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Research article

Qualitative and quantitative comparison of the essential oils of *Citrus medica* and *Salvia officinalis* prepared from fresh, freeze-dried and shade-dried plant materials

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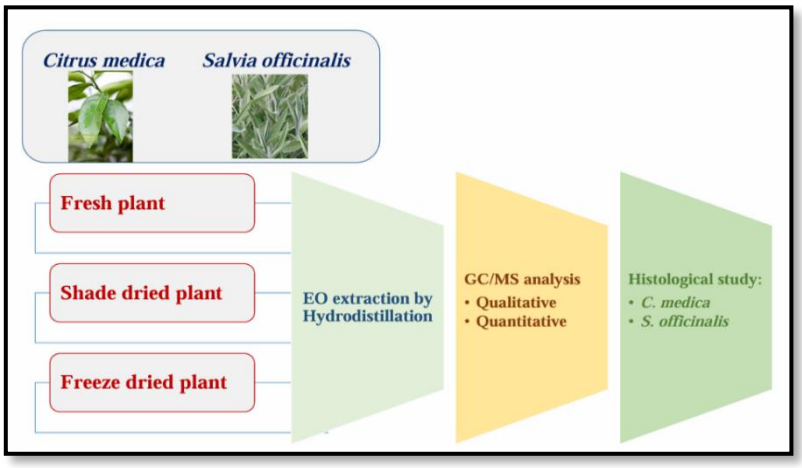
Abstract:



Plants bearing essential oils contribute to prevent or cure diseases and maintaining health. Essential oil (EO) prepared from the leaves of *Citrus medica* showed antimicrobial activity while EO obtained from *Salvia officinalis* (Sage) is used as carminative, antispasmodic, antiseptic, astringent, and for the treatment of many other diseases. Several studies were conducted to explore the effect of drying conditions on EO profile and quantity. In the current study, a comparison was performed for the EOs obtained from the fresh, freeze-dried, and shade-dried samples of *C. medica* leaves and *S. officinalis* herbs prepared by hydrodistillation and analyzed by GC/MS. *C. medica* expressed a greater loss in oil contents upon drying especially in the monoterpenes with lighter molecular weights. The histological study did not show any features for water and volatile content preservation as it revealed a thin cuticle, high stomatal index, and oil glands just beneath the epidermis.

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Although *S. officinalis* expressed some loss of the essential oil in the freeze-dried samples, the shade-dried samples interestingly, demonstrated an increase in the oil contents. Many factors participated in this phenomenon. Beside the histological features, slow drying permits the continuation of the enzymatic activity for more time expressing qualitative and quantitative impact on the oil contents.

Keywords: *GC-MS, Citrus medica*, *Salvia officinalis*, Essential oil, Fresh, Drying

1. Introduction

Plants bearing essential oils (EO's) contribute to prevent or cure diseases and maintaining health. They benefit all humanity by being a source of nutrition, body care products, perfumes, and traditional healing. These plants represent integral components of the traditional medicine systems found in local communities worldwide with great economic value [1].

The petroleum ether extracts of *C. medica* leaves possess dose-dependent anthelmintic activity *in-vitro* [2] and significant estrogenic activity [3]. EO prepared from the leaves showed antimicrobial activity against *Staphylococcus aureus*, *Propionibacterium acne,* and *Candida albicans* [4].

The EO obtained from *S. officinalis* (Sage) is used as carminative, antispasmodic, antiseptic, astringent and for the treatment of many diseases like those of the nervous system, heart, blood circulation, respiratory system, digestive system, metabolic and endocrine diseases [5,6]. The inhalation of *S. officinalis* fumes is applied traditionally for migraines, as ophthalmic anti-inflammatory and for rheumatism [7]. *S. officinalis* aroma proved to produce a significant enhancement effect in memory [8]. The oil also possesses antimicrobial and antifungal activities [9, 10]. *In vitro* study of *S. officinalis* EO revealed significant anti-inflammatory potential without any effect on the mammalian macrophages and keratinocytes viability [10].

Several studies were conducted to explore the effect of drying conditions on the EO contents and quantity. The EO contents of *Mentha longifolia* and the percentage of the main components were decreased as the plant material was exposed to higher temperature or sunlight [11]. Few reports studied the effect of freeze-drying on the quantity and contents of EO's such as the oils of *Coriandrum sativum*, *Ocimum basilicum* and *Satureja bachtiarica* [12-14]. In the case of *Satureja bachtiarica* the oven drying at 45 ^{0}C was recommended over freeze-drying for better quantity and quality of the obtained EO's [14].

The plants of *S. officinalis* were subjected to air drying at the shade and ambient temperature of 22 °C, hot-air oven at 45 °C, hot-air oven at 65 °C, microwave oven at 500 W, IR moisture analyzer at 45 °C and IR moisture analyzer at 65 °C. The study results indicated that the EO yield improved with drying at the lower temperatures used [15].

The current study compares the EO's obtained from the fresh, freeze-dried and shade-dried samples of *C. medica* leaves and *S. officinalis* herbs. The plants were selected to compare the effect of habitat on plant adaptation and histological features. *C. medica* is a cultivated plant while *S. officinalis* is a wild desert plant that lacks regular water supply. The study aims to explore the effect of drying on the quantity and composition of the oils obtained from *C. medica* leaves and *S. officinalis* using GC/MS. Such finding is important for selecting the best way to get the highest oil yield from the plants. The study also gave a scientific explanation for the variable results obtained from the two plants based on their histological characteristics.

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2. Materials and methods

2.1.Plant material:

The leaves of *C. medica* L. were collected from Hotat Bani Tamim south of Riyadh (voucher# 20220412-1) and the herbs of *S. officinalis* L. were purchased from the local market at Zarqa, Jordan (voucher# 20220305-4). Samples were authenticated by comparison with voucher specimen deposited at herbarium of the Medicinal, Aromatic and Poisonous Plants Research Centre (MAPPRC), College of Pharmacy, King Saud University, Riyadh, Saudi Arabia.

2.2.Preparation of the oils:

All plant samples were subjected to hydrodistillation for 8 hrs. using a Clevenger apparatus with 2 L rounded-bottomed flask. The condensate was extracted with ether. The ether extract was dehydrated over anhydrous sodium sulphate and evaporated to obtain the EO's.

Each plant was divided into three equal parts. The first parts of each plant were cut into small pieces and directly subjected to hydrodistillation. The second parts were subjected to freeze drying in a tray-type Lyophilizer (MILLROCK, STELLAR® Laboratory Freeze Dryer, Model NO. LD85B3-I) followed by hydro-distillation. The third parts of each plant were left for drying in the shade at room temperature (25°C) in a well-ventilated room. After complete drying (15 Days), the plant materials were subjected to hydro-distillation. The amounts of EO from each sample are presented in **Table 1**.

2.3.GC-MS analysis:

Aliquots of diluted oils (1 µL of 5 ppm concentration) were injected into the GC/MS apparatus using Autosampler. Samples were injected by split-less mode. Analysis was performed on GC/MS (Agilent Model 7890 MSD) equipped with a HP-5MS capillary column (30 m × 0.25 mm i.d., 0.25 µm coating) and temperature programming was performed at column temperature of 70°C for 5 min, programmed at the rate of 5°C/min to 290°C, and finally held isothermally for 5 min. The detector and injector temperatures were 290 and 280°C, respectively. The carrier gas used was Helium (99.999 % purity) at a flow rate of 1.0 mL/min. In addition to significant quadrupole MS operating parameters: Electrospray ionization at 70 eV with scan mass range of 30 to 600 m/z was applied. The components were identified by comparing their mass spectra with the National Institute of Standards and Technology (NIST 2017). The analysis and processing of the results were controlled using MASSHUNTER software.

2.4.GC analysis:

GC spectra obtained under the above-mentioned conditions were used for the identification of peaks by comparing their relative retention index (RRI) with a series of n-alkanes. The semi-quantitative estimation of each compound was carried out based on computerized peak area measurements in **Tables 2&3**.

Table 1. Percentage of essential oil obtained by hydro distillation from fresh, freeze and shade-dried samples of *C. medica* and *S. officinalis* [a,b].

	Fresh Sample			Freeze-dried sample			Shade dried sample		
	Sample	**Oil**	**Oil %**	**Sample**	**Oil**	**Oil %**	**Sample**	**Oil**	**Oil %**
C. medica	400	1.179	0.29	161	0.930	0.23	157	0.340	0.09
S. officinalis	456	1.635	0.36	186	1.321	0.22	151	1.985	0.44

[a] Sample and oil weight in grams.

[b] Oil percentages are relative to the fresh samples weight.

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Table 2. GC/MS analysis of the Essential oil of the fresh, freeze-dried and shade-dried samples of *Citrus medica*.

No	Name	Area %			RI	No	Name	Area %			RI
		Fresh	Freeze dry	Shade dry				Fresh	Freeze dry	Shade dry	
1	Methyl heptenone	1.46	2.21	0.71	932	22	Neral dimethyl acetal	1.51	4.28	8.94	1298
2	*β*-Myrcene	0.58	0.47	1.44	981	23	Geranial dimethyl acetal	2.27	2.58	5.10	**1319**
3	3-Carene **(1)**	4.12	3.21	1.88	1003	24	Citronellyl acetate	0.64	1.14	0.63	1356
4	*α*-Terpinene	0.06	0.15	0.07	1009	25	Neryl acetate **(6)**	4.36	5.51	4.29	1364
5	2-Furanmethanol	0.09	0.13	0.19	1021	26	Geranyl acetate	4.31	3.07	2.32	1387
6	Limonene **(2)**	38.38	36.55	17.77	1031	27	Caryophyllene **(7)**	1.15	5.39	9.94	1419
7	*β*-Ocimene **(3)**	3.08	2.72	1.79	1038	28	(1Z,4Z,7Z)-1,5,9,9 tetramethylcycloundeca-1,4,7-triene	0.12	0.42	0.82	1432
8	Terpinolene	0.79	-	0.63	1090	29	*cis*-*β*-Farnesene	0.06	0.16	0.50	1441
9	Citral dimethyl acetal	3.95	5.79	5.07	1050	30	*α*-Humulene	0.06	0.14	0.16	1452
10	Guaiacol	0.45	0.79	1.17	1095	31	Bicyclogermacrene	0.08	0.23	0.34	1495
11	Linalool	0.82	0.40	1.12	1100	32	*β*-Bisabolene **(8)**	0.27	1.29	3.40	1510
12	Allo-Ocimene	0.27	0.18	0.29	1130	33	Nerolidol	0.07	0.15	0.32	1531
13	Citronellal	0.71	1.45	1.17	1144	34	4-Ethoxy ethylbenzoate	0.16	0.20	0.12	1533
14	*α*-Terpineol	0.35	0.12	0.36	1175	35	Ledol	0.05	0.09	0.22	1563
15	Nerol	1.29	0.94	1.23	1227	36	Caryophyllene oxide	0.19	0.31	0.65	1574
16	*trans*-Cinnamaldehyde	0.24	0.34	0.43	1240	37	Spathulenol	0.07	0.11	0.19	1577
17	*Z*-Citral **(4)**	9.70	2.08	2.34	1251	38	(*Z*)-*epi*-*β*-Santalol	0.05	0.07	0.30	1711
18	Geraniol **(5)**	9.74	3.85	4.38	1257	39	Neophytadiene	0.07	0.62	1.629	1842
19	E-Citral	2.87	1.36	1.20	1266	40	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	0.128	0.15	0.06	2116
20	2-Isopropenyl-5-methylhex-4-enal	-	0.21	0.02	1274	41	Phytol	0.04	0.46	6.16	2120
21	Methyl nerolate	0.58	0.88	0.77	1282		**Total**	**95.19**	**90.20**	**90.12**	

Monoterpene hydrocarbons: 2- 4, 6- 8, 12.
Oxygenated Monoterpenes: 9- 11, 13- 15, 17- 26.
Sesquiterpenes: 27- 32.
Oxygenated sesquiterpenes: 33, 35- 38.
Diterpenes hydrocarbons: 39.
Oxygenated Diterpenes: 40- 41.
Others: 1, 5, 16, 34

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Table 3. GC/MS analysis of the Essential oil of the fresh, freeze-dried and shade-dried samples of *Salvia officinalis*

No	Name	Area %			RI	No	Name	Area %			RI
		Fresh	Freeze dry	Shade dry				Fresh	Freeze dry	Shade dry	
1	*α*-Thujene	0.56	0.59	0.21	932	23	Longifolene	0.25	0.26	0.24	1400
2	*α*-Pinene	2.72	2.70	1.92	939	24	*β*-Longipinene	-	0.12	-	1407
3	Camphene	0.80	0.87	0.97	951	25	(-)-Aristolene	0.28	0.18	0.05	1424
4	5-Methyl-3-heptanone	-	0.98	-	962	26	Caryophyllene **(7)**	7.24	9.25	9.71	1432
5	*β*-Pinene **(9)**	6.44	0.62	-	978	27	Aromandendrene	0.99	1.86	0.21	1437
6	*β*-Myrcene **(10)**	1.72	0.91	1.64	981	28	*α*- Maaliene	-	0.17	0.23	1442
7	*p*-Cymene	0.27	0.10	0.13	1021	29	*α*-Humulene **(15)**	1.97	1.86	1.47	1452
8	1,8-Cineole (Eucalyptol)**(11)**	38.10	35.84	48.57	1046	30	(1Z,4Z,7Z)-1,5,9,9-tetramethylcycloundeca-1,4,7-triene	2.67	3.57	3.36	1455
9	*γ*-Terpinene	0.32	0.72	0.72	1064	31	Alloaromadendrene	0.40	0.45	0.44	1460
10	Camphenilone	0.23	0.31	0.20	1085	32	*γ*-Muurolene	-	0.18	0.16	1474
11	Linalool	0.36	0.46	0.34	1100	33	*α*-Muurolene	-	0.13	0.09	1499
12	*cis*-Thujone **(12)**	3.59	3.4	2.87	1102	34	*γ*-Cadinene **(16)**	2.41	3.36	1.74	1513
13	*trans*-Thujone	1.63	1.68	1.48	1110	35	*trans*-Calamenene	-	0.257	0.14	1527
14	Camphor **(13)**	6.57	4.73	4.39	1145	36	4-Ethoxy ethylbenzoate	0.49	-	-	1533
15	Pinocamphone	0.66	0.78	0.52	1159	37	Caryophyllene oxide	1.10	1.21	0.80	1574
16	Borneol	0.60	0.27	0.33	1167	38	Spathulenol	1.15	1.86	0.63	1577
17	*δ*-Terpineol **(14)**	1.62	1.7	1.54	1170	39	Viridiflorol	0.85	0.77	0.31	1595
18	*α*-Terpineol	5.27	5.45	4.97	1175	40	*β*-Selinene	-	0.19	0.12	1746
19	Linalyl acetate	0.25	0.22	0.26	1253	41	Verticiol	-	0.12	0.03	2037
20	Thymol	0.24	0.23	0.30	1291	42	Manool	0.56	0.76	0.26	2056
21	Carvacrol	0.17	0.12	0.08	**1296**						
22	*α*-Terpinyl acetate	0.89	0.85	0.71	1367		**Total**	**93.37**	**90.08**	**92.14**	

Monoterpene hydrocarbons: 1- 3, 5- 7, 9.
Oxygenated Monoterpenes: 8, 10- 22, 13- 15, 17- 22.
Sesquiterpenes: 23- 35, 40.
Oxygenated sesquiterpenes: 37- 39.
Diterpenes hydrocarbons: 39.
Oxygenated Diterpenes: 41- 42.
Others: 4, 36.

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2.5.Stomatal index:

The number of stomata present in microscopic view field of 1 mm^2 was recorded for calculating the stomatal index by using the formula:

Stomatal index (%) = (S/S+E) x 100

Where S and E are the number of stomata and epidermal cells respectively. Ten fields were used for the determination [16].

3. Results and discussion

The effect of two drying conditions on the quantity and components of the EO's were explored. A comparison between the percentage of EO obtained from the same weight of the fresh plant subjected to various drying conditions was conducted. In both plants the shade-drying was more efficient in removing water from the plant as the dry samples weight resulted from the same weights of fresh plant samples were less **Table 1**. That may be due to the dry weather conditions at Al-Kharj city. The EO percentages in *C. medica* dramatically decreased from 0.29 in the fresh plants to 0.23 in the freeze-dried samples and 0.09 in the shade-dried plants **Table 1**. On the other hand, *S. officinalis* samples showed decrease in the EO percentage of the freeze-dried samples, while the shade-dried ones demonstrated increase in the EO percentage to 0.44 compared to 0.36 in the fresh samples. The process of freeze-drying will immediately stop the enzymatic activity in the plant tissues and the oil percentages notably decreased due to evaporation under the applied vacuum. In the case of shade-drying, the loss of water is gradual and enzymatic activity will continue for some time. The maximum enzymatic activity is maintained at 45% moisture contents [17]. Preservation of plant materials from enzymatic activity in some drugs requires keeping moisture contents as low as 5% [18]. Before the shade-drying process decreases the moisture contents to such levels enzymatic activity is expected to proceed. Some EO producing plants keep releasing their scent after cutting for some time, as do Jasmin flowers [19]. Moreover, some studies demonstrated that reduced water contents increased the carbon-based secondary metabolites in plant leaves [20]. These facts explain the increase in the EO contents of *S. officinalis* on shade-dried samples.

Regarding the EO composition, it was clear that changes in the relative concentrations of each component were observed. The more volatile monoterpene hydrocarbons and monoterpene alcohols expressed greater loss as observed for limonene, *β*-ocimene, 3-carene, and geraneol in *C. medica* oil **Table 2**. Other components of higher molecular weight showed increased relative concentrations in the oil such as the case of caryophyllene and phytol **Table 2**. Similar observation was recorded for the level of *β*-pinene in *S. officinalis* **Table 3**. The level of 1,8-cineole decreased to 35.84 % in the freeze-dried samples compared to 38.10 % in the fresh samples. Interestingly, its level increased in the shade-dried samples to 48.57%. This fact could be explained by continuation of the plant activity during the drying process and the increase of the secondary metabolites as reflection of the gradual decrease in water contents [19, 20]. The observed retention indices of the oil components in **Tables 2&3** are matching the reported literature values. The well-known markers components detected from fresh, frozen and shade-dried samples of *C. medica* and *S. officinalis* are presented in **Table 4**.

As most of the EO's components expressed considerable similarity, the greater loss of the oil in *C. medica* compared to *S. officinalis* can't be referred to the EO composition. To justify these experimental data, a histological study was conducted on the leaves of both plants. *C. medica* is a cultivated plant and water supply up to 30 m^3/ha daily must be

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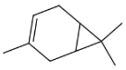
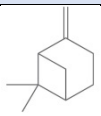
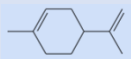
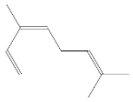
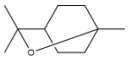
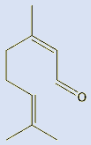
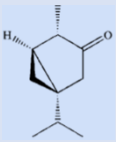
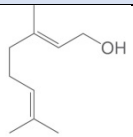
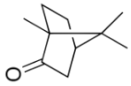
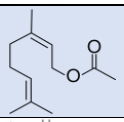
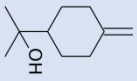
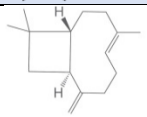
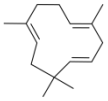
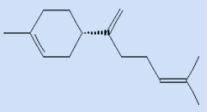
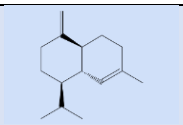
provided. During the rainy seasons, these amounts could be decreased [21]. Consequently, the plant histology didn't show any sign of water preservation. The cuticle of the leaves is very thin layer. Stomata are present in both upper and lower epidermis with stomatal index of 20.90 (reported 17.64 [22]). The oil glands are present just under the epidermal cells rendering the oil secretion liable to loss via the numerous stomata **Table 5-A**. The leaves surfaces are almost straight **Table 5-A1**. On the other hand, *S. officinalis* is originally a wild plant that grows in drought conditions [23]. Drought has positive influence on secondary metabolites in aerial parts of the plant [24]. Moderated stress condition enhances the synthesis of terpenes by the plant tissues [25]. The histology of the leaves showed many features for water preservation and consequently the EO secretions. Both leaf surfaces are covered with thick cuticle. The stomatal index is 6.18 with upper epidermis totally free from stomata. Epidermal cells and the layer beneath showed thick cell walls. The epidermal cells are occasionally covered with hairs. The leaves' surface is wavy creating deep grooves away from direct contact with external environment **Table 5-B**. All these features render *S. officinalis* more resistant to water and volatile contents loss.

Table 4. Main EO marker components detected from fresh, frozen and shade-dried samples of *C. medica* and *S. officinalis*.

No	Name	Chemical Structure	No	Name	Chemical Structure
1	3-Carene		**9**	*β*-Pinene	
2	Limonene		**10**	*β*-Myrcene	
3	*β*-Ocimene		**11**	1,8-Cineole	
4	*Z*-Citral		**12**	*cis*-Thujone	
5	Geraniol		**13**	Camphor	
6	Neryl acetate		**14**	*δ*-Terpineol	
7	Caryophyllene		**15**	*α*-Humulene	
8	*β*-Bisabolene		**16**	*γ*-Cadinene	

Compounds 1-8 from *C. medica*
Compounds 7, 9-16 from *S. officinalis*

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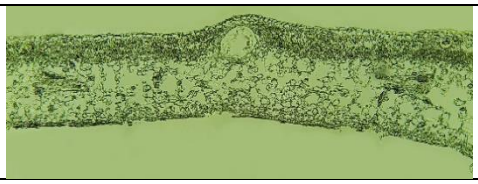
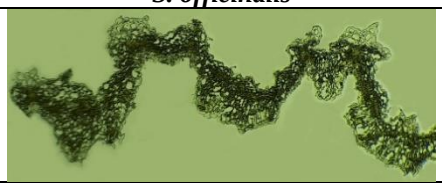
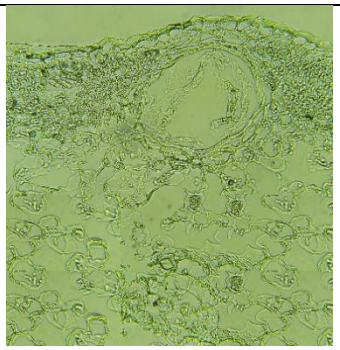
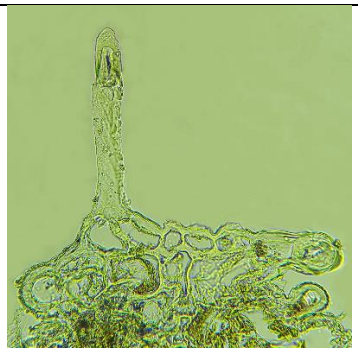
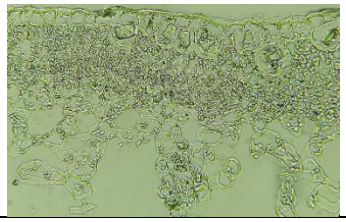
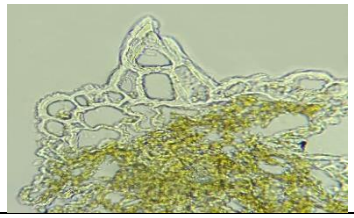
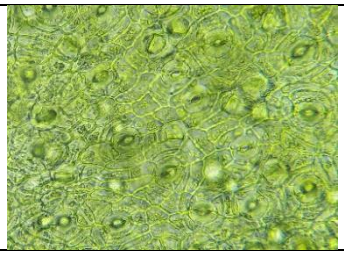
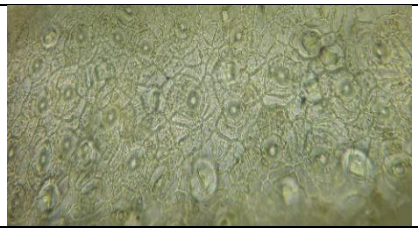
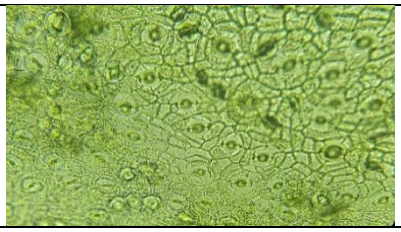
Table 5: Histological study of the leaves of *C. medica* (A) and *S. officinalis* (B).

	C. medica	***S. officinalis***
Leaves T.S.		
	A1	**B1**
T.S. Showing upper epidermis		
		
	A2	**B2**
Upper epidermis in surface view		
	A3	**B3**
Lower epidermis in surface view		
	A4	**B4**

4. Conclusion

The EO quantity and relative percentage of components in *C. medica* and *S. officinalis* were studied on fresh, freeze-dried, and shade-dried samples. EOs were prepared by hydrodistillation and analyzed by GC/MS. *C. medica* expressed a greater loss in EO contents upon drying and the histological study did not show any features for water and volatile content preservation as the plant is cultivated and should be irrigated regularly. Although *S. officinalis* expressed some loss of the EO in the freeze-dried samples, the shade-dried samples interestingly,

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demonstrated increase in the oil contents. Many factors participated in this phenomenon. Slow drying in shade allows enzymatic activity to take place; drought condition enhances the production of the secondary metabolites and the histological characters that enable the plant to preserve its water and volatile contents. The observed variations in EO yield and profile in *C. medica* and *S. officinalis* underscore the significance of selecting appropriate drying techniques tailored to specific plant species and intended applications. Changes in the EO profile due to drying methods may influence the therapeutic efficacy and industrial value of these oils. Tailoring drying techniques to maximize desired compounds could enhance their application in pharmaceuticals, cosmetics, and food industries. In addition, drying protocols should be developed with the end-user requirements, due to the diverse uses of EOs whether for antimicrobial properties, flavour enhancement, or therapeutic benefits. By aligning drying techniques with the biological and industrial potential of plants, we can better increase the benefits of essential oils while preserving their integrity and efficacy.

Credit author statement

MSA: Conceptualization, resources, reviewing and editing; HHZ: methodology, investigation, visualization, data curation, reviewing and editing; MAAS: methodology, investigation, writing original draft, soft wear; SMSA: soft wear, visualization, investigation; AKA: investigation, writing original draft, soft wear; HM: Conceptualization, methodology, data curation, visualization, reviewing and editing. All authors read and approved the final manuscript.

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Conflicts of interest

The authors declare that they have no conflict of interest.

Funding sources

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Data access and retention

Data are available with no restriction.

Ethical approvals

The authors declare no ethical approvals associated with this manuscript.

Highlights:

- Hydrodistilled EOs of *Citrus medica* and *Salvia officinalis* were analyzed by GC/MS.
- Different drying conditions significantly affected EO yield and profile.
- *C. medica* showed higher reduction in the EO percentage drying.
- Shade-dried *S. officinalis* unexpectedly showed increased EO content.
- *S. officinalis* leaves have low stomatal index and thick cuticle.

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