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Morphology, Anatomy and Chemical Composition of *Salvia fruticosa* Mill in Al-Marj (Libya)

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الملخص

درست الصفات المورفولوجية والتشريحية والكيميائية لنبات الميرمية اليوناني *Salvia ffruticosa* Mill النامي بريا في ليبيا. الهدف من الدراسة الحالية لتزويد صفات جديدة لتعريف ووصف هذا النوع. تعد صفات مورفولوجيا الورقة وعدد النورات اللولبية والقنابة والكأس والتويج والاسدية والشعيرات وصفوف الكولنشيمي والجزمة الوعائية وبنية النسيج الوسطي للورقة من الصفات المهمة لتمييز هذا النوع. لوحظت أنواع عديدة ومختلفة من الشعيرات الغدية. يمكن أن تزود نتائج دراسة تعرق الورقة معلومات دقيقة جدا لتعريف هذا النبات. احتوى الزيت العطري على السينيول (43.93%)، البتا-بينين (11,43%)، الفا-بينين (9.53%)، بيتا-ميرسين (9.08%)، والكاريوفيلين(8.75%)، كافور(5.12%) والفا- تيربينول(4.15%)..

الكلمات المفتاحية: *Salvia fruticosa*، الصفات المورفولوجيا والتشريحية، الشعيرات، الزيوت الطيارة، ليبيا.

Abstract

Macro, micromorphological and chemical characteristics of *Salvia fruticosa* Mill, wild growing in Libya were studied. The aim of the present study is to provide new properties for the identification and description of this species. The features of leaf morphology, verticillaster number, bract, calyx, corolla, stamens, trichomes, collenchyma's rows, vascular bundle and mesophyll structure are important characters to distinguish this species. In the study, we observe morphologically different types of glandular trichomes. The results of leaf architecture studies can provide a more accurate basis for the identification of this species. The essential oil contained cineol (43.93%), β-pinene (11,43%), α-pinene (9.53%), β-myrcene (9.08%), caryophyllene (8.75%), camphor (5.12%) and α-terpineol (4.15%).

Keywords: *Salvia Fruticosa*, Morphological & Anatomical Characteristics, Trichomes & Essential Oil, Libya.

1. INTRODUCTION

Salvia is the largest genus of the Lamiaceae family and includes about 900 species distributed worldwide [1]. In Libya, it is represented by 10 species; 3 of which are cultivated [2]. Many species of *Salvia* are used as herbal tea and in food, cosmetics, perfumery and the pharmaceutical industry, and other species are grown in gardens as ornamental plants [3]. The essential oils and extracts of *Salvia* species have shown antimicrobial, antioxidant, antidiabetic, antitumor, antiplasmodial and anti-inflammatory activities [4,5,6,7,8,9,10].

Ettingshausen et al. [11] have made the first comprehensive effort to systematize the description of the vegetative architecture of leaves with their classification of venation patterns. The architectural characteristics of leaves have proved valuable taxonomic and systematic data both in fossil and living plants [12,13,14]. Leaf architectural characters and venation patterns studied in different families of dicotyledons; amongst others, Compositae (Asteraceae) [15], Solanaceae [16], Bignoniaceae [17], Hamamelidaceae sensu lato [18], Leguminosae (Fabaceae) [19], Amaranthaceae [20], Ulmaceae [21], Fagaceae [22], and in some monocots [23]. Studies on the leaf architecture and anatomy of this genus are limited. Some studies indicated that anatomical studies are a good tool for grouping *Salvia* species [24]. *Salvia fruticosa* Mill. (Syn. S. *triloba* L., Greek sage), endemic species, native to the eastern Mediterranean basin.

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It is distributed from Italy and Cyrenaica through the South Balkan Peninsula to West Syria [25]. Libyan *S. fruticosa*, valued for medicinal and culinary properties due to essential oils produced in glandular trichomes, has been studied from micromorphological and ultrastructural points of view [26].

The present study aimed to carry out comprehensive research and provide data on the morphological, anatomical characteristics and chemical composition of essential oils of Libyan *S. fruticosa*.

2. MATERIALS AND METHODS

Fresh leaves of *S. fruticosa* Mill (Table 1) were collected at the end of February from a farm in the city of Al-Marj (32ʺ 55ʹ 59̊ E, 21ʺ 38ʹ 29̊ L) and identified by the Libyan flora [2]. The nomenclature of this plant followed IPNI.

2.1. Leaf architectural investigation

Fresh leaves of the studied species were soaked in a strong household bleach solution (sodium hypochloride less than 5%, sodium hydroxide less than 5% and water) until they turned white (which took 24 hours), and then they were transferred into water. The cleared leaves were then photomicrographed using (LM). The description of leaf veins was carried out by the Manual of Leaf Architecture [27].

2.2. Anatomical investigation

The leaves of the studied plant were prepared using hand microtome at 10-20 µm. They were then stained using safranin and light green and mounted in Canada balsam and examined using a light microscope [28].

2.3. Essential oils extraction

Fresh leaves of the studied plant were submitted for 2-3 h to water distillation using a Clevenger distillation apparatus [29].

2.4. Gas-chromatography–mass spectrometry (GC-MS) analysis

Quantitative and qualitative analysis of the essential oil was done using a GC-MS (Model GC-2010 plus, SHIMAD24, Japan) at the Faculty of Pharmacy, (ASU), Cairo, Egypt, equipped with an Rtx-5 MS(Cross bound 5% diphenyl/95% dimethyl polysiloxane capillary column (30 m × 0.25 mm i.d., film thickness 0.25 µm). The major components of oils are recognized by the National Institute of Standards Technology (NIST) V.11 GC–MS library, established by previous studies on *S. fruticosa* and different species of Salvia. The relative concentration of each compound in essential oils is counted based on the peak area integrated by the analysis program [29].

3. Results

3.1. Morphological characteristics (Fig. 1a,b,c, d).

Leaf venation is pinnate, the secondary vein category is festooned brochidromous with irregular spacing and the angle smoothly increases to the base. The interseconderies are weak, tertiary veins arising at an obtuse angle to the primary vein with a sinus course and are mixed opposite/ alternate percurrent category. The quaternary vein is a regular polygonal reticulate category and areolation is well developed and the marginal ultimate veins are looped (Fig.1c). Inflorescence is verticillaster (verticals 2-7 flowered). Bract ovate. Calyx is 0.7cm, 5-lobed, campanulate, brown, and tomentose. Corolla 2.3cm, upper lip bifid and lower lip 3-lobed, purple. Stamens 2, epipetalous, another two-celled. Style bifid.

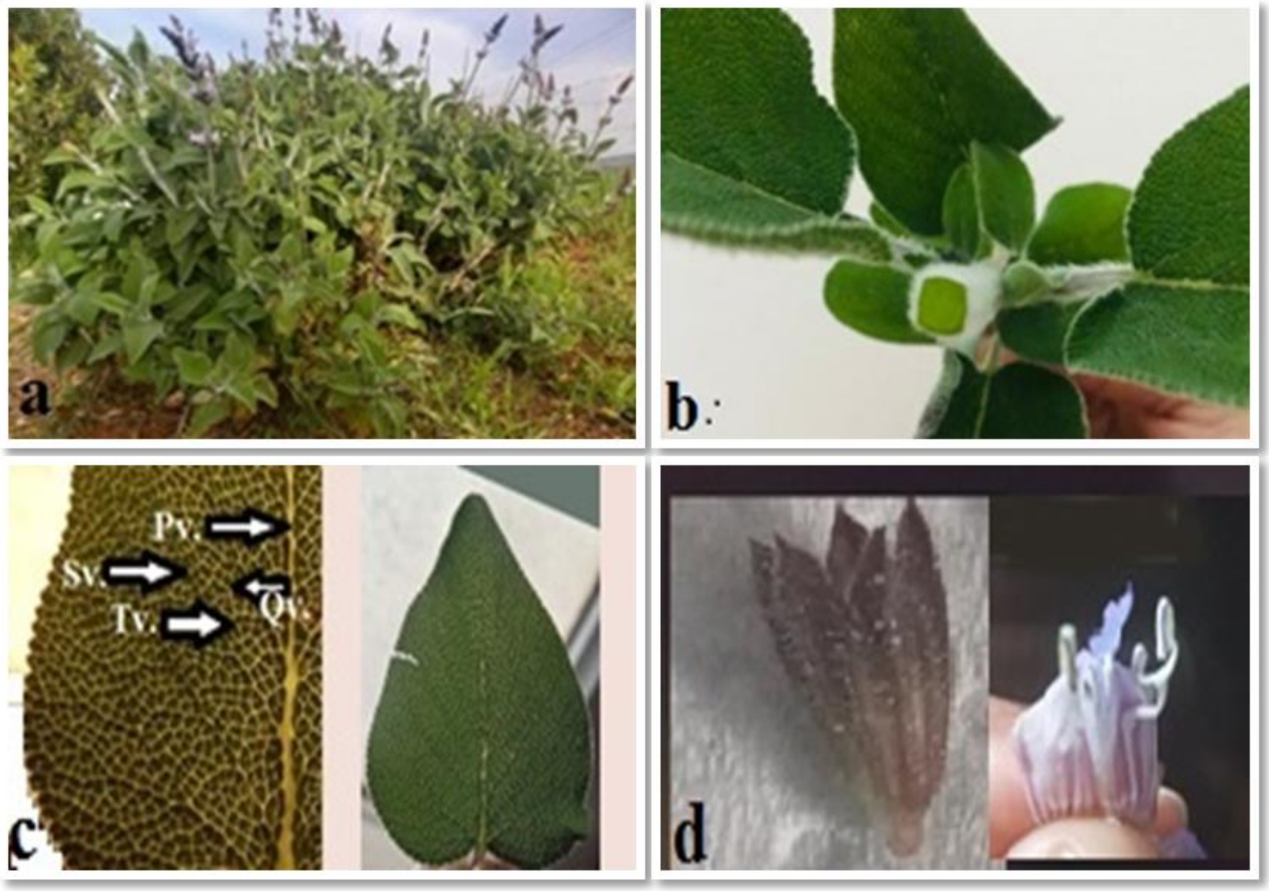

Figure 1. a - Whole plant. b- Stem morphology. c- Leaf morphology and leaf venation. d- Floral morphology. Pv.: Primary vein. Sv.: Secondary vein. Tv.: Tertiary vein. Qv.: Quaternary vein.

3.2. Lamina anatomy

Upper and lower epidermal cells are radial with thin cuticles. The non-glandular and glandular trichomes are on both sides of the leaves. The non-glandular trichomes consist of one (triangular), two or three cells (hornlike or flagelliform). Several types of glandular trichomes are distinguished. One type of peltate and 5 types of capitates. The peltate type has a secretory head with 3-4 cells. One capitate type exhibits a unicellular secretory head, a basal cell and a unicellular stalk (Fig. 2).

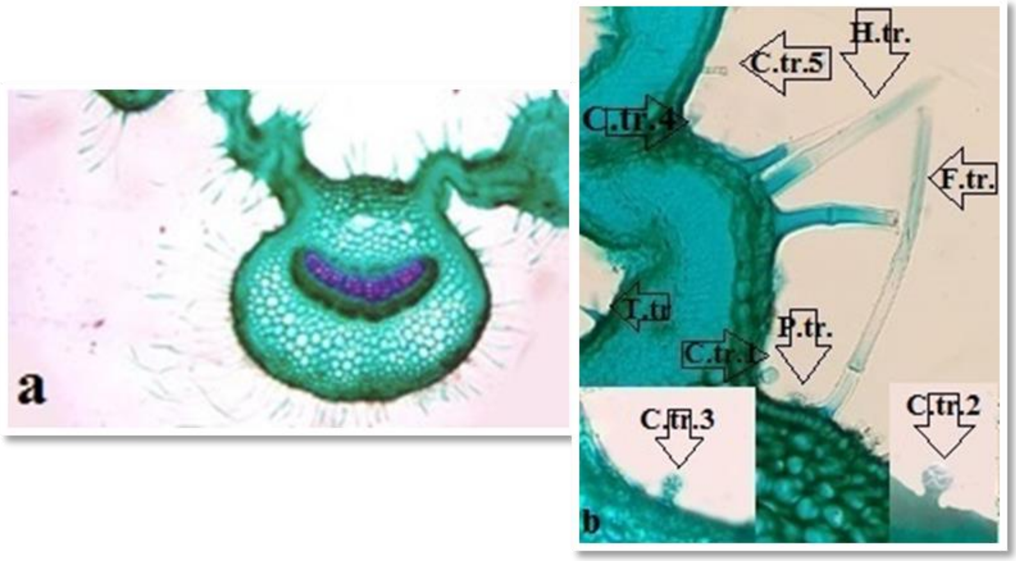

Figure 2. a- Leaf anatomy of S. fruticosa. b – Types of trichomes. C.tr.1: The first type of capitates. C.tr.2: The second type of capitate.C.tr.3: The third type of capitates. C.tr.4: The fourth type of capitates. C. tr.5: The fifth type of capitates. P.tr: Peltate trichome. F.tr.: Flagelliform trichome. H.tr.: Hornlike trichome. D.tr.: Dendritic trichome.

A second capitate type has a tricellula secretory head and a basal cell, and a unicellular stalk. The third capitate has a secretory head with 6-8 cells, a basal cell, and a unicellular stalk. The fourth capitate has a unicellular secretory head, a basal cell and a bicellular stalk. The fifth capitates have a unicellular secretory head, a basal cell and a tricellular stalk (Fig. 2). Midrib of the leaf is surrounded by 3-5 rows of annular collenchymas. The vascular bundle is single, arc-shaped and surrounded by 7-9 rows of polyhedral parenchyma. The mesophyll structure is isobilateral of 1-2 rows elongated rectangular palisade tissue, 2-3 rows of spongy tissue and followed by other palisade tissue.

3.3. Chemical compounds of essential oil

Table1. Chemical composition of essential oil of S. fruticosa.

Compound	R.Time	*S. fruticosa*
o-Xylene	5.637	1.19
Heptane,2,4-dimethyl	6.865	1.04
3-Ethyl-4-methylpentan-l-ol	7.293	1.47
(1R)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene (alpha-Pinene)	7.693	9.53
2,4,4-Trimethyl-lhexene	8.133	1.43
Bicyclo[2.2.1]heptane, 2,2-dimethyl-3-methylene-, (1S)- beta-Pinene)(	8.851	11.43
.beta.-Myrcene	9.344	9.08
Decane	9.888	1.39
Eucalyptol (Cineole)	10.437	43.93
Undecane	13.022	1.47
(+)-2-Bornanone (Camphor)	13.477	5.12
3-Cyclohexene-1-methanol, .alpha.,.alpha.,4-trimethyl-	14.325	1.59
alpha.-Terpineol	15.145	4.15
Dodecane	16.083	0.91
Caryophyllene	22.048	8.75
Humulene	22.916	1.15
Caryophyllene oxide	25.925	0.80
1-Naphthalenepropanol,.alpha-ethenyldecahydro-.alpha.,5,5,8a-tetramethyl-2methylene-,[1S-[1.alpha.(R*),4a.beta.,8a.alpha.]]	36.230	1.90

The essential oil of *S. fruticosa* was analyzed by GC-MS chromatography. Eighteen compounds were identified. The main component was cineol (43.93%)**.** The essential oil was dominated by oxygenated and non-oxygenated monoterpenes (84.79%). Monoterpenes that were present in high concentrations were cineol (43.93%), β-pinene (11,43%), α-pinene (9.53%), β-myrcene (9.08%), camphor (5.12%) and α-terpineol (4.15%). Sesquiterpenes were present in low concentration in *S. fruticosa*. Caryophyllene is one of the non-oxygenated sesquiterpenes and is found in a high concentration (8.75%) in oil.

4. DISCUSSION

There are limited studies on leaf architecture and venation patterns of *Salvia*, although these characteristics are useful in the classification of plants. Some features of leaf venation can be of taxonomic value for the identification and classification of species [12]

Most of the floral features exhibited in our results were noted by [2], but some of them were not mentioned, such as the presence of the bract and the position of stamens. In previous studies on *Salvia*, morphological characters such as stamen type, verticillaster number, calyx shape, corolla shape, corolla length and upper lip shape are distinctive characters in the identification of species [30,31].

This study showed two main types of trichomes, which are glandular and non-glandular. These characters are often used in plant taxonomy [32,33,34,35]. Leaves of *S. fruticosa* were covered with non-glandular trichomes variable in morphology and length. Variation in cell number and morphology of non-glandular trichomes can be considered a good taxonomic marker [35]. Midrib is surrounded by collenchyma cells in *the Salvia* species [36]. This is in agreement with our results. Also, the characteristics of the vascular bundle are in agreement with [37], who observed that *S. glutinosa* contains a single vascular bundle surrounded by parenchymal tissue. According to the mesophyll structure of *S. halophila* [38] is isobilateral. This result is consistent with this study.

The obtained results showed that the main components of the oil were cineol (43.93%), β-pinene (11,43%), α-pinene (9.53%), β-myrcene (9.08%), and camphor (5.12%). These results are in agreement with [39] who reported that 1,8-cineole was the most abundant compound, followed by camphor, β-pinene, myrcene and α-pinene of *S. fruticosa* essential oil from Libya. Other studies by [40] indicated that cineol was the main component of *S. fruticosa* from Turkey. Giweli [39] also found caryophyllene but with less concentration than our result (4.13%). In general, in this study, there are differences in the number of chemical components of the essential oil and the concentration of these components, compared to previous studies. The variation in the components of the essential oil could be attributed to geographical origin, seasonal maturity, genetic variation, growth stages, part of plant utilized and postharvest drying and storage which may influence the essential oil composition [41,42].

5. CONCLUSION

The paper showed some new features that were mentioned by Jafri and El-Ghadi [2]. Morphological, leaf architectural, and anatomical characteristics are very important in describing, identifying and classifying *Salvia* species. The analysis of the essential oil was largely compatible with Jafri and El-Ghadi [39]. Our results suggest studying the antibacterial effect of *Salvia fruticosa*, and studying this species with a group of other plants of the same genus and comparing them.

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