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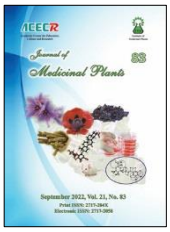

Research Article

Isolation and purification of apigenin, quercetin and apigenin 7-O-glycoside from *Apium graveolens* L., *Petroselinum crispum* (Mill.) Fuss, *Allium cepa* L., respectively

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ABSTRACT

Background: Flavonoids, a group of natural substances, are found in flowers, fruits, roots and stems. These natural products are well known for their health-beneficial effects. Hence, isolation and purifying of flavonoids from the ingredients is worthwhile. Flavonoids are now considered indispensable in various nutraceutical, pharmaceutical, medicinal and cosmetic applications. Apigenin, quercetin, and apigenin-7-O-glycoside have therapeutic properties that these two are in the flavonoid group. **Objectives:** In this study, apigenin, quercetin, and apigenin 7-O-glycoside were purified by column chromatography and identified by NMR and HPLC UV-visible techniques. **Methods:** Total extracts of three plants (*Apium graveolens*, *Petroselinum crispum*, *Allium cepa*) were divided into different solvent fractions. Column chromatography is used to purify these fractions. Finally, ^{1}H-NMR, ^{13}C-NMR, and mass spectrometry techniques were used for identification. Also, the HPLC technique was used for identifying these compounds and standardized. **Results:** In the present work, apigenin was extracted from ethyl acetate fraction of *Apium graveolens* seeds. High purity of apigenin 7-O-glycoside isolated from the butanol fraction of *Petroselinum crispum* aerial parts. Also, ethyl acetate fraction isolated quercetin from the *Allium cepa*. Identification of the data obtained from ^{1}H-NMR and ^{13}C-NMR, which were proofed by comparison with the sources of purified compounds. **Conclusion:** The methods used in this research were able to produce these three compounds with a high percentage of purity to be used in industry as standards.

Abbreviations: ^{1}H-NMR, Proton Nuclear Magnetic Resonance; ^{13}C-NMR, Carbon Nuclear Magnetic Resonance; HPLC, High-Performance Liquid Chromatography

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1. Introduction

Plant secondary metabolite is a generic term used for substances that play essential roles in plant survivability [1]. Some metabolites have spatial and temporal variation in plant species, and under a given experimental condition, within a short period, the metabolome of a plant can completely change [2].

Flavonoids are polyphenolic secondary metabolites with a common Phenyl benzopyrone structure (C6-C3-C6). According to variations in their heterocyclic C-ring, flavonoids may be categorized as flavones, flavanols, anthocyanidins, isoflavones, or chalcones [3]. Flavanols are the most abundant natural yellow colorants. There are various flavanols, but only a few produce a sufficient quantity of dyes with relatively good color fastness [4]. Due to neighboring hydroxyl–keto functional groups, Flavanols can easily chelate with metal cations [5]. Quercetin and apigenin are the predominant flavanols, followed by morin, myricetin, isorhamnetin, and glycoside derivatives. Flavanols usually have brilliant yellow color and have been mainly used to dye wool and silk fabric since ancient times [6].

Among the wide variety of phenolic compounds, apigenin is among the most renowned, with countless nutritional and organoleptic characteristics. Apigenin (4′,5,7-trihydroxyflavone), a natural product belonging to the flavone class, is found in many plants and is the aglycone of several naturally occurring glycosides. It is a yellow crystalline solid used to dye wool [7]. Various pharmacological activities of apigenin and its mechanism of action deepen the importance of human health, and it would be of the utmost importance for possible nutraceutical applications [8-9].

Apigenin 7-O-glycoside is a flavone belonging to the polyphenols class and is commonly found in the *Thymus* species. It shows a broad spectrum of pharmacological properties such as antispasmodic, bactericidal, expectorant, anthelmintic, astringent, antitussive, and carminative agents [8]. *In vitro* exhibits substantial antiproliferative activity against B16F10 melanoma cells after 24 and 48 hours of incubation. Apigenin-7-O-glycoside increases the proportion of subG0/G1, S, and G2/M phase cells, with a significant decrease in cell proportion in G0/G1 phases. Api7G specifically induced the differentiation of CD34+ cells towards the erythroid lineage and inhibited myeloid differentiation [10].

Quercetin is reported to have protective effects in reducing the risk of Cardiovascular disease [11-13]; it functions as an anticancer agent [14] and promises to be an antioxidant agent because of its antiprotonic, anti-inflammatory responses, and decreased DNA degradation rate. The quercetin levels in red and yellow onions are higher than in white onions [15]. The amounts of Quercetin in onions vary with bulb color, type, and variety [16].

In this study, we aimed to purify the onion quercetin by column chromatography and identify it by NMR, HPLC UV-visible techniques. Also, the combination of apigenin and apigenin 7-glycoside extracted from parsley was purified by column chromatography and identified by NMR, UV-visible techniques, and then standardized using HPLC to use it in industry.

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2. Materials and Methods

2.1. Sample preparation

First, plants were bought (*Apium graveolens* L., *Petroselinum crispum* (Mill.) Fuss, *Allium cepa* L.) from the authentic store. Then the scientific names were approved by the Herbarium of the Medicinal Plants Research Institute. It registered with the herbarium code IMPH-7131 for *Allium cepa*, IMPH-7130 for *Petroselinum crispum*, and the herbarium code IMPH-0000 for *Apium graveolens*. Then the plants were dried in the shade after removing the excess flakes. Then 50 grams of the completely ground dried plant was powdered by milling and passed through 18-145 mesh. Extraction was done by a maceration method with 96 % ethanol. In this step, 0.5 litters of 96 % ethanol were poured into the plant powder thrice after 48 hours. The Heidolph rotary evaporator evaporated the solvent, and the resulting extract remained in the flask. The extract was stored in a freezer for the purification process.

2.2. Solvent fractionation

2.2.1. Allium cepa L.

First, the extract was dispersed in 50 ml of water. 100 ml of chloroform was poured into the extract, and the resulting mixture was poured into a 500 ml decanter. After shaking, the two phases of chloroform and water were separated. The upper step was water at this stage, and the lower grade was chloroform. The chloroform phase contains chlorophyll pigments, fatty and non-polar compounds of the plant that was separated from the extract and discarded. Ethyl acetate was added to the remaining aqueous phase and transferred the mixture back to the decanter. The mixture was stirred thoroughly for a few minutes. Then the upper phase was separated and entirely dried by a rotary evaporator.

2.2.2. Petroselinum crispum (Mill.) Fuss

First, the extract was dispersed in 200 ml of water. Then adding 300 ml of chloroform, the mixture was poured into a 1-liter decanter. After shaking, allow the two phases of chloroform and water to be separated. At this stage, the upper phase was water, and the lower phase was chloroform. The chloroform phase contains chlorophyll pigments, fatty and non-polar compounds of the plant that was separated from the extract and discarded. Butanol solvent was added to the aqueous phase and transferred the mixture back to the decanter. The mixture was shaken thoroughly for a few minutes. At this stage, the upper phase was separated, containing butanol, and dried entirely by a rotary evaporator.

2.2.3. Apium graveolens L.

To decolorize and degrease the resulting extract, after dissolving at 300 ml of distilled water, 500 ml of chloroform was added, and poured the resulting mixture into a 2-liter decanter. After shaking for 1 hour, the two phases of chloroform and water were separated. At this stage, the upper phase was water, and the lower phase was chloroform. The chloroform phase contains chlorophyll pigments, fatty and non-polar compounds of the plant that was separated from the extract and discarded. Ethyl acetate was added to the aqueous phase, and the mixture was transferred back to the decanter. Then, the mixture was stirred thoroughly for a few minutes. Apigenin and some compounds with the same polarity as ethyl acetate were moved from the aqueous phase to the ethyl acetate phase. At this stage, the upper phase containing ethyl acetate was separated and entirely dried by a rotary evaporator.

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2.3. Column chromatography

2.3.1. Apigenin

Column chromatography of ethyl acetate fraction resulted on separation of apigenin. This method fills a column with a 3 cm diameter and a 120 cm height with Sephadex LH-20 with 80% methanol solvent. 400 mg of the ethyl acetate fraction was dissolved in 80 % methanol solvent and poured over the column. The mobile phase was 80 % methanol at a flow rate of 1.0 ml/min. The effluents were collected (fraction size 100 ml). After adding 500 ml of methanol to the column, the first compounds were removed from the column.

2.3.2. Apigenin 7- glycosides

Column chromatography was applied to separate apigenin 7-O-glycoside from the butanol fraction. In this method, a column with a diameter of 1 cm and a height of 100 cm was filled with Sephadex LH-20 and used methanol as solvent. During the next step, 200 mg of the butanol fraction was dissolved in 100 % methanol and loaded over the column. The first compounds were removed from the column after adding 300 ml of methanol.

2.3.3. Quercetin

Column chromatography was used to separate quercetin from ethyl acetate fraction. In this method, a column with a diameter of 1 cm and a height of 100 cm, filled with Sephadex LH-20 and 80 % methanol, was the solvent. Then 300 mg of ethyl acetate fraction dissolved with 80 % methanol solvent and was loaded on the column. The mobile phase was 80 % methanol. After adding 280 ml of 80 % methanol, the first compounds were withdrawn from the column.

2.4. Structure elucidation

2.4.1. Nuclear magnetic resonance (NMR) analysis

Proton nuclear magnetic resonance (^{1}H-NMR) and carbon nuclear magnetic resonance (^{13}C-NMR) spectra were recorded on a Bruker 500 MHz AVANCE III HD NMR Spectrometer, using tetramethyl silane (TMS) as the internal standard. The solvents were hexadeuterodimethyl sulfoxide (DMSO-d6).

2.4.2. High-performance liquid chromatography

The studies were conducted with the Knauer separation model with UV K2501: 254 nm. The analysis was performed on Eclipse –XBD-C18 15 cm column having 4.6 mm i.d.; with 5 µm particle size using an isocratic program. The mobile phase was glacial acetic acid, acetonitrile, and water (6:30:64 V/V/V). The flow rate was kept at 1.5 ml/min with a column temperature of 25 °C, and the eluent was monitored at 254 nm, with an injection volume of 20 µl. The mobile phases and all solutions and samples were filtered through a 0.45-mm Millipore membrane filter (Bedford, MA) and degassed by an ultrasonic bath before use. The quantification was conducted by integrating the peaks using the standard external method. The chromatographic peaks were confirmed by comparing their retention times and UV spectra with the reference standards. All chromatographic operations were conducted at room temperature and in triplicate.

2.4.3. Preparation of standards solution

Quercetin, apigenin, and apigenin 7-O-glycoside reference standards solutions were prepared in acetonitrile water (1:1, v/v).

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Standard calibration solutions at five levels were prepared by a serial dilution of a stock solution at concentrations of 10-500 mg/ml.

3. Results

3.1. Column chromatography

3.1.1. Apigenin

A total of 28 fractions with a volume of 10 ml were collected from the column. These fractions were monitored by the aluminum back TLC with 80 % chloroform and 20 % methanol as the mobile phase. The apigenin standard observed the apigenin compound in fractions 17 to 19. A rotary evaporator dried these three fractions. Reloaded this fraction on the Sephadex LH-20 column with a diameter of 1 cm and a height of 100 cm. 30 fractions were obtained from this column. The composition of apigenin in the fraction was 19 to 21.

3.1.2. Apigenin 7-glycoside

Eighteen fractions with a volume of 5 ml were withdrawn from the column. TLC monitored these fractions with 80:20 (chloroform:methanol) as a mobile phase. The composition of apigenin 7-O-glycoside was observed in fractions 11 to 13 by the standard apigenin 7-O-glycoside. A rotary evaporator dried these three fractions. Again, this fraction was poured on the Sephadex LH-20 column with a diameter of 1 cm and a height of 100 cm. 26 fractions were obtained from this column. The composition of apigenin 7-O-glycoside in the fraction was 14 to 19.

3.1.3. Quercetin

Twenty-three fractions with a volume of 5 ml were taken from the column. TLC's fractions are monitored with a solvent containing 80 % chloroform and 20 % methanol. The Quercetin compound was observed in fractions 16 to 18 compared to the Quercetin standard. These three fractions were dried by using a rotary evaporator. This fraction was applied again on the Sephadex LH-20 column with a 1 cm diameter and a height of 100 cm for further purification. Eventually, 32 fractions were separated by 2.5 ml from this column. Quercetin was observed in fractions 21 to 24. This column for separating our desired composition into four completely pure fractions was suitable.

3.2. Identification of apigenin, apigenin 7-O-glycoside, and Quercetin by HPLC

The HPLC spectra of standard samples and purified compounds quercetin, apigenin, and apigenin 7-glycoside are given in Fig. 1.

The range of standard samples showed strong peaks at the following retention times. The standard peaks of apigenin, apigenin 7-O-glycoside, and quercetin at different retention times with resolutions of 5.01, 10.34, and 22.99 min, respectively. Purified fractions were injected into a high-performance liquid chromatography (HPLC) apparatus using the identical protocol described above. In Fig. 1, chromatogram A is related to apigenin's composition, which shows the purified compound's purity. The sample peak overlaps well with the standard peak in retention time and peak area. The chromatogram B is related to the quercetin combination and its standard, the standard chromatogram with green colour, and the purified sample's blue chromatogram. Chromatogram C is related to the combination of apigenin 7-O-glycoside with its standard, where the peak with pink is associated with the sample, and the blue peak is related to the standard. Finally, the purified samples showed high purity compared to the standard chromatograms.

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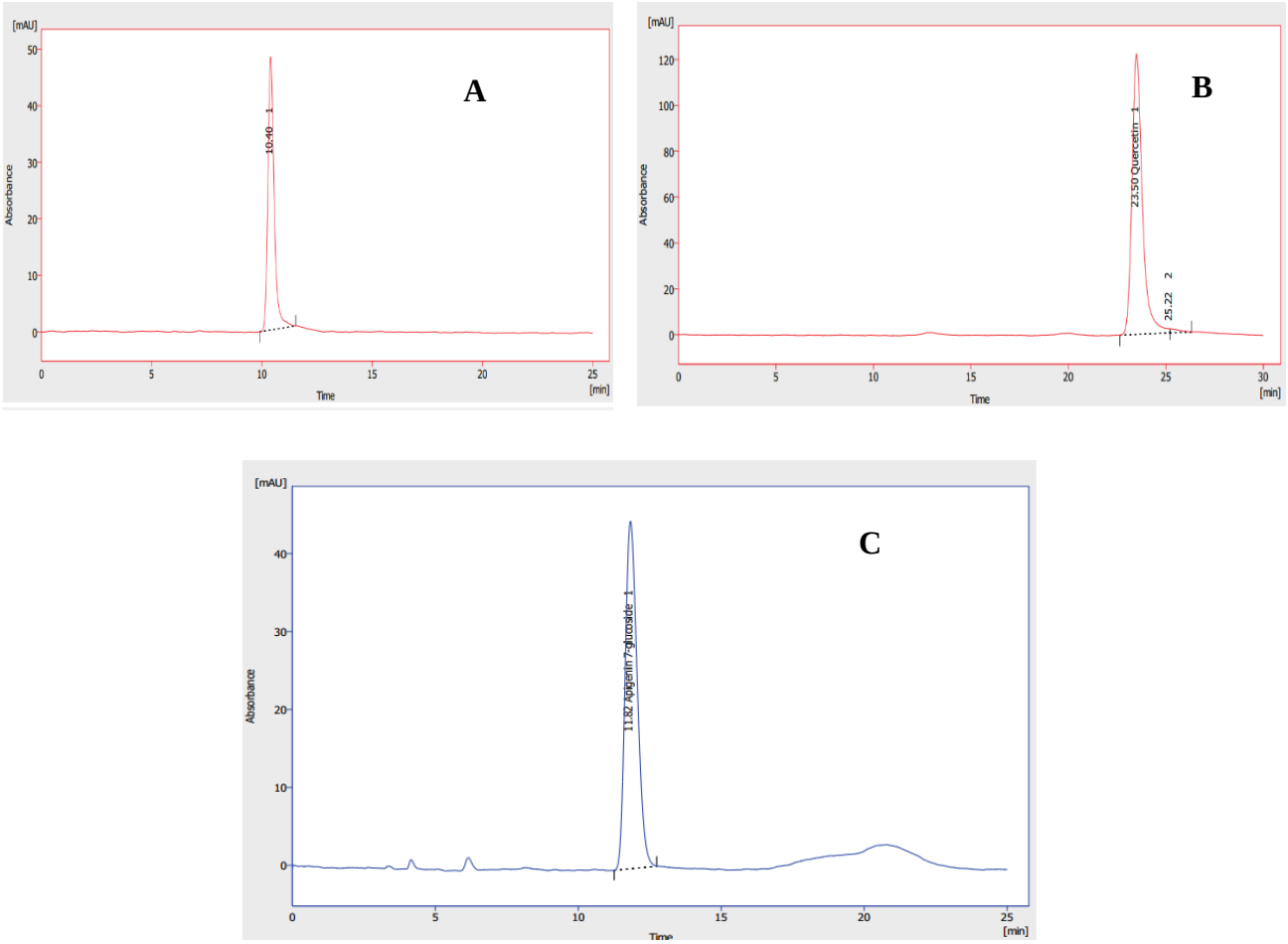

Fig. 1. The HPLC spectra of standard and compounds (A) apigenin, (B) quercetin, and (C) apigenin 7-O-glycoside

3.3. Structure elucidation

3.3.1. Quercetin

Quercetin (Fig. 2) is a yellow crystal obtained from the Sephadex LH-20 column. The empirical formula was $C_{15}H_{10}O_7$. The molecular mass of this compound was 302.23 g/mol, measured by LC-Mass. In the ^{1}H-NMR spectrum (Fig. 3), this combination of 6 peaks with chemical Shifts of 6.19, 6.41, 6.88, 7.54, 7.69, and 12.50 ppm. The isolated compound ^{1}H-NMR spectrum showed aromatic hydrogen groups from 6.18-7.66 ppm and phenolic-OH groups from 9.36-12.48 ppm. The two bifurcated peaks, with an integral one, 2Hz J-coupling, and a chemical Shift of 6.41 and 6.19 ppm, belong to the aromatic ring A hydrogens, each split by a proton in its ortho position. The bifurcation peak with integral one, chemical displacement of 6.88 ppm, and fission of 8.5 Hz are related to the proton of the aromatic B ring. The bifurcation peak with integral one, chemical displacement of 7.69 ppm, and fission of 2.2 Hz are associated with the other hydrogen in the aromatic B ring. The singlet peak in the 12.50 ppm chemical displacement corresponds to the OH group (hydroxy) on carbon No. 5 in the A ring, which forms an intramolecular hydrogen bond with the carbonyl group.

In the ^{13}C-NMR spectrum (Fig. 4), this compound also has 15 carbons, and their chemical shifts are shown in Table 1. The peak with the highest chemical shift at 175.89 ppm

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belongs to the carbonyl group in the structure. Carbon peaks for aromatic groups were shown in the chemical shift of 93.40-163.93 ppm. The corresponding ^{1}H-NMR and ^{13}C-NMR showed the position of peaks for isolated compounds (Table 1). The peaks present in the NMR spectrum showed a resemblance with the pure quercetin, which was also confirmed by previous literature [11, 17, 18]. Thus, it can ensure that the isolated compound is quercetin.

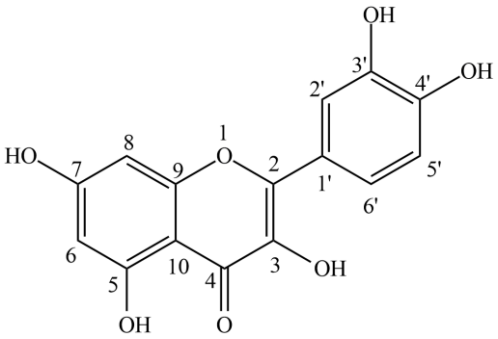

Fig. 2. Quercetin

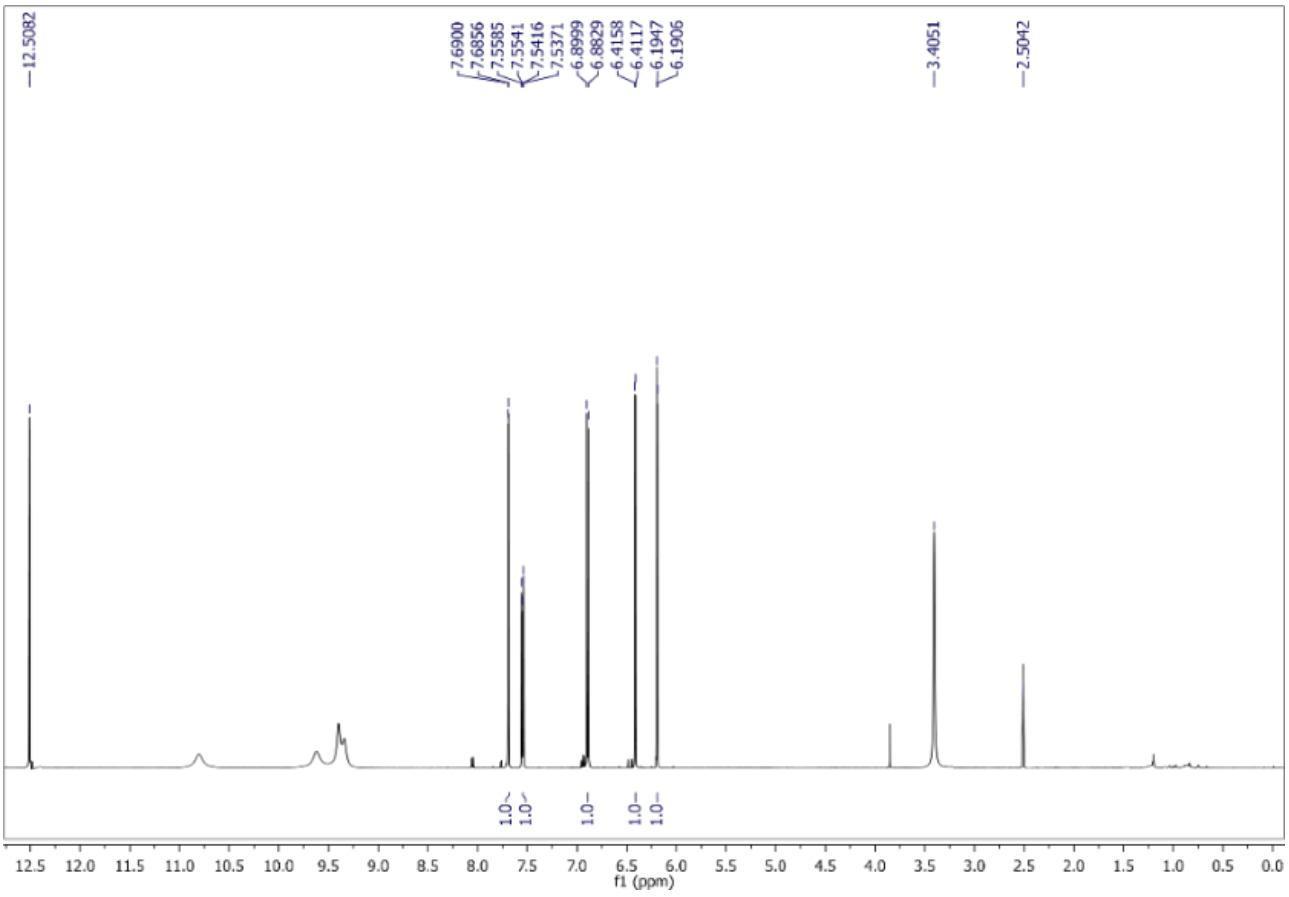

Fig. 3. ^{1}H-NMR spectrum of the isolated compound: quercetin

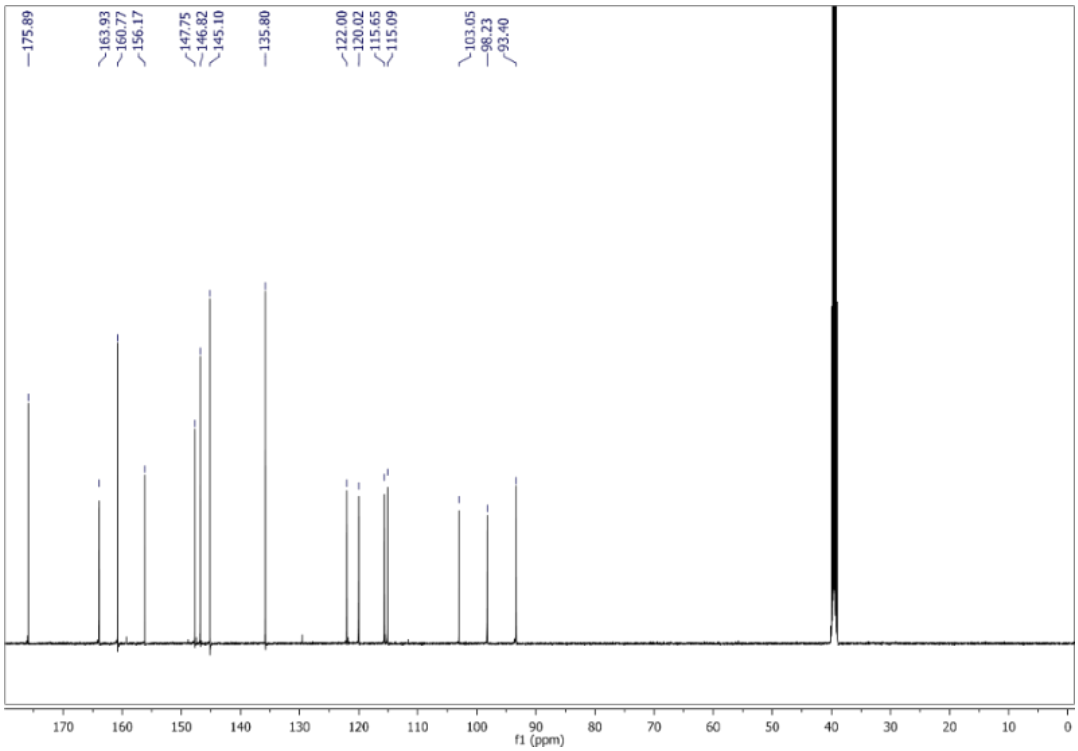

Fig. 4. ^{13}C-NMR spectrum of the isolated compound: quercetin

Table 1. ^{1}H-NMR and ^{13}C-NMR data for the isolated compound: quercetin

^{1}H NMR spectrum of Quercetin	**^{13}C NMR spectrum of Quercetin**
6.19 (d, 1H, *J=2 Hz, Ar-H*)	93.40, 98.23, 115.09, 115.65, 120.02 (*Ar-C*)
6.41 (d, 1H, *J=2 Hz, Ar-H*)	120.40, 122.00, 135.80 (*Ar-C*)
6.88 (d, 1H, *J=8.5 Hz, Ar-H*)	145.10 (*Ar-C*)
7.54 (q, 1H, *J=8.45 Hz, Ar-H*)	146.82 (*Ar-C*)
7.69 (d, 1H, *J=2.2 Hz, Ar-H*)	147.75 (*Ar-C*)
9.36 (s, 2H, *Ar-OH*)	156.17 (*Ar-C*)
9.65 (s, 1H, *Ar-OH*)	160.77 (*Ar-C*)
10.87 (s, 1H, *Ar-OH*)	163.93 (*Ar-C*)
12.50 (s, 1H, *Ar-OH*)	175.89 (*Ar-C=O*)

3.3.2. Apigenin-7-O-glycoside

The empirical formula $C_{21}H_{20}O_{10}$ was detected as Apigenin; the LC-Mass device determined the molecular mass of this compound as 432.40 g/mol (Fig. 5). ^{1}H-NMR spectra (Table 2 and Fig. 6) further confirmed apigenin and glucose moieties in the structure. The occurrence view of an apigenin skeleton from a hydroxyl δH at 12.97 (s, OH-5), two doublets δH at 6.43 (d, J = 2.2 Hz, H-6) and δH at 6.82 (d, J=2.2 Hz, H-8) on the A-ring; A2B2-type aromatic δH at 7.92 (d, J = 8.2 Hz, H-2', H-6') and δH at 6.90 (d, J = 8.15 Hz, H-3', H-5'). 1H NMR data fit exactly with previously reported data in the literature [19-21]. Besides this, glycosidic δH at 5.44 (d, J = 7.4 Hz, H-1")

and δC at 99.9 (C-1") were evident in the 1H and ^{13}C-NMR spectra. The multiple δH at 3.27-3.47 (5H, m, H-3"–H-6") was assignable to the coupling between protons and methylene protons of the glucosyl ring. Proton δH at 3.71 (m, H-2"), hydroxyl δH at 5.12 (s, OH-2"), δH at 5.07 (s, OH-3"), δH at 5.05 (s, OH-4"), and δH at 4.65 (s, OH-6") were assigned in the glycosidic ring. Analyses of the ^{13}C-NMR spectrum (Table 2 and Fig. 7) revealed the existence of 21 carbons, including a hexose moiety at δC (99.9, 73.5, 77.6, 69.9, 76.9, and 63.5). The ^{13}C-NMR spectrum also exhibited the presence of δC (157.4, 100.3, 164.7, and 95.3) for the A-ring, δC (163.4, 103.5, 182.5, 161.9, and 105.8) for the C-ring, and δC (121.4, 129.1, 116.5, and 161.6) for the B-ring of the flavone. H chemical shifts (Fig. 6). Therefore, the purified compound structure was elucidated as apigenin-7-O-glycoside, following the literature's reported data [21-23].

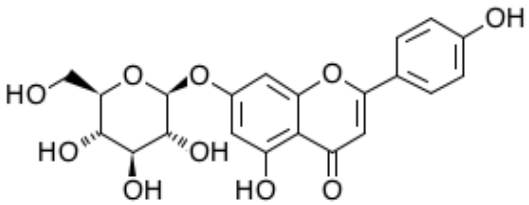

Fig. 5. Apigenin-7-O-glycoside

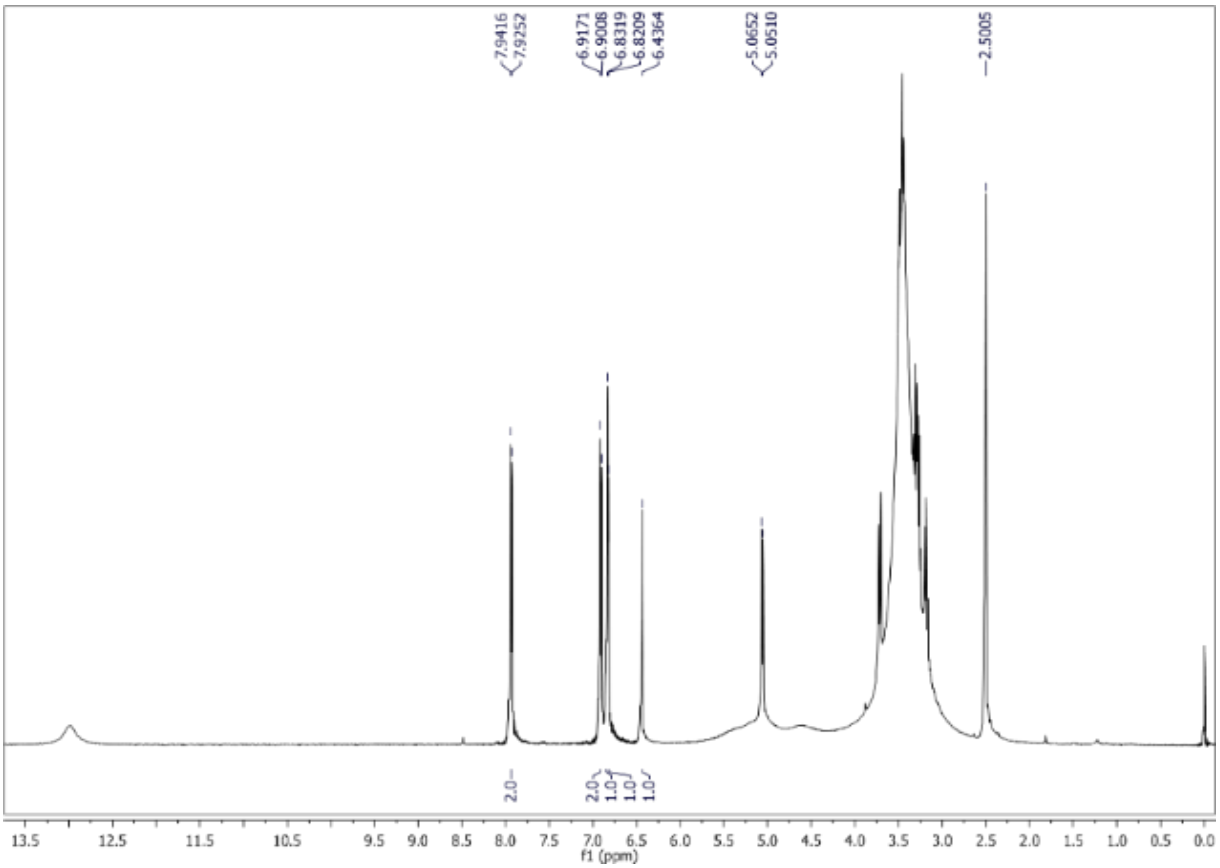

Fig. 6. ^{1}H-NMR spectrum of the isolated compound: apigenin-7-O-glycoside

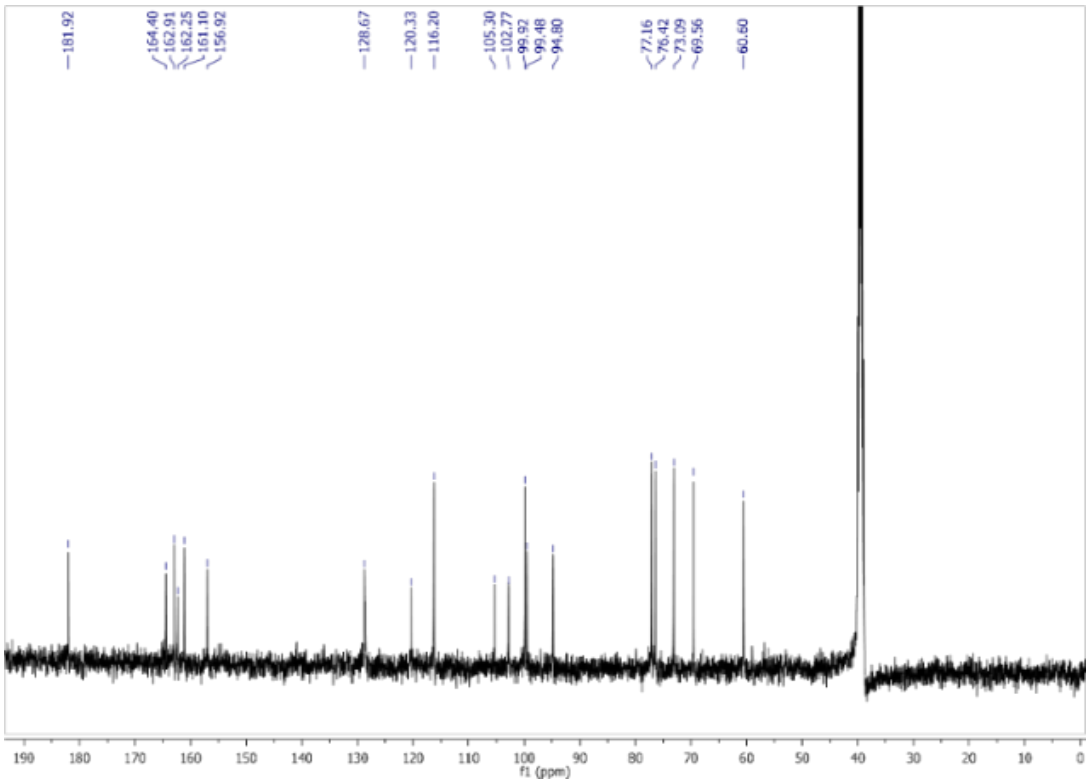

Fig.7. ^{13}C-NMR spectrum of an isolated compound: apigenin-7-O-glycoside

Table 2. ^{1}H-NMR and ^{13}C-NMR data for the isolated compound: apigenin-7-O-glycoside

^{1}H-NMR spectrum of apigenin-7-O-glycoside	**^{13}C-NMR spectrum of apigenin-7-O-glycoside**
-	163.40
6.83 (1H, s)	102.77
-	181.92
-	156.92
6.43 (1H, d, *J*=2.0 Hz)	100.30
-	164.40
6.82 (1H, d, *J*=2.0 Hz	94.80
-	162.91
-	105.30
-	120.33
7.92 (1H, d, J=8.2 Hz)	128.67
6.90 (1H, d, J=8.15 Hz)	116.20
-	162.2
6.93 (1H, d, J=8.9 Hz)	116.5
7.95 (1H, d, J=8.9 Hz)	129.1
12.97 (1H, s, OH-5)	
10.51 (1H, s, OH-4')	
5.44 (1H, d, J=7.4 Hz)	99.92
3.71 (1H, d, J=10.3 Hz, H-2")	73.09
3.14-3.86 (5H, m, J=6.8 Hz, glucose H-3", -4", -5", -6")	77.16
5.12 (1H, s, OH-2"), 5.07 (1H, s, OH-3"), 5.05 (1H, s, OH-4"), 4.65 (1H, s, OH-6")	69.56
-	76.42
-	60.60

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3.3.3. Apigenin

Using mass spectroscopy (is not mentioned), apigenin was identified by empirical formula $C_{15}H_{10}O_5$ and molecular mass 270.23 g/mol (Fig. 8). The ^{1}H-NMR spectrum of apigenin (Table 3, Fig. 9) showed six aromatic protons at δH 7.92 (2H, d, J = 8.80 Hz) and 6.91 (2H, d, J = 8.80 Hz), indicating the presence of AA'XX' ring system in the B ring. Besides, two doublet signals at δH 6.18 (1H, d, J = 2.00 Hz) and 6.47 (1H, d, J = 2.00 Hz) showed the meta position correlation. This means two proton signals are in the same ring system (ring A). The ^{13}C-NMR spectrum of apigenin showed 13 carbon resonances (Table 3, Fig. 10), including a downfield carbonyl signal at δ_C 81.73 ppm (C-4) could observe five oxyaryl carbons at 157.34; 161.21; 161.49; 163.76, and 164.17, two quaternary sp2 carbons at δC 103.73 and 121.17, and seven methines sp2 carbons at δC 94.00, 98.86, 128.52 (2C), 115.99 (2C).

The HMBC (Fig. 11) correlation between δH 6.19 with δC 161.43, 164.10, 103.56, and 94.06, together with correlations showed by proton δH 6.47 with carbons at δ_C 157.27, 103.56, 164.57, and 98.96 confirmed the location of δH 6.19 and 6.47 at H-8 and H-6 of the A-ring, respectively. Another set of HMBC correlations between δH 6.90 with quaternary sp2 carbon at δC 121.17 and δH 7.93 with quaternary sp2 carbon at δC 161.15 confirmed the presence of the AA'XX' ring system in the B ring.

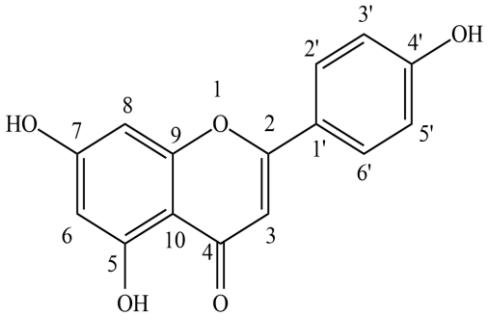

Fig. 8. Apigenin

Table 3. ^{1}H-NMR and ^{13}C-NMR data for the isolated compound: apigenin

^{1}H-NMR spectrum of apigenin	**^{13}C-NMR spectrum of apigenin**
-	164.17
6.78 (1H, s)	103.73
-	181.80
-	161.49
6.47 (1H, d, *J*=2.0 Hz)	98.86
-	163.76
6.18 (1H, d, *J*=2.0 Hz)	94.00
-	157.34
-	105.83
-	121.63
7.92 (1H, d, J=8.8 Hz)	128.52
6.91 (1H, d, J=8.8 Hz)	115.99
-	161.21

[DOR: 20.1001.1.2717204.2022.21.83.7.7]
[DOI: 10.52547/jmp.21.83.72]

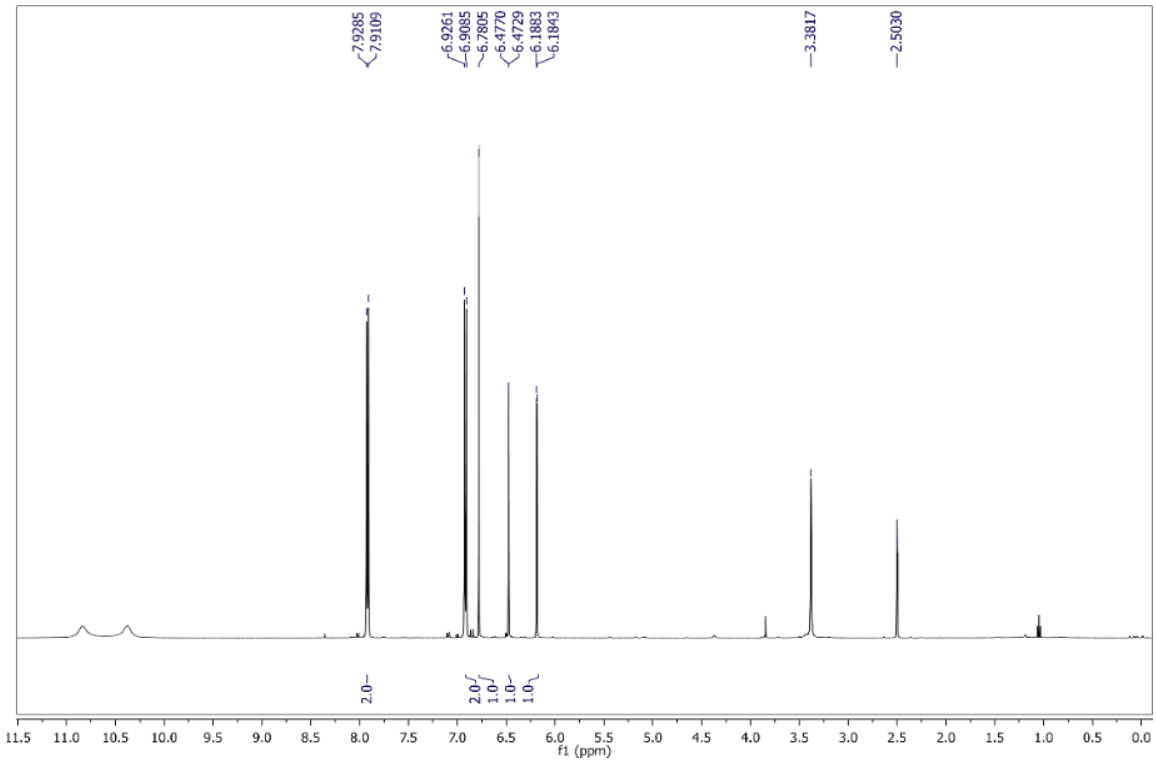

Fig. 9. ^{1}H-NMR spectrum of the isolated compound: apigenin

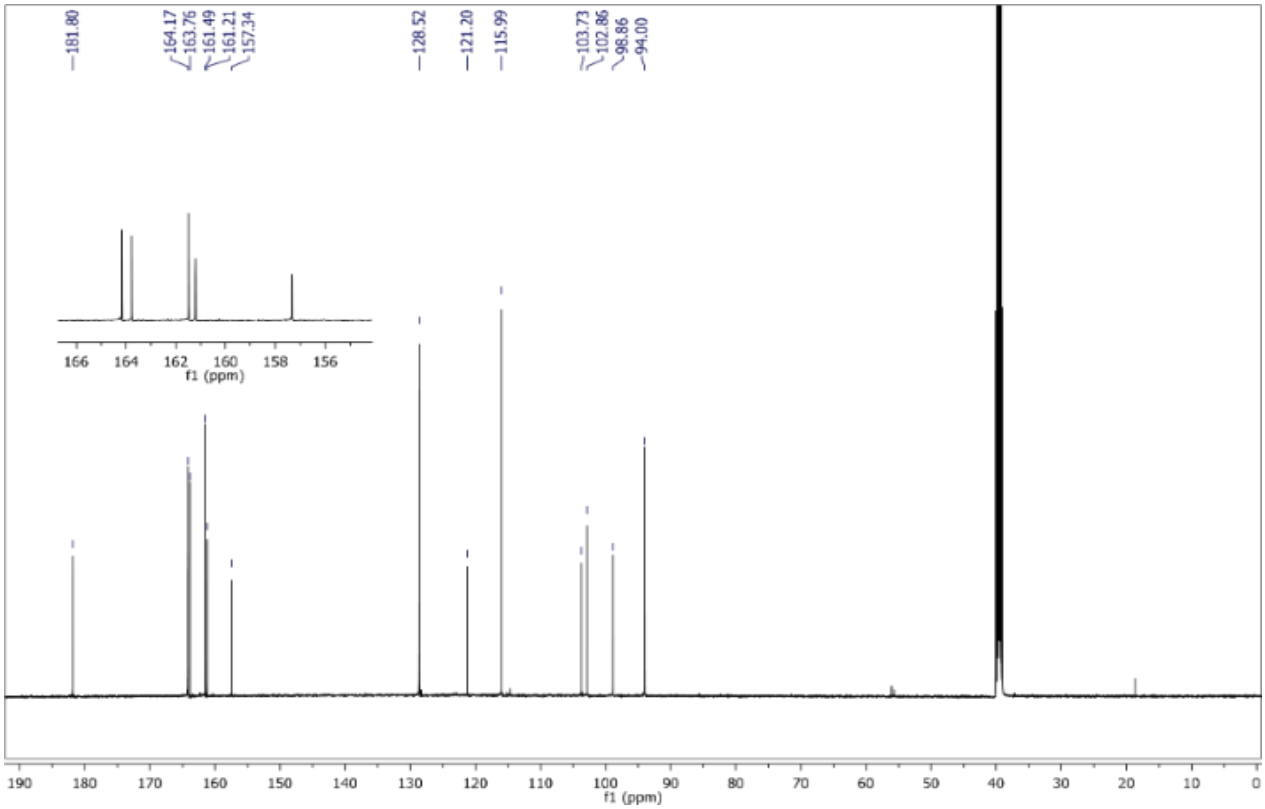

Fig. 10. ^{13}C-NMR spectrum of the isolated compound: apigenin

[DOR: 20.1001.1.2717204.2022.21.83.7.7]
[DOI: 10.52547/jmp.21.83.72]

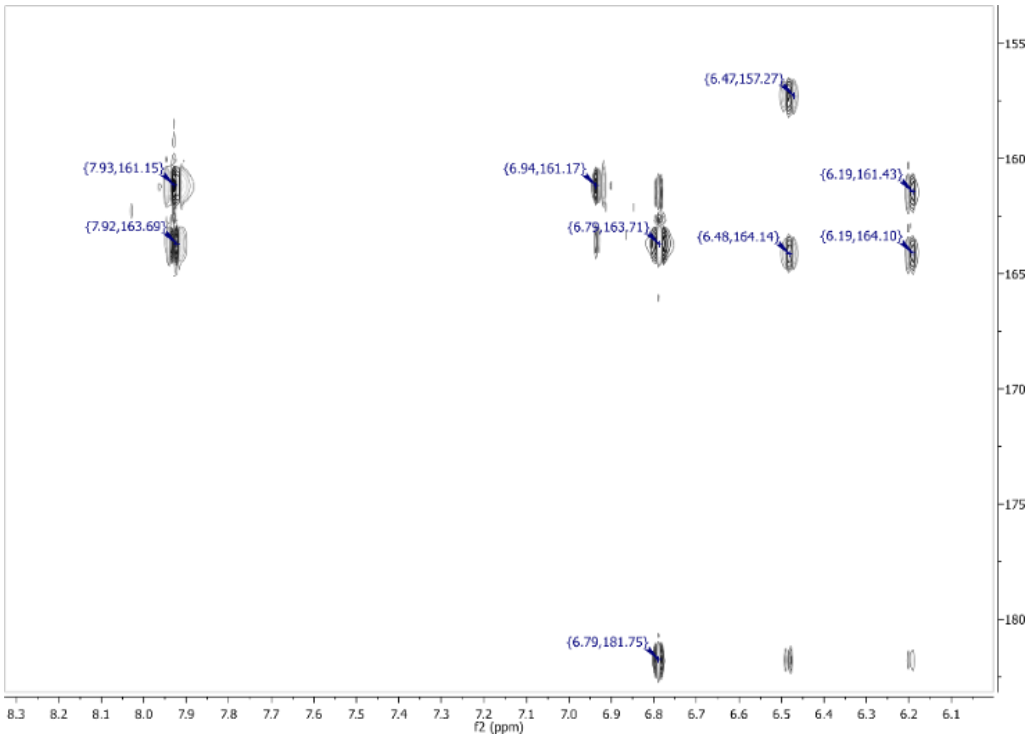

Fig. 11. HMBC spectrum of the isolated compound: apigenin

4. Discussion

Flavonoids, a group of natural substances with variable phenolic structures, are found in fruits, vegetables, grains, bark, roots, stems, flowers, tea, and wine. These natural products are well known for their health benefits. Flavonoids are attributed to their anti-oxidative, anti-inflammatory, anti-mutagenic, and anti-carcinogenic properties and their capacity to modulate critical cellular enzyme function [3].

The isolation of flavonoids includes the separation of apigenin, quercetin, and apigenin 7-O-glycoside, and obtaining monomeric compounds is a critical approach in contemporary research. The choice of solvent for extraction (polarity of solvents), isolation methods, polarity, acidity, molecular weight differences, and unique structure are chosen. There are many articles about the isolation of flavonoids from different plants. Chromatography is still the first choice to isolate diverse flavonoids [4].

This study purified apigenin, quercetin, and apigenin-7-O-glycoside from *Petroselinum crispum, Apium graveolens, and Allium cepa*. Because of the differences between the structures, various solvents were used for the extraction, different methods and columns were used for separation and purification, identity and structure elucidation, and the NMR methods and LC-MS and HPLC were used for standardization.

5. Conclusion

In conclusion: There is a growing interest in extracting and isolating natural products and their applications. In the present work, apigenin has been extracted successfully from *Apium graveolens* ethyl acetate fraction seeds. High purity of apigenin 7-O-glycoside isolated from the butanol fraction of *Petroselinum crispum* aerial parts. Also isolated is quercetin from the ethyl acetate fraction of *Allium cepa*.

Author contributions

S. T: contributed to the study design and conducted the data analysis, R. H: contributed to the conducted the data analysis as well as the writing and reviewing of the manuscript, R. Gh: contributed to the study design and the writing and reviewing of the manuscript, M. Y: contributed to the NMR and HPLC data analysis as well as the writing of the manuscript, F. K.S: contributed to the conducted the data analysis as well as the writing and reviewing of the manuscript, A, GH: contributed to the HPLC data analysis as well as the writing of the manuscript, N. KHD: contributed to the data analysis as well as the writing of the manuscript.

Conflicts of interest

The authors declare that there is no conflict of interest.

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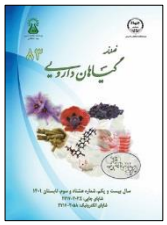

مقاله تحقیقاتی

خالص‌سازی و شناسایی آپیژنین، کوئرستین، آپیژنین ۷-O-گلیکوزید به ترتیب از گیاهان کرفس، جعفری و پیاز

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اطلاعات مقاله

گل‌واژگان:
کوئرستین
آپیژنین
آپیژنین گلیکوزید
کروماتوگرافی ستونی
کرفس
جعفری
سیر

چکیده

مقدمه: فلاونوئیدها، گروهی از ترکیبات طبیعی، در گل‌ها، میوه‌ها، ریشه‌ها و ساقه‌ها یافت می‌شوند. این دسته از ترکیبات طبیعی به دلیل اثرات دارویی مفید شناخته شده‌اند، از این جهت، جداسازی و شناسایی فلاونوئیدها از گیاهان ارزشمند است. فلاونوئیدها در حال حاضر کاربردهای گسترده‌ای در صنایع غذایی، دارویی و آرایشی دارند. آپیژنین، کوئرستین و آپیژنین-۷-O-گلیکوزید دو گروه از فلاونوئیدها هستند که دارای خواص درمانی نیز هستند. **هدف:** هدف از این مقاله خالص‌سازی سه ترکیب آپیژنین، کوئرستین و آپیژنین-۷-O-گلیکوزید از گیاهان و شناسایی آنها با کمک NMR, HPLC UV-visible است. **روش بررسی:** در این مطالعه عصاره تام سه گیاه کرفس، جعفری و پیاز با کمک حلال‌هایی با قطبیت مختلف به بخش‌های مختلف حلالی تقسیم شدند. برای خالص‌سازی این فراکسیون‌ها از کروماتوگرافی ستونی استفاده شد. در نهایت از تکنیک‌های ^{1}H-NMR، ^{13}C-NMR و طیف‌سنجی جرمی برای شناسایی استفاده شد. همچنین برای شناسایی این ترکیبات از تکنیک HPLC و استانداردسازی استفاده شد. **نتایج:** در کار حاضر، آپیژنین از فراکسیون اتیل استات دانه کرفس استخراج شد. آپیژنین-۷-O-گلیکوزید با خلوص بالا از عصاره بوتانولی قسمت‌های هوایی جعفری خالص‌سازی شد. همچنین، کوئرستین از فراکسیون اتیل استاتی پیاز جدا شد. آنالیز داده‌های به دست آمده از ^{1}H-NMR، ^{13}C-NMR و مقایسه این داده‌ها با منابع خلوص ترکیبات اثبات شد. **نتیجه‌گیری:** روش‌های به کار رفته در این پژوهش توانسته‌اند این سه ترکیب را با درصد خلوص بالا، جهت استفاده در صنعت به عنوان استاندارد تولید کنند.

مخففها: ^{1}H-NMR، تشدید مغناطیسی هسته‌ای پروتون؛ ^{13}C-NMR، تشدید مغناطیسی هسته‌ای کربن؛ HPLC، کروماتوگرافی مایع با عملکرد بالا

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