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Isolation and identification of the chemical composition of *Salvia* Species growing in Iraq by GC-MS

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ABSTRACT

In this research, the chemical composition of six species of *Salvia* were studied, wild grown (*Salvia multicaulis, Salvia indica, Salvia spinosa, Salvia reuterana,* and *Salvia russellii*) collected from different cities in the Kurdistan region, and cultured (*Salvia officinalis*) collected from Ramadi city. Study of the chemical components of the aerial parts (stem and leaves) was achieved by Gas Chromatography-Mass Spectrometer (GC-MS), the chemical study showed important variations in the content of chemical compounds and their concentration, the most quantitative chemical compounds in *Salvia multicaulis* were (9-Octadecenoic acid 20.15%, cis-Vaccenic acid 14.2% and Ethanone 9.24%), in *Salvia indica* (Methyl β-D-galactopyranoside 18.3%, 9-Octadecenoic acid 17.4%, and cis-Vaccenic acid 11.0%), in *Salvia spinosa* (Oleic acid 30.1%, 11-Octadecenoic acid 6.4% and β-Eudesmol 6.3%), in *Salvia reuterana* (9-Octadecenoic acid 19.1%, Oleic acid 13.2% and Hexadecanoic acid 6.41%) in *Salvia russellii* (N,N,N-Trimethyl-1,4- phenylenediamine 16.5%, Ethyl α-D-glucopyranoside 9.33% ، cis-Vaccenic acid 5.86%،), and in *Salvia officinalis* (Camphor 22.84%, 1-Naphthalenepropanol 20.81% and 4-Amino-3-hydroxybenzoic acid 9.64%).

1. Introduction

The *Salvia* is colloquially known as sage and is considered the largest genus in the family Lamiaceae according to [1], the Lamiaceae is well-known throughout the world for its economic significance in the food and pharmaceutical industries, there are more than 700 species of *Salvia* plants that are found worldwide [2,3], in the flora of Iraq, *Salvia* is present 33 species [4], also recorded a new species, so the number of species in Iraq is 34 [5]. The name *Salvia* means to save and is derived from the Latin word salvere. This probably refers to its healing properties, as some of its species have historically been used in traditional medicine.

Salvia is an important and useful plant, some are famous for their medicinal, aromatic, and antioxidant where they have been used as volatile compounds and aqueous extracts of some types of *Salvia* for the treatment of characteristics [6], some *Salvia* species such as *S. officinalis*, *S. viridis*, and *S. multicaulis* possess antioxidant properties sore mouth and throat, excessive sweating, indigestion, and stomach disorders [1,7].

According to [8] *Salvia* species exhibit great chemical diversity, [9] pointed out *Salvia* is chemically rich in various kinds of natural compounds, especially flavonoids, monoterpenoids, triterpenoids, polysaccharides, and phenolic acids. This may be due to the nature of *Salvia* growth, as some species of *Salvia* can be grown in gardens as ornamental plants, and some of them grow in the wild [10].

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One of the most crucial techniques for figuring out the taxonomic placements of taxa is chemotaxonomic research. Today, even individual genotypes and low and high taxonomic levels can have their chemical profiles studied [11].

The purpose of the current study was to evaluate the chemical diversity of the Salvia genus and its usefulness in the chemotaxonomic of *Salvia*.

2. Material and methods:

2.1 Plant material collection:

Samples were gathered in several locations around the Iraqi Kurdistan area: the wild grown of *Salvia* was *S. indica, S. multicaulis, S. spinosa, S. reuterana, S. russellii,* and *S. officinalis* was cultivated in Ramadi, during the months of May and June (Figure 1).

After cleaning and drying the samples at room temperature, they were identified using specimens kept in the College of Science's herbarium at the University of Baghdad for subsequent use.

2.2 Preparation of plant extract:

The samples (aerial part) were extracted at the Desert Studies Center University of Anbar, according to the method mentioned by [12]. Where the dry samples were crushed by the electric grinder, 2g of the dry powder samples were taken with 20 ml of absolute ethyl alcohol, and the samples remained for 48 hours at room temperature, then added 10 ml distilled water to each sample, and put in an Ultrasonic bath for 20min to increase the extraction efficiency, the samples were filtered with filter papers and evaporated using a hot plate at 50˚C.

2.3 Analysis of extracts by GC-MS:

The examination of Chemical compounds present in the sediment was carried out using the GC-MS device in the laboratories of Ibn Al-Baytar Center of the Ministry of Industry and Minerals, Gas Chromatograph: Agilent (7820A) USA GC Mass Spectrometer Analytical Column: Agilent HP-5ms Ultra unit (30 m length x 250 µm inner diameter x 0.25 µm film thickness), Injection volume 1 µl, Pressure 11.933 psi, GC Inlet Line Temperature: 250 ˚C, Aux heaters Temperature 300 ˚C, Carrier Gas: He 99.99%. (Figure 2).

The degree of similarity between the studied species was determined by analyzing the hierarchical cluster using the Past program (Figure 3).

3. Results and Discussion:

Table (1) shows some of the important chemical compounds that were obtained from the analysis of plant sample extracts with the (GC-MS) device.

17 chemical compounds were identified from *S. multicaulis* and *S. officinalis*, additionally, 16 chemical compounds in *S. reuterana*. The main compounds that were the most quantitative in *S. multicaulis* were 9-Octadecenoic acid 20.15%, cis-Vaccenic acid 14.2%, and Ethanone 9.24%. In addition to other chemical compounds is Hexadecanoic acid 6.74%, β-Eudesmol 5.01%, and n-Hexadecanoic acid 4.52% (Figure 4).

While in *S. officinalis* were Camphor 22.84%, 1-Naphthalenepropanol 20.81%, and 4-Amino-3-hydroxybenzoic acid 9.64%, as well as other important chemicals were Viridiflorol 5.37%, phenol 3.95%, and Eucalyptol 3.37% (Figure 5). As well in *S. reuterana* were 9-Octadecenoic acid 19.1%, Oleic acid 13.2%, and Hexadecanoic acid 6.41%, additionally, some significant chemicals were 3-Buten-1-ol 6.29%, β-Eudesmol 6.0%, and Methyl stearate 4.85% (Figure 6).

In *S. spinosa* and *S. russellii*, the number of the chemical compounds have been identified as (18,22) in *S. spinosa* and *S. russellii* respectively. The main compounds that were the most quantitative in *S. spinosa* were Oleic acid 30.1%, 11-Octadecenoic acid 6.4%, and β-Eudesmol 6.3%, less of which was Ethyl α-D-glucopyranoside 5.3%, 5-Hydroxymethylfurfural 5.0% and n-Hexadecanoic acid 4.8% (Figure 7). While in *S. russellii* were N,N,N-Trimethyl-1,4- phenylenediamine 16.5%, Ethyl α-D-glucopyranoside 9.33%, and cis-Vaccenic acid 5.86%, in addition to smaller amounts of chemical compounds were 9-Octadecenoic acid 5.68%, n-Hexadecanoic acid 4.57%, and 1,3,5-Triazine-2,4,6-triamine 4.31% (Figure 8).

In *S. indica* the number of compounds was 21, Methyl β-D-galactopyranoside 18.3%, 9-Octadecenoic acid 17.4%, and cis-Vaccenic acid 11.0%, in addition to smaller amounts of chemical compounds were n-Hexadecanoic acid 5.3%, Hexadecanoic acid 5.29% and β-Eudesmol 4.2% (Figure 9).

The results also showed the presence of chemical compounds in certain types and the absence of them in other types such as 4-Hydroxypyrimidine found only in *S. multicaulis* and *S. indica* (1.18%, 1.0%) respectively, as well as piperazine found only in *S. indica*, *S. spinosa*, and *S. russellii* (0.80%, 1.0%,1.23%) respectively, also Ethyl (trimethylsilyl)acetate found only in *S. spinosa* and *S. reuterana* (0.7%, 1.81%) respectively. Additionally, we found the 4-Cyclopentene-1,3-dione in *S. spinosa* and *S. russellii* (0.65%,1.26%) respectively. As well 2-pentadecanone found in *S. indica* and *S. officinalis* (0.9%,1.08%) respectively.

In addition, there are some chemical compounds present in only one species and not others, such as Dodecanoic acid and Hydrazinecarboxylic acid found in *S. multicaulis* only (4.13%,1.82) respectively, also 5-Methoxypyrimidine and β-D-Glucopyranose found in S. indica only (2.2%,2.8%) respectively, too, Methyl 6,11-Octadecadienoate and Methyl 9,12-heptadecadienoate found in *S. spinosa* only (3.57%,1.6%) respectively, and in S. reuterana found Trimethylhydroxylamine and D-Talonic acid lactone (2.73%,3.69%) respectively, as well, in *S. russellii* found N,N,N'-Trimethyl-1,4-phenylenediamine and Imidazole (16.5%,3.54%) respectively.

There is also what distinguishes *S. officinalis* as it contains only chemical compounds such as camphor, 1-Naphthalenepropanol, 4-Amino-3-hydroxybenzoic acid, and Viridiflorol (22.8%, 20.81%,9.64%,5.37%) respectively.

Depending on the presence of chemical compounds between the studied species, a diagram was drawn showing the degree of convergence and similarity between the studied species in Figure (3), where it was divided into three clusters: the first cluster (*S. multicaulis, S. indica,* and *S. russellii*), the second cluster (*S. spinosa* and *S. reuterana*) and finally the third cluster (*S. officinalis*).

Chemical characteristics have been adopted in the diagnosis of plant families, genera, and species, such as the presence or absence of various chemical compounds that were important taxonomic characteristics in the diagnosis of genus and species, and from these studies [13,14,15]. This study showed clear and important variations in the content of chemical compounds and their concentration, in all samples, it was found that n-Hexadecanoic acid was the dominant component, but in a varying percentage between the studied species. some chemical compounds appeared in one species only and did not appear in the other species, so they may be the distinguishing signs of this type, and we believe that it is possible to rely on them in distinguishing morphologically similar species.

In addition, the species of the genus *Salvia*, especially the most famous *S. officinalis*, have been proven to possess a wide range of biological and pharmaceutical substances and have antimicrobial activity for many bacteria and fungi [16,17,18,19].

4. Conclusion

we have significant differences in terms of the presence or absence of different chemical compounds between species, which indicates that it is possible to rely on these results to support the classification between species, especially as there is a morphological similarity between the *S. reuterana* and *S. spinosa*.

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عزل وتحديد التركيب الكيميائي لأنواع *Salvia* التي تنمو في العراق بواسطة GC-MS

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الخلاصة:

في هذا البحث تم دراسة التركيب الكيميائي لستة أنواع من نبات *Salvia* ، وهي خمسة أنواع تنمو بريا (*S. multicaulis*، *S. indica*، *S. spinosa*، *S. reuterana* و*S. russellii*) جمعت من مناطق مختلفة في إقليم كردستان ، ونوع مستزرع هو (*S. officinalis*) جمع من مدينة الرمادي . تم دراسة المكونات الكيميائية للأجزاء الهوائية (الساق والأوراق) بواسطة GC-MS ، أظهرت الدراسة الكيميائية اختلافات واضحة وهامة في محتواها من المركبات الكيميائية وتركيزها ، وكانت المركبات الكيميائية الأكثر كمية في *S. multicaulis* (9-Octadecenoic acid 20.15%، cis-Vaccenic acid 14.2%، Ethanone 9.24%) وفي *S. indica* (Methyl β-D-galactopyranoside 18.3%، cis-9-Octadecenoic acid 17.4%، Oleic acid 30.1%) وفي *S. spinosa* ، (Vaccenic acid 11.0%) وفي ، (β-Eudesmol 6.3%، 11-Octadecenoic acid 6.4%) وفي *S. reuterana* (9-Octadecenoic acid 19.1% ، Oleic acid 13.2% ، Hexadecanoic acid 6.41%) وفي *S. russellii* (cis-Vaccenic acid 5.86%، Ethyl α-D-glucopyranoside 9.33%، N,N,N-Trimethyl-1,4- phenylenediamine 16.5%) وفي *S. officinalis* (Camphor 22.84% ، 1-Naphthalenepropanol 20.81% ، 4-amino-3-hydroxybenzoic acid 9.64%).

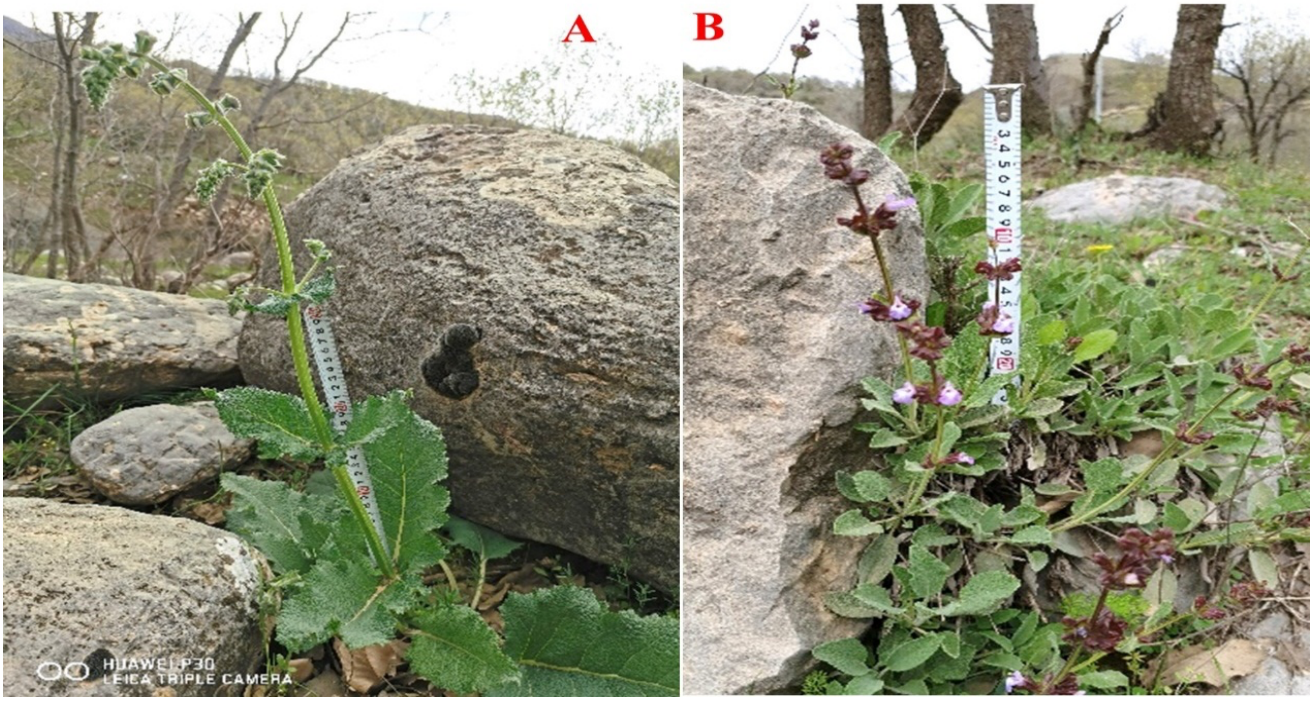

Figure (1): *Salvia* species, A= *S. indica* (Erbil-Mergasor), B= *S. multicaulis* (Erbil-Shaqlawa)

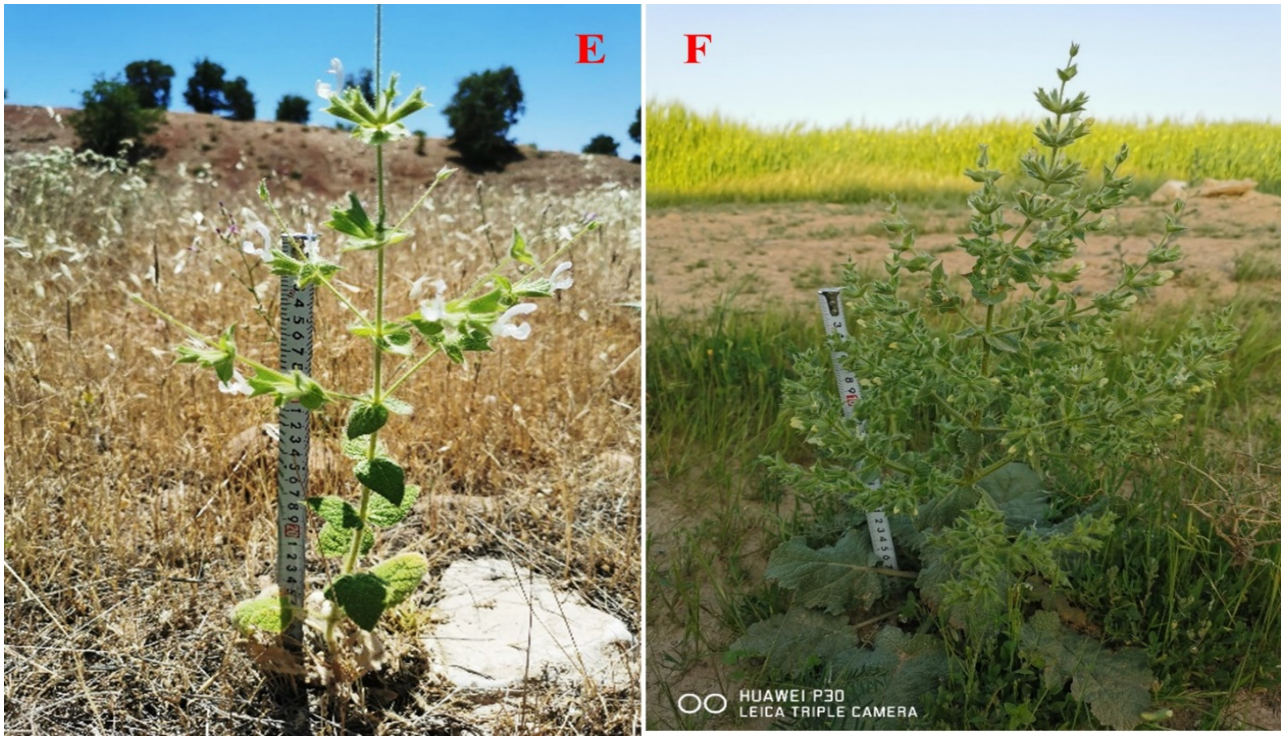

Figure (1): continued: *Salvia* species, E= *S. reuterana* (Sulaymaniyah-Dokan), F = *S. spinosa* (Sulaymaniyah- Qaradagh)

Figure (1): continued: *Salvia* species, C= *S. officinalis* (Ramadi), D= *S. russellii* (Dohuk-Sarsang),

Figure (2): GC-Mass chromatography apparatus

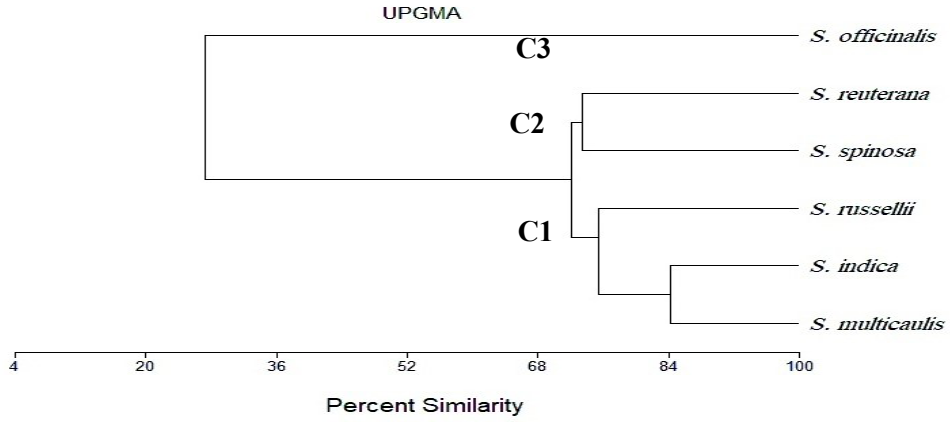

Figure (3): Dendrogram obtained from the agglomerative hierarchical cluster analysis of *Salvia* Species

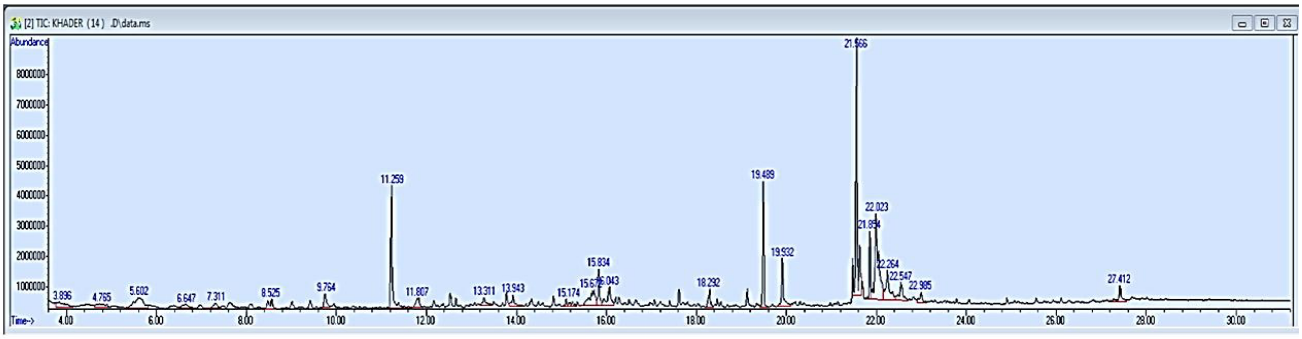

Figure (4) GC-MS chromatogram of *S. multicaulis*

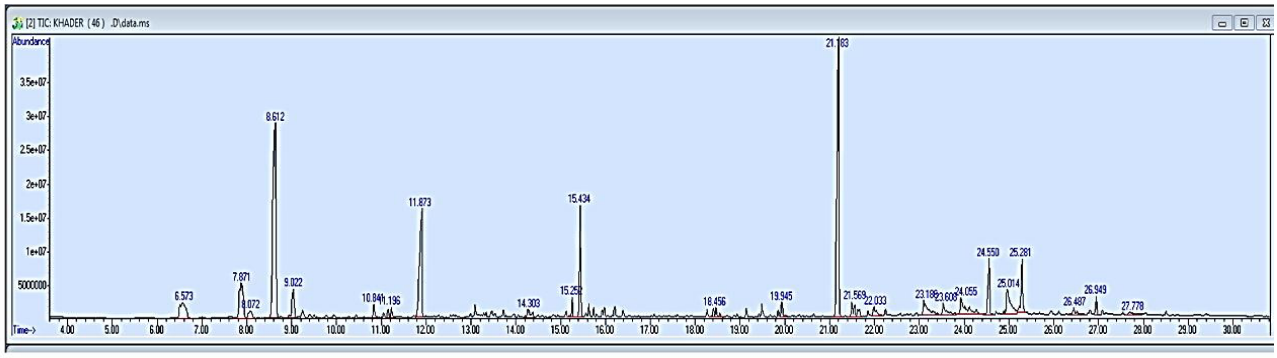

Figure (5) GC-MS chromatogram of *S. officinalis*

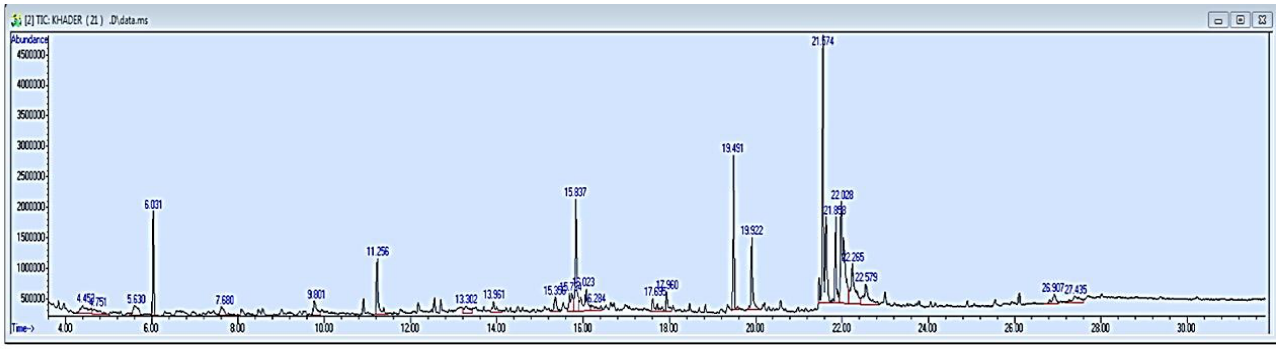

Figure (6) GC-MS chromatogram of *S. reuterana*

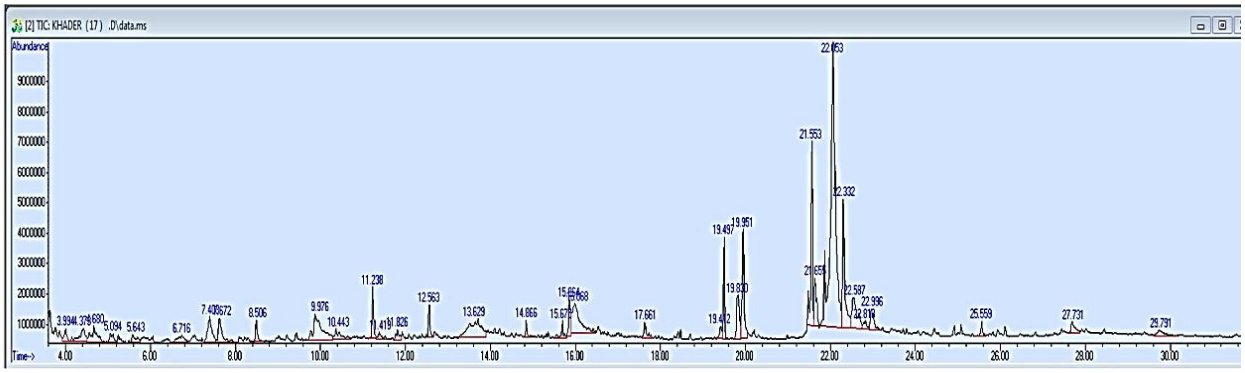

Figure (7) GC-MS chromatogram of *S. spinosa*

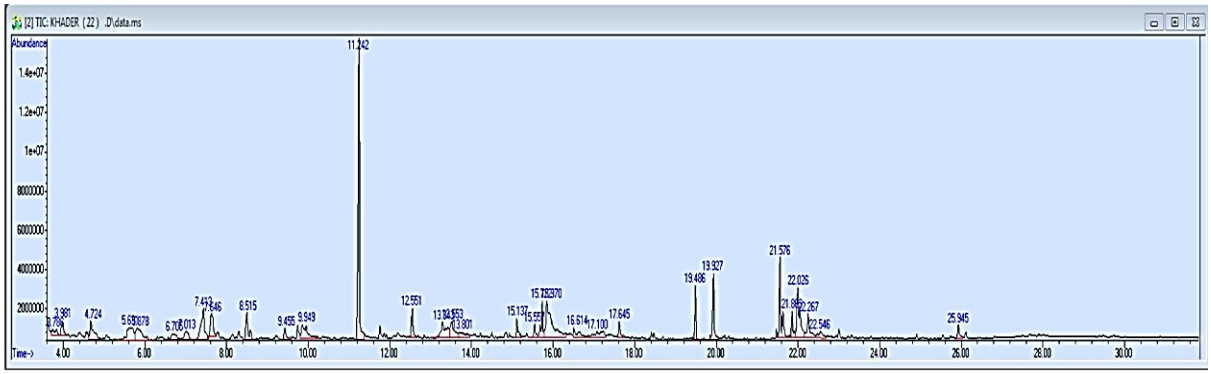

Figure (8) GC-MS chromatogram of *S. russellii*

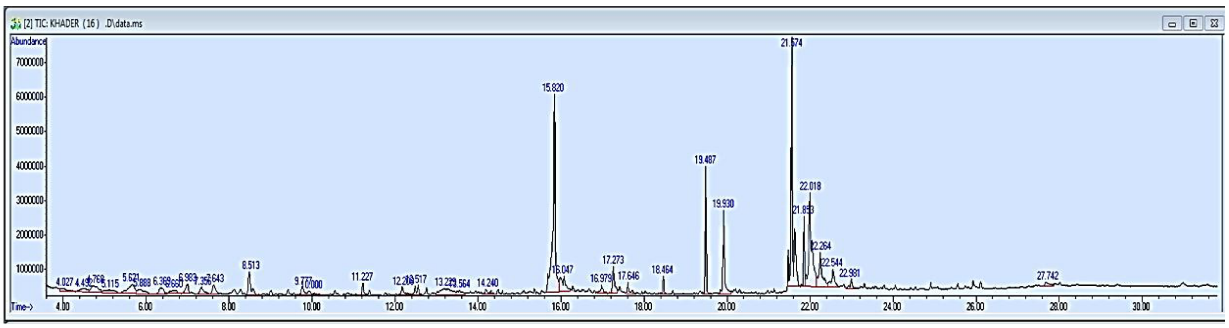

Figure (9) GC-MS chromatogram of *S. indica*

Table (1): Chemical composition of aerial part of *Salvia* species as revealed by Gas Chromatography-Mass Spectrometry (GC-MS)

Title	Chemical compound	Retention time	*S. multicaulis*	*S. indica*	*S. spinosa*	*S. reuterana*	*S. russellii*	*S. offianalis*
			Area %	Area %	Area %	Area %	Area %	Area %
1.	Hydrazinecarboxylic acid	3.8	1.82					
2.	4-Cyclopentene-1,3-dione	3.9			0.65		1.26	
3.	Glyceraldehyde	4.02		0.8				
4.	1,2-Hydrazinedicarboxaldehyde	4.45				2.01	1.0	
5.	2-Cyclopenten-1-one	4.76	1.22	1.7	2.1		2.61	
6.	Benzaldehyde	5.29						
7.	Ethyl (trimethylsilyl)acetate	5.64			0.7	1.81		
8.	Ethyl(dimethyl)ethoxysilane	5.63	4.36					
9.	Succindialdehyde	5.88		1.4				
10.	Trolamine	6.03				4.11		
11.	1H-Pyrrole-1-ethanamine	6.37		1.2				
12.	Eucalyptol	6.57						3.37
13.	Piperazine	6.71		0.8	1		1.23	
14.	4-Hydroxypyrimidine	7.35	1.18	1				
15.	Methyl 2-furoate	7.36						
16.	1,3,5-Triazine-2,4,6-triamine	7.41					4.31	
17.	1,3-Propanediamine	7.64					2.83	
18.	Benzoic acid	7.68				1.55		
19.	Thujone	8.07						1.04
20.	Succinimide	8.32						
21.	4H-Pyran-4-one	8.51	1.8	2.3	0.9		2.42	
22.	Camphor	8.61						22.84
23.	Bicyclo[2.2.1]heptan-2-ol	9.02						2.53
24.	Catechol	9.45					1.09	
25.	Benzofuran	9.78	1.67	0.8		1.87		
26.	5-Hydroxymethylfurfural	9.97			5			
27.	Bornyl acetate	10.83						0.88
28.	Ethanone	11.23	9.24	0.7	1.6	3.85		
29.	N,N,N'-Trimethyl-1,4-phenylenediamine	11.24					16.5	
30.	Eugenol	11.85						
31.	4-Amino-3-hydroxybenzoic acid	11.87						9.64
32.	Naphthalene	12.55					1.81	
33.	β-D-Glucopyranose	13.23		2.8				

34.	Imidazole	13.36					3.54	
35.	Cyclohexanol	15.14					1.45	
36.	Caryophyllene oxide	15.25						0.81
37.	Viridiflorol	15.43						5.37
38.	Dodecanoic acid	15.67	4.13					
39.	Megastigmatrienone	15.72					2.7	
40.	Trimethylhydroxylamine	15.73				2.73		
41.	Ethyl α-D-glucopyranoside	15.91			5.3		9.33	
42.	Methyl β-D-galactopyranoside	15.82		18.3				
43.	3-Buten-1-ol	15.83	2.44			6.29		
44.	Decanoic acid	15.86						
45.	D-Talonic acid lactone	16.02				3.69		
46.	Trehalose	16.04		2.9				
47.	1H-Indole, 3-methyl	16.1	2.92					
48.	5-Methoxypyrimidine	17.27		2.2				
49.	Cyclohexanone	17.64					1.18	
50.	2-Pentadecanone	18.46		0.9				1.08
51.	Hexadecanoic acid	19.41	6.74	5.29	2.5	6.41	2.23	
52.	Pentadecanoic acid	19.83			2.8			
53.	n-Hexadecanoic acid	19.93	4.52	5.3	4.8	4.73	4.57	0.87
54.	1-Naphthalenepropanol	21.18						20.81
55.	11-Octadecenoic acid	21.55			6.4			2.02
56.	9-Octadecenoic acid	21.57	20.15	17.4	1.98	19.1	5.68	
57.	Methyl stearate	21.85	3.79	3		4.85	1.74	
58.	cis-Vaccenic acid	22.03	14.2	11			5.86	
59.	Oleic acid	22.05			30.1	13.2		1.15
60.	β-Eudesmol	22.27	5.01	4.2	6.3	6.04	2.63	
61.	9,12-Octadecadienoic acid	22.54	2.99	2.2			1.29	
62.	2(3H)-Oxazolone	22.57				3.93		
63.	Ethyl 6,9,12-hexadecatrienoate	22.57						
64.	Methyl 6,11-octadecadienoate	22.58			3.57			
65.	Methyl 9,12-heptadecadienoate	22.99			1.6			
66.	Trimethylsilylestrone	24.05						3.22
67.	Phenol	25.01						3.95
68.	5-Pregnen-3β-ol-20-one	25.28						3.18
69.	2-Phenanthrenol	26.94						0.95
70.	13-Octadecenal	27.73			0.91			
71.	B-Sitosterol	29.72						
Total			17	21	18	16	22	17