Physiological Studies on some Medicinal Plants of the Family Lamiaceae Grown Wildly in Saint Catherine Peninsula

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Abstract: Comparative physiological studies were carried out on five wild medicinal plant species of Lamiaceae, namely (Qartam) Stachys aegyptiaca Pers., (Attan) Lavandula pubescens L., (Rosemary) Rosmarinus officinalis L., (Sharma) Ballota kaiserii Tackh.and (Marmaria) Salvia multi caulis Vahl. The results showed that, Salvia multi caulis had the highest essential oil content (7.46%) which might be attributed to the increased thickness of midrib, lamina, length and width of both phloem and xylem tissues as well as to the number of xylem arms/bundle in the 3rd leaf. The highest value of free amino acids and phenolic compounds (8.76 and 12.16 mg/100 g FW, respectively) were found in Stachys aegyptiaca. The maximum stomatal number (650 stoma/mm²) was found in Ballota kaiserii. However, Lavandula pubescens had the maximum length and width of stomatal aperture (22.85 and 2.85 μm, respectively). Analysis of essential oils by GC-MS showed 87 different essential oil constituents in all investigated species. Essential oils showed high efficiency as an antimicrobial agent against some pathogens. Salvia multi caulis had a high potential against Salmonella typhimurium and Staphylococcus aureus with maximum clear zones of microbes (22 and 30mm, respectively). Lavandula pubescens had a positive effect against Escherichia coli with a maximum clear zone of 13 mm, due to the high total antioxidants (72.4%) in leaves. Rosmarinus officinalis showed high impact against Pseudomonas aeruginosa and Candida albicans with maximum clear zone of 25 and 22 mm, respectively. The high efficiency of essential oils against pathogens was correlated with specific bioactive constituents.

Keywords: Mint family, leaf anatomy, phytochemicals, GC-MS, essential oils, antipathogens

INTRODUCTION

Lamiaceae (Labiatae), the mint family, contains about 239 genus and 6900-7200 species distributed around the world (Bekut et al., 2018). Also, it has about 200 genus and about 4000 species in Egypt (Hassanein et al., 2020). About 20 genus and 42 species recorded in Saint Catherine peninsula (Boulos, 1995). Saint Catherine peninsula is one of the richest areas with wild medical, rare, and endemic plants. It is characterized by high and rugged mountains that range between 1500 and 2624 m above sea level.

High Medicinal value of lamiaceae plants is due to its high content of bioactive phytochemicals such as essential oils and antioxidants (Elless et al., 2000). Al-Badani et al. (2017) showed high differences in oil composition according to environmental conditions in Yemen Lavandula Pubescens which mainly grown in slopes and wadis. Moreover, they found that the concentration of β-pinene was decreased with latitude while the concentration of myrcene increased with rainfall. Dadach and Mehadi (2018) found that, germination percentage of Ballota hirsuta seed was 78% at 20°C, while high temperature and salinity reduced the seed germination which considered the principal factor for flora limitation in the arid regions.

Baser (1993) classified lamiaceae plants according to concentration of essential oil as oil-rich genera (>2 %), moderately oil-content (2%-0.5%) and poor oil-content (<0.5%). Rosmarinus, Lavandula and Salvia classified as oil-rich genera while Calamintha and Cyclorchium were moderately genera. Ballota and Stachys were low oil genera. Ali et al. (2011) separated 13 constituents from essential oil in leaves of Lavandula pubescens, by GC-MS as carvacrol (70.0%), caryophyllene oxide (5.5%), β-copaene (3.7%), β-bisabolene (2.5%), thymol methyl ether (2%), borneol (1.9%), piperitone oxide (1.5%) and unidentified compounds (4.4%). Akhlaghi et al. (2011) analyzed the essential oil of leaves of wild Iranian Stachys pubescens using GC-MS and found total volatile compounds 0.06% (v/w) and 17 constituents were detected. The main components of the oil were thymol (35.5%), linalool (23.7%) and geranial (9.0%). Monoterpenoids and oxygenated monoterpenes consisted of (81.0 %) while monoterpene hydrocarbons were (9.6%).

Morteza-Semnani et al. (2006) found that essential oil of Stachy balansa and S. recta had β-caryophyllene (24.3%) and 1-octen-3-ol (33.8%), respectively, while S. aegyptiaca had α-pinene (54.5%). Fu et al. (2013) used hydro-distillation and found that essential oils of Salvia contained monoterpens, oxygenated monoterpens, sesquiterpenes, diterpenes, not iso-prenoidal compounds and oxygenated sesquiterpenes. Alipour and Saharkhiz (2016) found the oil of Rosmarinus officinalis had α-pinene (25.8–27.7%), camphor (8.6–9%), camphene (6.5–7.7%) and 1,8-cineole (9.4–9.6%). Al-Badani et al. (2017) separated 56 compounds from the essential oils of Lavandula pubescens cultivated in Yemen. It was containing carvacrol (4.0–11.4%), caryophyllene oxide (2.1 – 6.8%) and terpinolins (0.6–9.2%). Ghomi et al. (2012) found that, essential oils derived from the flowers and leaves of Salvia had beneficial effect against Candida albicans. Al-Badani et al. (2017) found that essential oils of Lavandula pubescens had antibacterial activity against Staphylococcus aureus, Micrococcus luteus and

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Escherichia coli, with minimal inhibition concentration (MIC) value 0.078 μg/mL.

Due to global changes and overgrazing, wild plants as Lamiaceae species are endangered (Moustafa, 2001). Also, abiotic stressors as high temperature, drought, high light intensity, speed wind, radiation, salinity, pH of soil solution and deficiency of nutrient elements affected on the quality and quantity of essential oil of aromatic and medical values of medicinal plants (Abdelmajeed et al., 2013). Therfore, this study aimed to investigate some physiological parameters of five wild medicinal plant species of Lamiaceae naturally grown in Saint Catherine peninsula as well as its essential oil composition by GC-MS and its potency against some microbial organisms.

MATERIALS AND METHODS

Species of Lamiaceae:

Five wild species of Lamiaceae, were collected from Saint Catherine peninsula, mountains of Tafah Valley, in April 2019. This site was located at 33°57' to 34°00' South, and 28°26' to 28°34' East and attitude of mountains was 1500 and 2624 m above sea level. Five species include, qartam (Stachys aegyptiaca Pers.); attan (Lavandula pubescens L.) and rosemary (Rosmarinus officinalis L.); shaarma (Ballota kaiseri Tachk.) and bardaqash (Salvia multicaulis or acetabulosa) Vahl.). Species identification was done according to (Täckholm, 1974; El-Gazzar and Watson, 1968).

Soil analysis:

Soil samples were collected from a depth of about 30 cm under each plant and analyzed according to (Pharande and Sonar, 1997) as shown in Table (1). Particle size distribution was determined using the pipette method as described by (Tributh, 1970). Electrical conductivity (EC) of the saturated soil pastes extracts expressed as dSm⁻¹ was measured using conductivity meter model 710 according to (Allison and Richards, 1954). The pH of soil sample was determined according to (Page et al., 1982). Soluble anions of HCO₃⁻, Cl⁻ and soluble cations of Ca²⁺, Mg²⁺, Na⁺ and K⁺ were determined according to (Allison and Richards, 1954). Sulfate (SO₄²⁻) was precipitated by barium chloride as barium sulfate and gravimetrically determined (Jackson, 1967).

| Sample            | EC  dSm⁻¹ | pH  | Cations | Anions | Gravels | Sand  | Silt  | Clay  | Tex.         |
|-------------------|-----------|-----|---------|--------|---------|-------|-------|-------|--------------|
| Ballota kaiseri    | 0.73      | 7.97| 2.8     | 2.2    | 1.9     | 0.4   | 2.3   | 3.8   | 1.2          |
| Stachys aegyptiaca| 0.79      | 7.83| 2.8     | 2.3    | 2.0     | 0.9   | 3.1   | 2.6   | 2.3          |
| Lavandula pubescens| 0.67    | 8.07| 2.5     | 1.6    | 2.0     | 0.6   | 2.1   | 2.0   | 2.6          |
| Salvia multicaulis| 0.75     | 7.57| 19.0    | 15.0   | 23.3    | 0.7   | 22    | 8.0   | 28.0         |
| Rosmarinus officinalis| 0.46      | 8.23| 2.80    | 1.0    | 1.0     | 0.2   | 1.8   | 2.1   | 1.1          |

Biochemical determinations:

Photosynthetic pigments (Chl. a, b and carotenoids) were extracted with 80% of acetone and estimated spectrophotometrically at 662, 644 and 440.5 nm (Arnon, 1949). Soluble protein was determined by Bradford method (Bradford, 1976) at 595 nm. Free amino acids, reducing sugars, free phenolics and total antioxidant were extracted according to (Abdel-Rahman et al., 1975). Free phenolics were determined by a modified Folin-Ciocalteu method and measured at 650 nm according to (Horwitz et al., 1970). Free amino acids were estimated using the method of (Rosen, 1957) with ninhydrin reagent. The blue colored was measured against blank sample at 570 nm. Reducing sugars were determined by Nelson’s method (Moore, 2012) at 540 nm. Total antioxidants (%) were estimated by determine the inhibition % of DPPH (2,2-diphenyle-1-picyrillyhydrazy) according to (Hatano et al., 1988). All spectrophotometric analyses were done using UV/VIS spectrophotometer, PG instrument Ltd, USA. All biochemical compounds estimated as mg 100 g⁻¹ FW.

Extraction of essential oil:

For essential oil extraction, air-dried powdered aerial parts of each species were percolated several times in hexane at room temperature till complete exhaustion, and then filtrated and removed the hexane by distillation under reduced pressure (Danh et al., 2013). The extract was then weighed and stored at –20°C to test the biological activity and GC-MS analysis. The essential oil % was calculated according to (Danh et al., 2013).

GC-MS analysis:

The essential oils composition was analyzed with Gas chromatography–Mass spectrometry (GC-MS) method, using Trace GC-ISQ mass spectrometer (Thermo Scientific, Austin, TX, USA) with a direct
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Capillary column TG–5MS (30 m x 0.25 mm x 0.25 μm film thickness). The column oven temperature was initially held at 50°C and then increased by 5°C/min to 200°C hold for 2 min. Temperature increased to the final 300°C by 25°C/min and hold for 2 min. The injector and MS transfer line temperatures were kept at 270, 260°C respectively; Helium was used as a carrier gas at a constant flow rate of 1 ml/min. The solvent delay was 4 min and diluted samples of 1 μl were injected automatically using Autosampler AS1300 coupled with GC in the split mode. El mass spectra were collected at 70 eV ionization voltages over the range of m/z 50–650 in full scan mode. The ion source temperature was set at 250°C. The components were identified by comparison among its retention times and mass spectra with those of WILEY 09 and NIST 14 mass spectral database (Salem et al., 2016).

Antimicrobial activity assay:

The antimicrobial activity of essential oils was determined by well diffusion method according to (Atlas and Unterman, 1999). 100 μL of essential oil were added into the well against the following indicator strains: Escherichia coli strain ATCC 25922, Staphylococcus aureus strain ATCC 25923, Candida albicans strain ATCC 10231, Salmonella typhimurium strain ATCC 14028 and Pseudomonas aeruginosa strain ATCC 27853. The samples were incubated for 24 hours at 37°C, effective inhibitory was estimated by measuring diameters of clear zones.

Anatomy of leaves:

Tissues of 3rd leaf specimens were fixed by formalin acetic acid (F.A.A), then dehydrated in ethanol series, then ethanol with xylene series, embedded in paraffin wax, sectioned to thickness of 15 μ, double stained with safranin and light green, cleared in xylene, and mounted in Canada balsam according to (Willey, 1971). Measurements and photomicrographs were achieved using research microscope (LEICA, DM500) fitted with digital camera (LEICA, ICC50). The measurements were taken and average of 10 reading from 3 slides were calculated and evaluated comparatively (Sass, 1958).

Stomatal measurements:

According to (Willey, 1971), the number of stomata was determined and the dimensions of guard cells on adaxial surface of 3rd leaf was measured using LEICA, DM500 microscope.

Statistical analysis:

All data were statistically analyzed as randomized complete block design (Steele et al., 1997); using the MSTAT-C statistical package (Mstat-C, 1990) and means were separated by LSD test, P ≤ 0.05.

RESULTS AND DISCUSSION

Photosynthetic pigments:

Five Lamiaceae species under study were significantly different in the concentrations the photosynthetic pigments (Chl. a, b and carotenoids) as shown in Table (2). Leaves of Salvia multicaulis contained the highest values of Chl. a and Chl. a+b (64.1 and 108.8 mg/100 g FW) as compared to other species. This increment may be correlated with increasing of Mg2+ cation in cultivated soil (15 mEq/l) as shown in Table (1) and as compared to other soil types. The results agreed with those of Kochhar and Sukhbir (2020) who reported that Mg2+ was essential macro-elements, responsible for chlorophyll biosynthesis. However, Salvia multicaulis and Rosmarinus officinalis had the highest values of Chl. b without any significant differences. Increment of Chl. b compared to Chl. a in Rosmarinus officinalis and Lavandula pubescens may be a physiological adaptation to increase the range of absorption spectrum for maximum photosynthesis. Ohtsuka et al. (1997) reported a high ratio of photosystem II (Chl. a equal Chl. b) to photosystem I (which had more Chl.a) in shade adapted chloroplasts. However, leaves of Stachys aegyptiaca had the highest content of carotenoids (4.12 mg/100g FW) as compared to other species. High content of carotenoids may contribute with its protective role against high sunlight radiation (Taiz and Zeiger, 2004), as this species is distributed at high altitudes on Sant Catrine Mountain.

| Species            | Chl.a | Chl.b | Carotenoids | Chl.a +b | Chl.a : Chl.b | Total Chl.s: carotenoids |
|--------------------|-------|-------|-------------|----------|--------------|------------------------|
| Ballota kaiser     | 46.4b | 35.8c | 3.47b       | 82.3bc   | 1:0.8        | 1:0.04                 |
| Stachys aegyptiaca | 50.9b | 38.9b | 4.12a       | 89.8b    | 1:0.8        | 1:0.05                 |
| Lavandula pubescens| 38.3c | 40.8b | 2.82c       | 79.1c    | 1:1.1        | 1:0.04                 |
| Salvia multicaulis | 64.1a | 44.7a | 3.69b       | 108.8a   | 1:0.7        | 1:0.03                 |
| Rosmarinus officinalis | 40.2c | 45.1a | 2.77c       | 85.3bc   | 1:1.1        | 1:0.03                 |

Phytochemical compounds and total antioxidants:

Estimated phytochemicals differed according to species as shown in Table (3). Leaves of Rosmarinus officinalis had the highest concentration of reducing sugars (2.81 mg/100 g FW), while the highest values of free amino acids and phenolic compounds (8.76 and 12.16 mg/100 g FW, respectively) were found in Stachys aegyptiaca. Ballota kaiser had the maximum
value of total soluble protein (61.45 mg/100 g FW). Extract of *Lavandula pubescens* had the highest value of total antioxidants (72.4% inhibition of DPPH). The maximum percentage of essential oil (7.46%) was recorded in *Salvia multicaulis*. The high percentage of essential oil in *Salvia multicaulis* may be attributed to the high content of Chl. a and total Chl. a+b, which may increase the photosynthetic assimilates and therefore increase the carbon skeleton for oil biosynthesis Kochhar and Sukhbir (2020).

**Anatomical characters of 3rd leaf:**

Rolling of leaves downward and sunken stomata as observed in *Rosmarinus officinalis*, multiseriate and isobilateral palisade tissue in all investigated species were obvious features of desert or xerophytes plants (Table 4 and Fig. 1). Previous features of modification of leaves in *Rosmarinus officinalis* may be the cause of increment of reducing sugars content (Table 3) as a final product of photosynthesis. Leaves of *Salvia multicaulis* recorded the maximum values of most estimated parameters as thickness of midrib (567 µm), thickness of lamina (242 µm), thickness of phloem length and width (83.3 and 350 µm, respectively).

![Fig. (1): Histological characteristics of the 3rd leaf of the five wild Lamiaceae species. Uep, upper epidermis, Lep, Lower epidermis, Pa, palisade tissue, S, spongy tissue, X, xylem, Ph, phloem, Co, collenchyma, Tr, Trichomes](image)
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### Table (3): Biochemical constituents of 3rd leaf of different species of *Lamiaceae* collected from different locations in Saint Catherine during April-2019

| Species                | Reducing sugars | Free amino acids | Total protein | Free Phenolic compounds | Total antioxidants (Inhibition % of DPPH) | Essential oil (%) |
|------------------------|-----------------|------------------|--------------|-------------------------|-------------------------------------------|-------------------|
| Ballota kaiseri        | 1.31b           | 6.93ab           | 61.45a       | 6.11b                   | 52.6c                                     | 1.12              |
| Stachys aegyptiaca     | 2.08b           | 8.76a            | 38.23b       | 12.87a                  | 48.9d                                     | 3.48              |
| Lavandula pubescens    | 1.28b           | 0.83c            | 33.34c       | 6.26b                   | 72.4a                                     | 2.76              |
| Salvia multicaulis     | 1.82b           | 4.71b            | 34.59c       | 12.16a                  | 58.9b                                     | 7.46              |
| Rosmarinus officinalis | 2.81a           | 0.08c            | 26.81d       | 10.89a                  | 46.6e                                     | 3.05              |

### Table (4): Anatomical characters of different tissues of 3rd leaf of *Lamiaceae* species

| Species                | Thickness (µm) of epidermis with cuticle | Number of xylem arms/bundle | Number of xylem vessels/arm |
|------------------------|------------------------------------------|-----------------------------|-----------------------------|
|                        | epidermis | collenchyma | parenchyma | Palisade tissue | Spongy tissue | Phloem tissue | Xylem tissue | xylem arms/bundle | xylem vessels/ |
|                        | upper | lower | upper | lower | Upper | lower | length | width | Length | width | arm |
| Ballota kaiseri        | 160e | 74e   | 13.3d | 13.3d | 10.0d | 10.0d | 40.0d | 60.0d | 29.0d | 18.0e | 26.6e | 113e | 46.6d | 86.6d | 6d | 6b |
| Stachys aegyptiaca     | 242d | 183c  | 16.6c | 16.6c | 36.3c | 36.3c | 118.1b | 81.1c | 40.0c | 33.3d | 125d | 50c | 100c | 8c | 4b |
| Lavandula pubescens    | 407c | 207b  | 35.7a | 28.5a | 171a | 100b | - | - | 100a | 78.5a | 42.8c | 143b | 57.1b | 129b | 18b | 8a |
| Salvia multicaulis     | 567a | 242a  | 16.6c | 16.6c | 109.5b | 234.5a | 66.6c | 166.6a | 59.0c | 29.4d | 83.3a | 350a | 91.6a | 283a | 21a | 5b |
| Rosmarinus officinalis | 469b | 154d  | 25.0b | 23.0b | - | - | 285.7a | 142.8b | 66.6b (3 layers) | 50.0b (6 layers) | 53.8b | 131c | 46.1d | 100c | 8c | 4b |
The thickness of xylem length and width (91.6 and 283 µm, respectively) and number of xylem arms / bundle (21) as well as the thickness of lower parenchyma (166.6 µm). Stachys aegyptiaca leaves had palisade tissue on both side of lamina, as a type of modification in desert leaves. Maximum thickness of upper parenchyma (285.7 µm) was found in Rosmarinus officinalis leaves. The highest thickness of palisade tissue (100 µm), thickness of spongy tissue (78.5 µm), number of xylem vessels/ arm (8), thickness of both lower and upper epidermis with cuticle (28.5 and 35.7 µm, respectively), thickness of upper and lower collenchyma (171 and 100 µm, respectively), were observed in Lavandula pubescens leaves. However, the minimum values of most previous estimated parameters were found in leaves of Ballota kaiseri, as thickness of midrib was (160 µm), thickness of lamina (74 µm), thickness of upper and lower parenchyma (40 and 60 µm, respectively), thickness of palisade tissue (29 µm), thickness of spongy tissue (18 µm), width of xylem tissue (86.6 µm) and number of xylem arms (6).

Stomatal measurements:

Adaxial surface of leaves of Ballota kaiseri had the maximum stomatal number (650 of stoma/mm²) as compared to other species (Table 5). The maximum length and width of guard cells (27.14 and 8.75 µm, respectively) were found in Lavandula pubescens. The maximum length and width of stomatal aperture (22.85 and 2.85 µm, respectively) were found in Lavandula pubescens and Salvia multicaulis.

| Species                | Guard cells (µm) | Stomatal aperture (µm) | Number of stomata (mm²) |
|------------------------|------------------|------------------------|------------------------|
|                        | Length           | Width                  | Length                 | Width                  |
| Ballota kaiseri        | 23.07c           | 7.69b                  | 13.84d                 | 3.07a                  | 650a                   |
| Stachys aegyptiaca     | 22.61c           | 5.69d                  | 15.92c                 | 2.07b                  | 300c                   |
| Lavandula pubescens    | 27.14a           | 8.75a                  | 22.85a                 | 2.85a                  | 250d                   |
| Salvia multicaulis     | 25.71b           | 5.71d                  | 22.85a                 | 2.85a                  | 450b                   |
| Rosmarinus officinalis | 22.84c           | 6.76c                  | 18.85b                 | 1.80b                  | 250d                   |

Constituents of volatile oils:

Analysis of shoot essential oils of different species by gas chromatography-mass spectrometry detected AV different compounds in all investigated species as shown in (Table 6 and Figs. 2-7). These compounds were classified as essential hydrocarbons, essential oxygenated hydrocarbons, and terpenes. The highest number of essential hydrocarbons (28) was found in Ballota kaiseri, while the maximum number of essential oxygenated hydrocarbons (22) was detected in Stachys aegyptiaca, and the maximum number of essential terpenes (22) were found in Lavandula pubescens. Essential hydrocarbons compounds as undecane, dodecane, dodecane, tridecane, tridecane, cetane, cetane, heptadecane, octadecane, norphytane, heneicosane, docosane, heptacosane, octacosane, dotriacontane and hexatriacontane were found in all investigated species.

Essential oxygenated hydrocarbons as dodecanol, tridecanal, tetradecanol, cetanol and ethyl hexadecanoate were detected in all investigated species. Compounds as jasmonate lactone (0.08%), dodecanal (0.39%), ionone (0.24%), ionol (0.09), methyl linoleate (0.26), and retinol acetate (0.09) were recorded only in Lavandula pubescens. While methyl jasmonate (0.09), cinnamodial (0.04%), methyl eperuate (0.04%) and retinol acetate (0.07%) were found only in Stachys aegyptiaca, while hexadecanoic acid (1.25%) was detected in Ballota kaiseri. Essential terpenes as menthan, decane, pentadecane, pentadecanoene, pentadecanol, eicosane, phyto, eicosanol and pentatriacontane were separated from all studied species. Terpenes as cadalene (0.03%), scarel (0.02%) and ɑ-amyrin (0.04%) were observed in Stachys aegyptiaca, while totarol (0.50%), gerany-p-cymene (0.47%), abietol (0.12%) and 4,8,13-duvatriene-1,3-dio (0.50%), were detected in Lavandula pubescens. Similar constituents were separated using GC in the same genera with different researchers (Morteza-Semnani et al., 2006; Ali et al., 2011; Akhlaghi et al., 2011; Ghomi et al., 2012; Fu et al., 2013; Alipour and Saharkhiz, 2016; Al-Badani et al., 2017). Arcila-Lozano et al. (2004) reported that the essential oil composition depends on the climate, altitude, time of collection, and the stage of growth.
Table (6): Essential constituents as detected by GC-MS in different studied species

| Group                        | Sub-group                        | Ballota kaiseri | Stachys aegyptiaca | Lavandula pubscens | Salvia multicaulis | Rosmarinus officinalis |
|------------------------------|----------------------------------|-----------------|--------------------|-------------------|-------------------|------------------------|
| Hydrocarbons                 |                                  | 28              | 26                 | 25                | 25                | 25                     |
| Oxygenated hydrocarbons      |                                  | 14              | 22                 | 19                | 15                | 9                      |
|                              | monoterpenoids (C10)             | 2               | 2                  | 3                 | 2                 | 3                      |
|                              | oxygenated monoterpenoids        | 1               | 1                  | -                 | 1                 | -                      |
|                              | sesquiterpenes (C15)             | 2               | 3                  | 2                 | 2                 | 2                      |
|                              | oxygenated sesquiterpenes        | 6               | 5                  | 5                 | 5                 | 4                      |
| Terpenes                     |                                  | 17              | 21                 | 22                | 18                | 15                     |
|                              | Total terpenes                   | 17              | 21                 | 22                | 18                | 15                     |
|                              | Total constituents               | 59              | 69                 | 66                | 58                | 49                     |

Fig. (2): Constituents of volatile oil of Ballota kaiseri separated by GC-MS. 1.menthane 2.decane 3.undecyne 4.undecane 5.decen-1-ol 6.dodecene 7.dodecane 8.methyl biphenyl 9.tridecane 10.tridecane 11.chamazulene 12.dodecanol (lauryl alcohol) 13.ionol 14.tetradecene 15.tridecane 16.tetradecane 17.tridecanol 18.â-longipinene 19.phenyl propyl isobutanoate 20.tetradecanal (myrist aldehyde) 21.pentadecane 22.tetradecanol (myristyl alcohol) 23.(bisabolene) caryophyllene oxide spathulenol 24.cetene (hexadecene) 25.cetane (hexadecane) 26.pentadecane 27.pentadecanol 28.â-longipinene 29.platamin (geranyl isoalterate) 30.heptadecane 31.menthol isoalterate 32.cetanol (hexadecanol) 33.octadecene 34.cyclohexadecanolide 35.octadecane 36.â-nonadecene 37.norphytane (pristanne) 38.hexadecanoic acid 39.juvabione 40.ethyl hexadecanoate 41.stearol (octadecanol) 42.eicosane 43.heneicosane 44.phytol 45.eicosanol 46.docosene 47.docosane 48.ethyl octadecanoate 49.tricosane 50.1-docosanol 51.pentacosane 52.hexacosane 53.heptacosane 54.octacosane 55.nonacosane 56.dotriacontane 57.pentatriacontane 58.hexatriacontane 59.tetratetracontane.
Fig. (3): Constituents of volatile oil of *Stachys aegyptiaca* separated by GC-MS
1. nonane 2. menthane 3. decane 4. undecane 5. decen-1-ol 6. dodecane 7. dodecene 8. methyl biphenyl 9. tridecene 10. dodecadienal 11. tridecane 12. chamazulene 13. dodecanol (lauryl alcohol) 14. ionol 15. tetradecene 16. tridecanal 17. cadalene 18. tetradecane 19. tridecanol 20. á-longipinene 21. phenyl propyl isobutanote 22. tetradecan (myristaldehyde) 23. pentadecane 24. tetradecanol (myristyl alcohol) 25. (bisabolene) caryophyllene oxide 26. cetene (hexadecene) 27. methyl jasmone 28. cetane (hexadecane) 29. pentadecanone 30. limonen-6-ol 31. heptadecene 32. menthol isovalerate 33. cetanol (hexadecanol) 34. octadecene 35. cyclohexadecanolide 36. octadecane 37. norphytane (pristane) 38. methyl dehydroabietae 39. norphytane (pristane) 40. juvabione 41. ethyl hexadecanoate 42. steanol (octadecanol) 43. eicosane 44. nonadecanol 45. retinal 46. manool 47. heneicosane 48. phytol 49. eicosanol 50. cinnamodal, agandencial 51. sclareol 52. docosane 53. ethyl octadecnoate 54. methyl dehydroabietae 55. methyl eperuate 56. tricosane 57. 1-docosanol 58. retinol acetate 59. phytol acetate (erucic acid) 60. idebenone 61. tetracontane 62. pentacosane 63. hexacosane 64. heptacosane 65. octacosane 66. á-amyrist 67. dotriacontane 68. pentatriacontane 69. hexatriacontane.

Fig. (4): Constituents of volatile oil of *Lavandula pubescens* separated by GC-MS
1. menthene 2. menthane 3. decane 4. undecyne 5. undecane 6. dodecene 7. dodecene 8. methyl biphenyl 9. tridecene 10. dodecadienal 11. tridecane 12. jasmone lactone 13. chamazulene 14. dodecanol (lauryl alcohol) 15. ionol 16. tetradecene 17. tridecanal 18. tridecanol 19. á-longipinene 20. ionone 21. ionol 22. pentadecanone 23. tetradecanol (myristyl alcohol) 25. (bisabolene) caryophyllene oxide 26. cetene (hexadecene) 27. cetane (hexadecane) 28. pentadecanone 29. pentadecanone 30. limonen-6-ol 31. platambin (geranyl isovalerate) 32. heptadecane 33. cetanol (hexadecanol) 34. cyclohexadecanolide 35. octadecane 36. 1-nonadecene 37. norphytane (pristane) 38. juvabione 39. ethyl hexadecanoate 40. steanol (octadecanol) 41. gerany-p-cymene 42. eicosane 43. nonadecanol 44. retinal 45. retinol 46. manool 48. manool 49. phytol 50. phytol 51. totolel 52. eicosanol 53. 4,8,13-duvatriene-1,3-dio 54. docosene 55. docosane 56. tricosane 57. methyl nitoresedate 58. phytol acetate (erucic acid) 59. idebenone 60. pentacosane 61. hexacosane 62. heptacosane 63. octacosane 64. dotriacontane 65. pentatriacontane 66. hexatriacontane.
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**Salvia multicaulis**

![Graph](image1.png)

**Fig. (5): Constituents of volatile oil of *Salvia multicaulis* separated by GC-MS**

1. nonane 2. menthane 3. decane 4. undecyne 5. undecane 6. decen-1-ol 7. dodecene 8. dodecane 9. methyl biphenyl 10. tridecene 11. tridecane 12. chamazulene 13. dodecanol (lauryl alcohol) 14. ionol 15. tetradecene 16. tridecanol 17. tetradecane 18. tridecanol 19. á-longipinene 20. tetradecanol (myrist aldehyde) 21. pentadecane 22. tetradecanol (myristyl alcohol) 23. (bisabolene) caryophyllene oxide 24. dodecanol (lauryl alcohol) 25. tetradecene 26. pentadecane 27. pentadecanol 28. limonen-6-ol 29. platambin (geranyl isoalvaretate) 30. heptadecane 31. cetanol (hexadecanol) 32. cyclohexadecanolide 33. octadecane 34. 1-nonadecene 35. norphytane (pristane) 36. juvabione 37. ethyl hexadecanoate 38. stearol (octadecanol) 39. eicosane 40. nonadecanol 41. retinal 42. retinol 43. manool 44. heneicosane 45. phytol 46. eicosanol 47. docosane 48. methyl dehydroabietate 49. tricosane 50. 1-docosanol 51. phytol acetate (erucic acid) 52. pentacosane 53. hexacosane 54. heptacosane 55. octacosane 56. dotriacontane 57. pentatriacontane 58. hexatriacontane.

**Rosmarinus officinalis**

![Graph](image2.png)

**Fig. (6): Constituents of volatile oil of *Rosmarinus officinalis* separated by GC-MS**

1. nonane 2. menthene 3. menthane 4. decane 5. undecyne 6. undecane 7. dodecene 8. dodecane 9. methyl biphenyl 10. tridecene 11. tridecane 12. chamazulene 13. dodecanol (lauryl alcohol) 14. tetradecene 15. tridecanol 16. tetradecane 17. á-longipinene 18. tetradecanol (myrist aldehyde) 19. pentadecane 20. tetradecanol (myristyl alcohol) 21. (bisabolene) caryophyllene oxide 22. dodecanol (lauryl alcohol) 23. tetradecene 24. pentadecane 25. pentadecanol 26. platambin (geranyl isoalvaretate) 27. heptadecane 28. cetanol (hexadecanol) 29. octadecane 30. 1-nonadecene 31. norphytane (pristane) 32. ethyl hexadecanoate 33. stearol (octadecanol) 34. eicosane 35. nonadecanol 36. retinal 37. heneicosane 38. phytol 39. eicosanol 40. docosane 41. 1-docosanol 42. tetrasose 43. pentacosane 44. heptacosane 45. octacosane 46. dotriacontane 47. pentatriacontane 48. hexatriacontane 49. tetracontane
Antimicrobial activity of essential oils:

Essential oils from all studied species had antimicrobial positive effect on Candida albicans, compared to other pathogens. All essential oils had positive effect on Staphylococcus aureus except those from Ballota kaiseri. The essential oils of Stachys aegyptiaca and Lavandula pubescens had efficient antimicrobial effect on Escherichia coli with high clear zone (13mm) for Lavandula pubescens. The high activity of Stachys aegyptiaca against Escherichia coli may be due to its high content of terpenes as cadalene (0.03%), sciarol (0.02%) and α-amyrin (0.04%). The results were in agreement with (Khanavi et al., 2009) who observed that Stachys species contained polyphenols as a major antioxidant in its essential oil. Also, (Shafaghat and Oji, 2010) found that the essential oil of Stachys byzantina had antimicrobial activity against Pseudomonas aeruginosa, Candida albicans and Staphylococcus aureus. Hyldgaard et al. (2012) found also that the presence of hydrophobic bioactive compounds in essential oils can alter cell permeability, disturb ion homeostasis, and lead to microbe death. High efficiency of Lavandula pubescens as antipathogen may be due to its high content of oxygenated hydrocarbons (0.91% ionol) and terpenes (0.50% totarol), as well as, a high value of total antioxidants that inhibited 72.4% of 2,2-diphenyl-1-picrylhydrazyl. The results were compatible with those of Al-Badani et al. (2017), who showed that essential oils of Lavandula pubescens had antibacterial activity against Salmonella enterica and Staphylococcus aureus, antifungal activity against Candida albicans. Essential oils of Salvia multicaulis and Rosmarinus officinalis had effective antimicrobial effect on Salmonella typhimurium with high clear zone (22 mm) for Salvia multicaulis. Essential oil of Salvia multicaulis and Rosmarinus officinalis had effective antimicrobial effect on Pseudomonas aeruginosa with high clear zone (25 mm) for Rosmarinus officinalis under laboratory conditions as shown in Table (7) and Figure (7). The high efficiency of essential oil of Salvia multicaulis may be correlated with the high content of terpenes as manool (2.68%), oxygenated hydrocarbons as ionol and docosanol (0.11% and 0.25%, respectively), as well as the high value of total phenolics (12.16 mg/100 g FW). Also, it had high antioxidants % (oils inhibited 58.9% of 2,2-diphenyl-1-picrylhydrazyl) and had high percent of essential oils (7.46%) compared to other species.

The Results were in agreement with those of Jassbi et al. (2012), who separated more than 100 active compounds from Salvia, including hydrocarbon monoterpenes, oxygenated monoterpenes, sesquiterpene hydrocarbons, sesquiterpene oxygenated terpenes and diterpenes which exhibit different microorganisms. Also, Lambert et al. (2001) found that the presence of phenolic substances in plant essential oils, including carvacrol, eugenol and thymol, can damage the plasmalemma and coagulate the cellular components of microbes. Essential oils of Rosmarinus officinalis had high capacity against pathogens due to its high content of terpenes as eicosanol (1.66%) and oxygenated hydrocarbons as cetanol (1.66%), also contain high concentration of phenolic compounds (10.89 mg/100 g FW). The results are closely attuned with those of Bozin et al. (2007), who found that essential oils of rosemary had positive effect against a wide spectrum of bacteria and fungi through peroxidation of membrane lipids in microbes. Suryanti et al. (2020) reported also that eicosanol is a natural compound with an antioxidant activity.

Table (7): Effect of different essential oils (100 µl) as antimicrobial agent on different pathogenic microorganisms

| Species           | Escherichia coli | Salmonella typhimurium | Pseudomonas aeruginosa | Staphylococcus aureus | Candida albicans |
|-------------------|------------------|------------------------|------------------------|-----------------------|-----------------|
| -ve control       | -ve              | -ve                    | -ve                    | -ve                   | -ve             |
| Ballota kaiseri   | -ve              | -ve                    | -ve                    | -ve                   | 14              |
| Stachys aegyptiaca | 12               | -ve                    | -ve                    | 15                    | 15              |
| Lavandula pubescens | 13               | -ve                    | -ve                    | 15                    | 16              |
| Salvia multicaulis | -ve              | 22                     | 22                     | 30                    | 20              |
| Rosmarinus officinalis | -ve     | 20                     | 25                     | 20                    | 22              |
Physiological Studies on some Medicinal Plants of the Family *Lamiaceae* Grown Wildly in Saint Catherine

**Escherichia coli**

**Salmonella typhimurium**

**Pseudomonas aeruginosa**

**Staphylococcus aureus**

**Candida albicans**

*Fig. (7):* Diameter of inhibitory clear zone (mm) of different pathogenic microbes after application of essential oils of *Lamiaceae* species. -ve control, negative control (Hexane); CL, Clear zone meaning the lethal effect of essential oil. 6- *Ballota undulata* 7- *Stachys aegyoica* 8- *Lavandula pubescens* 9- *Salvia multicaulis* 10- *Rosmarinus officinalis*
CONCLUSION

Salvia multicaulis, the most studied species of Lamiaceae, contained antioxidant activities and had a high concentration of beneficial phytochemicals against Staphylococcus and Salmonella pathogens. The essential oils of Rosmarinus officinalis can be used for treating Pseudomonas aeruginosa and Candida albicans due to its high concentration of terpenes and oxygenated hydrocarbons. The essential oils of Lavandula pubescens and Stachys aegyptiaca can be used against Escherichia coli. The chemical properties of the soil had an obvious effect on the physiological characteristics and composition of essential oils of the medicinal plants of the family Lamiaceae grown in Saint Catherine Peninsula.

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دراسات فسيولوجية لبعض الأنواع البرية الطبية للعائلة الشفوية النامية في محمية سانت كاترين

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قسم النبات والميكروبيولوجي - كلية العلوم - جامعة السويس - السويس - مصر
كلية الصيدلة - جامعة سيناء - الإسماعيلية - مصر

أجريت دراسة مقارنة فسيولوجية على خمسة أنواع بحرية طبية من العائلة الشفوية هي: Stachys aegyptiaca Pers., Lavandula pubescens L., Rosmarinus officinalis L., Ballota kaiser Tackh. and Salvia multicaulis Vahl. وحولت على أساس نسبة من الزيت العطري (27.44%) الذي أرتبط ب⁻¹⁵ بالنتر الساطع والصمل وزن وطول وعرض كلاً من نسيج الخشب واللحم وعدد أذرع الخشب لكل حزمة في الورقة الثالثة للنباتات. سجل أعلى قيمة للأحماض الأمينية الحرة والمركبات الفينولية (8.76) وسجل أعلى عدد للنغم (150 تغ لكل ميتر مربع) في Stachys aegyptiaca 12.16 مجم/100 جم وزن طازج، على التوالي) في نبات القرطس (GC-MS) الغاز-الطيف الكتلي للزيت العطري للأنواع المختلفة وجود 72 مركب طيار. كما اظهر الزيت العطري لهذه الأنواع كفاءة عالية كمضاد للميكروبات على بعض مسببات الأمراض البكتيرية والفطريات في الدراسة. حيث اظهر الزيت العطري للمرمية كفاءة عالية ضد بكتيريا الاستيفالوكوكس و بكتيريا السالسياليا وظهر أيضاً فاعلية ضد بكتيريا الابكاليا وظاهر أيضاً فاعلية ضد بكتيريا السالسياليا وظاهر أيضاً فاعلية ضد بكتيريا السالسياليا وظاهر أيضاً فاعلية ضد بكتيريا الابكاليا وظاهر أيضاً فاعلية ضد بكتيريا الابكاليا وظاهر أيضاً فاعلية ضد بكتيريا الابكاليا وظاهر أيضاً فاعلية ضد بكتيريا الابكاليا وظاهر أيضاً فاعلية ضد بكتيريا الابكاليا وظاهر أيضاً فاعلية ضد بكتيريا الابكاليا وظاهر أيضاً فاعلية ضد بكتيريا الابكاليا وظاهر أيضاً فاعلية ضد بكتيريا الابكاليا وظاهر أيضاً فاعلية ضد بكتيريا الابكاليا وظاهر أيضاً فاعلية ضد بكتيريا الابكاليا وظاهر أيضاً فاعلية ضد بكتيريا الابكاليا وظاهر أيضاً فاعلية ضد بكتيريا الابكاليا وظاهر أيضاً فاعلية ضد بكتيريا الابكاليا وظاهر أيضاً فاعلية ضد بكتيريا الابكاليا وظاهر أيضاً فاعلية ضد بكتيريا الابكاليا وظاهر أيضاً فاعلية ضد بكتيريا الابكاليا وظاهر أيضاً فاعلية ضد بكتيريا الابكاليا وظاهر أيضاً فاعلية ضد بكتيريا الابكاليا وظاهر أيضاً فاعلية ضد بكتيريا الابكاليا وظاهر أيضاً فاعلية ضد بكتيريا الابكاليا وظاهر أيضاً فاعلية ضد بكتيريا الابكاليا وظاهر أيضاً فاعلية ضد بكتيريا الابكاليا وظاهر أيضاً فاعلية ضد بكتيريا الابكاليا وظاهر أيضاً فاعلية ضد بكتيريا الابكاليا وظاهر أيضاً فاعلية ضد بكتيريا الابكاليا وظاهر أيضاً فاعلية ضد بكتيريا الابكاليا وظاهر أيضاً فاعلية ضد بكتيريا الابكاليا وظاهر أيضاً فاعلية ضد بكتيريا الابكاليا وظاهر أيضاً فاعلية ضد بكتيريا الابكاليا وظاهر أيضاً فاعلية ضد بكتيريا الابكاليا وظاهر أيضاً فاعلية ضد بكتيريا الابكاليا وظاهر أيضاً فاعلية ضد بكتيريا الابكاليا وظاهر أيضاً فاعلية ضد بكتيريا الابكاليا وظاهر أيضاً فاعلية ضد بكتيريا الابكاليا وظاهر أيضاً فاعلية ضد بكتيريا الابكاليا وظاهر أيضاً فاعلية ضد بكتيريا الابكاليا وظاهر أيضاً فاعلية ضد بكتيريا الابكاليا وظاهر أيضاً فاعلية ضد بكتيريا الابكاليا وظاهر أيضاً فاعلية ضد بكتيريا الابكاليا وظاهر أيضاً فاعلية ضد بكتيريا الابكاليا وظاهر أيضاً فاعلية ضد بكتيريا الابكاليا وظاهر أيضاً فاعلية ضد بكتيريا الابكاليا وظاهر أيضاً فاعلية ضد بكتيريا الابكاليا وظاهر أيضاً فاعلية ضد بكتيريا الابكاليا وظاهر أيضاً فاعلية ضد بكتيريا الابكاليا وظاهر أيضاً فاعلية ضد بكتيريا الابكاليا وظاهر أيضاً فاعلية ضد بكتيريا الابكاليا وظاهر أيضاً فاعلية ضد بكتيريا الابكاليا وظاهر أيضاً فاعلية ضد بكتيريا الابكاليا وظاهر أيضاً فاعلية ضد بكتيريا الابكاليا وظاهر أيضاً فاعلية ضد بكتيريا الابكاليا وظاهر أيضاً فاعلية ضد بكتيريا الابكاليا وظاهر أيضاً فاعلية ضد بكتيريا الابكاليا وظاهر أيضاً فاعلية ضد بكتيريا الابكاليا وظاهر أيضاً فاعلية ضد بكتيريا الابكاليا وظاهر أيضاً فاعلية ضد بكتيريا الابكاليا وظاهر أيضاً فاعلية ضد بكتيريا الابكاليا وظاهر أيضاً فاعلية ضد بكتيريا الابكاليا وظاهر أيضاً فاعلية ضد بكتيريا الابكاليا وظاهر أيضاً فاعلية ضد بكتيريا الابكاليا وظاهر أيضاً فاعلية ضد بكتيريا الابكاليا وظاهر أيضاً فاعلية ضد بكتيريا الابكاليا وظاهر أيضاً فاعلية ضد بكتيريا الابكاليا وظاهر أيضاً فاعلية ضد بكتيريا الابكاليا وظاهر أيضاً فاعلية ضد بكتيريا الابكاليا وظاهر أيضاً فاعلية ضد بكتيريا الابكاليا وظاهر أيضاً فاعلية ضد بكتيريا الابكاليا وظاهر أيضاً فاعلية ضد بكتيريا الابكاليا وظاهر أيضاً فاعلية ضد بكتيريا الابكاليا وظاهر أيضاً فاعلية ضد بكتيريا الابكاليا وظاهر أيضاً فاعلية ضد بكتيريا الابكاليا وظاهر أيضاً فاعلية ضد بكتيريا الابكاليا وظاهر أيضاً فاعلية ضد بكتيريا الابكاليا وظاهر أيضاً فاعلية ضد بكتيريا الابكاليا وظاهر أيضاً فاعلية ضد بكتيريا الابكاليا وظاهر أيضاً فاعلية ضد بكتيريا الابكاليا وظاهر أيضاً فاعلية ضد بكتيريا الابكاليا وظاهر أيضاً فاعلية ضد بكتيريا الابكاليا وظاهر أيضاً فاعلية ضد بكتيريا الابكاليا وظاهر أيضاً فاعلية ضد بكتيريا الابكاليا وظاهر أيضاً فاعلية ضد بكتيريا الابكاليا وظاهر أيضاً فاعلية ضد بكتيريا الابكاليا وظاهر أيضاً فاعلية ضد بكتيريا الابكاليا وظاهر أيضاً فاعلية ضد بكتيريا الابكاليا وظاهر أيضاً فاعلية ضد بكتيريا الابكاليا وظاهر أيضاً فاعلية ضد بكتيريا الابكاليا وظاهر أيضاً في الدراسة اللاحقة.