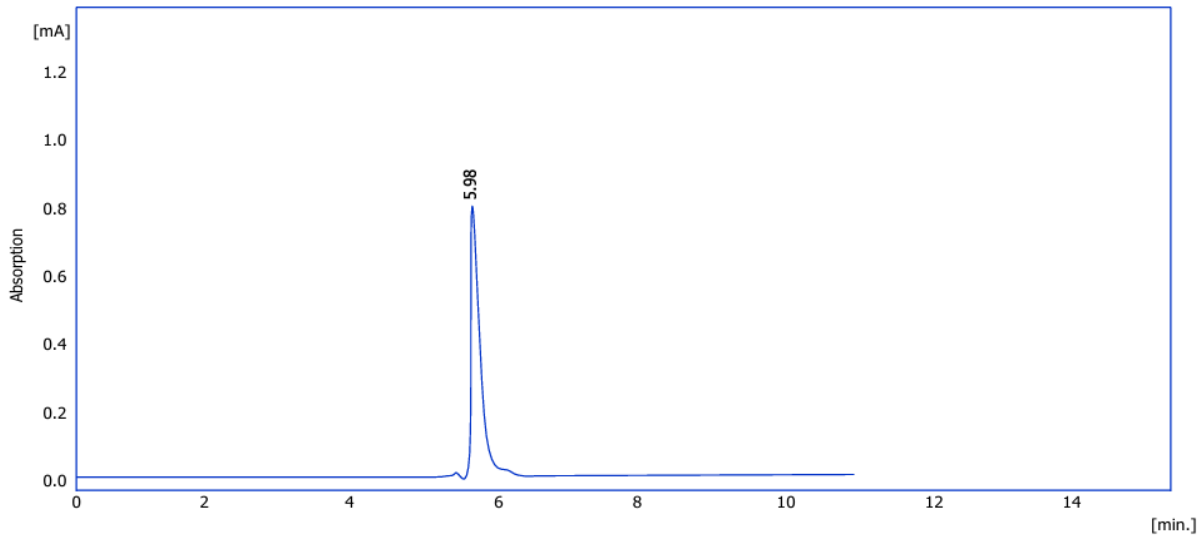

Figure 9. Chromatogram of HPLC isolated P-coumaric acid spiked with P-coumaric standard HPLC chromatogram

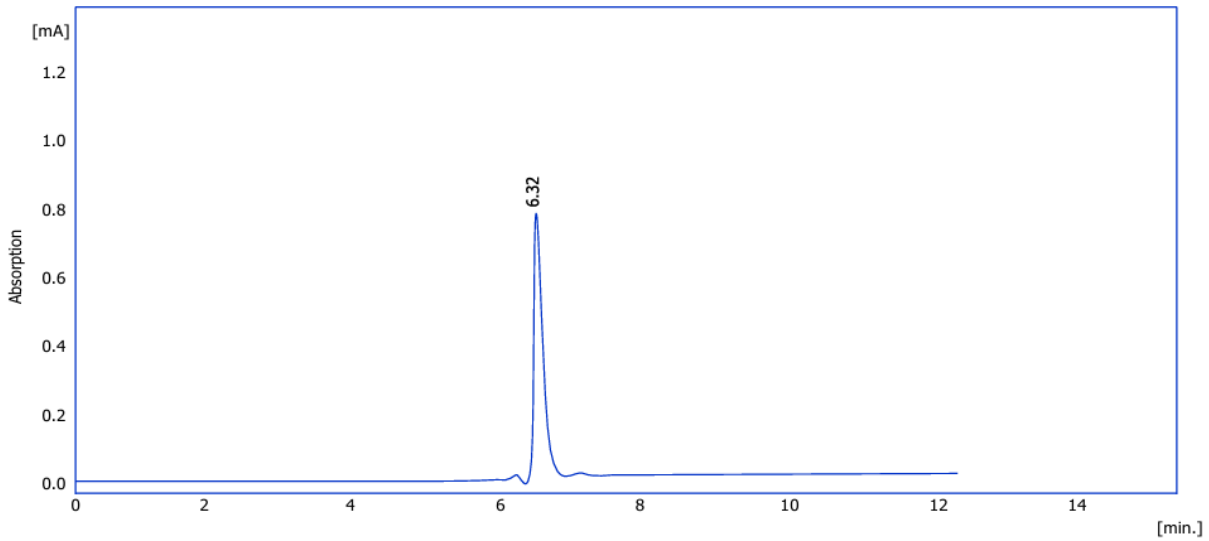

Figure10. Chromatogram of HPLC for isolated vitexin

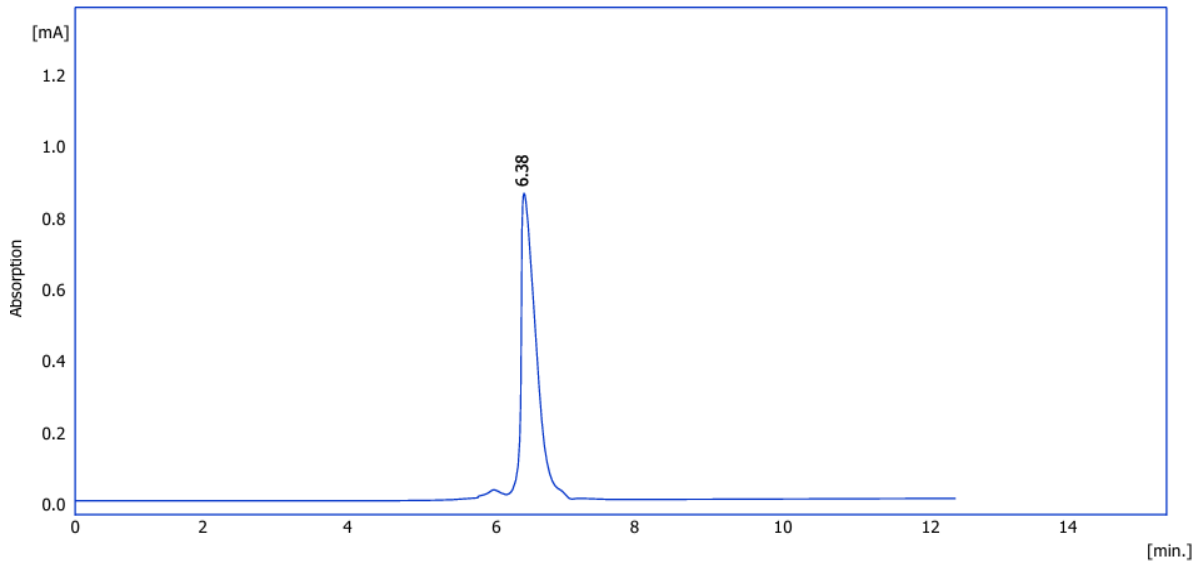

Figure11. Chromatogram of HPLC for Vitexin spiked with Vitexin standard HPLC chromatogram.

Quantification of the compounds detected by HPLC results:

The amount of the proposed p -coumaric acid and vitexin in the ethyl acetate fraction was determined by the regression equation obtained from the calibration curve, as shown

Table6. Quantitative analysis of ethyl acetate fraction

compound	**Result**
p-coumaric acid	144 µg/g
vitexin	220 µg/g

Table 7. Equations of calibration curves obtained for compound standards

Standard	Equations	
p-coumaric acid	Y=102.50667* x	0.9998
vitexin	Y 105.66667*X	0.9998

FT-IR

Infrared spectroscopy using the Fourier transform (FTIR) analysis was carried out on isolated compounds; Figure 15 shows the FTIR spectrum of isolated p-coumaric acid, and Table 8 provides the band interpretation.

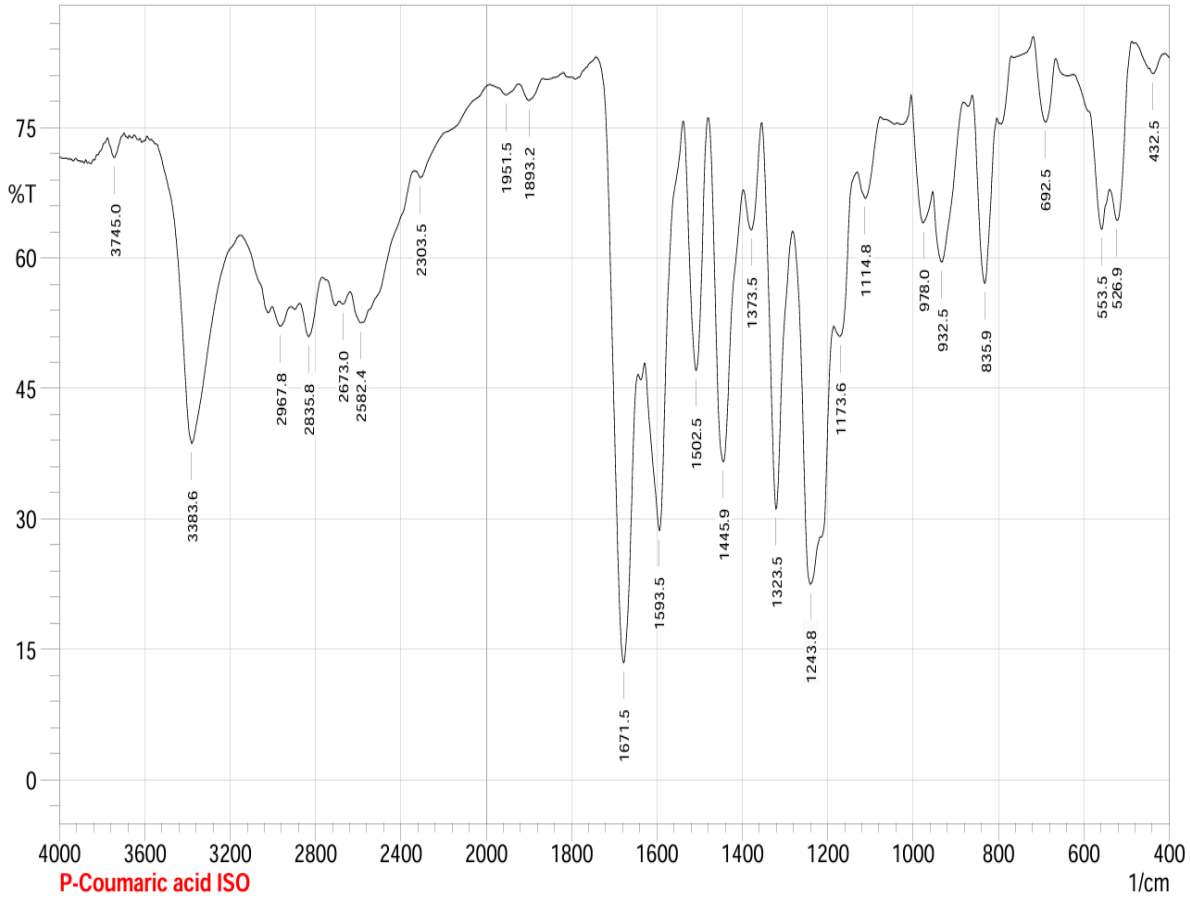

Figure 12. FTIR spectrum of isolated p-coumaric acid .

Table 8. Interpretation of the isolated p-coumaric acid's FTIR bands[24]

Functioal groups	Wave-number cm-1	Interpretation
O-H	3383.6	O-H stretching vibration
C-H	2967.8	C-H stretching vibrations
C=C	1593.5	Alkene C=C stretching vibration
C=C	1502.5 . 144.9, 1373.5	Aromatic C=C stretching vibration
C-O	1243.8	C-O stretching vibration of phenol
C=O	1617.5	C=O stretching vibration

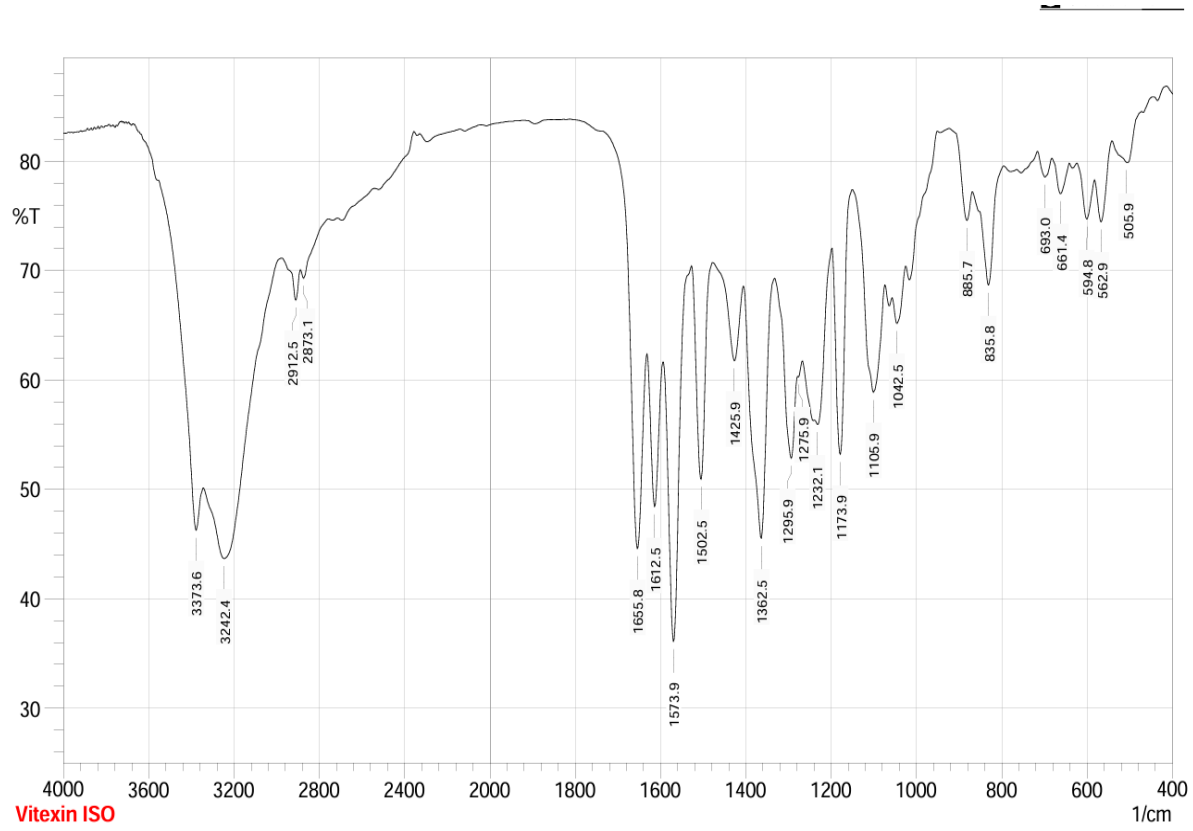

Figure 13. FTIR spectrum of isolated vitexin .

Table 9. interpretation of the isolated Vitexin's FTIR bands[25]

Functional groups	Wave-number cm-1	Interpretation
OH	3242.4	OH stretching vibrations
CH	2912.5,2873.1	C-H stretching vibrations
C=C	1655.8 1612.5,1573.9	CH stretching of aromatic alkene
C=O	1573.9,1502.5	C=O stretching vibrations
CO	1232.1,1042.5	CO two stretching vibrations of phenyl alkyl ether
CO	1173.5	CO stretching vibrations of cyclic ether

Liquid chromatography mass spectrometry (LC-MS/MS)

LC MS/MS was performed on the isolated chemicals in order to further identify and characterize them. Figure 14 showed the isolated p-coumaric acid's ion fragmentation spectrum[26,27].

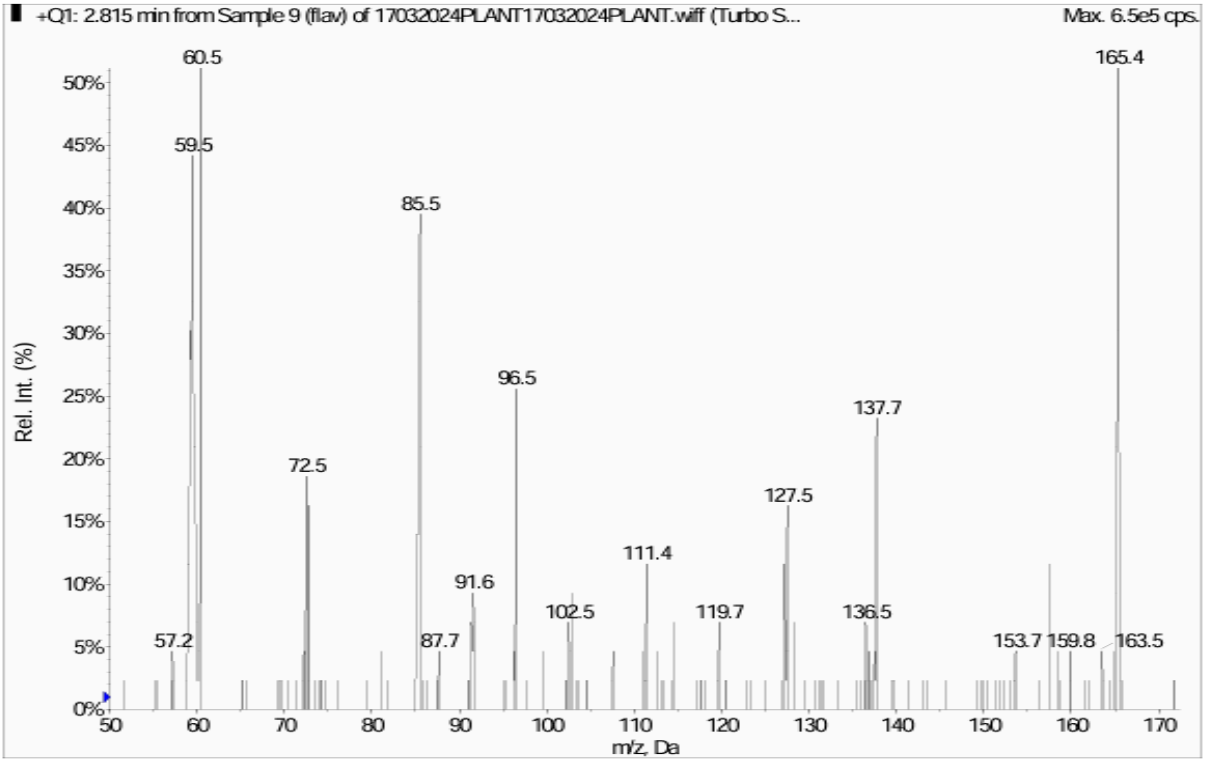

Figure 14. ion mass fragmentation spectra of p-coumaric acid that were separated

According to data in Figure. 14, the [M+H] ion with m/z 165.06, was considered as a molecular ion peak, where the molecular ion equal to m/z 164.04 .

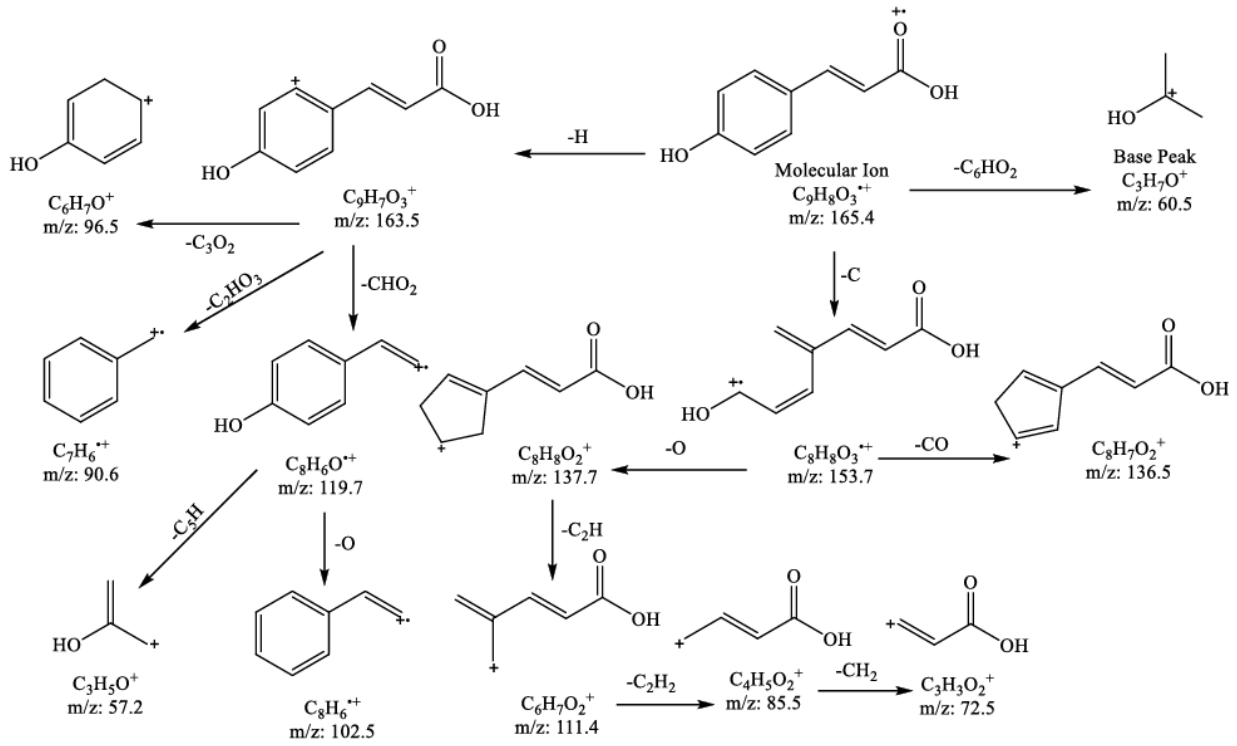

Figure15 .LC- MS/MS fragmentation of p-coumaric acid.

The protonated molecular weight of p-coumaric acid is confirmed by the molecular ion [M+H]+ at m/z 165.4.The base peak at m/z 60.5 ($C_3H_7O^+$), which represents a stable tiny fragment, most likely originates from side chain cleavage and loss of aromatic structure.The loss of one hydrogen atom (–H) from the molecular ion causes the fragment at m/z 163.5 ($C_9H_7O_3^+$). The loss of carbon monoxide (–CO), a frequent cleavage in phenolic acids, is shown by the fragment at m/z 136.5 ($C_8H_8O_2^+$). When $–CHO_2$ is lost, the carboxylic group breaks down, as seen by m/z 119.7 ($C_8H_7O^+$). The existence of the phenylpropanoid structure is supported by m/z 102.5 and 96.5, which are indicative of cleavage in the aromatic ring with side chain loss. The smaller pieces from the side chain's continuous cleavage are represented by m/z 85.5, 72.5, and 57.2 in line with the hydroxycinnamic acid structure breaking down. The fragmentation pattern validates the phenylpropanoid biosynthesis route and shows that the chemical is derived from phenylalanine via common enzyme conversions such as PAL and C4H.

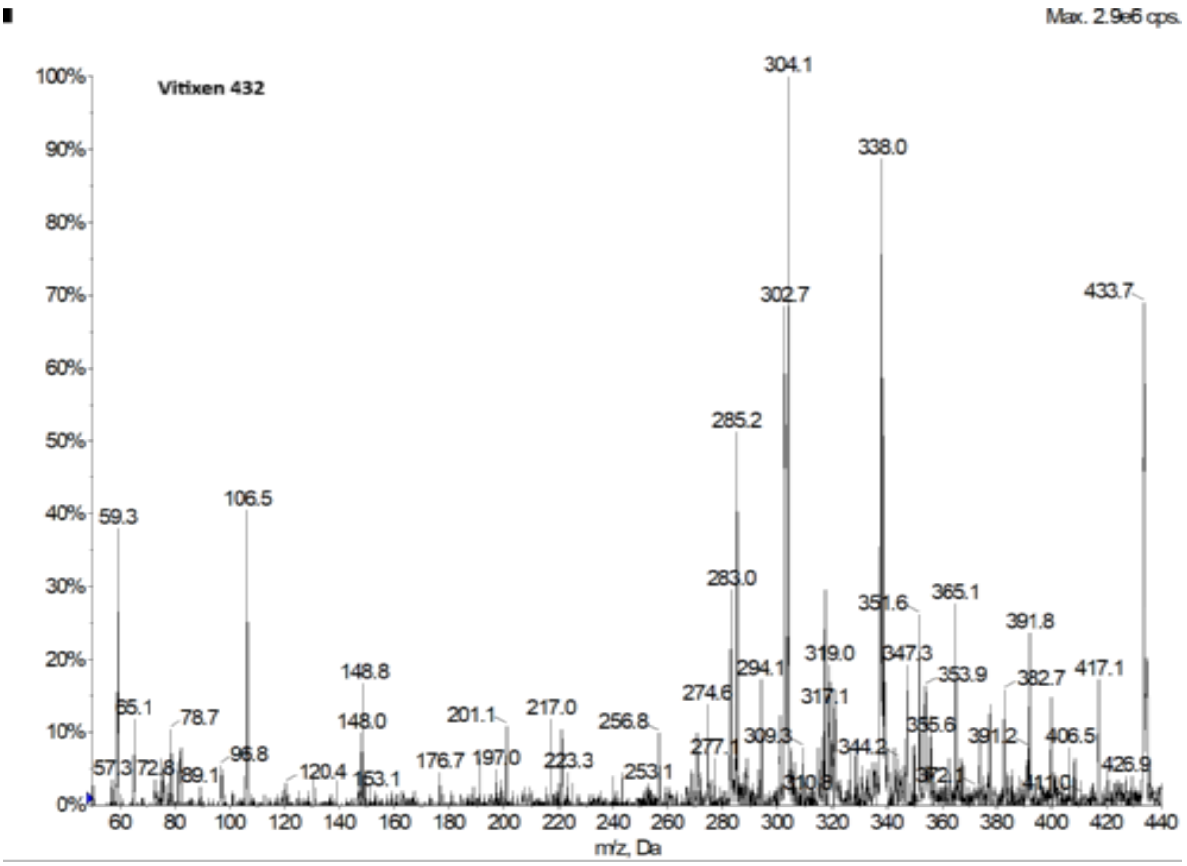

Figure 16. ion mass fragmentation spectra of Vitexin that were separated

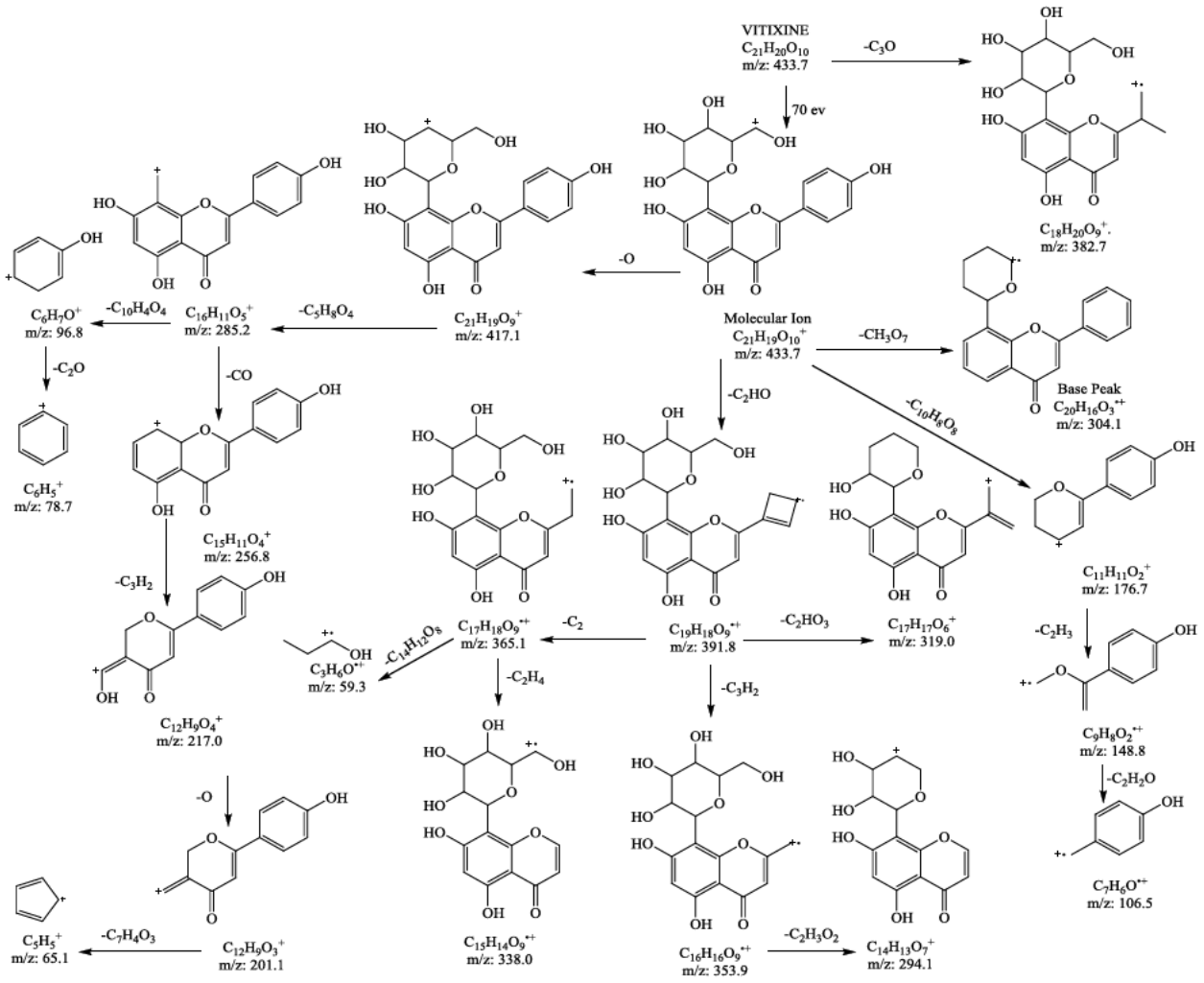

Figure 17. LCMS/MS fragmentation of Vitexin.

Vitexin's MS/MS fragmentation pattern (m/z 433.7) showed distinctive fragment ions that were the consequence of successive losses of carbonyl-containing fragments, methyl groups (-CH_3), and sugar moieties. Like with C-glycosyl flavonoids, the breaking of the C-glycosidic bond is represented by the main fragment at m/z 304.1 (base peak). The breakdown of the flavone core is supported by other notable ions (such as m/z 285.2, 217.0, and 176.7), which also validate the existence of methoxy and hydroxyl substituents. These fragmentation routes mirror the production of vitexin through the flavonoid branch of the phenylpropanoid pathway, which involves important enzyme steps like flavone synthase and C-glycosyl transferases, and offer compelling structural evidence for its identification[28,29].

^{1}H-NMR Spectroscopy of the Isolated Compound

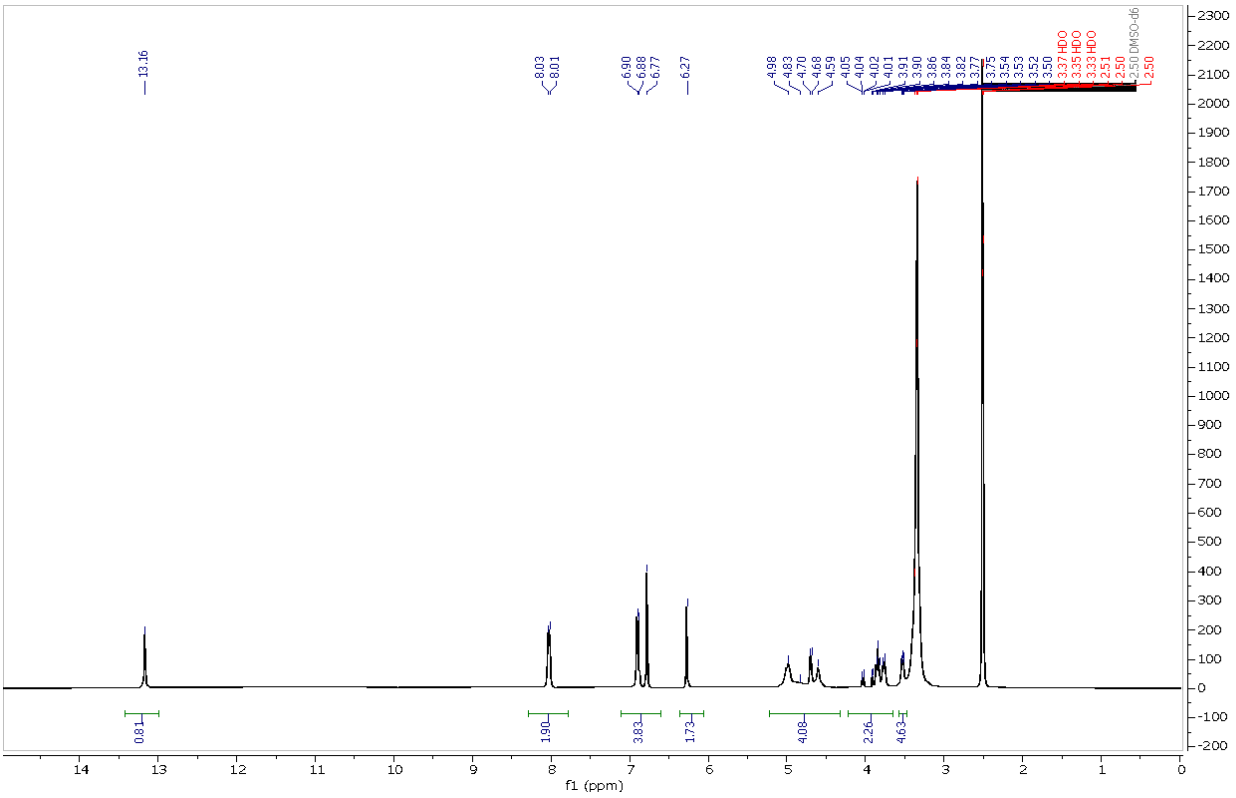

Figure18. ^{1}H-NMR spectrum of vitexin.

The ^{1}H-NMR spectrum showed a multiplet signal at a chemical shift of δ = 2.50–2.51 ppm, corresponding to the DMSO-d_6 solvent, and a signal at δ = 3.33–3.37 ppm attributed to HDO (residual water in DMSO). Doublet signals at δ = 3.50–3.54 ppm (d, 5H) were observed, corresponding to the methyl group protons and the protons of a saturated ring. Another doublet signal appeared at δ = 3.75–3.91 ppm (d, 2H), attributed to the C-H and O-H protons in the saturated ring and methylene-linked hydroxyl group. A singlet at δ = 4.01–4.98 ppm (s, 4H) indicated the presence of hydroxyl protons attached to the saturated ring.

Additionally, a singlet at δ = 6.27 ppm (s, 2H) was assigned to aromatic and olefinic protons, while a doublet at δ = 6.77–6.90 ppm (d, 4H) corresponded to aromatic ring protons. A singlet at δ = 8.01–8.03 ppm (s, 2H) indicated phenolic hydroxyl protons, and a singlet at δ = 13.16 ppm (s, 1H) was also attributed to a phenolic hydroxyl proton[(26)].

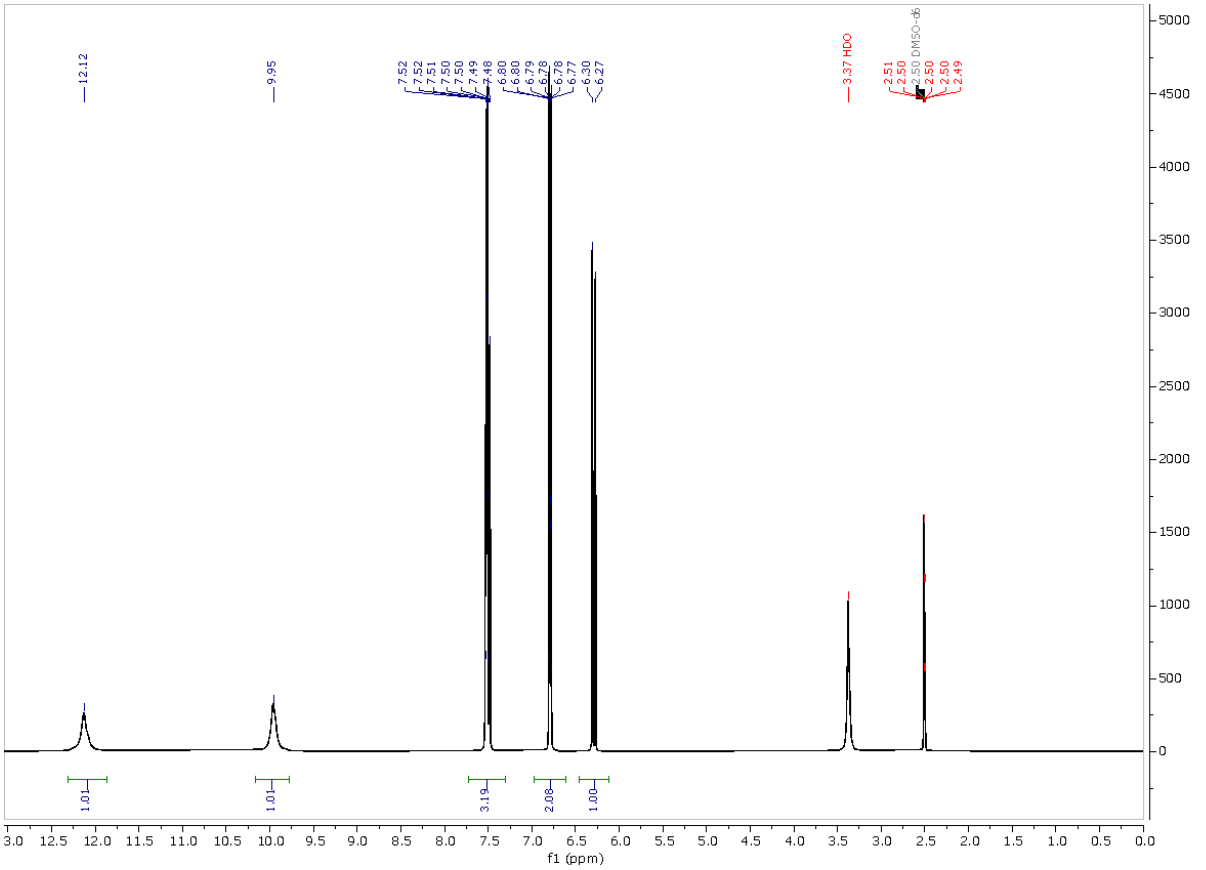

Figure19. ^{1}H-NMR spectrum of p-coumariic acid.

The ^{1}H-NMR spectrum exhibited a multiplet signal at a chemical shift of δ = 2.49–2.51 ppm corresponding to the DMSO-d_6 solvent, and a signal at δ = 3.37 ppm attributed to residual water (HDO). A singlet appeared at δ = 6.27–6.30 ppm (s, 1H), corresponding to the olefinic proton (CH=). Another singlet was observed at δ = 6.77–6.80 ppm (s, 2H), indicating aromatic protons (Ar–H). Additionally, a singlet at δ = 7.48–7.52 ppm (s, 3H) was attributed to aromatic and olefinic protons in the compound.A singlet at δ = 9.95 ppm (s, 1H) indicated the phenolic hydroxyl proton (O–H phenol), and another singlet at δ = 12.12 ppm (s, 1H) was assigned to the hydroxyl proton of the carboxylic acid group (O–H carboxylic acid)[30] .

Disscusion

As reported in previous studies, the presence of various secondary metabolites in methanolic plant extracts often complicates the direct isolation of specific active compounds. In our study, the separation of p-Coumaric acid and Vitexin from Basella alba also required chromatographic techniques such as HPLC, as direct purification from the crude extract was not effective. This supports the importance of using advanced analytical tools like LC-MS and NMR for accurate identification and structural confirmation of bioactive compounds[31]. The results of this study confirm the presence of secondary metabolites in *Basella alba*, specifically vitexin, a flavonoid, and p-coumaric acid, a phenolic compound, with concentrations of 220 µg/g

and 144 µg/g, respectively. P-coumaric acid was measured only in the stem of Basella alba in earlier investigations, and its reported content was 59.65 µg/g, or 0.05965 mg/g. In contrast, the concentration of p-coumaric acid in the current investigation, which employed the complete plant, was 220 µg/g, or 0.22 mg/g[28]. A prior research that exclusively extracted the vitexin from the leaves of Basella alba "Tsurumurasaki" found that the concentration was 14.1 mg/g of dry weight. By extracting the whole plant, however, the current study achieved a lower concentration of 0.22 mg/g of dry weight. In addition to possible differences in extraction techniques and environmental conditions, the different plant sections employed for extraction might be the cause of the concentration differences[32]. The shikimate pathway uses chorismic acid as an intermediary to transform shikimic acid into aromatic amino acids. One of these amino acids, L-phenylalanine, is essential for the production of flavonoids and phenolic acids. The first phenolic acid produced from L-phenylalanine is p-coumaric acid [33]. After being activated, 4-coumaroyl-CoA is created and enters the phenylpropanoid pathway. Via this pathway, 4-coumaroyl-CoA is an essential substrate for the biosynthesis of flavonoids, which results in the synthesis of a number of flavonoids, including vitexin [34].In this study, Soxhlet extraction was utilized due to its efficiency in extracting bioactive compounds from *Basella alba*. This method has been widely recognized for its high extraction performance compared to conventional techniques, providing larger yields with minimal solvent usage .Despite its advantages, Soxhlet extraction may not be ideal for thermolabile compounds due to prolonged exposure to heat. However, its continuous solvent reflux mechanism enhances the extraction of phenolic compounds and flavonoids, making it a suitable choice for the current study[35,36]. The hydroxy derivative of cinnamic acid, p-coumaric acid, has a number of important biological properties, including as cardioprotective, antibacterial, anticancer, anti-inflammatory, and anti-diabetic actions. It is a strong contender for treating a number of illnesses, including cancer, heart disease, and metabolic disorders, because of these qualities[37]. A flavonoid C-glycoside called vitexin has also shown encouraging therapeutic benefits, especially in the prevention of cancer. Cancers of the breast, liver, and colon are particularly susceptible to its anti-carcinogenic qualities. Additionally, vitexin has substantial anti-inflammatory and antioxidant properties and can prevent angiogenesis, making it a promising therapeutic agent for the treatment of cancer. P-coumaric acid and vitexin work together to draw attention to Basella alba's therapeutic potential and indicate possible uses in the creation of medicines and nutraceuticals in the future[38]. To accurately identify, isolate, and characterize these bioactive compounds, multiple advanced analytical techniques were employed.Although *B. alba* contains bioactive compounds with potential health benefits, caution should be exercised regarding its continuous intake. The presence of flavonoids and phenolic acids suggests possible pharmacological effects, but further clinical studies are required to evaluate their impact on human health and to establish safe consumption guidelines. Standardization of B. alba intake is essential to maximize its therapeutic potential while minimizing potential risks.

Conclusion

The effective isolation, identification, and characterization of vitexin and P-coumaric acid will be useful for standardizing herbal formulations that contain these substances. With a shorter run time and excellent efficiency, the suggested HPLC approach is linear, sensitive, accurate, and precise and can be used to determine the concentration of P-coumaric acid and vitexin in a variety of samples from different herbs and formulations. LC-MS/MS and NMR played a crucial role in the structural elucidation and molecular characterization of the isolated compounds, confirming their identity with high specificity and sensitivity.

Conclusion

The effective isolation, identification, and characterization of vitexin and P-coumaric acid will be useful for standardizing herbal formulations that contain these substances. With a shorter run time and excellent efficiency, the suggested HPLC approach is linear, sensitive, accurate, and precise and can be used to determine the concentration of P-coumaric acid and vitexin in a variety of samples from different herbs and formulations. LC-MS/MS and NMR played a crucial role in the structural elucidation and molecular characterization of the isolated compounds, confirming their identity with high specificity and sensitivity.

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استخلاص وتقدير مركبي الفيتيكسين وحمض الكوماريك من نبات الباسلا ألبا المزروع في العراق

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الخلاصة

هدفت هذه الدراسة إلى عزل وتوصيف وتحديد كمي لمادتين فعالتين حيوياً من نبات باسلا ألبا (سبانخ الملابار) Basella alba، وهو نبات ذو قيمة غذائية وعلاجية. تم عزل وتحديد كميات مادة فيتكسين وحمض p-كوماريك باستخدام كروماتوغرافيا السائل عالية الأداء (HPLC). كما تم التوصيف باستخدام مطيافية الأشعة تحت الحمراء (FT-IR)، والرنين المغناطيسي النووي (NMR)، وكروماتوغرافيا السائل المزدوجة مع مطيافية الكتلة (LC-MS/MS)، بالإضافة إلى طريقة الإضافة (spiking) لمقارنة المركبات المعزولة مع المعايير القياسية للتأكد من صحتها تمثل هذه الدراسة أول تحقيق مفصل لهذه المركبات في نبات باسلا ألبا (سبانخ الملابار) المزروع في العراق، مما يبرز الأثر المحتمل للموقع الجغرافي وتركيب التربة على تركيز المركبات الفعالة. استخدمت طريقة استخلاص سوكسليت لعزل المركبات واستعادتها بكفاءة عالية، متوافقة مع نتائج الدراسات السابقة. تؤكد هذه النتائج على أهمية نبات باسلا ألبا (سبانخ الملابار) كمصدر غني بالمركبات النشطة، وتشجع على إجراء المزيد من الدراسات لاستكشاف تطبيقاته في مجالات المكملات الغذائية والصناعات الدوائية