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Isolation and identification of phenolic compound from *Salvia palaestina*widley grown in north of Iraq

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Introduction

Phytotherapy continues to play an important role in the prevention and treatment of diseases (communicable and non-communicable). Amazingly, more than 80% of the global populations nowadays adopt phytotherapy as a basic source of maintaining good healthy conditions, owing to the pronounced side effects, non-availability, and expensive nature of conventional treatment options. . A number of medicinal plants had been resourceful (effective) against a range of diseases, with few developed into drugs based on the available phytotherapeutics, quite a large number of them are yet to scale through clinical trials to determine their safety and efficacy. The origin of the medicinal plants use had been since time immemorial and traced back to Europe and Egypt many centuries ago. The first records of knowledge documentation were, however, produced by Shen Nung (a Chinese emperor) 2500 BC ago, describing different recipes of drug preparation from 300 medicinal plants for the management of numerous human diseases [(1)].

Labiatae Family

Labiatae (Lamiacea) Family contain about 200 genera and 3000 species one of the largest genus of the family *Salvia* L., is wide distributed in a lot of regions of world[(2)].

This family is widely distributed in temperate places of the world such as the Mediterranean and Irano- Turanian biogeographic zones of Jordan [(3)]. There are many representatives of this family and each one of these representatives have various pharmacological activities among these are analgesic, anti-inflammatory [(4)], anti- oxidant, anti-bacterial [(5)], anti-tumour and central nervous system depressant activities [(6)]. These activities are mainly related to terpenoids and flavonoids; both substances are often detected in plants belonging to this family [(7)]. **Genus *Salvia* L.**

The name of *Salvia* is derived from the Latin word (Salvare) which means use for healing wound or to be safe and unharmed. The genus *Salvia* (Family Lamiacea) is composed of 900 herb and shrub, growing is warm zones of word. The plants belong to this genus show high diversity in secondary metabolite [(8)].

Salvia palaestinaBenth

Figure 1: ***Iraqi Saliva palaestina pictures***

Taxonomy of *Salvia palaestina*

Kingdom: Plantae
Phylum: Magnoliophyta
Class: Angiospermae
Category: Lamiids
Order: Lamiales
Family: Lamiaceae
Subfamily: Nepetoideae
Genus: *Salvia*
Species: *Salvia palaestina* [9]

Materials and methods

Collection of plant material

The widely aerial parts of *Salvia palaestina* were collected from Kalopazyan Mountain near Sulaymaniya city at its flowering season in March 2019. The taxonomic identification of the plant is done by Prof. Dr. Sukiena Saeed Department of Biology / College of Science / University of Baghdad.

Experimental work

Extraction method (cold method)

About 300 gm. of the powdered of the aerial part of the plant material was first defatted with hexane for 24hr to remove chlorophyll and waxy material then soaked in 1500ml of 80%

ISSN 2515-8260 Volume 08, Issue 02, 2021

methanol, with occasional shaking, at room temperature. After 3 day, the methanol soluble materials were filtered off. And the residue was suspended with another equal volume of 80 % methanol as the first volume get saturated and left at room temp. In another 3 days again methanol soluble materials were filtered off. Then the filtrate was evaporated to dryness under vacuum using rotary evaporator. The residue was suspended in 200ml water and partitioned successively with hexane, chloroform, ethyl acetate, and n-butanol (3x350ml) for each fraction. The ethyl acetate fraction dried over anhydrous sodium sulfate, filtered, and evaporated to dryness [(10).]

Fractionation of cold method

The dried extract which was obtained from the extraction method (hot method) suspended in 350ml water and partitioned successively with hexane, chloroform, ethyl acetate, and n-butanol (3x350ml) for each fraction. The ethyl acetate fraction dried over anhydrous sodium sulfate, filtered, and evaporated to dryness under reduced pressure using rotary evaporator. Each fraction was weighted and assigned for further analysis.

Isolation and purification of phenolic and flavonoid compounds from ethyl acetate fraction

The isolation of the samples (A1), (A2), (A3), (A4), and (A5) from the ethyl acetate is done by preparative layer chromatography (PLC).

Preparative layer chromatography done was comprised of the following steps:

- Stationary phase preparation for preparative layer chromatography (PLC):Sixty grams of silica gel GF254 was suspended in 120 mL distilledwater and mixed thoroughly then spread on a clean, dried, 20x20 cm glass plates with a thickness of 0.5 mm using the Jobling Laboratory Division TLC coater. The plates were left-hand to dry at room temperature and solidify for about one hour, then placed in a rack, and activated in a heated oven at 120 ^{0}C for one hour then left to cool to be ready to apply samples.

- Preparation of mobile phase for preparative layer chromatography (PLC):100 mL of the mobile phase was introduced in a clean, dried, 20x20 cm jar. The jar was lined with filter paper, closed firmly, and left for saturation for about half-hour away from sunlight and air current. The mobile phase used for separation of compounds from the n-butanol fraction is of chloroform: Acetone: Formic acid (75:16:8).

- Application of the sample in preparative layer chromatography (PLC): About 1gm from each fraction was dissolved in about 3 mL of absolute methanol, applied in the form of a streak on the preparative layer using a capillary tube, left to dry at room temperature, then introduced in the full jar, the jar is closed forcefully, and left for development.

- Detection of the separated bands:After development, the plates were taken out of the jar, left at room temperature to dry, and the separated bands were allocated by a needle under UV light using a wavelength of 254 nm.

- Scrapping and elution of the separated bands:The marked bands were scrapped out of the preparative plate on separate papers using a spatula. Each band's powder was introduced in an individual clean and dry conical flask, a sufficient quantity of methanol was added, and the flasks were shaken on a warm water bath, filtered through the double special funnel filter for silica gel, the solvent was evaporated under reduced pressure using a rotary evaporator. The purity of the separated bands were checked using an analytical TLC. Five bands were isolated from the ethyl acetate fraction by the cold method and symbolized as (M1), (M2), (M3), (M4), and (M5).
- **Liquid chromatography-mass mass spectrometry (LC-MSMS)**
- Depending on the type of mass spectrometer used, information is provided on the molecular mass of a detected compound.

Results and discussion

Extraction of different active constituents

Extraction is the first essential step in this study; it relies on the kind of phytochemicals that are investigated. extraction methods are done, the cold method the selection of a suitable method and solvent for extraction depends on the TLC analysis of crude extract for constituents.

Fractionation of different active constituent

The phytochemical screening for the present plant fractionation is required to explain the main classes of the plant constituent which isolated from each other according to difference in polarity and solubility before chromatographic analysis is performed. Fractionations of crude extracts of the plant done with different solvents (chloroform, ethyl acetate, and n-butanol). Each fraction was subjected to different chromatographic technique for further separation, purification, identification, and isolation of major phytochemical constituents.

Isolation and purification of phenolic and flavonoid compounds by preparative layer chromatography (PLC)

Different mobile phase and standards were used for the identification to obtain the best separation in TLC after that PLC plate was used to isolate and purify flavonoid and phenolic compounds in which one gram of ethyl acetate was dissolved in 2 ml of methanol and put on PLC plate of 1cm by use of chloroform: Acetone: Formic acid (75:16:8) as mobile phase.

Identification and qualitative analysis by LC/MSMS

This mass spectrometric (MSMS) detection for LC becomes particularly useful. LC-MSMS is nowadays becoming a routine technique [11, 12], with ease of use comparable to UV/Vis or fluorescence detection (FLD). Depending on the type of mass spectrometer used, information is provided on the molecular mass of a detected compound, and, moreover, structural information is also gained from the interpretation of the specific fragmentation pattern observable under certain instrumental conditions, or with particular types of mass spectrometers [13].Although the initial costs of such instrumentation were for a long time prohibitive for conservation science laboratories, LC-MSMS is now becoming popular as a tool for the investigation of natural organic[14].

ISSN 2515-8260 Volume 08, Issue 02, 2021

By using multiple reactions monitoring mass spectrometer (MRM) the identification and assignment of peak within sample chromatograms.

The use of selective detector and spectrometer can greatly increase the confidence in the peak assignment detector system as mass spectrometers are able to record unique spectra for each peak within the sample chromatogram.

- **Identification of the isolated compound Rutin (A1) by LC/MSMS**

Depending on the type of mass spectrometer used, information is provide on the molecular mass of a detected compound (A1) as shown in Table (1) & figuer (2).

Table (1): MRM for the isolated compound (A1):

Q1Mass (Da)	Q3 Mass (Da)	Dwell (msec)	ID
609.419	299.000	50.00 DP	Rutin
609.419	270.900	50.00 DP	Rutin
609.419	178.700	50.00 DP	Rutin

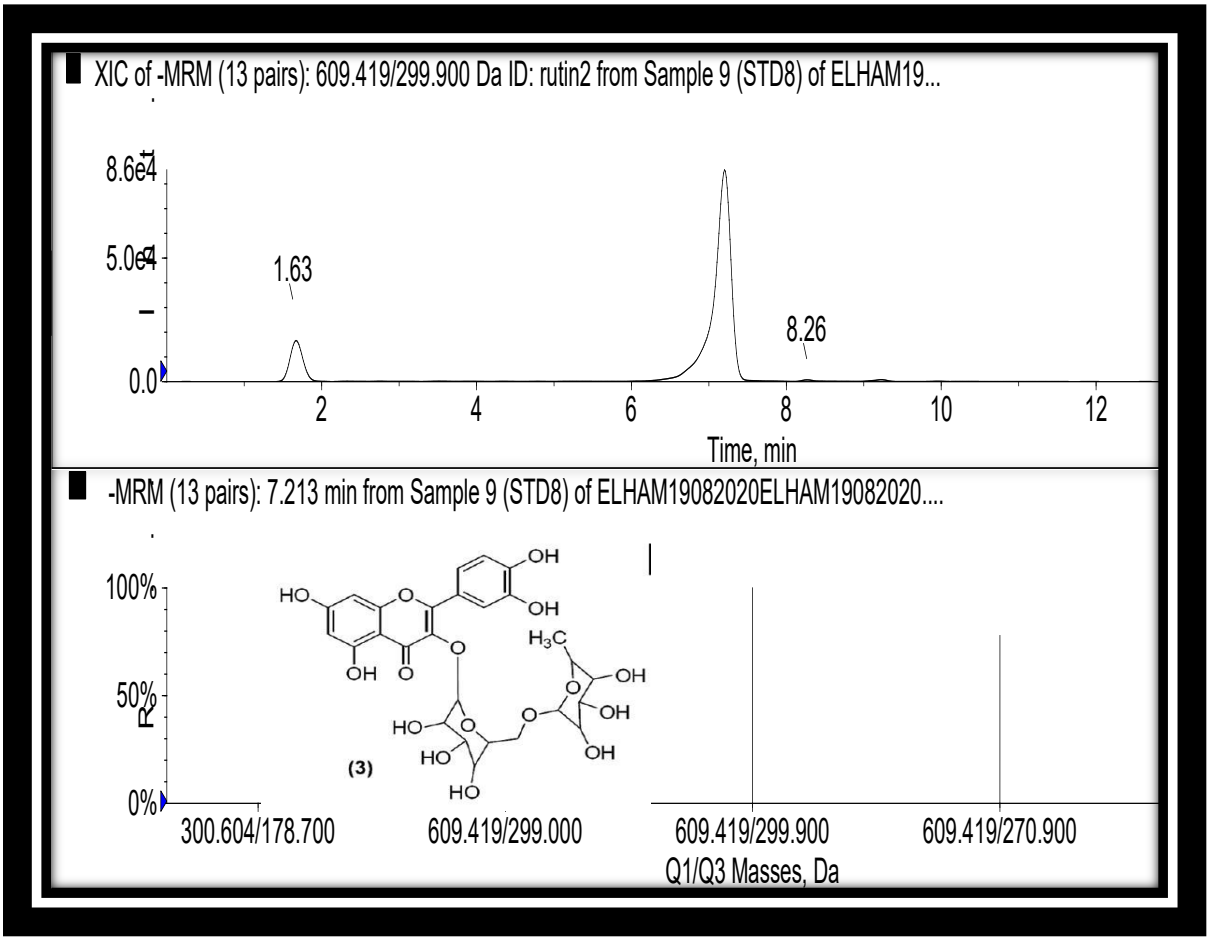

Figure (2): LC/MSMS for the isolated compound (A1) Rutin

- **Rutin($C_{27}H_{30}O_{16}$) m/z 609.152, m/z 300.028, m/z 271.025, m/z255.030, m/z151.02**[15]
- **Identification by LC/MSMS for A2 (Luteolin)**

Depending on the type of mass spectrometer used, information is provided for the molecular mass of a detected compound (A2) as in Table (2) and Figure (3).

ISSN 2515-8260 Volume 08, Issue 02, 2021

Table (2): MRM for isolated compound (A2)

Q1 Mass (Da)	Q3 Mass (Da)	Dwell (msec)	ID
285.000	217. 000	50.00 DP	Luteolin
285.000	199.000	50.00 DP	Luteolin

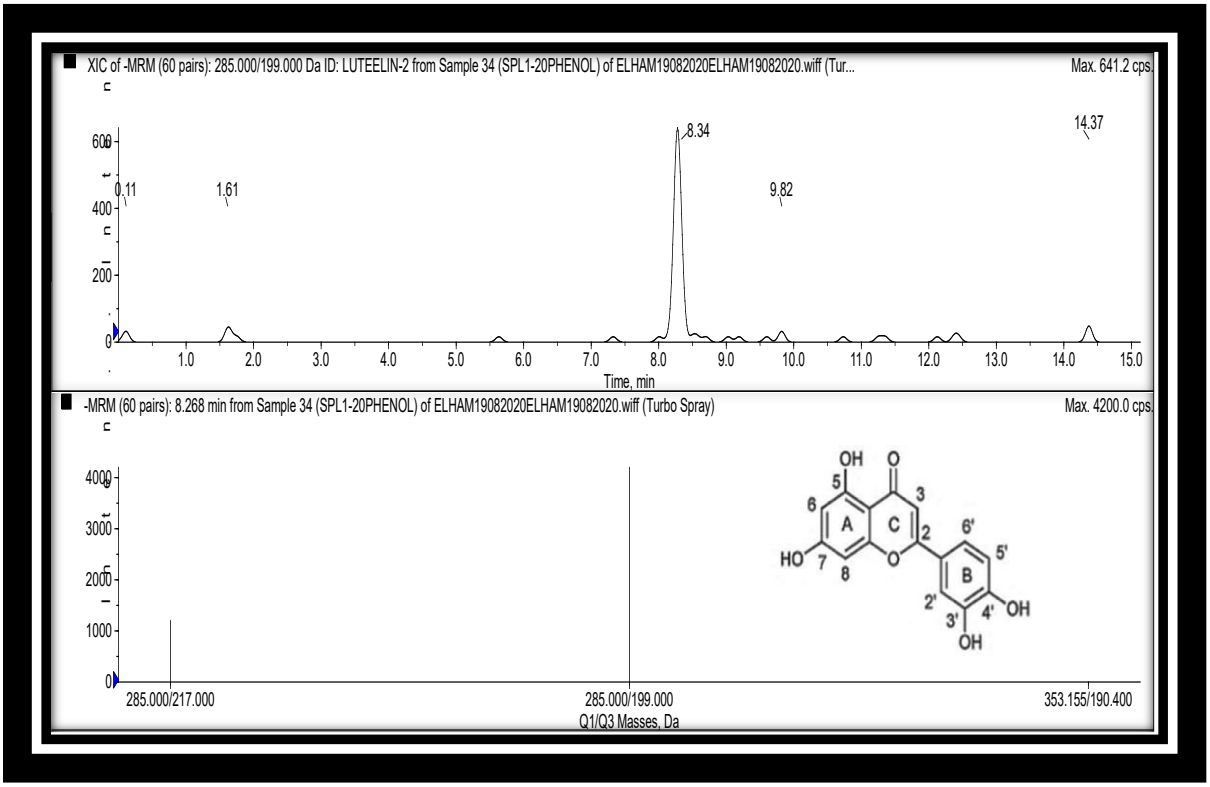

Figure (3): LC/MSMS for isolated compound (A2) luteolin.

- **Luteolin($C_{15}H_{10}O_6$)*m/z* 285, *m/z* 133, *m/z*107, *m/z*151** [16]

- **Identification of isolated compound (A3) Quercetin by LC/MSMS**

Depending on the type of mass spectrometer used, information is provide on the molecular mass and retention time of the detected compound (A3) as in Table (3) and Figure (4)

Table (3): MRM for compound (A3)

Q1 Mass (Da)	Q3 Mass (Da)	Dwell (msec)	ID
300.604	150.400	50.00	Quercetin
300.604	178.700	50.00	Quercetin

ISSN 2515-8260 Volume 08, Issue 02, 2021

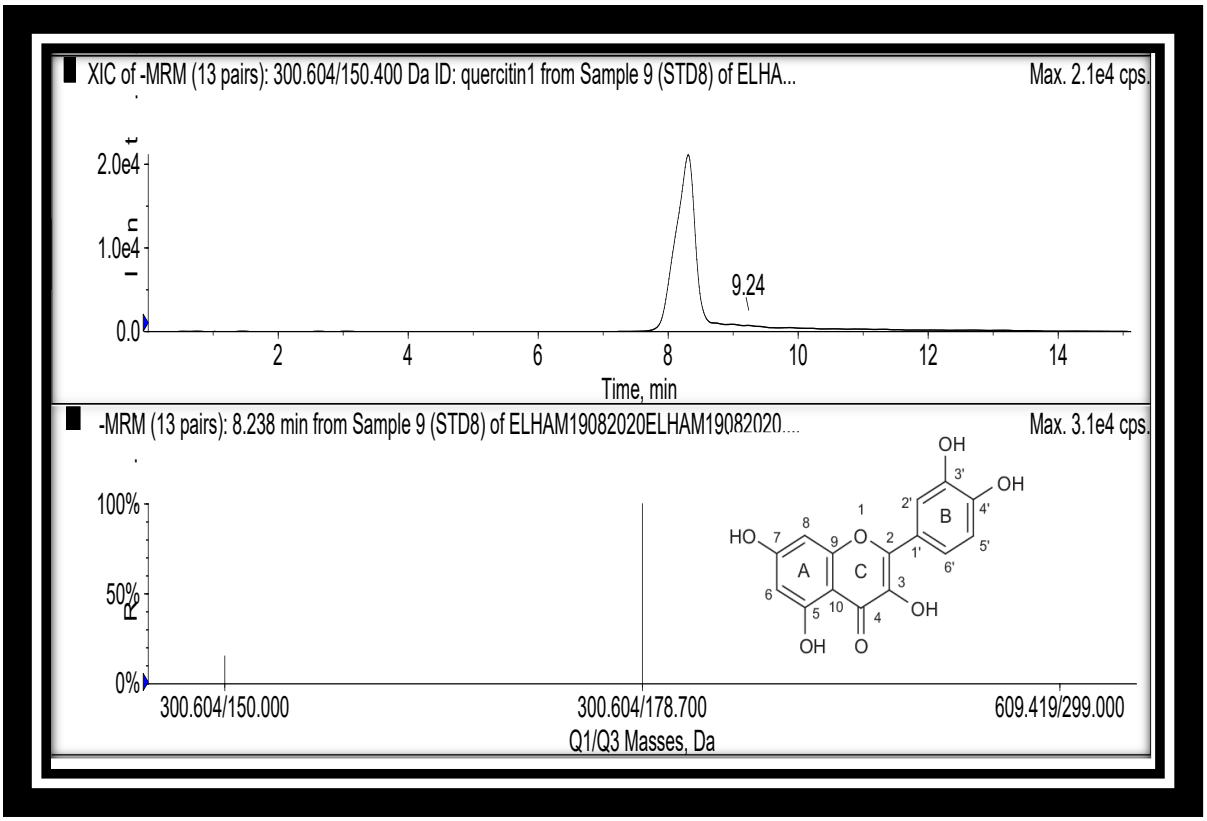

Figure (4): LC/MSMS for (A3) Quercetin

Quercetin ($C_{15}H_{10}O_7$)301.036 *m/z* 273.042, *m/z*243.028, m/z 178.998, *m/z* 151.004, *m/z*121.030 [(15)]

Identification of isolated compoundApeginin (A4) by LC/MSMS

Depending on the type of mass spectrometer used, information is provide the molecular mass and retention time of a detected compound (A4) as shown in Table (4) and Figure (5).

Table (4): MRM for isolated compound (A4)

Q1 Mass (Da)	Q3 Mass (Da)	Dwell (msec)	ID
269. 000	151.000	50.00 DP	Apigenin 1
269.000	117.000	50.00 DP	Apigenin 2

ISSN 2515-8260 Volume 08, Issue 02, 2021

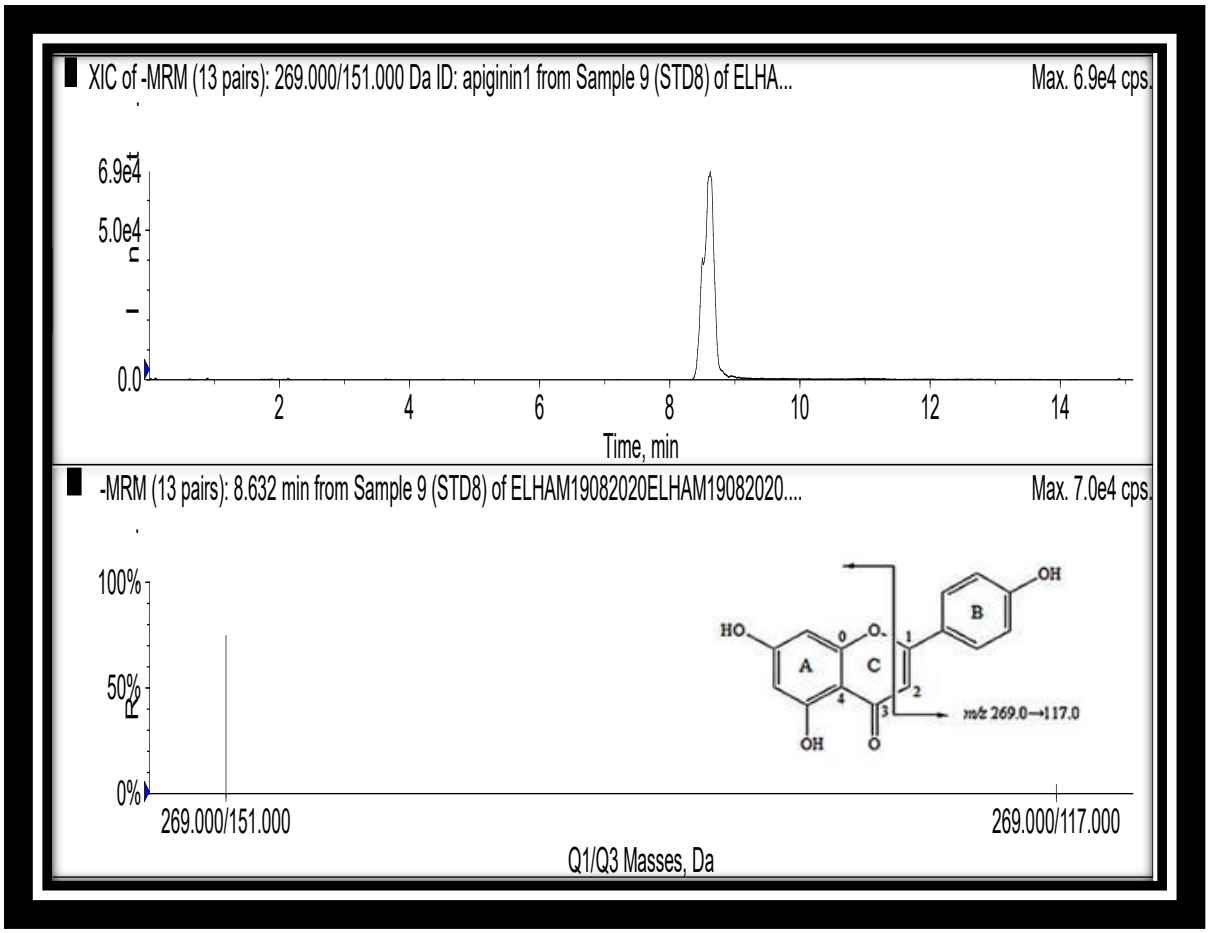

Figure (5): LC/MSMS for isolated compound (A4) apigeninApigeninC15H10O5 269, m/z 117, m/z 107, m/z 151 [16]

- **Identification of isolated compound Caffeic acid (A5) by LC/MSMS**

Depending on the type of mass spectrometer used, information is provide the molecular mass and retention time of a detected compound(A5) as shown in Table (5) and Figure (6).

Table (5): MRM for isolated compound (A5)

Q1 mass (Da)	Q3 Mass (Da)	Dwell (msec)	ID
178.465	134.200	50.00 DP	Caffeic acid
178.465	106.400	50.00 DP	Caffeic acid

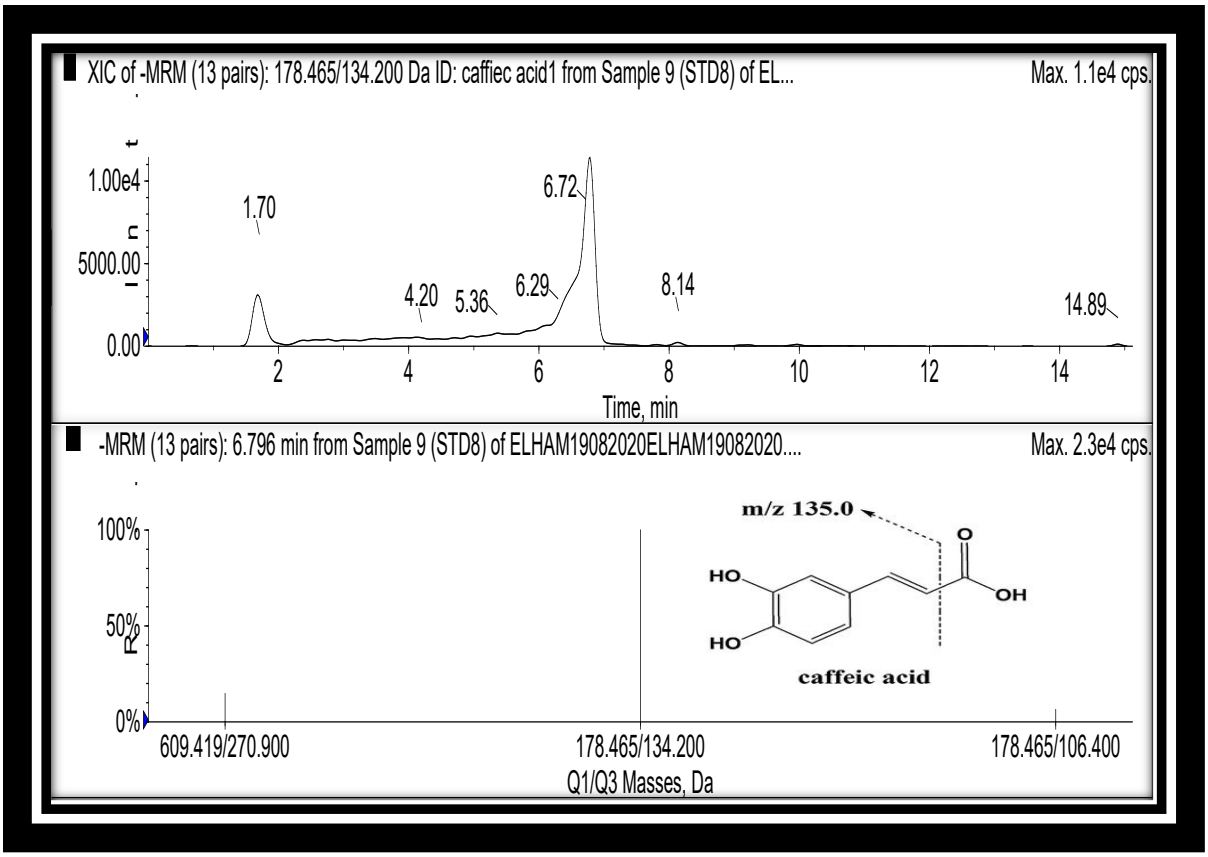

Figure (6): LC/MSMS for isolated compound (A5) caffeic acid
Caffeic acid ($C_9H_8O_9$) *m/z*179,*m/z*161,*m/z*135.1[(15)]

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