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RESEARCH ARTICLE

Isolation and Characterization of New Flavonoids from *Fumaria Parviflora* Cultivated in Iraq

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Abstract

Objective: The aim of this study was to investigate chemical constituents of aerial parts of *Fumaria parviflora* since no phytochemical investigation had been done previously in Iraq. Methods: Phytochemical screening of the extracts obtained from this plant indicated the presence of flavonoids, saponins, steroids, phenols, and alkaloids. Results: The different chromatographic and spectroscopic results revealed the presence of new flavonoids isolated from *Fumaria parviflora* Conclusion: The isolation and purification afforded white crystalline powder which was subjected to chemical and spectral identification by infrared and H^1- C^{13} nuclear magnetic resonance.

Introduction

Medicinal plants are very ancient and true natural medicines which are useful for the treatment of different diseases. They can be used directly or in extracted forms for the management of various ailments due to the presence of various secondary metabolites [1]. Iraqi *Fumaria parviflora* Figure-1 is a species of flowering plant known by the common names fine leaf fumitory, fine-leaved fumitory and Indian fumitory. It is native to Europe, Asia, and Africa, but it is common and widely distributed in many other parts of the world [2].

The pharmacological studies showed that *Fumaria parviflora* possess hepatoprotective, antidiabetic, anti-inflammatory, antipyretic, analgesic, prokinetic, laxative, dermatological, antimicrobial, anti-parasitic, reproductive, anti-cholinesterase and smooth muscle relaxant effects [3] *Fumaria parviflora* was used traditionally in dermatological diseases, in stimulation of liver function and gall bladder and also as antiscabies, antiscorbite, antibronchite, diuretic, expectorant, antipyretic, diaphoretic, appetizer and laxative [4]. In folk medicine of Turkey it was used against hepatobiliary dysfunction, while, in the Unani traditional system it was prescribed to treat gut and respiratory disorders, abdominal cramps, indigestion and asthma [5].The preliminary phytochemical analysis of *Fumaria parviflora* revealed the presence of flavonoids, glycosides, tannins, saponins, steroids, triterpenoids, phenols, alkaloids and anthraquinones [3].From these chemical groups, flavonoids and phenolic acids are the most important.

The flavonoids have 15 carbon skeleton (benzopyran) C6-C3-C5 backbone structure and it is composed of two benzene rings, namely, ring A and ring B connected by heterocyclic ring called ring C. It is subdivided to many classes such as flavones, flavonols, flavanones, flavanonols, isoflavones, and other, depending on the degree of oxidation, type, and manner of substitution of ring C; the difference between individual flavonoids depends on type and manner of substitution on rings A and B.

It appears in nature as aglycones or glycosides in which the carbohydrates such as D-glucose and galactose attached to carbon number 3 or 7 and as methylated compounds [6, 8]. Flavonoids have several biological activities such as antioxidant, hepatoprotective, antibacterial, anti-

inflammatory, anticancer, and antiviral activity [9]. Flavonoid is potential as antibacterial agents against *Staphylococcus epidermidis* and therefore justifies their usage in traditional medicine for the treatment of body odor [10], and phenolics and polyphenolics compounds have considerable value with respect to antioxidant activities [11].

Figure1: Iraqi *Fumaria parviflora*

Methods

Collection of Plant Materials

Iraqi Fumaria parviflora plants were collected from Erbil North of Iraq, in September 2017. The plant was identified and authenticated by Prof. Dr. Sukaena Abass, Department of Biology, College of Sciences University of Baghdad. Plant materials were washed thoroughly, dried under shade, and ground in a mechanical grinder to a fine powder, and the seeds were cleaned from unwanted materials, dried in an oven at 40°C for 2 days, and ground in a mechanical grinder to a fine powder.

Equipment and Chemical

The instruments used were rotary evaporator (BÙCHI Rotavapor R-205, Swiss), sonicator (Branson Sonifier, USA), high-performance liquid chromatography (HPLC) (SYKAM Germany), Preparative HPLC (PHPLC)((JASCO FC-2088-30-Jasco/Japan), Elemental microanalysis (CHN) (Euro EA Elemental analyzer-IRMS/ Italy), Fourier transforms infrared spectra (FT-IR) spectra were scanned on Shimadzu FT-IR-8400S Infrared Shimadzu /Japan and H^1-C^{13} NMR (Euro-vectorEA3000A Italy) All chemicals and solvents used were of analytical grade and obtained from Riedel-de Haen, Germany, methanol which is HPLC grade was purchased from Sigma-Aldrich, Germany. The term Preparative HPLC is usually associated with large columns and high flow rates. The objective of an analytical HPLC run is the qualitative and quantitative determination of a compound, while preparative HPLC run, it is the isolation and purification of a valuable product.

With increasing demand for production of highly pure valuable compounds in varying amounts in the chemical and pharmaceutical industry, the field of operation for preparative HPLC is increased [12], so preparative HPLC (Figure-2) was used in this study to isolate in a very pure one compound from Fumaria plant.

Figure 2: Preparative HPLC apparatus

Extraction

- 300 g of shade-dried pulverized aerial parts of *F. parviflora* were defatted by maceration with hexane for 24 h and then allowed to dry at room temperature. The defatted plant materials were extracted by Soxhlet using aqueous methanol 85% as a solvent for 24 h. The extract was filtered, and the solvent was evaporated under reduced pressure using a rotary evaporator to get a dry extract. The residue was suspended in 400 ml water and partitioned successively with petroleum ether (B.P. 30-60), chloroform and ethyl acetate (3×300 ml) for each fraction. The three fractions were dried over anhydrous sodium sulfate, filtered, and evaporated to dryness.

Preparations of Standards and Samples for Analysis

Standard solutions for HPLC of quercetin, rutine, myricetin, apiginine, and kampferol were prepared by dissolving 0.04 mg in 1 ml of methanol HPLC grade. Dried samples were prepared for HPLC analysis by dissolving them in methanol and subjecting them to ultrasonication at 60% duty cycles for 25 min at 25°C followed by centrifugation at 7500 rpm for 15 min. The clear supernatant of each sample was evaporated under vacuum. The residues were re suspended individually, in 1 ml of methanol HPLC grade, homogenizing using vortex mixer, and passing them through 2.5 µm disposable filter, and stored at 4°C for further analysis. 20 µl of the sample was injected into the HPLC system for analysis.

Standards used for HPLC analysis were prepared by dissolving 1 mg of each standard in 1 ml methanol, while the samples were prepared by dissolving few milligrams from ethyl acetate fraction sample in 1 ml methanol.

Preliminary Phytochemical Investigation [13]

Test for Flavonoids

Few milligrams of aqueous methanol plant extracts were suspended in ethanol and few drops of 5% ethanolic KOH were added, and then, few drops of 5% HCl were added. The changes in colors were recorded [13].

Test for Saponins:

Froth test:

About (2 gm) of the powdered sample from the leaves and seeds was boiled in (20 ml) of distilled water in a water bath and filtered. (10 ml) of the filtrate was mixed with (10 ml) of distilled water in a test tube and shaken vigorously. And observed the formation of froth that persists for 15 minutes that indicates the presence of saponins.

Test for Sterols

0.5 g of each plant extract was shaken with petroleum ether to remove the coloring material. The residue was extracted with 10 ml chloroform and the chloroform layer was dried over anhydrous sodium sulfate. 5 ml of the chloroform layer was mixed with 0.25 ml of acetic anhydride and then two drops of concentrated sulphuric acid were added. Green color indicated the presence of sterols.

Test for Phenols

Few milligrams of aqueous methanol plant extracts were treated with few drops of 1% FeCl3. Formation of dark greenish-blue color indicates the presence of phenols.

Test for Alkaloids

2 ml of sample solution was taken into a test tube and treated separately with a few drops of both reagents (Mayer's and Dragendorff's) reagents and shaken. After which it was observed whether the alkaloids were present or absent in the turbidity or precipitate formation.

Identification of Flavonoid and Phenolic Derivatives from Ethyl acetate Fraction by HPLC

HPLC technique (SYKAM, Germany) was applied for the detection of different constituents found in the ethyl acetate fractions as flavonoids aglycone and flavonoids glycoside for aerial parts of the plant using a mobile phase composed of solvent A: Methanol: Solvent B: 0.05% trifluoroacetic acid in water for HPLC, gradient program from A=70% (0–5 min), A=40% (5–8 min), and A=90% (8-15 min).Column used: C18-ODS (25 cm x 4.6 mm), Detector: UV-280 nm, Injection volume: 20 µL, Flow rate: 1.0 ml/min.

Isolation of (ME) from Ethyl Acetate Fraction by Preparative HPLC

2 g from the ethyl acetate fraction was dissolved in 5 ml gradient methanol for the isolation of specific compound named (ME), using the same conditions for HPLC analysis mentioned above, and the target peak was collected by fractions collector after monitoring it according to the time, i.e., (time from the beginning of each peak appearance until disappearance of peak).

Results

Preliminary examination of the ethyl acetate fraction results is shown in Table 1.

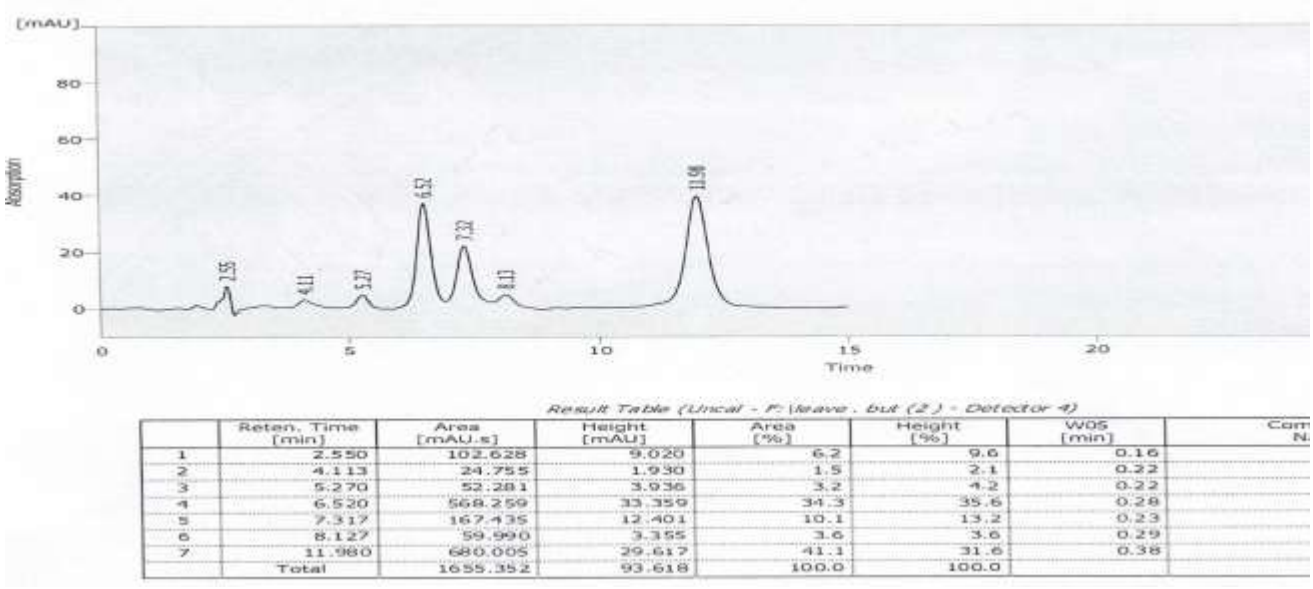
Table 1: Preliminary examination of the aqueous methanol extract for phenols and flavonoids

Phytochemicals	Flavonoids	Saponins	Sterols	Phenols	Alkaoids
Ethyl acetate fraction	+	+	+	+	+

HPLC results for ethyl acetate fraction are shown in the following figures, revealed the presence of rutin, myrecetin, quercetin, kaempferol and apiginine in addition to other compound which is named (ME) depending on matching of retention time with standard used .(Figures 3-5).

Table 2: Retention times of the detected compounds in minutes in ethyl acetate and n-butanol fractions corresponding to the related standards

Ethyl acetate	Standard compound	Retention time in a min for standards
2.55	Rutin	2.55
4.11	Myrecetin	4.11
5.27	Quercetin	5.27
6.52	Unknown (ME)	6.52
7.32	Kaempferol	7.32
8.13	Apiginine	8.21



	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	Com N
1	2.550	102.628	9.020	6.2	9.6	0.16	
2	4.113	24.755	1.930	1.5	2.1	0.22	
3	5.270	52.281	3.936	3.2	4.2	0.22	
4	6.520	568.259	33.359	34.3	35.6	0.28	
5	7.317	167.435	12.401	10.1	13.2	0.23	
6	8.127	59.990	3.355	3.6	3.6	0.29	
7	11.980	680.005	29.617	41.1	31.6	0.38	
	Total	1655.352	93.618	100.0	100.0		

Figure 3: HPLC of ethyl acetate fraction

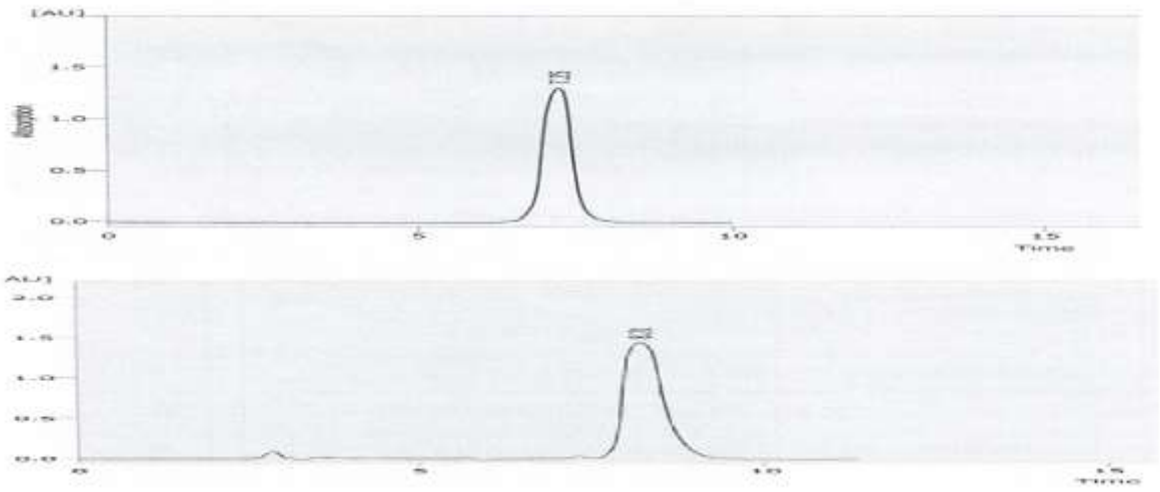


Figure 4: HPLC of Kaempferol & Apiginin standards

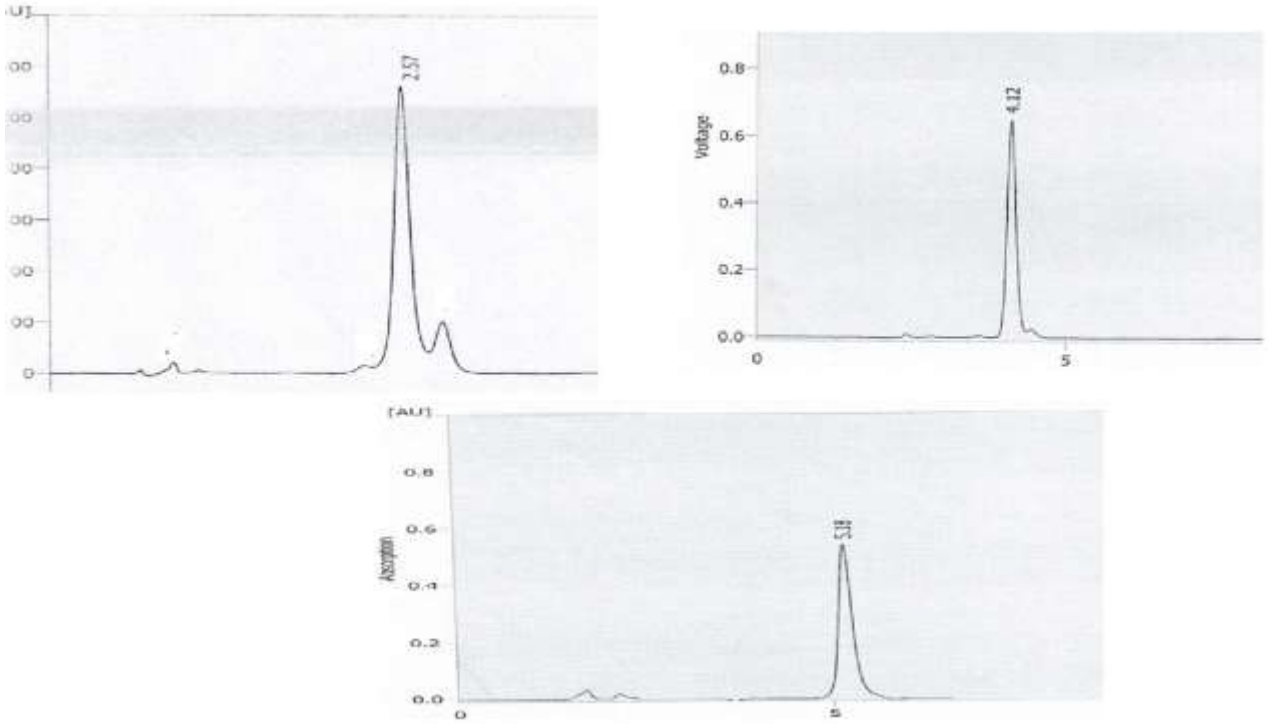


Figure 5: HPLC of Rutin, Myrecetin & Quercetin standards

Preparative HPLC was used to separate specific compound named (ME) from ethyl acetate fraction

Characterization and Identification of the Isolated ME

FTIR Spectra

The characteristic IR absorption bands revealed by (ME0 are listed in table-3, since absorption bands at 1675 and 3197 nm of the IR spectrum indicated where the molecule harbors conjugated carbonyl and hydroxyl groups, respectively.

Table3 Characteristic FT-IR absorption band (cm-1) of the isolated ME [14]

Functional group	Group frequency wave number (cm-1)	Assignment
O-H	broad band (3600-3083) central at 3334	O-H stretching of phenol
C-H	3052, 3142	stretching of aromatic ring
C=O	1660	C=O stretching of keton conjugated
C=C	1614-1569	C=C stretching of aromatic ring
O-H	1363	O-H bending of phenol
C-O-C	1130, 1124	C-O-C stretching
O-H	1296	O-H bending of alcohol
C-H	975, 885, 848	C-H of aromatic group out of plane

CHN Analysis

Elemental microanalysis was performed for unknown isolated compounds (ME), and the data of this analysis indicated that this compound consist of carbon, hydrogen and oxygen in different percentage, (Table-4).

Table 4: Elemental Microanalysis of the Unknown Isolated compound ME

Compound	C% (calculator)	H% (calculator)	O% (calculator)
ME	56.21% 58.3%	4.46% 4.6%	39.25% 37.03%

H^1 &C^{13} NMR Analysis

The 1H NMR spectrum of the compound (ME) gave two aromatic hydrogen signals with 'meta coupling' at δ 6.20 (1H, *s*) and 6.42 (1H, *s*) which was predicted by the hydrogens at C-6 and C-8 of the A ring of the flavone skeleton. Accordingly, this compound was suggested to have a hydroxyl group at C-5 and C-7. Furthermore, its 1H NMR spectrum revealed two signals with 'ortho coupling' at δ 6.8 (2H, *d*) and 8.0 (2H, *d*), the signals of which were approximated from the hydrogens at C-2′, C-3′, C-5′ and C-6′ of the B ring. The absence of a specific signal for an olefinic hydrogen at C-3 and the presence of an anomeric hydrogen signal at δ 5.24 (1H, *d*) suggested that the compound was a flavonol glycoside. The appearance of an anomeric

carbon signal at δ 93.5 in the 13C NMR spectrum indicated the presence of a sugar moiety. Due to a correlation between the anomeric hydrogen signal (δ 5.24) and the anomeric carbon signal (δ 93.5) that was revealed by analysis of the heteronuclear multiple bond correlation (HMBC) spectral data obtained from other research [15], the position of the sugar moiety was assigned to the C-3 hydroxyl group.

The methyl signal observed at δ 0.93 (3H, *s*) in the 1H NMR spectrum and at δ 17.19 in the 13C NMR spectrum indicated that the sugar moiety was rhamnose. Figures (6 a & b, 7a & b).

Based on the Accumulated Data Above, the Compound (ME) was identified as kaempferol-3-O-rhamnoside

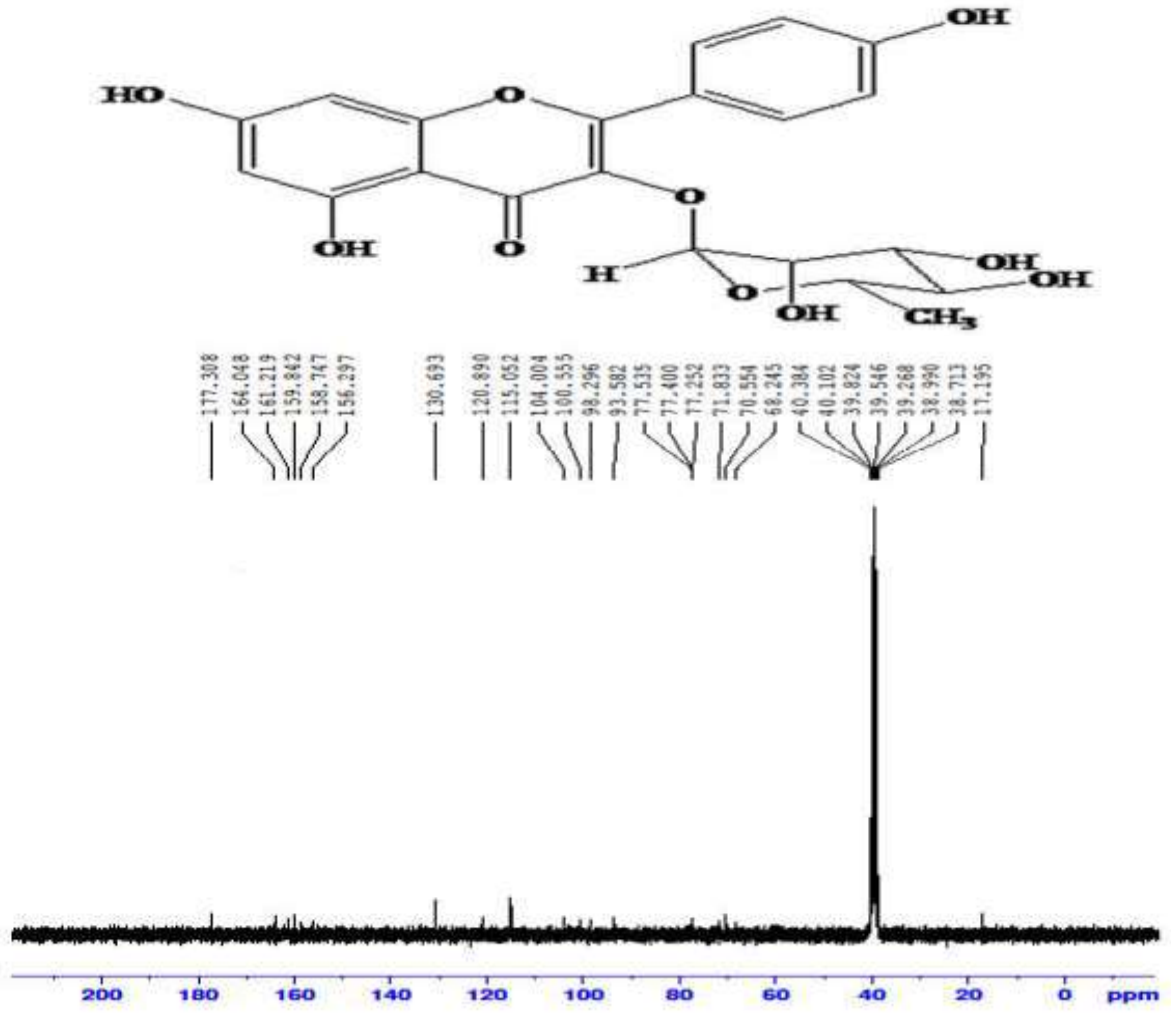


Figure 6a: 13C-NMR analysis of the isolated ME compound

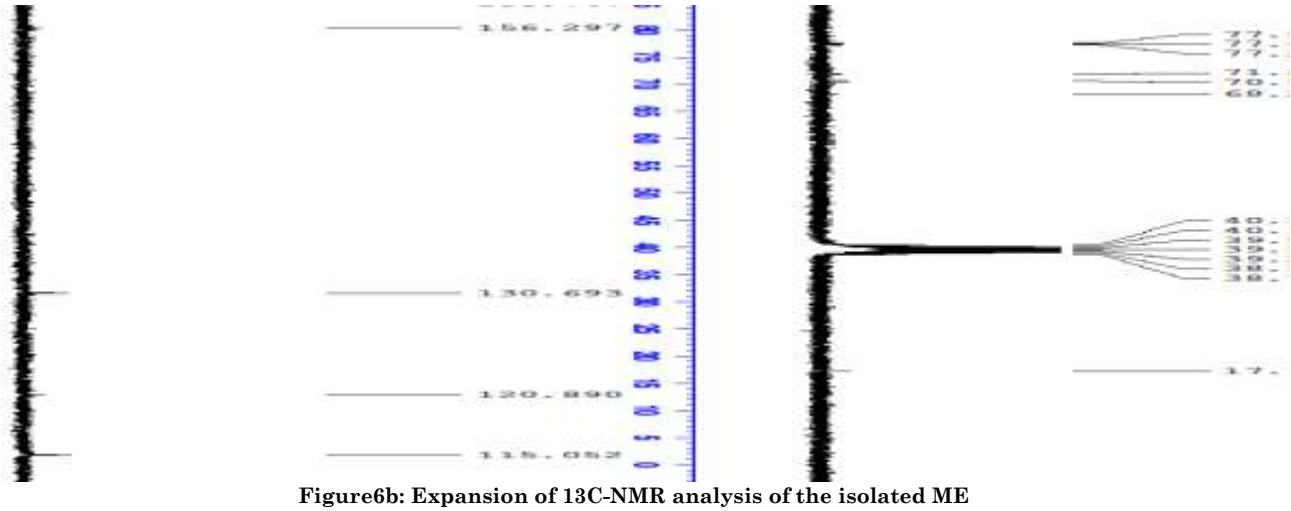


Figure6b: Expansion of 13C-NMR analysis of the isolated ME compound

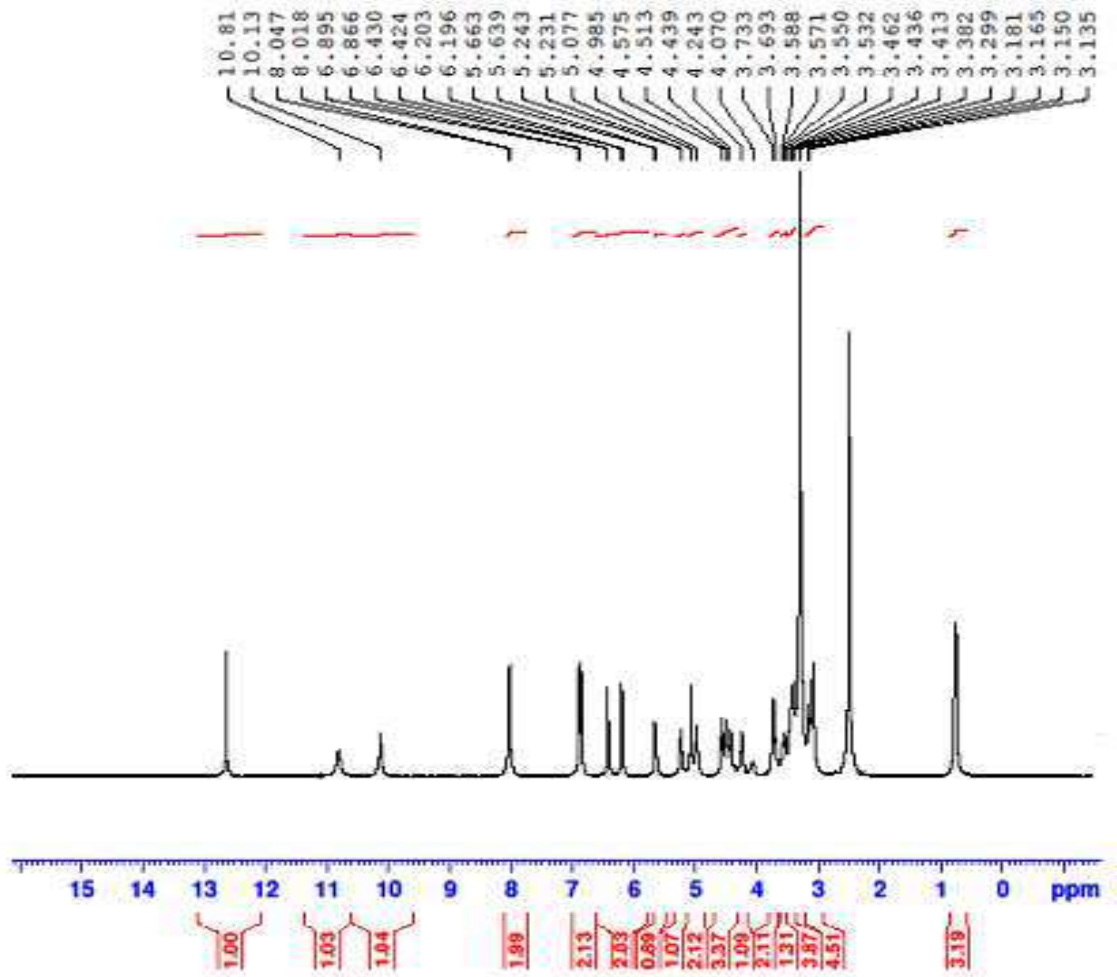


Figure 7a:1H-NMR analysis of the isolated ME compound

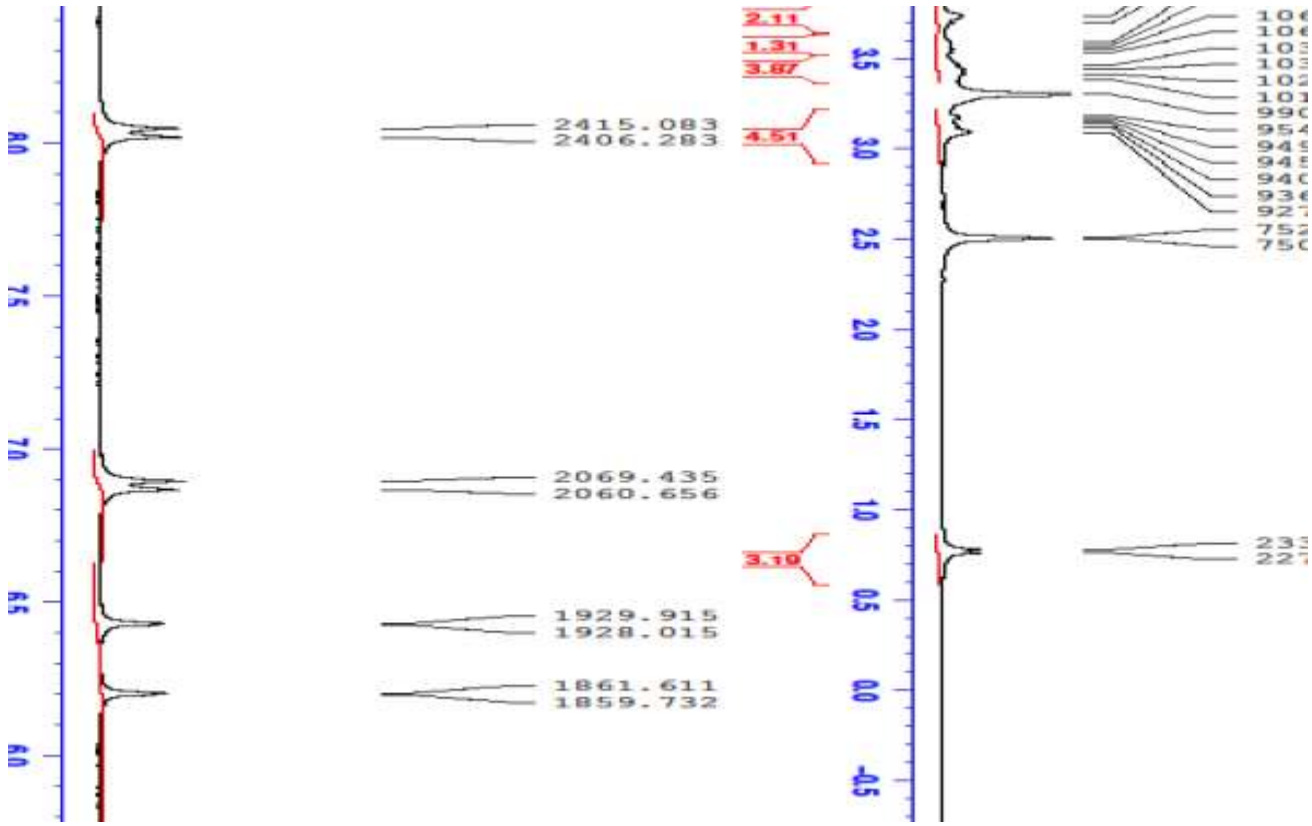


Figure 7b: Expansion of 1H-NMR analysis of the isolated ME compound

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