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Detection of Important Compounds from The Aerial Part of *Callistemon viminalis* Plant Cultivated in Iraq

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ABSTRACT

Background: Medicinal plants are a generous source of our drugs and remedies, coming from their fabulous secondary metabolites, which involve different classes such as alkaloids, glycosides, essential oils, phenolic compounds, and steroids. *Callistemon viminalis* is a small tree cultivated in Iraq, that owns very interesting bioactive compounds especially, essential oils, phenolic derivatives, and terpenoids.

Objective: To investigate the bioactive compounds from the aerial part of the Iraqi *Callistemon viminalis* plant.

Material and methods: The plant was extracted by Soxhlet apparatus and fractionated with solvents that differ in polarity, the n-butanol and ethyl acetate fractions were applied to Liquid Chromatography-Tandem mass spectrometer, while the hexane defatting extract was applied to Gas Chromatography-Mass spectrometer analysis to discover to components of the plant.

Results: The results show the presence of many glycosides, flavonoids, terpenoids, and alkaloids like Kaempferol, Astragalin, Quercitrin, Scutellarin alpha-Phellandrene, 3-Carene, (-)-Spathulenol and, Dihydroepinatalensine.

Conclusion: *Callistemon viminalis* aerial part involves diverse types of important that own important medical activity, they are mostly flavonoids, phenolic derivatives, terpenoids, terpenes, and glycosides. Also, the study supports the presence of powerful anti-inflammatory flavonoids and terpenoids that are involved in the traditional use of the plant in the relief of tissue inflammations.

Keywords: medicinal plants, phenolic compounds, flavonoids, liquid chromatography.

INTRODUCTION

Natural surround is always a very attractive field to explore new treatments and remedies. The plants are mostly revealed as the premier source for our drug discoveries. Deeply, when we take a look at the plant, we see the primary and the secondary metabolites, the primary metabolites are proteins, sugars, fatty acids, and whatever plants need to grow, they also act as a food source for both humans and animals. Secondary metabolites are what plants use to survive in their environment, just like protection from insects; they are also considered to have the role of appreciable pharmaceutical activity [(1)]. Secondary metabolites are versatile in their structure and activity. Also, many biosynthetic pathways give rise to different natural products. Alkaloids, glycosides, terpenes, essential oils, and phenolics are some examples of these hopeful bioactive compounds [(1,2)].

Ancient history reveals many medicinal plants that were used by people, the oldest evidence was a Sumerian clay slab found in Nagpur about 5000 years in old. It involved 12 preparations from 250 different plants which include poppy and henbane alkaloids [(3)]. Also, Indian Vedas holy books have mentioned numerous treatments from plants that grow in nature [(4)]. Even in this time we use treatments with plant parts like ginseng and camphor written in "Pen T'Sao", the Chinese book on roots and grasses, that listed 365 drugs from plants and belonged to 2500 BC [(5,6)].

Medicinal plant products not only can treat and prevent diseases but also give template structures that can be modified and developed as active molecules against existing illnesses [(7)]. *Callistemon viminalis* primarily involves essential oils as well as phenolic acids, phloroglucinol derivatives, tannins, flavonoids, and triterpenoids. Active molecules in plant extraction show exceptional applications in nanoparticle drug manufacturing, medical solutions, and cosmetic concepts [(8,9)].

Plant phenolic compounds are a very broad class of secondary metabolites, which obtain more deliberation in the last years due to their high occurrence in the plant kingdom in addition to their powerful antioxidant activity. Natural roles may be diverse from securing the plant from predators to sensorial, structural, and reproductive functions. Chemically, plant phenolics involve an aromatic ring bonded with at least one hydroxyl group that is generally derived from the shikimic acid pathway and acetate coenzyme A or its active forms. With their obvious radical scavenging ability, they play an important role in protection from degenerative diseases as well as the strong ability to suppress tumor formation and inflammatory response [(10)].

MATERIAL AND METHODS

Ethical approval: The study was approved by the Ethics Board of Baghdad University.

Plant material:

Callistemon viminalis aerial part was collected from Baghdad city on separate periods extended from the end of October 2021 AD to February 2022 AD. The plant was identified and authenticated by the Department of Biology / College of the Science / University of Baghdad. The plant component was

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properly cleaned, allowed to dry at room temperature, and then ground into a fine powder using an electric grinder.

Extraction method:

Sixty grams of the plant aerial part powder was macerated with hexane for three consecutive days for defatting, then filtered and dried with a rotary evaporator and 6 grams of the extract has been collected. The defatted plant was dried at room temperature, then extracted by Soxhlet apparatus with 2.2 liters of 85% ethanol for 24 hours. The extract was filtered and dried under reduced pressure using a rotary evaporator to get a dried extract of about 38.5 grams. 3.2 grams of the extract was taken to do general chemical tests and the rest was suspended with 0.75 liters of warm distilled water and fractionated with chloroform, ethyl acetate, and n-butanol respectively, (2x350 mL) for each fraction. These three fractions were collected, dried, and weighted to be further analyzed and studied [11,12].

Preliminary qualitative phytochemical analysis of crude extract and fractions:

Phytochemical analysis for screening and identification of plant secondary metabolites was applied to the crude extracts, as well as for plant arial part powder, using standard general chemical tests described by Harborne [13].

Test for Saponins:

Froth test:

Two grams of dried, grinded aerial part of the plant was taken, then 20 mL of distilled water was added. The combination was heated to 100 degrees Celsius in a water bath, filtered, and the filtrate was then mixed with 10 milliliters of distilled water, Shaked, and waited for 10 minutes after the shaking to observe the formation of foam.

Test for Alkaloids:

Mayer reagent:

A little amount of dried plant crude extract was dissolved in 1 mL ethanol, then 4 drops of Mayer reagent were added, and white precipitate indicate the presence of alkaloids.

Dragendroff reagent:

A little amount of dried plant crude extract was dissolved in 1 mL ethanol, then 4 drops of Dragendroff reagent was added, and white precipitate formation indicates the existence of alkaloids.

Test for Cardiac glycosides:

killer Killani test:

A little amount of dried plant crude extract was dissolved in 5 mL ethanol, then mixed with 2 mL of glacial acetic acid containing 1 drop of ferric chloride, lastly, 1 mL of concentrated sulfuric acid was added, and a brown ring emerged at the interface indicating the presence of cardiac glycosides.

Test for Terpenoids:

Salkowski test:

A little amount of dried plant crude extract was dissolved in 5 mL ethanol, then combined with 2 mL of chloroform, and 3 mL of concentrated sulfuric acid was added to the mixture; the appearance of a brown ring on the mixture's surface denotes the existence of terpenoids.

Test for Sterols:

Half of a gram of dried plant crude extract was taken and shaken with petroleum ether to get rid of the plant pigment. Then 10 mL of chloroform was added to the residue, then a chloroform layer was formed, and anhydrous sodium sulfate was applied to dry this layer. 5 mL of the chloroform layer was taken, and 0.25 mL of acetic anhydride and two drops of concentrated sulfuric acid were added successively. The green color indicated the presence of sterols.

Test for Phenols:

Ten mL of distilled water was used to dissolve 0.25 g of crude dried plant extract before filtering. To the filtrate, 1% aqueous ferric chloride (FeCl3) solution was added. The presence of tannins in the extract is indicated by the emergence of vivid green, blue, or black color.

Test for Flavonoids:

Petroleum ether was mixed with 0.5 g of dried plant crude extract to remove the fatty components (lipid layer). The defatted residue was filtered after being dissolved in 20 mL of 80% ethanol. The following test was conducted using the filtrate:

In a test tube, 3 mL of filtrate was combined with 4 mL of 1% potassium hydroxide and color change was detected. The presence of flavonoids was indicated by a dark yellow tint.

Test for Coumarins:

In a test tube, 0.5 g of dried plant crude extract was added. Filter paper that had been exposed to a 1 N NaOH solution was placed over the tube's mouth. After briefly immersing the test tube in boiling water, the filter paper was taken out and inspected under a UV light to check for yellow fluorescence, which showed the presence of coumarins.

Test for Anthraquinones:

6 mL of 1% HCl was added to a little amount of plant-dried crude extract, then boiled and filtered. 5 mL of benzene was added to the filtrate with shaking, and two layers were formed, the organic benzene layer was eliminated and 10% of 10% NH_4OH was added. Red color or pink-violet in the ammoniacal alkaline layer reveals the presence of anthraquinones.

Identification of n-butanol fraction components from the plant extract:

The compounds were identified using LC-MS/MS (SCIEX X500R QTOF) connected to the spectral library (SCIEX All-In-One HR-MS/MS Library Version 2.0 with NIST 2017 Library Bundle) at the following conditions:

- Column: Phenomenex Gemini 5 µm NX-C18 110 Å, LC Column 250 x 4.6 mm, Ea.
- Injection volume: 10 µL.
- Autosampler: AC Autosampler version 2.12.
- Pump: binary gradient AC pump A: version 2.11, B: version 2.12.
- Column oven: AC column oven version 2.10, temperature, 40 ˚C.
- Flow rate: 1 mL/min.
- Detector: PDA detector version 4.02, wavelength 254 nm.
- System controller: controller version 3.61.
- Solvent system: A: 1% formic acid in the water, B: Acetonitrile.
- Mass ion source: TurbolonSpray, temperature: 500 ˚C.
- Scan type: SWATCH, polarity: positive and negative.
- TOF start mass (Da): 50, TOF stop mass (Da): 1000.

The gradient program is mentioned in **Table (1).**

Table (1): LC-MS/MS gradient program.

Fraction	Time (min)	Solvent A%	Solvent B%
n-butanol	0	0%	0%
	5	3%	97%
	18	90%	10%
	18-23	90%	10%
	27	3%	97%
	27-30	3%	97%

Identification of the chemical composition of the hexane defatting extract using gas chromatography (GC/MS):

The GC-MS system Agilent (7820A) USA with NIST 11 Library was used under the following conditions:

- Analytical Column: Agilent HP-5ms Ultra Inert (30 m length x 0.25 mm diameter x 0.25 µm inside diameter).
- Injection volume:1 µL.
- Pressure 11.933 psi.
- GC Inlet Line Temperature: 250 ˚C.
- Aux heaters Temperature: 310 ˚C.
- Carrier Gas: He 99.99%.
- Injector Temperature: 250 ˚C Scan, Range: m/z 50-500.
- Injection Type: Splitless.

The oven temperature program is shown the **Table (2)**

Table (2): The temperature program of the GC-MS oven.

Ramp	Temperature	Hold time/Rate
Ramp 1	55 ˚C	hold to 2 min
Ramp 2	55 ˚C to 180˚C	7 ˚C/min
Ramp 3	180 ˚C to 280 ˚C	8 ˚C/min
Ramp 4	280 ˚C	hold to 2 min

RESULTS

Extraction yield:

The Soxhlet 85% ethanolic extract of the aerial part give a weight of 38.5 g and the hexane defatting extract was 8 g. The wights and (W/W) percent of the fractions are shown in **Table (3).**

Table (3) The wights and (W/W) percent of the fractions

Fraction	Weight and (W/W) percent
Chloroform	3.1 g (8.051%)
Ethyl acetate	1.83 g (4.753%)
n-butanol	3.28 g (8.519%)

Preliminary qualitative phytochemical analysis of crude extracts:

Phytochemical screening using the standard chemical test for the main class of plant secondary metabolites is an important simple step that provides knowledge about the presence of the components to direct the study expects toward further analysis and choosing the proper conditions, solvents, standards, and experimental methods.

The results of the preliminary phytochemical tests are given in **Table (4).**

Table (4): Results of phytochemical tests for *Callistemon viminalis* plant.

Phytochemicals		Extract/Fraction	Result
Saponins		Aerial part powder	-
Terpenoids		Crude	+
Sterols		Crude	+
Anthraquinones		Crude	-
Alkaloids	Mayer	Crude	-
	Dragendorff	Crude	+
Cardiac glycosides		Crude	+
Coumarins		Crude	-
Phenols		Crude	+
Flavonoids		Crude	+

Extract contents:

LC-MS/MS:

The identified compound's chromatograms and mass fragmentation appear in **Figures (1-11),** and the information is mentioned in **Table (3).** The structures are in figure (12). The chromatograms on the right of the figures below show detected peaks of the compounds with intensity and retention time, while the lower fragmentation on left shows the acquired fragmentation from the sample, and upper to it, the blue fragmentation of the sample is put together slightly upper the grey matching compound fragmentation in the library. The (Fit%) in the upper right reveals the percent of matching of library fragmentation with the acquired one.

Table (5) The compounds detected by LC-MS/MS from n-butanol and ethyl acetate fractions.

Name of compound	Class	RT (min)	Area	M.W. g/mol	Molecular formula	Fraction
Gallic acid	Phenolic acid	13.25	1.663e+05	170.12	$C_7H_6O_5$	n-butanol
Quercitrin	Flavonoid glycoside	13.29	1.752e+06	448.4	$C_{21}H_{20}O_{11}$	
Quercetin	Flavonoid	14.95	1.955e+06	302.23	$C_{15}H_{10}O_7$	
Scutellarin	Flavonoid glycoside	13.17	2.708e+06	462.4	$C_{21}H_{18}O_{12}$	
3,5-Di-O-caffeoylquinic acid	Hydroxycinnamic acids derivatives	10.87	7.562e+05	516.4	$C_{25}H_{24}O_{12}$	
Hyproside	Flavonoid glycoside	11.81	1.423e+06	464.4	$C_{21}H_{20}O_{12}$	
Isoquercetin	Flavonoid glycoside	12.80	1.648e+05	464.4	$C_{21}H_{20}O_{12}$	
Astragalin	Flavonoid glycoside	12.76	1.021e+05	448.4	$C_{21}H_{20}O_{11}$	
Kaempferol	Flavonoid	15.90	2.790e+05	286.24	$C_{15}H_{10}O_6$	
Maslinic acid	Triterpene	20.86	3.567e+04	472.7	$C_{30}H_{48}O_4$	
Loganin	Monoterpenoid glycoside	13.02	2.100e+05	390.4	$C_{17}H_{26}O_{10}$	Ethyl acetate

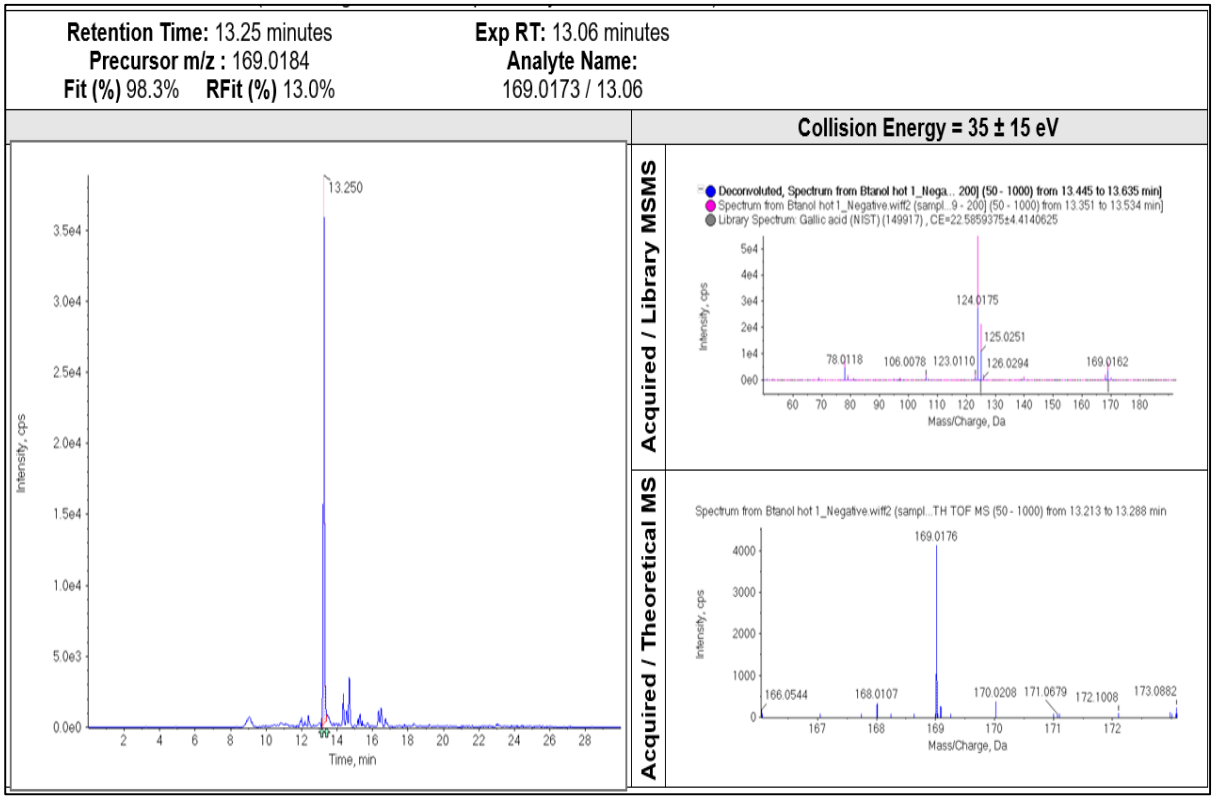

Figure (1) Chromatogram and fragmentation of n-butanol compound match with Gallic acid.

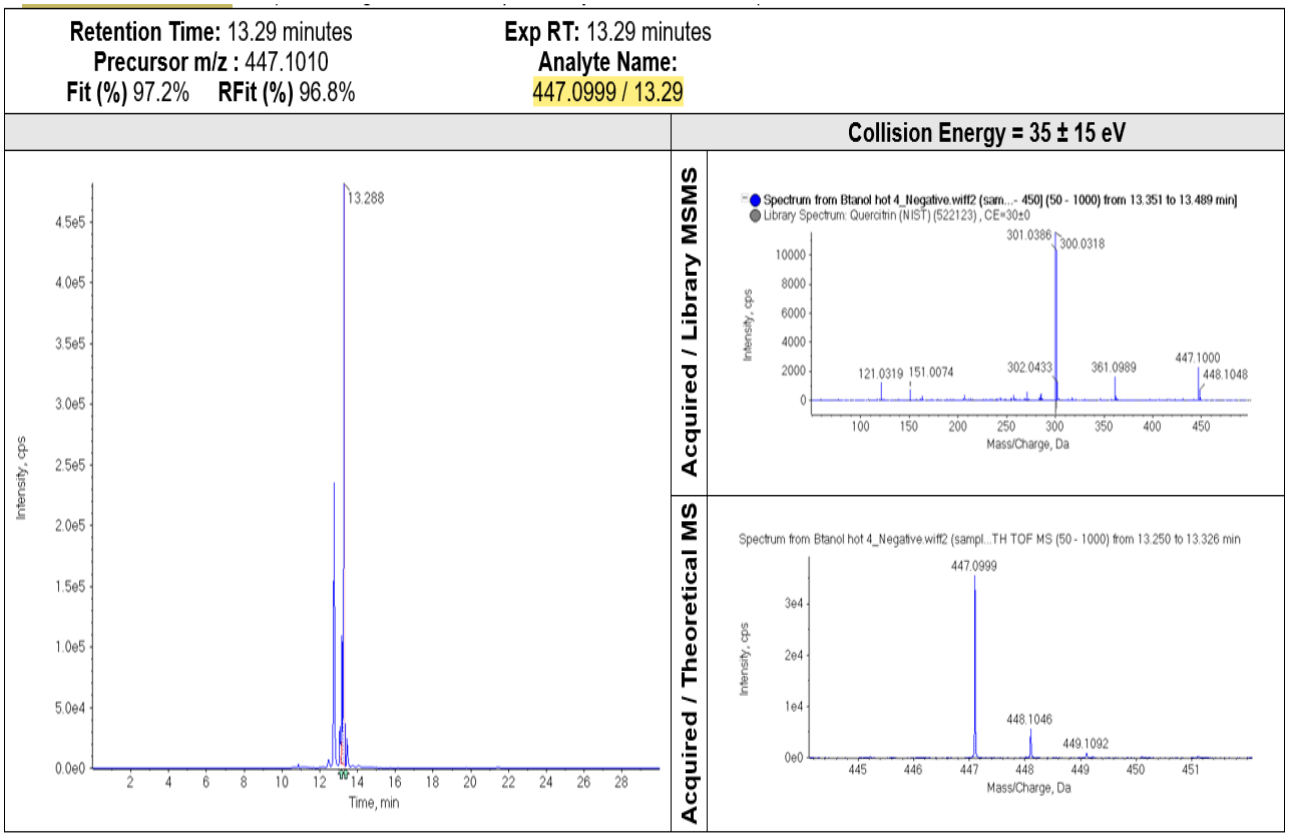

Figure (2) Chromatogram and fragmentation of n-butanol compound match with Quercitrin.

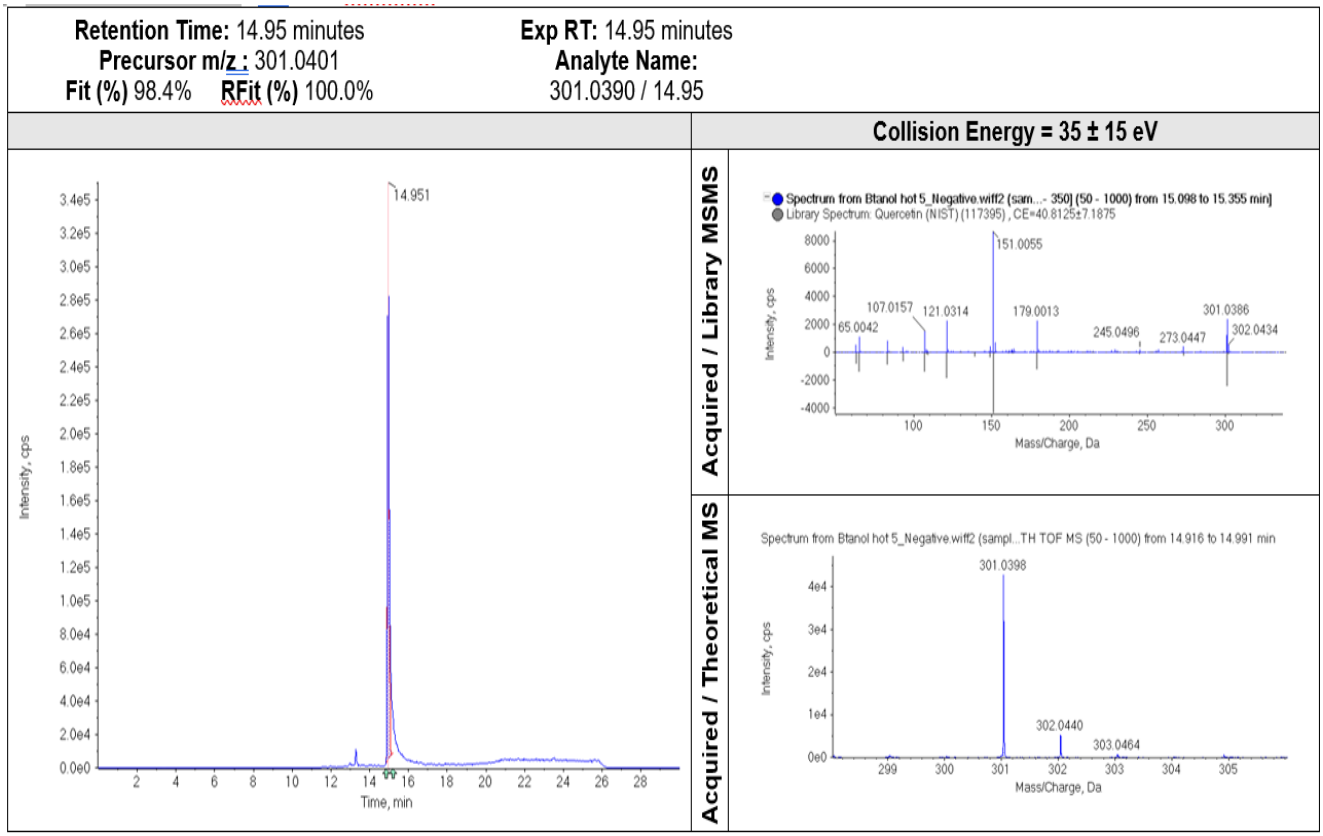

Figure (3) Chromatogram and fragmentation of n-butanol compound match with Quercetin.

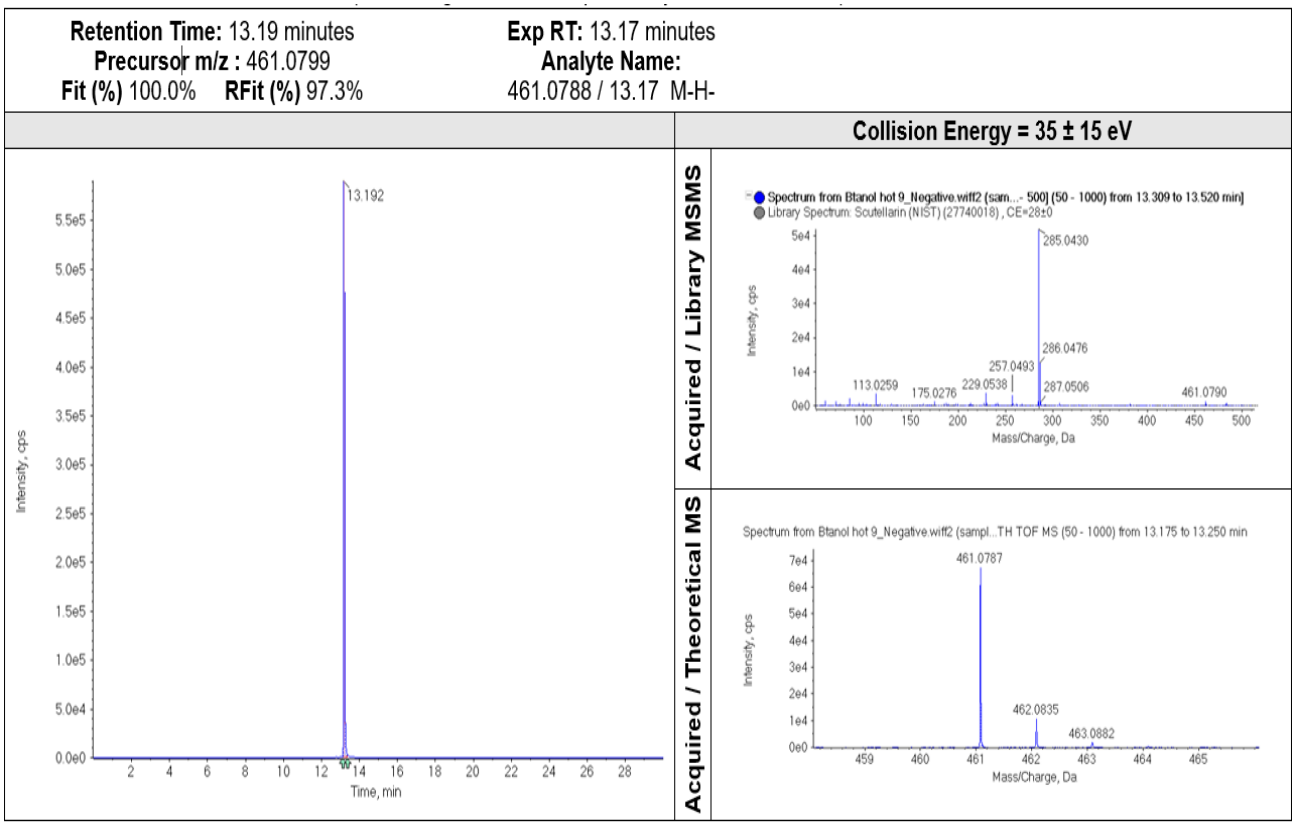

Figure (4) Chromatogram and fragmentation of n-butanol compound match with Scutellarin.

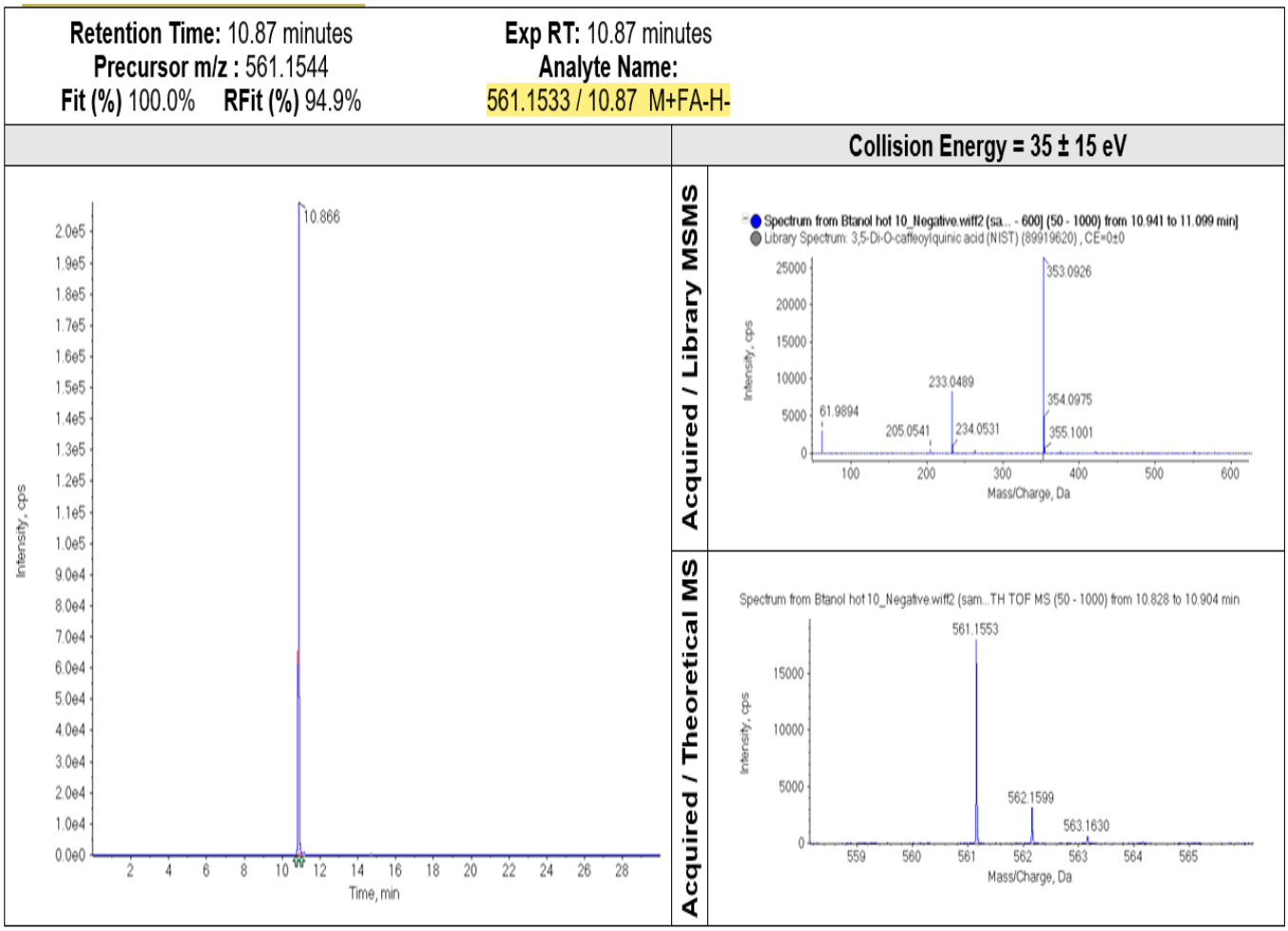

Figure (5) Chromatogram and fragmentation of n-butanol compound match with 3,5-Di-O-caffeoylquinic acid.

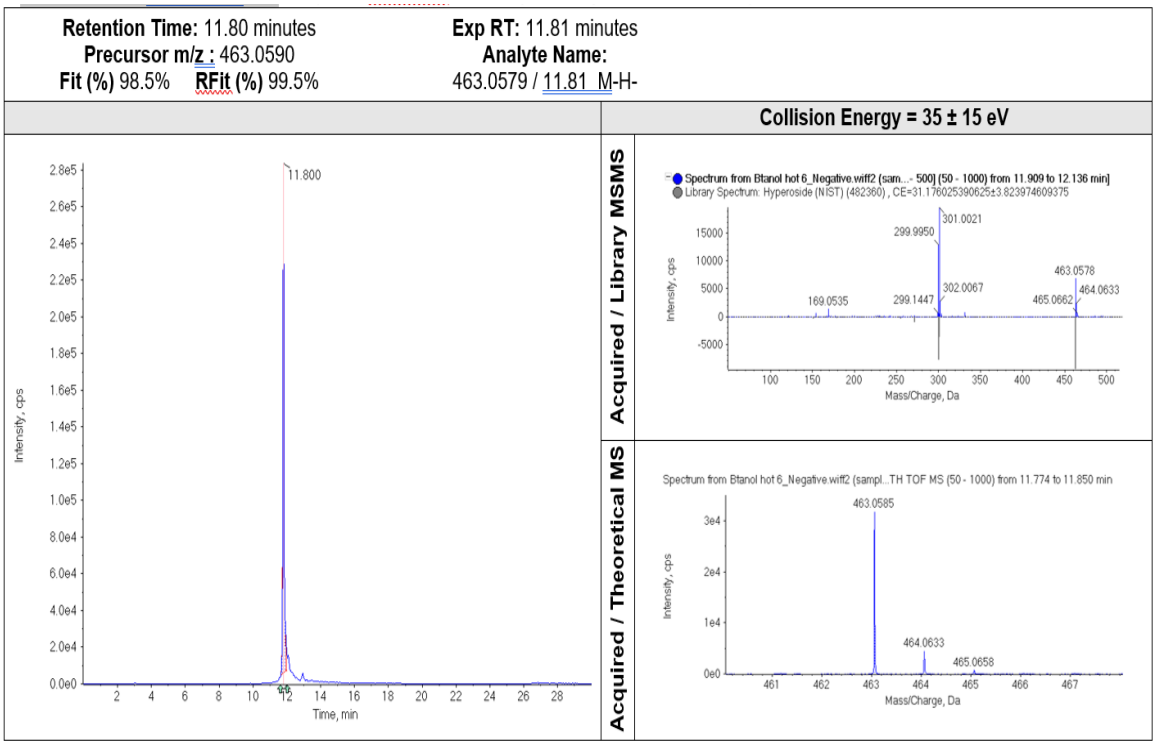

Figure (6) Chromatogram and fragmentation of n-butanol compound match with Hyproside.

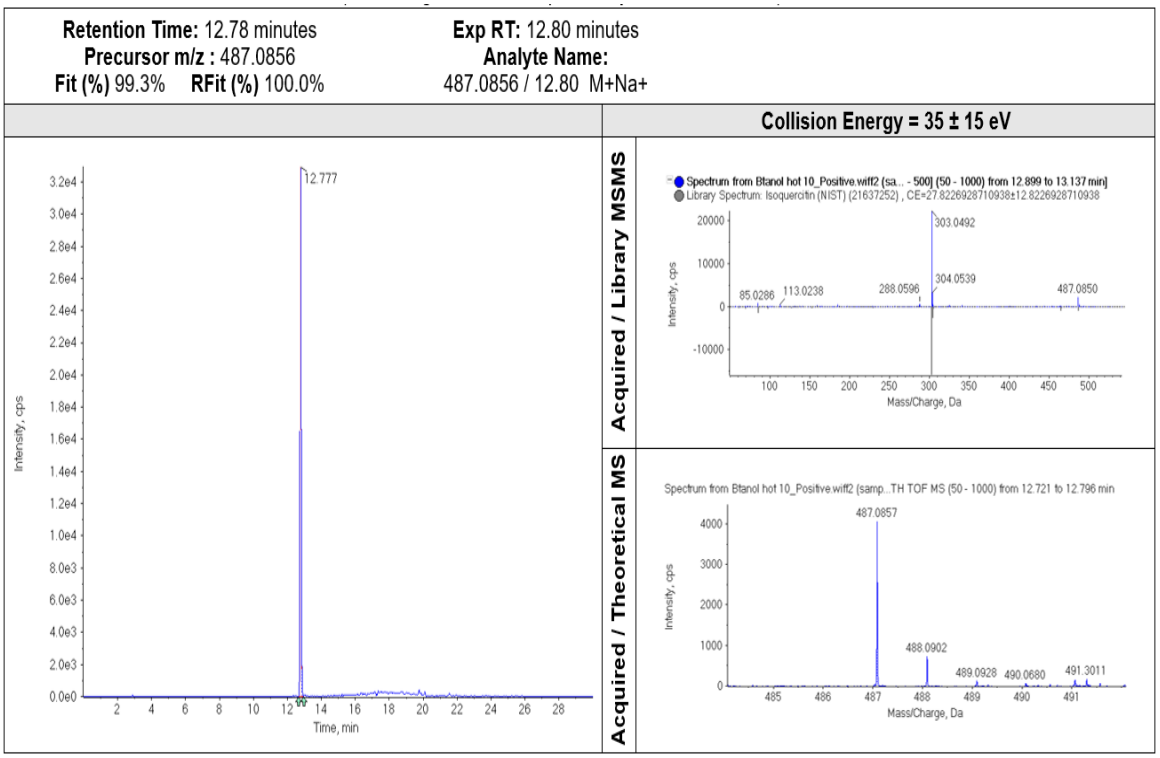

Figure (7) Chromatogram and fragmentation of n-butanol compound match with Isoquercetin.

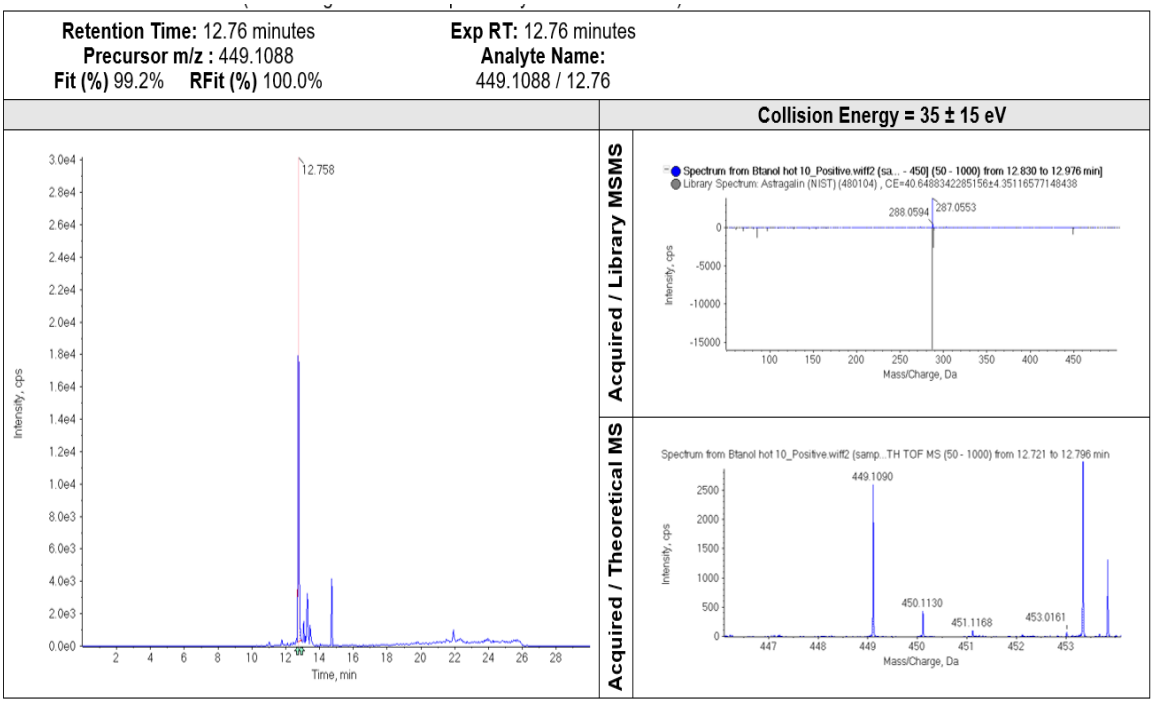

Figure (8) Chromatogram and fragmentation of n-butanol compound match with Astragalin.

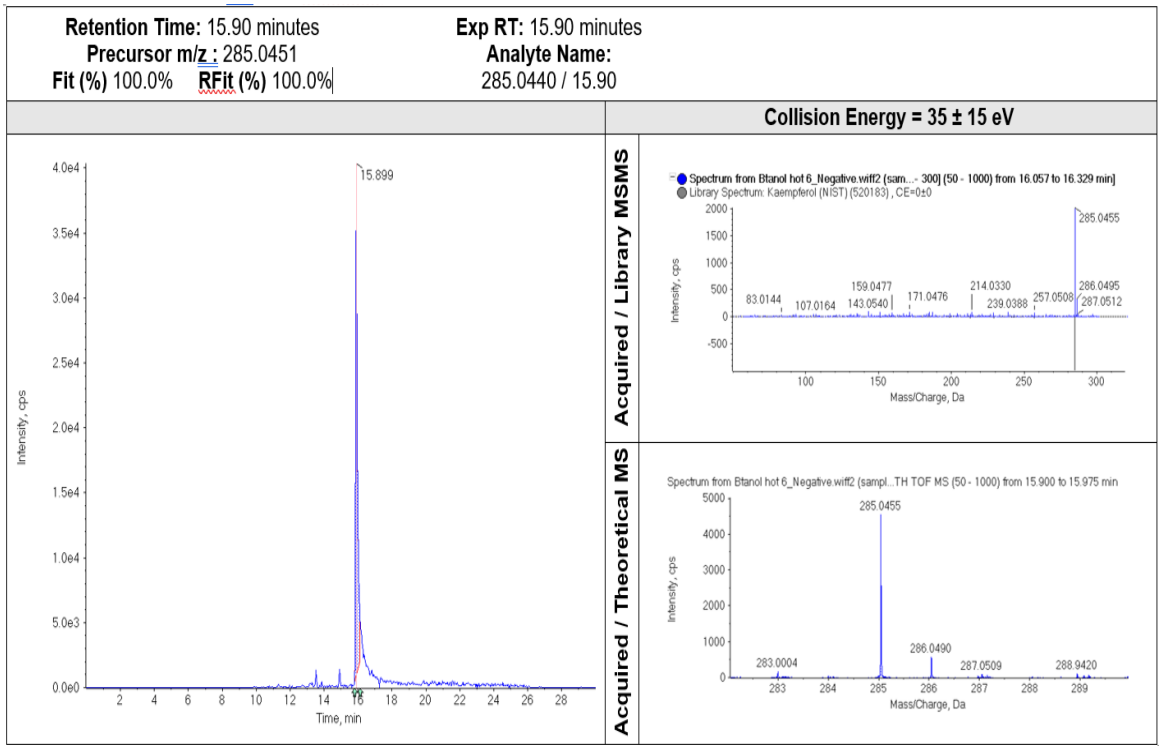

Figure (9) Chromatogram and fragmentation of n-butanol compound match with Kaempferol.

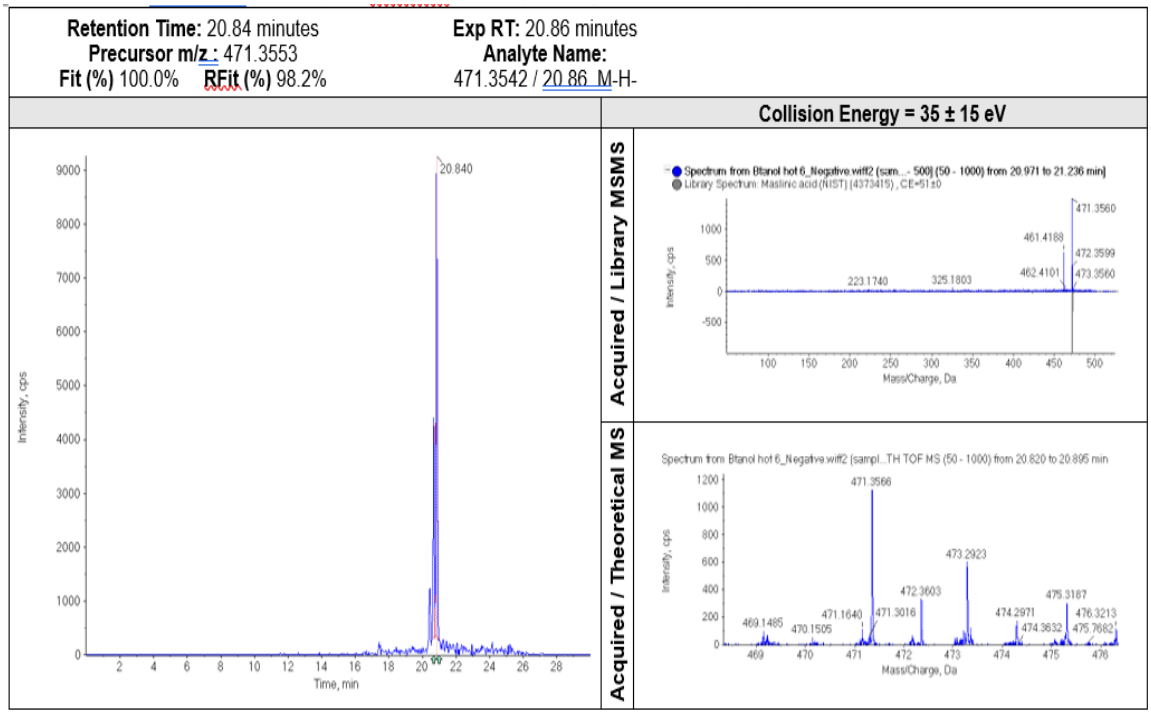

Figure (10) Chromatogram and fragmentation of n-butanol compound match with Maslinic acid.

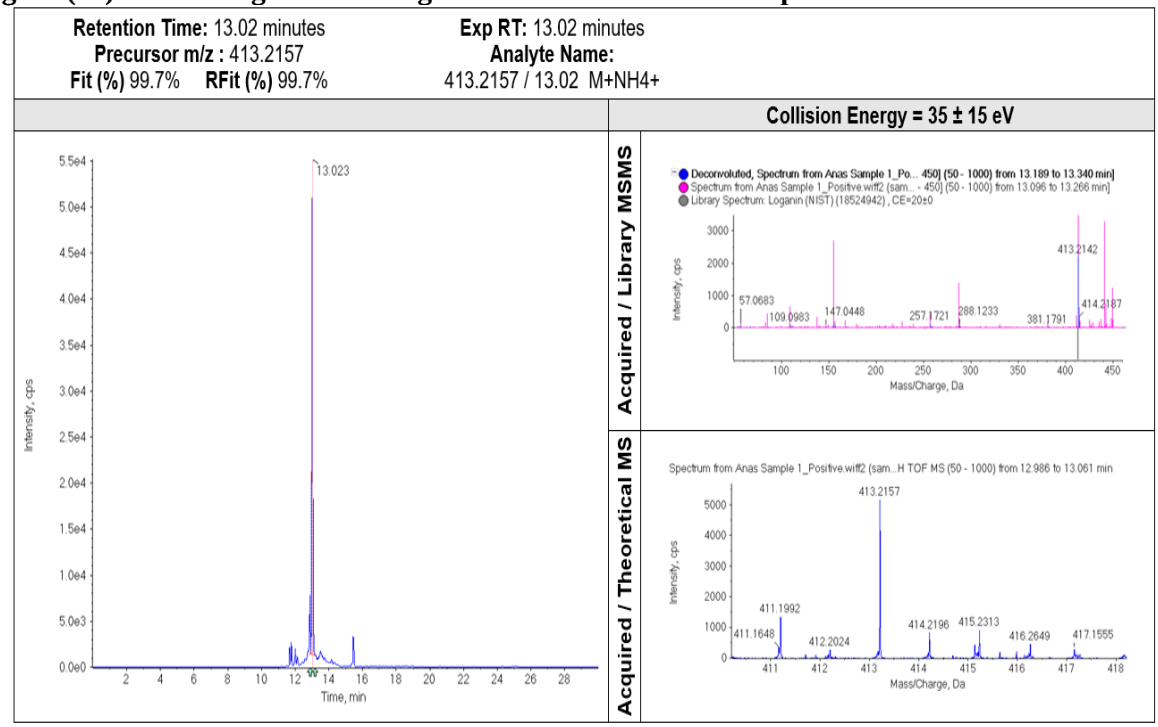

Figure (11) Chromatogram and fragmentation of Ethyl acetate compound match with Loganin.

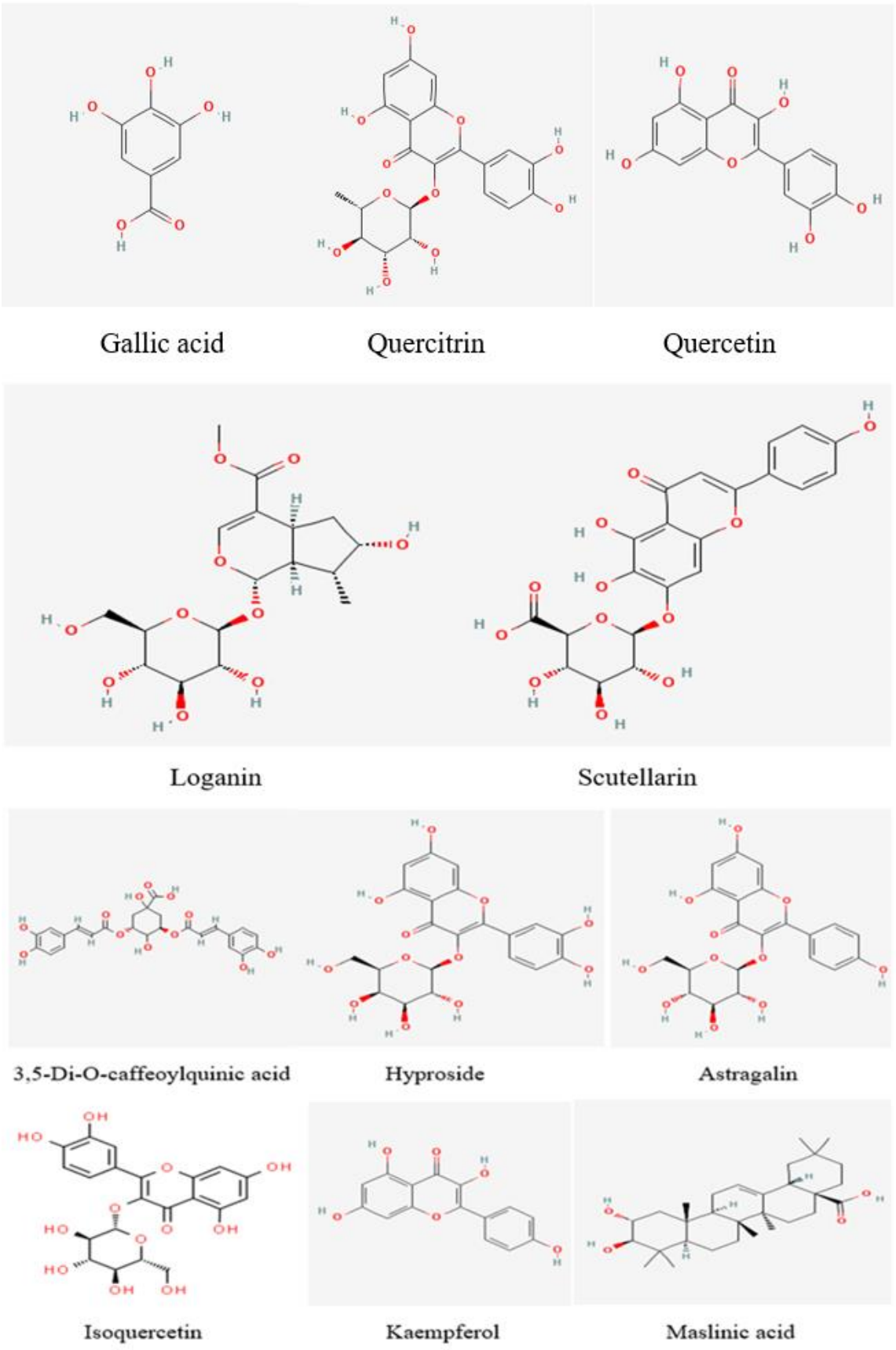

Figure (12) structure of compounds detected by LC-MS/MS.

Detection by GC-MS for hexane defatting extract:

The defatting hexane extract was applied to GC-MS for the identification of components, the detected compounds are mentioned in **Table (6)** and the chromatogram appears in Figure (13).

Table (6) Compounds detected by GC-MS from hexane defatting extract.

Name of compound	Class	Retention time (min)	Area %	Molecular weight (g/mol)	Molecular formula
alpha-Phellandrene	Monoterpene	6.998	3.23	136.23	$C_{10}H_{16}$
3-Carene	Monoterpene	7.363	1.23	136.23	$C_{10}H_{16}$
(-)-Spathulenol	Sesquiterpene	17.616	5.86	220.35	$C_{15}H_{24}O$
Dihydroepinatalensine	Alkaloid	23.283	4.07	303.35	$C_{17}H_{21}NO_4$
Phytol	Diterpene	24.288	4.67	296.5	$C_{20}H_{40}O$
gamma-Terpinene	Monoterpene	25.638	2.02	136.23	$C_{10}H_{16}$
Geranyl phenylacetate	Fatty alcohol esters	27.457	2.54	272.4	$C_{18}H_{24}O_2$
Squalene	Triterpene	31.227	32.38	410.7	$C_{30}H_{50}$

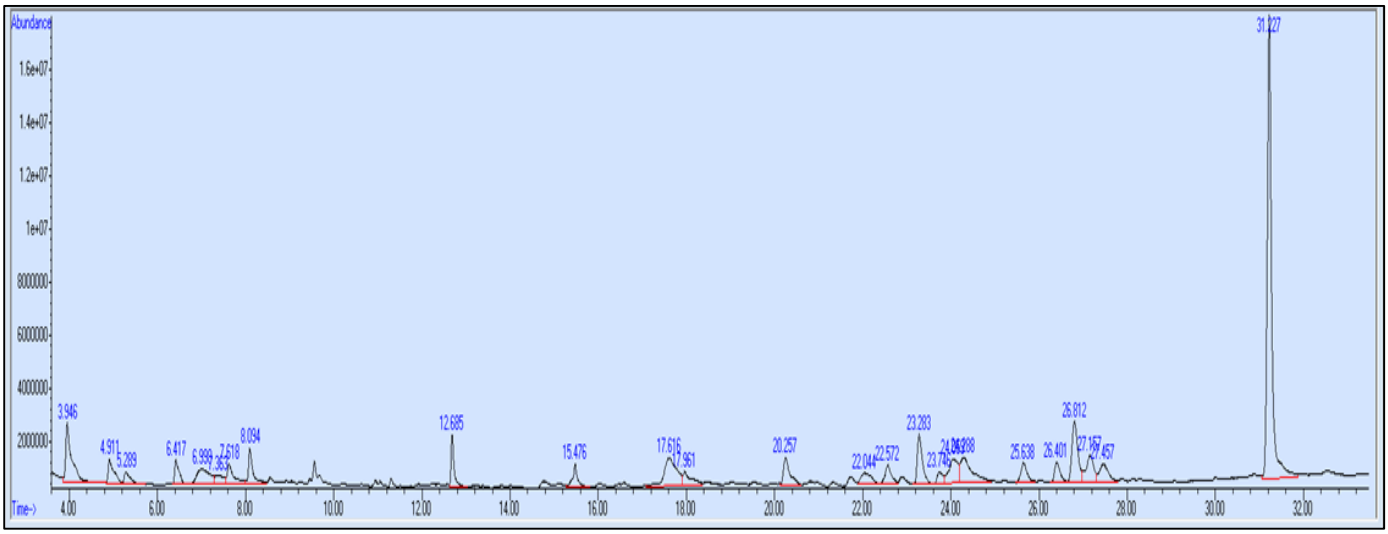

Figure (13) Chromatogram of gas chromatography for defatting hexane extract.

DISCUSSION

The results of extraction fractions yield of *Callistemon viminalis* aerial part (n-butanol >Chloroform> Ethyl acetate), the n-butanol was the higher yield which indicates high phenolic and sugar components due to their polar properties, while chloroform represent second higher yield, which indicates the presence of terpenoids, pigments, and plant fats, and finally, ethyl acetate fraction is the lower yield which represents the component which has polar properties lower than from those attract to n-butanol, in other words, the glycosides, the sugar-rich compounds are more dominant than the free compounds in the plant and due to their high polar properties, they preferred n-butanol fraction. So, the solvents play a major role in the type of secondary metabolites found in the extracts [14, 15].

The identified compounds were mostly flavonoids and phenolic derivatives and terpenoids, also terpenes, fatty alcohol, and one alkaloid were detected in hexane defatting extract. Many studies show similar compounds presented in the *Callistemon viminalis* plant [16,17]. The compounds have important biological activity against many diseases' pathways, plant phenolic compounds, terpenes, and terpenoids have a significant application in anticancer and antioxidant research [18,19]. The presence of powerful antioxidants and anti-inflammatory components like Kaempferol, Astragalin, Quercitrin, Scutellarin, and other detected molecules support the evidence of medicinal properties of the plant and support its use in traditional medicine for digestive system inflammations [20].

CONCLUSION

The *Callistemon viminalis* aerial part involves diverse types of flavonoids, phenolic derivatives, terpenoids, and glycosides, many of these components are detected in the n-butanol fraction of the extract due to their high polarity properties in addition to hexane defatting important terpenes constituents. These components are involved in many beneficial biological activities and support human health. Also, the study supports the presence of powerful anti-inflammatory flavonoids and terpenoids that are involved in the

traditional use of the plant in the relief of tissue inflammations.

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Conflicts of Interest: Regarding the publishing of this paper, the authors state that they have no conflicts of interest.

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