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Morphological , Anatomical and Chemical Studies of *Salvia Rosmarinus* Spenn. Growing in Al- Marj (Libya)

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Abstract: *Salvia rosmarinus* Spenn (synonym: *Rosmarinus officinalis* L.) is economically and medically important and grows naturally in Al-Marj (Libya). Macro, micro morphological and chemical characteristics were studied. Leaf venation, leaf anatomy and chemical compounds of essential oils were carried out according to traditional methods. The objective of the present study is to provide detailed description of the characteristics of this species. Morphological charactersistics of leaves, calyx, corolla, stamens are useful for sectional and specific delimitations in *Salvia*. The leaf architecture characters of primary, secondary, tertiary and quaternary veins are good diagnostic markers for the identification and classification of species. Anatomical characters such as glandular and non- glandular trichomes , mesophyll structure, presence of hypodermis and structure of vascular bundles have been found to have taxonomic value. Cineol (32.38%) is main component of essential oil, followed by Camphor, β-pinene, borneol, Caryophyllene, α-terpineol and α-pinene. Chemical data in essential oils carried taxonomic value of *Salvia* species.

دراسات مورفولوجية و تشريحية و كيمائية لـ . *S. rosmarinus* Spenn النامية فى المرج (ليبيا)

الكلمات المفتاحية: *Salvia rosmarinus*، الصفات الكبيرة و الدقيقة، الشعيرات و الزيوت الطيارة، المرج (ليبيا).

المستخلص: النوع *S. rosmarinus* Spenn (المرادف: *Rosmarinus officinalis* L) هو نبات مهم اقتصاديًا وطبياً، وينمو بشكل طبيعي في المرج (ليبيا). تمت دراسة الصفات الكبيرة، والدقيقة المورفولوجية، والكيميائية لنوعين. تم إجراء تعرق الأوراق، وتشريح الأوراق، وتحليل المركبات الكيميائية للزيوت الطيارة وفقًا للطرق التقليدية. الهدف من هذه الدراسة تقديم وصف مفصل لهذا النوع. وجد أن الصفات المورفولوجية، كالأوراق، الكأس، التويج، والأسدية صفات مفيدة في تحديد الأنواع على مستوى النوع، والقسم. تمييز الأنواع: تعد الصفات الهندسية لتعرق الأوراق كعروق الأول، الثاني، الثالث، والرابع علامات تشخيصية جيدة لتحديد الأنواع. تعد الصفات التشريحية كالشعيرات الغدية، والغير غدية، بنية النسيج الأوسط للورقة، وجود طبقة تحت البشرة، وتركيب الحزم الوعائية صفات ذات قيمة تصنيفية. السنيول Cineol هو المكون الرئيسي للزيوت الأساسية، يليه الكافور، بيتا–بينين، بورنيول، كاريوفيلين، ألفا تربينول وألفا بينين. أيضا البيانات الكيميائية في الزيوت الأساسية تحمل قيمة تصنيفية لأنواع جنس *Salvia*.

INTRODUCTION

The genus *Salvia* L. (Lamiaceae), comprises approximately 1000 species widely distributed (Walker & Sytsma, 2007). According to (Jafri & El-gadi, 1985) Flora of Libya comprises 10 *Salvia* L. species, out of which 3 are cultivated. Recently, *Salvia rosmarinus* Spenn., also known as rosemary, the most used name, *Rosmarinus officinalis L.*, has to be considered a synonym of the actual name, because molecular investigations evidenced

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as *Rosmarinus* L. is nested in *Salvia* L.(Drew et al., 2017).

Salvia L. can be distinguished from the other Lamiaceae genera by unique stamina architecture, having only two stamens which are separated by a significantly elongated connective tissue (Hu et al., 2018; Kriebel et al., 2019; Will & Claßen-Bockhoff, 2017). Based on the calyx, corolla and stamen morphology, Benthams first created an infrageneric classification of *Salvia* L. (Hu et al., 2018). Molecular studies have demonstrated that *Salvia* is not monophyletic (Will & Claßen-Bockhoff, 2017). Regarding classifications of the genus *Salvia* L., different opinions are proposed, one option is to treat the five embedded genera (*Dorystaecha Meriandra*, *Perovskia*, *Rosmarinus*, and *Zhumeria*) as subgenera and maintain *Salvia* in a broad sense (Drew et al., 2017; Kriebel et al., 2019) and the other is to split *Salvia* L. into six smaller genera (Will & Claßen-Bockhoff, 2017).

Salvia species are usually composed of perennial, herbaceous, semi-shrubby or shrubby plants and leaves, calyx and trichomes on the flower are important characteristics used to distinguish species (Davis et al., 1982). Also in the revision studies on Salvia; morphological features such as stamen type, verticillaster number, calyx shape, corolla shape, corolla length, length of the corolla tube, whether or not annulus or upper lip is flat or helmet shaped, are distinctive characters in the determination of species (Doğan et al., 2008; Kahraman et al., 2009b).

Leaf characters are considered as important morphological features for taxonomic investigations (Swaminathan et al., 2012). These include leaf architectural characters, venation patterns (Pulan & Buot Jr, 2014). Although leaves are generally plastic, mostly to environmental conditions, and are sometimes neglected in taxonomy, the leaf architecture and venation patterns have long been considered useful for the taxonomy of many different dicotyledonous families, including, Rubiaceae (Banaticla & Buot Jr, 2004), Solanaceae (Inamdar, 1978), Apocynaceae (Mohan & Inamdar, 1982), and even monocotyledonous families (Inamdar et al., 1983; Obico et al., 2007).

Some studies indicated that anatomical studies is a good tool for grouping *Salvia* species (Celep & Dirmenci, 2017). Recently, several anatomical investigations on *Salvia* species have been done (Bagherpour et al., 2010; Buyukkartal et al., 2011; Celep et al., 2014; Kahraman, Celep, & Dogan, 2010; Kahraman, Celep, & Doğan, 2010; Kahraman & Dogan, 2010; Polat et al., 2017; Polat et al., 2010). Studies on the anatomy of this species in Libya are limited.

Various *Salvia* L. species are used in traditional medicine worldwide, for food flavoring, in cosmetics, perfumery and pharmaceutical industry (Dweck, 2000), due to the production of the essential oils which are proved to exhibit considerable biological activities (Alimpić et al., 2015; Alimpić Aradski et al., 2015). *S. rosmarinus* is a rich source of essential oil (Loizzo et al., 2013). The essential oil of *S. rosmarinus* was demonstrated to possess antibacterial, antioxidant, antifungal, and anti-inflammatory properties (Kačániová et al., 2017).

S. rosmarinus grow naturally in Al-Marj and has economic and medical importance. There are not enough studies on this species in Libya, so the aim of this work is to study the morphological, anatomical characteristics and chemical compounds of essential oil, and provide new set of characters to description this species.

MATERIALS AND METHODS

Fresh materials of *S. rosmarinus* were collected from farm on Al-Marg city (Bata). The plant samples were identified using the Libyan flora (Jafri & El-gadi, 1985). Morphological characteristics of studied species, flower, stem, leaf morphology and lamina architecture were determined from fresh materials.

Leaf architectural investigation: Fresh leaves of studied species were accomplished by soaking the fresh samples in a strong household bleach solution (sodium hypochloride less than 5%, sodium hydroxide less than 5% and water) until it turns white (took 24 hours), then transferred into water. Then, the cleared leaves were photomicrographed using (LM) and mobile camera (Galaxy A10s, 8.0 Mega Pixels). The description of leaf veins was carried out by Manual of Leaf Architecture(Ash, 1999).

Micro morphological investigation: The leaves of the studied species were prepared using hand microtome at 10-20 µm. Then were double stained using safranin and light green and mounted in Canada balsam according to (Johansen, 1940) then, examined using light microscope. Photomicrographs were taken using digital camera (Canon power-shot A720, 8.0 mega pixels), the magnification power was expressed by (x).

Essential oils extraction: Fresh leaves of the examined species were submitted for 2 h to water- distillation using a Clevenger distillation apparatus (Clevenger-type) (Su et al., 2012).

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 Faculty of Pharmacy, (ASU),Cairo, Egypt, equipped with a 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 recognized by National Institute of Standards Technology (NIST) V.11 GC–MS library, established by (Adams, 2007) and previous studies on *S. rosmarinus* and different species of Salvia. The relative concentration of each compound in essential oils counted based on the peak area integrated by the analysis program (Su et al., 2012).

RESULTS

S. rosmarinus Spenn., Handb. Angew. Bot. 2: 447 (1835) (IPNI). Replaced synonym: *Rosmarinus officinalis* L., Sp. Pl. 1: 23 (1753). Common name: Kleel. Collection date: 30-1-2022.

Macro-Morphological characteristics (Fig1&2): Evergreen aromatic shrub, up to 85cm tall. Stem square, grey and glabrous. Leaves 5x0.2cm, opposite, sessile. Lamina simple, linear, shiny green, rough on upper surface and white, tomentose on lower surface, revolute margin , acute apex and attenuate base.

Leaf venation pinnate. Secondary vein category festooned brochidromous with spacing irregular and angle one pair acute on base**.** The interseconderies weak. Tertiary vein arise at obtuse angle to primary vein with sinus course and is regular polygonal reticulate category. Quaternary vein regular polygonal reticulate category. Areolation well developed. The marginal ultimate vein are looped. Inflorescence verticillaster (verticals 2-8 flowered). Bract ovate. Calyx 0.5cm, 3-lobed, campanulate, brown-green, tomentose. Corolla 1.2cm, upper lip bifid and lower lip 3-lobed, pale purple-pale blue. Stamens 2, anther one- celled .Style bifid.

Figure (1): *S. rosmarinus*

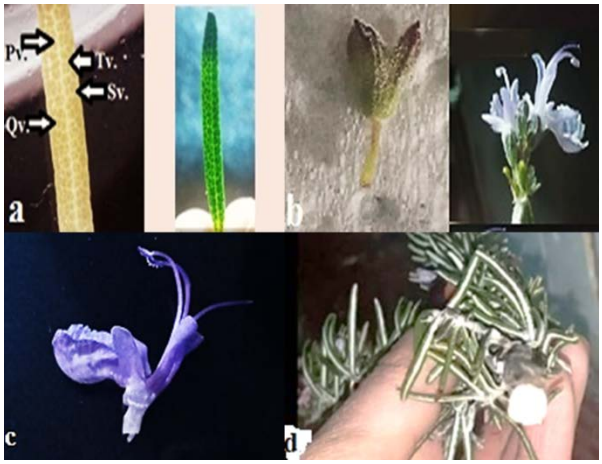
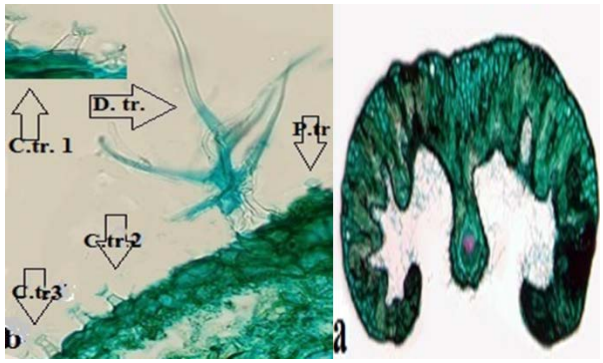


Figure:(2). a-Leaf morphology. **b&c-** Floral morphology. d-Stem morphology

Micro-morphological characteristics (Lamina anatomy Fig3): Epidermis is uniserate and covered by a thick cuticle on the adaxial surface and a thin cuticle on the abaxial. The epidermial cells are radially to tangential on both surfaces. The non-glandular and glandular trichomes are on the abaxial surface only. The non-glandular trichomes (dendritic trichomes) consist of one cell, followed by a bifurcated series of cells. Several types of glandular trichoms are distinguished.

One type of peltate and three types of capitates. Peltate type has a secretory head with 3-4 cells, a basal cell and large stalk. One capitate type exhibits a unicellular secretory head, abasal cell and a unicellular stalk. A second capitate type has a unicellular secretory head, a basal cell and a bicellular stalk. The third capitate has a unicellular secretory head, a basal cell and a tricellular stalk. A hypodermis occurs under the adaxial epidermis. The midrib contain rounded single vascular bundle surrounded by 2-3 rows of polyhedral parenchyma. The leaf is dorsiventral of 2-3 raws elongated rectangular palisade tissue and 1-2 raws of spongy tissue.

Chemical compounds of essential oil (Table1): Thirty six components was identified in essential oil of *S. rosmarinus*. The main component was cineol (32.38%)**.** Essential oil was dominated by oxygenated and non- oxygenated monoterpenes (83.53%). Monoterpenes that were present in high concentrations were cineol (32.38%), Camphor (12.32%), β-pinene (6.49%), borneol (5.84%), α-terpineol (4.04%) and α-pinene (3.20%). Sesquiterpenes were present in low concentration in *S. rosmarinus*. Caryophyllene is one of non- oxygenated sesquiterpenes and found in a good amount of about (4,80%) in oil.



Figure(3): a. Micromorphology. **b.** Types of trichomes **C.tr.1:** The First type of capitate. **C.tr.2:** The second type of capitate.**C.tr.3**: The third type of capitates. **D.tr.:** Dendritic trichome

Table (1). Chemical composition of essential oil of *S. rosmerinus*.

Coumpound	R.Time	*S. rosmerinus*
o-Xylene	5.637	0.19
Heptane,2,4-dimethyl	6.865	0.16
Tricyclo[2.2.1.0(2,6)heptane, 1,7,7-trimrthyl-	7.340	0.58
Bicyclo[3.1.0]hex-2-ene, 2-methyl-5-(1-methylethyl)- (alpha-Thujene)	7.504	0.32
(1R)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene (alpha-Pinene)	7.693	3.20
Camphene	8.046	8.50
2,4,4-Trimethyl-lhexene	8.133	0.33
Bicyclo[2.2.1]heptane, 2,2-dimethyl-3-methylene-, (1S)- beta-Pinene)	8.851	6.49
.beta.-Myrcene	9.344	1.61
Decane	9.888	0.20
alpha-Terpinene	10.080	0.74
o-Cymene	10.181	1.04
Eucalyptol (Cineole)	10.437	32.38
trans-.beta.-Ocimene	10.723	0.25
.gamma.-Terpinene	11.346	1.01
5-Isopropyl-2-methylbicyclo[3.1.0]hexan-2-ol	11.448	0.22
(+)-4-Carene (Terpinolene)	12.264	0.42
Bicyclo[3.1.0]hexan-2-ol, 2-methyl-5-(1-methylethyl)-, (1.alpha.,2.beta.,5.alpha- (beta-Terpineol)	12.364	0.12
Linalool	12.515	0.18
Undecane	13.022	0.33
(+)-2-Bornanone (Camphor)	13.477	12.32
Pinocarvone	14.021	1.30
Bicyclo[2.2.1]heptane-2-ol, 1,7,7-trimethyl-, (lS-endo)- (Borneol)	14.391	5.84
3-Cyclohexene-1-ol,4methyl-1-(1-methylethyl)-, (R)-	14.789	1.60
alpha.-Terpineol	15.145	4.04
Bicyclo[2.2.1]hep-3-en-2-one, 4,6,6-trimethyl-,(1S) (Verbenone)	15.320	0.65
Endo-Borneol (Borneol)	15.548	0.72
Dodecane	16.083	0.15
Bicyclo[2.2.1]heptane-2-ol, 1,7,7-trimethyl-, (lS-endo)- Bornyl acetate)(	18.005	0.29
Benzen,1,2-dimethoxy-4-pr0penyl-(Z) (Methyleugenol)	21.903	0.61
Caryophyllene	22.048	4.80
Humulene	22.916	0.30
Caryophyllene oxide	25.925	1.13
4a(2H)-Naphtalenol,1,3,4,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-,(1S,4R,4aS,8aR)-	26.899	0.37
Tau-candol (gamma-Muurolene)	27.310	0.55
Androstan-17-one,3-ethyl-3-hydroxy-,(5.alpha)-	27.655	0.36

DISCUSSION

In present study, most of the morphological characteristics agreed with the description recorded in the Flora of Libya (Jafri & El-gadi, 1985). Some of the characteristics differed, namely the size of the leaves and the calyx, and other characteristics not mentioned by Jafri, 1985, which are the number of verticils and bract shape. Morphological attributes such as bract shape and calyx shape have been recorded in other species of *Salvia* (Kaplan & Çakir, 2019). (Walker et al., 2015) also observed that the stamen morphology of the genus *Rosmarinus* remarkably similar to some species of *Salvia.*

A study of leaf venation for the *Salvia* genus is limited. There is a rich diversity of vena-

tion patterns in dicotyledonous plants, leaf venation can be classified into some characters that may have taxonomic value for the identification and classification of species (Hickey, 1973). In this work, the characteristics of primary, secondary, tertiary and quaternary veins were studied. The primary vein category is pinnate. This result is in agree with (Alimpić Aradski et al., 2021) indicated that primary vein of some species of *Salvia* is pinnate.

In *S. rosmarinus*, the non-glandular and glandular trichomes are on the abaxial surface of leaves. The non-glandular trichomes are unicellular dendroid form. Several types of glandular trichoms are distinguished, Peltate type and three types of capitates. These results in accordance with (Boix et al., 2011). Trihomes have often been used in plant taxonomy (Atalay et al., 2016; Gul, Ahmad, Zafar, Bahadur, Celep, et al., 2019; Gul, Ahmad, Zafar, Bahadur, Sultana, et al., 2019; Wagner, 1991). Also our results showed the presence of a hypodermis and this is in harmony with (Boix et al., 2011; KiliÇ & KiliÇ, 2022), found this layer in four species of *Salvia*. The structure of vascular bundles in the leaf anatomy of Salvia species can be used as a very useful marker for distinguishing the species. *S. blepharochlaena* has one vascular bundles in the middle region and two small bundles on the sides (Ozkan & Soy, 2007), while S. halophila has two large bundles in the middle in the midrib (Kaya et al., 2007). According to our result *S. rosmarinus* has single vascular bundle in the midrib surrounded by parenchymatic tissue (Fig3a). This result in agreement with (Kahraman et al., 2009b), who observed these characteristics of *S. glutinosa*. The leaves of *S. indica* (Kahraman et al., 2009a) are bifacial, however those of *S. halophila* (Kaya et al., 2007) are monofacial. Our result in harmony with (Kaya et al., 2007).

The results obtained in this research showed that peltate, capitates glandular hairs are present on the abaxial surface of S. rosmarinus (Fig3b). Capitate trichomes are commonly composed of basal cell, 1-2 stalk cells and 1-2 cells forming a round or pear-shaped secretory head (Fahn, 1988; Werker et al., 1985). Plant species that contain glandular hairs produce relatively large amounts of bioactive compounds which include highly concentrated phytochemicals with biological activities of interest to many industries (Dyubeni & Buwa, 2012).

In our study, the essential oil of *S. rosmarinus* were analyzed by GC-MS chromatography. 36 compounds were detected. The main volatiles were oxygenated and non-oxygenated monoterpenes. The cineole, camphor, α-terpineol, borneol, α-pinene, β-pinene, and caryophyllene are the most abundant compounds of essential oils. The studies of (Leporini et al., 2020) indicated that oxygenated and non-oxygenated monoterpenes are the main volatiles of *S. rosmarinus* at two different coasts of Italy, and main compounds were cineole, α-pinene, camphor, and caryophyllene. (Leporini et al., 2020) found β-pinene of low concentation but borneol was not detected. In a study at Al-Jabal Al-Akhdar (Libya) by (El-Ageeli et al., 2020)recorded that main component of essential oil of *S. rosmarinus* is camphor this result disagree with our results. Cineol, α-pinene, β-pinene and caryophyllene were detected by (El-Ageeli et al., 2020) but in different concentrations. The variation in the essential oils components 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 (Anwar et al., 2009; Hussain et al., 2008; Marotti et al., 1994).

(Giweli et al., 2013) indicated that cineol is the main component of essential oil and compounds such as camphor, β-pinene, myrcene, α-pinene, caryophyllene and α-terpineol were in high concentrations when studying the species of *Salvia*. These compounds were discovered in our current study. (Coassini Lokar

& Moneghini, 1990) indicated that the chemical data are useful for determining the degree of affinity between species in the genus *Salvia*.

CONCLUSION

In conclusion, The morphological characteristics of *S. rosmarinus* show some differences with those reported in the flora of Libya (Jafri & El-gadi, 1985). Also some morphological characters of this species are absent in the flora of Libya. Macro & micro morphological characteristics are a very useful key for the identification and classification of species. The cineole, camphor, α-terpineol, borneol, α-pinene, β-pinene, and caryophyllene are the most abundant compounds of essential oils of *S. rosmarinus*. Further study might include studying *S. rosmarinus* with a large number of species and studying the antibacterial activity of the essential oils of plants.

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