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Chemical comparison and Trichomes types of *Salvia* species growing in Anbar Governorate – Iraq

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ABSTRACT

The trichomes and chemical composition of three species of the genus *Salvia* wild-grown (*Salvia lanigera*, *Salvia spinosa*) and cultured (*Salvia officinalis*) were studied in the Anbar governate, the chemical components of the stem and leaves were studied by Gas chromatography–mass spectrometry(GC-MS), in addition to studying the trichomes of the epidermis in the stem and leaves (upper and lower epidermis) by Light microscope. Important differences appeared to us in the chemical study, where it was found that some compounds were found in species without others, which gives them taxonomic importance, also, the trichomes were important in distinguishing the studied species, the species *S. spinosa* was distinguished by the presence of glandular trichome with funnel form head on the lower epidermis of the leaves and absence non-glandular multicellular-uniseriate flagellated trichomes. *S. officinalis* was distinguished by the density of non-glandular multicellular-uniseriate –flagellated trichomes, while *S. lanigera* was distinguished by non-glandular multicellular-uniseriate –branched trichomes, addition to the presence non-glandular multicellular-uniseriate – straight, curved and glandular trichome with globular head in all studied species.

مقارنة كيميائية وأنواع شعيرات البشرة لأنواع *Salvia* النامية في محافظة الأنبار – العراق

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الملخص

تمت دراسة التركيب الكيميائي وشعيرات البشرة لثلاثة أنواع من جنس *Salvia* التي تنمو بريا(*S. lanigera* ، *S. spinosa*) ونوع مستزرع (*S. officinalis*) في محافظة الانبار، تمت دراسة المكونات الكيميائية للأجزاء الهوائية (الساق والأوراق) باستخدام جهاز GC-MS ، إضافة الى دراسة الشعيرات في بشرة الساق وبشرة الأوراق (العليا والسفلى) بواسطة المجهر الضوئي. ظهرت لنا اختلافات مهمة في الدراسة الكيميائية ، حيث وجد أن بعض المركبات موجودة في أنواع دون غيرها مما يعطيها أهمية تصنيفية ، كما أن شعيرات البشرة كانت مهمة في تمييز الأنواع المدروسة ،، حيث تميز النوع *S. spinosa* بوجود شعيرات غدية متعددة الخلايا ذات راس قمعي الشكل على البشرة السفلى للأوراق ، و *S. officinalis*

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تميزت بكثافة وجود الشعيرات غير الغدية السوطية متعددة الخلايا ، بينما في *S. lanigera* تميزت بوجود الشعيرات غير الغدية المتفرعة متعددة الخلايا ، إضافة الى وجود الشعيرات غير الغدية المستقيمة والمنحنية متعددة الخلايا والشعيرات الغدية ذات الراس الكروي في جميع الأنواع المدروسة.

Introduction

Salvia L. is the largest member of the Lamiaceae family and contains many species, about 700 species, distributed in different places around the world, *Salvia* L. is sometimes known as sage, its name comes from the word salvere in Latin, which means saving, this probably refers to its healing properties. [1,2,3], in Iraq's flora, *Salvia* is present in 33 species[4].

Salvia is used as a culinary ingredient, herbal tea, and spice, in addition to its medicinal value [5,6]. *Salvia* species are frequently planted as decorative plants in gardens and parks [7].

Most of the aromatic genera are present in the family Lamiaceae, including the subfamily Nepetoideae, which includes the genus *Salvia*, which contains the largest number of trichomes in this family [8,9].

Trichomes are external growths or appendages or extensions to the outside that arise from the cells of the epidermis, they differ in their shapes, structure, and function. They may take the form of papillae, hairs, or scales. Hairs are of different types, including unicellular hairs, or they may be multicellular, and they are of two types, either multicellular, non-glandular, or multicellular. Glandular cells are characterized by a glandular head at the end of the filaments, in addition to other types, including plat filaments or branched multicellular filaments, Trichomes can protect plant buds from insect harm, lower leaf temperature, boost light reflection, stop water loss, and lessen leaf abrasion [10].

The essential oil that glandular hairs produce is one of the Lamiaceae family's most distinguishing features, and two basic forms of glandular hair capitate and peltate, which may be separated by stalk length and head size, are present in practically all species under study [11], additionally, it's crucial for the pharmaceutical, cosmetic sectors, flavoring and fragrance [12,13].

Salvia has many types and numbers of glandular hairs, these hairs might be a location of secondary metabolite production and play an important role in how plants interact with herbivorous and pathogenic creatures [8,14].

[15] pointed out the taxonomic importance of epidermal hairs (glandular trichomes) in distinguishing between types of *Salvia* in their study of forty-six species in Iran, additionally, they considered that the trichome features to be systemically significant, trichome characteristics differ between *Salvia* species, even when they are placed in the same section, but they are consistent amongst different populations of the same species.

The chemical compounds that are produced and stored in various glandular trichomes may be used as dependable taxonomic signs to determine taxonomy [16]. These substances are very significant since some of the chemical and essential oils from plants in this genus have demonstrated remarkable antibacterial and antioxidant properties, as a result, these plants' extracts are frequently employed in traditional medicine [17,18].

It is possible to identify medicinal plants chemotaxonomically with the use of intricate morphological and phytochemical analyses that are conducted concurrently, where [19] referred to the phytochemical may be effectively utilized to analyze the relationships between *Salvia* species.

The aim of this research is to study the chemical components and epidermal hair types of three species of *Salvia* growing in Anbar Governorate and to compare the wild species (*S. spinosa*, *S. lanigera*) with the cultured (*S. officinalis*) that is widely used in traditional medicine.

Materials and methods

1- Plant collected

Samples were collected from different places in the Anbar governorate of Iraq: the wild-grown of *Salvia* were *S. spinosa* (collected from Haditha), *S. lanigera* (collected from Alzawia village), and the cultured *Salvia* was *S. officinalis* (collected from Ramadi), during the month of June (Figure 1). The samples were cleaned and dried at room temperature for later use according to the method of [20], the species was diagnosed based on the species preserved in the herbarium of the College of Science - University of Baghdad.

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Fig. 1: *Salvia* species, A= *S. spinosa* (Anbar-Haditha), B= *S. lanigera* (Anbar-Alzawia village), C= *S. officinalis* (Anbar-Ramadi)

2- Plant extract and Chemical study

The stem and leaf samples were taken for each species and extracted at the Desert-Studies Center - University of Anbar, according to the method mentioned by [21], after crushing, 2g of the dry powder samples were taken, with 20ml of absolute ethyl alcohol, and the samples remained for 48hours at room temperature, then added 10ml 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, identify chemical compounds present in the sediment by using the GC-MS device in the laboratories of Ibn AlBaytar Center in Ministry of Industry and Minerals (Gas Chromatograph:Agilent(7820A) USA, GC-MS Spectrometer Analytical Column : Agilent HP - 5ms Ultra unit(30m length x250µm inner diameter x0.25µm film thickness.Injection volume 1µl, Pressure 11.933psi , GC-Inlet Line Temperature:250 ˚C .Aux heaters Temperature 300˚C Carrier Gas:He 99.99%).

By utilizing the Past software to analyze the hierarchical cluster, it was possible to assess how similar the investigated species were to one another.

3- Study of trichomes

The trichomes were studied based on samples collected from field trips after drying them, the stem and leaf (lower and upper Epidermis) samples were taken for each species and put in a beaker containing lactic acid at a concentration of 3%, then put the baker on the hot plate to reach the boiling point, then the plant sample is taken by forceps and placed on a clean slide under a dissection microscope, then the epidermis is flayed using a fine needle and forceps and the epidermis is placed on another clean slide

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[22]. The safranin stain is applied [23], then the sample is covered with a cover slide and the information about the sample is written on the tip of the slide and placed in the slide case until it is examined and the trichomes are photographed using a light microscope with a camera.

Results and discussion

In this study, Table (1) lists significant chemical compounds discovered by using the GC-MS to analyze plant sample extracts.

The results showed that there (15,17,18) chemical compounds were identified from *S. spinosa*, *S. officinalis*, and *S. lanigera* respectively, the substances with the highest quantitative levels in *S. spinosa* were 11-Octadecenoic acid 19.13%, Cis-Vaccenic acid 14.79% and Decanoic acid 14.34%, less of it was Hexadecanoic acid 7.07%, Octadecanoic acid 5.78% and n-Hexadecanoic acid 4.35% (Figure 2). As well as in *S. officinalis* were Camphor 22.8 % ,1 -Naphthalenepropanol 20.8 % ,and 4- Amino- 3-hydroxybenzoic acid 9.6 %, together with additional significant substances Viridiflorol 5.3 % ,phenol 3.9 % ,and Eucalyptol 3.3 %(Figure 3). While in *S. lanigera* were Ethanone 16.64%, 11 - Octadecenoic acid 15.83%, and Hexanoic acid 12.34%, in addition to founding Cis-Vaccenic acid 9.49%,Hexadecanoic acid 5.26%, and Trehalose 4.26% (Figure 4).

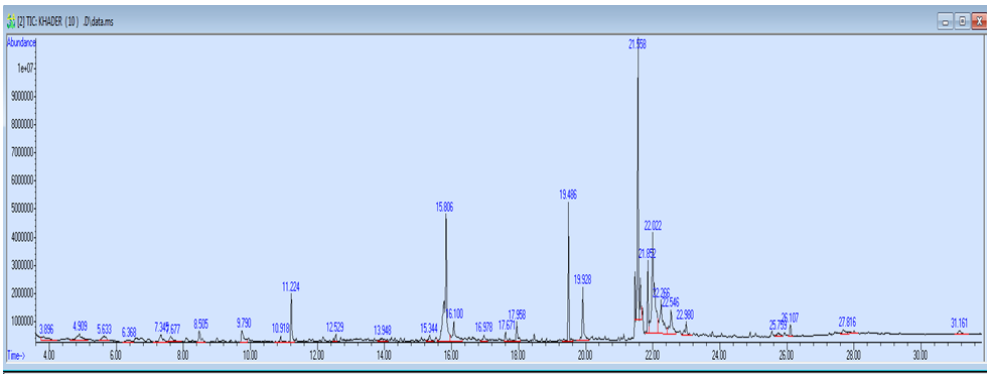

Fig. 2: GC-MS chromatogram of *S. spinosa*

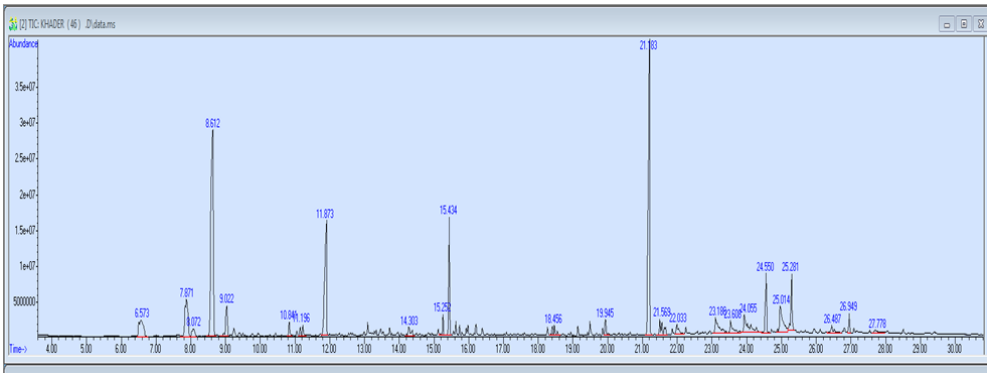

Fig. 3: GC-MS chromatogram of *S. officinalis*

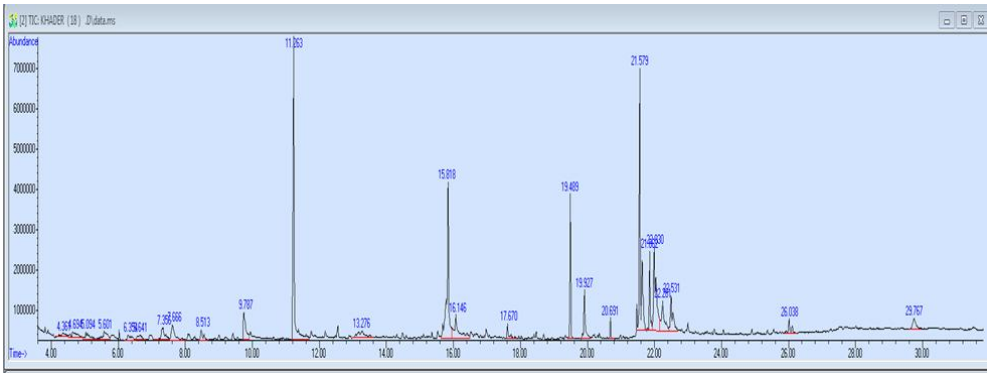

Fig. 4: GC-MS chromatogram of *S. lanigera*

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

Title	Chemical compound	Retention Time	*spinosa*	*lanigera*	*officinalis*
			Area %	Area %	Area %
1.	1,2-Hydrazinedicarboxaldehyde	3.9	1.17		
2.	Glyceraldehyde	4.36		0.88	
3.	2-Cyclopenten-1-one	4.67		1.52	
4.	Ethyl (trimethylsilyl)acetate	5.64	1.12		
5.	Tetrahydro-3-furanmethanol	6.3	0.68		
6.	Eucalyptol	6.57			3.3
7.	Benzoic acid	7.65	1.13	2.32	
8.	Thujone	8.07			1.0
9.	4H-Pyran-4-one	8.50	1.3	1.06	
10.	Camphor	8.61			22.8
11.	Bicyclo[2.2.1]heptan-2-ol	9.02			2.5
12.	Benzofuran	9.76	1.73	3.01	
13.	Bornyl acetate	10.83			0.8
14.	Ethanone	11.23		16.64	
15.	4-Amino-3-hydroxybenzoic acid	11.87			9.6
16.	2-Penten-1-ol	13.27		2.21	
17.	Caryophyllene oxide	15.25			0.8
18.	Viridiflorol	15.43			5.3
19.	Decanoic acid	15.8	14.34		
20.	Hexanoic acid	15.82		12.34	
21.	Trehalose	16.14		4.26	
22.	Cyclohexane	17.66		1.16	
23.	trans-2-Dodecenoic acid	17.95	1.57		
24.	2-Pentadecanone	18.45			1.0
25.	Hexadecanoic acid	19.50	7.07	5.26	
26.	n-Hexadecanoic acid	19.95	4.35	3.16	0.8
27.	1,6,10-Dodecatrien-3-ol	20.68		0.82	
28.	1-Naphthalenepropanol	21.18			20.8
29.	11-Octadecenoic acid	21.55	19.13	15.83	2.0
30.	Methyl stearate	21.85	3.6	3.62	
31.	Cis-Vaccenic acid	22	14.79	9.49	
32.	Oleic Acid	22.05			1.1
33.	Octadecanoic acid	22.33	5.78	3.8	
34.	9,12-Octadecadienoic acid	22.53	3.59	3.44	
35.	Trimethylsilylestrone	24.05			3.2
36.	Phenol	25.01			3.9
37.	5-Pregnen-3.beta.-ol-20-one	25.28			3.1
38.	2-Phenanthrenol	26.94			0.9

The findings also revealed that some types of chemical compounds were present in some species while others were not, in *S. spinosa* and *S. lanigera* found Benzoic acid, 4H-Pyran-4-one, Benzofuran, Hexadecanoic acid, Methyl stearate, Cis-Vaccenic acid and Octadecanoic acid (1.13%, 2.32%, 1.3%, 1.06%, 1.73%, 3.01, 7.07%, 5.26%, 3.6%, 3.62%, 14.79%, 9.49%, 5.78%, 3.8%) respectively.

Additionally, certain chemical components are exclusive to one species and absent from others, such as 1,2-Hydrazinedicarboxaldehyde (1.17%), Ethyl (trimethylsilyl)acetate (1.12%), Decanoic acid (14.34%) and trans-2-Dodecenoic acid (1.57%) found only in *S. spinosa*. Also, in *S. lanigera* found only Glyceraldehyde (0.88%), 2-Cyclopenten-1-one (1.52%), Ethanone (16.64%), 2-Penten-1-ol (2.21%), Hexanoic acid (12.34%), Trehalose (4.26%) and Cyclohexane (1.16%).

S. officinalis is particularly unique in that it solely includes chemical components such as thujone, viridiflorol, camphor, 4-Amino-3-hydroxybenzoic acid, eucalyptol, and oleic acid (1.0%, 5.37%, 22.8%, 9.6%,3.3%,1.15%) respectively.

Additionally, there are some chemical compounds present in all study species such as n-Hexadecanoic acid and 11-Octadecenoic acid.

The difference in the presence of chemical compounds between the studied species may give taxonomic importance to distinguishing between similar species, in addition to that some compounds

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have medicinal importance, such as Octadecenoic acid., which was mentioned by many researchers as an anti-inflammatory agent [24,25].

A diagram was created to indicate the level of similarity between the species in accordance with the existence of chemical compounds between the species (Figure 5), which was divided into two clusters: the first cluster (*spinosa* and *lanigera*), the second cluster (*officinalis*)

In our study, the stems and leaves of the three species have diverse glandular and non-glandular hairs, to illustrate the types of trichomes, we drew them by hand for all the studied species (Figure 6,7,8).

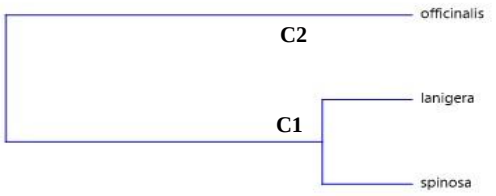

Fig. 5: Dendrogram obtained from the agglomerative hierarchical cluster analysis of *Salvia* Species

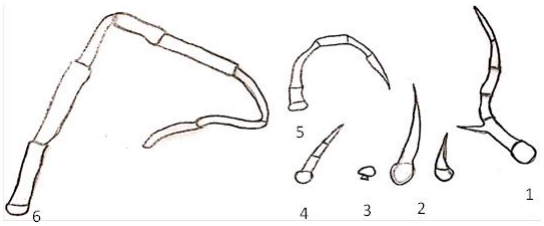

Fig. 6: The *Salvia lanigera*. trichomes in leaves and stem

1- non-glandular multicellular-uniseriate –branched trichomes
2- non-glandular unicellular trichomes
3- glandular trichome with globular head
4-non-glandular multicellular-uniseriate –straight trichomes
5- non-glandular multicellular-uniseriate –curved trichomes
6- non-glandular multicellular-uniseriate –flagellated trichomes

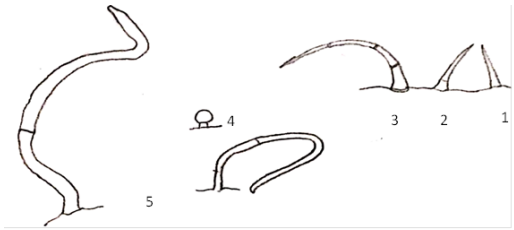

Fig. 7: The *Salvia officinalis*. trichomes in leaves and stem

1- non-glandular unicellular trichomes
2- non-glandular bicellular- trichomes
3- non-glandular multicellular-uniseriate –curved trichomes
4- non-glandular multicellular-uniseriate –flagellated trichomes
5- glandular trichome with globular head

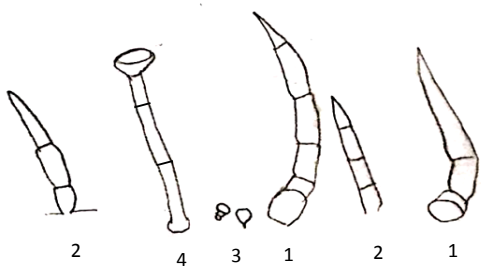

Fig. 8: The *Salvia spinosa*. trichomes in leaves and stem

1- non-glandular multicellular-uniseriate – curved trichomes
2- non-glandular multicellular-uniseriate – straight trichomes
3- glandular trichome with globular head
4- glandular trichome with funnel form head

Generally, our study showed the presence of non-glandular multicellular-uniseriate straight, curved and glandular trichome with globular heads in leaves and stems for all studied species, as well as present the non-glandular multicellular-uniseriate flagellated trichomes in *S. lanigera*, *S. officinalis* and their absence in *S. spinosa*. While non-glandular multicellular-uniseriate –branched trichomes were a distinctive feature of the *S. lanigera* (Figure 9,10,11).

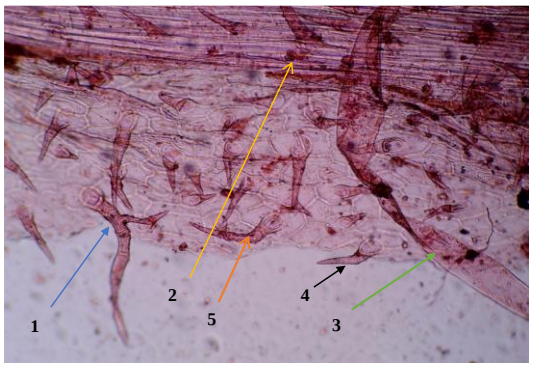

Fig. 9: The Light microscope slide of leaves *Salvia lanigera*. Upper epidermis. (x10)

1- non-glandular multicellular-uniseriate –branched trichomes
2- glandular trichome with globular head
3- non-glandular multicellular-uniseriate –flagellated trichomes
4- non-glandular unicellular trichomes
5- non-glandular multicellular-uniseriate-curved

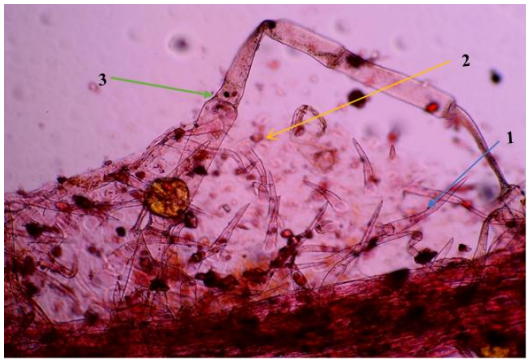

Fig. 10: The Light microscope slide of leaves *Salvia lanigera*. Lower epidermis. (x10)

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1- non-glandular multicellular-uniseriate –straight trichomes
2- glandular trichome with globular head
3- non-glandular multicellular-uniseriate –flagellated trichomes

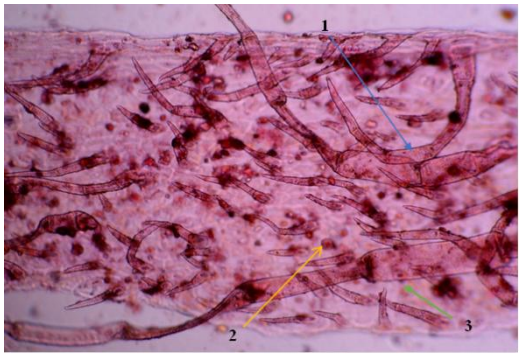

Fig. 11: The Light microscope slide of stem *Salvia lanigera*. (x10)

1- non-glandular multicellular-uniseriate –curved trichomes
2- glandular trichome with globular head
3- non-glandular multicellular-uniseriate –flagellated trichomes

In *S. officinalis* the non-glandular multicellular-uniseriate flagellated trichomes were dense in the leaves on both surfaces and stem, in comparison with *S. lanigera* while their absences in *S. spinosa* (Figure 12,13,14).

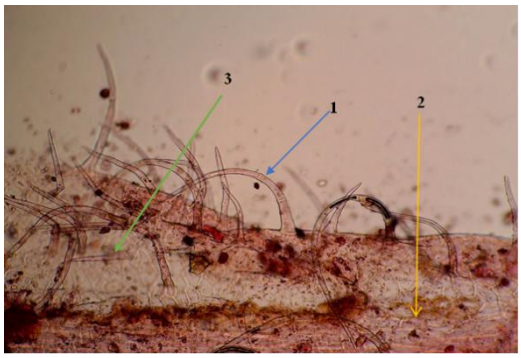

Fig. 12: The Light microscope slide of leaves *Salvia officinalis*. Upper epidermis (x10)

1- non-glandular multicellular-uniseriate –curved trichomes
2- glandular trichome with globular head
3- non-glandular bicellular- trichomes

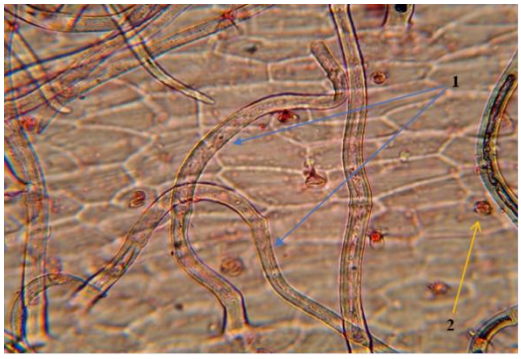

Fig. 13: The Light microscope slide of leaves *Salvia officinalis*. Lower epidermis (x40)

1- non-glandular multicellular-uniseriate –flagellated trichomes
2- glandular trichome with globular head

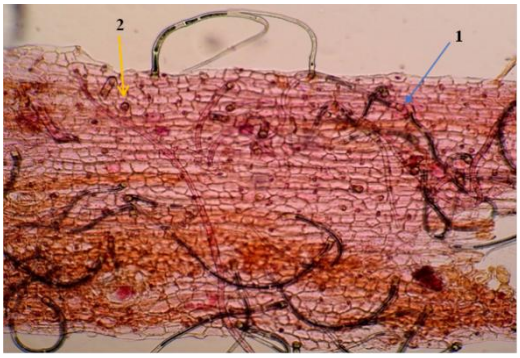

Fig. 14: The Light microscope slide of stem *Salvia officinalis*. (x10)

1- non-glandular multicellular-uniseriate –flagellated trichomes
2- glandular trichome with globular head

S. spinosa are distinguished by the presence of glandular trichome with funnel form head in the lower epidermis (Figure 15,16,17), this may be due to this species having rather large leaves and its ability to grow in drier environments compared to other species, the glandular trichome (peltate) is thought to be the location of essential oil synthesis and storage, whereas the glandular trichome (capitate) mostly hold carbohydrates and a smaller amount of essential oils [26,27].

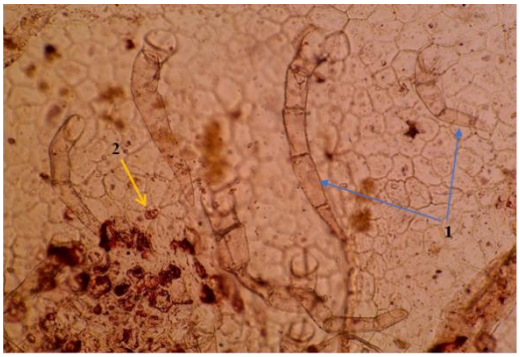

Fig. 15: The Light microscope slide of leaves *Salvia spinosa*. Upper epidermis (x10)

1- non-glandular multicellular-uniseriate – curved trichomes
2- glandular trichome with globular head

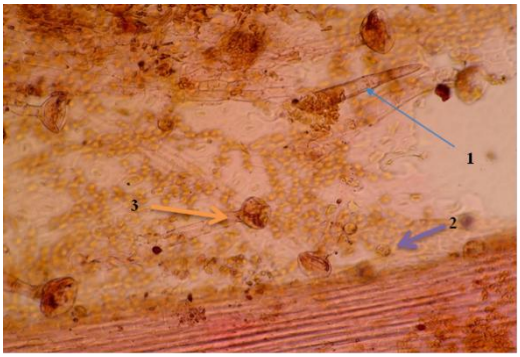

Fig. 16: The Light microscope slide of leaves *Salvia spinosa*. Lower epidermis (x10)

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1- non-glandular multicellular-uniseriate – straight trichomes
2- glandular trichome with globular head
3- glandular trichome with funnel form head

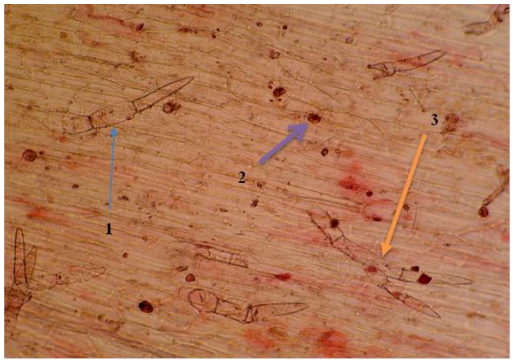

Fig. 17: The Light microscope slide of stem *Salvia spinosa*. (x10)
1- non-glandular multicellular-uniseriate – straight trichomes
2- glandular trichome with globular head
3- non-glandular multicellular-uniseriate – curved trichomes

Conclusion and recommendations

Our current study showed the presence of various chemical compounds, some of which are present in one species without the other, and this could give a distinctive characteristic that supports the morphological classification, and there are compounds present in more than one species, and this also could give an idea about the similarity of these types in appearance or in the environment.

And some compounds have medical importance, as mentioned by some research [24, 25], so we recommend studying the species that contain these compounds more to find out the effect of their extracts on microorganisms in the laboratory.

Also, the presence of trichomes was varied and gives a distinctive characteristic to the studied species, *S. lanigera* was distinguished by the presence of non-glandular multicellular-uniseriate –branched trichomes, additionally to the presence of non-glandular multicellular - uniseriate – flagellated trichomes in *S. lanigera* and *S. officinalis* and their absence in *S. spinosa*. While *S. spinosa* was distinguished by the presence of glandular trichome with funnel form head in the lower epidermis for leaves. We recommend studying the trichomes in other parts of the plant, such as the flower, and comparing them with the trichomes in the stem and leaves.

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