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 kaiseri* Tackh.and (Marmaria) *Salvia multicaulis* Vahl. The results showed that, *Salvia multicaulis* 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/mm^2) was found in *Ballota kaiseri.* 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 multicaulis* 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 Mehdadi (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 *Cyclotrchium* 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 geraniol (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 balansae* 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 monoterpenes, oxygenated monoterpenes, 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 terpinols (0.6–9.2%). Ghomi *et al.* (2012) found that, essential oils derived from the flowers and leaves of *Salvia* had benefical effect aginst *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 µl/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 Talah 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.)*;* sharma (*Ballota kaiseri* Tackh.) and bardaqoash *(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 HCO^{3-}, Cl^- and soluble cations of Ca^{2+}, Mg^{2+}, Na^+ and K^+ were determined according to (Allison and Richards, 1954). Sulfate (SO_4^{2-}) was precipitated by barium chloride as barium sulfate and gravimetrically determined (Jackson, 1967).

Table (1): Soil physical and chemical properties of the growing sites of different *Lamiaceae* species for the upper 30 cm soil depth (April-2019)

Sample	Chemical properties mEq/L									Physical properties %				
	EC dSm^{-1}	pH	Cations				Anions			Gravels	Sand	Silt	Clay	Tex.
			Ca^{2+}	Mg^{2+}	Na^+	K^+	Cl^-	HCO_3^-	SO_4^{2-}					
Ballota kaiseri	0.73	7.97	2.8	2.2	1.9	0.4	2.3	3.8	1.2	46.1	36.9	6.3	10.6	Sandy Loam
Stachys aegyptiaca	0.79	7.83	2.8	2.3	2.0	0.9	3.1	2.6	2.3	41.2	47.7	2.8	8.2	Sandy Loam
Lavandula pubescens	0.67	8.07	2.5	1.6	2.0	0.6	2.1	2.0	2.6	52.7	35.3	3.8	8.2	Sandy Loam
Salvia multicaulis	5.75	7.57	19.0	15.0	23.3	0.7	22	8.0	28.0	26.4	51.8	15.6	6.2	Sandy Loam
Rosmarinus officinalis	0.46	8.23	2.80	1.0	1.0	0.2	1.8	2.1	1.1	40.8	54.2	1.7	3.3	sand

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-picryllhydrazyl) 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

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. EI 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 stain* ATCC 14028 and *Pseudomonas aeruginosa* strain ATCC 27853. The samples were incubated for 24 hours at 37^0C, 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, $P \leq 0.05$.

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 Mg^{2+} 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 Mg^{2+} 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.

Table (2): Concentrations of photosynthetic pigments in 3rd leaves of different species of *Lamiaceae* collected from different locations in Saint Catherine during April 2019

Species	Chl.a	Chl.b	Carotenoids	Chl.a +b	Chl.a : Chl.b	Total Chl.s: carotenoids
	mg / 100 g FW					
Ballota kaiseri	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

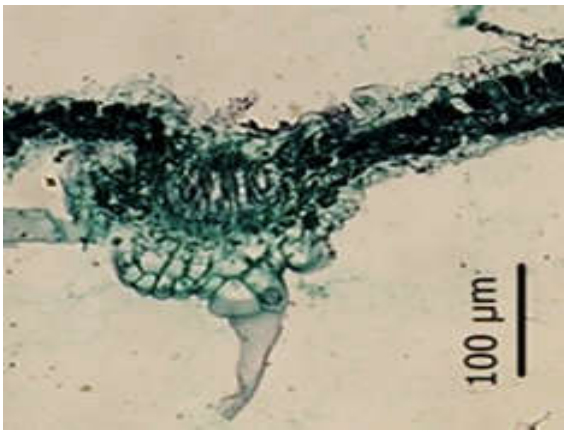
Phytochemical compounds and total antioxidants:

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 kaiseri* had the maximum

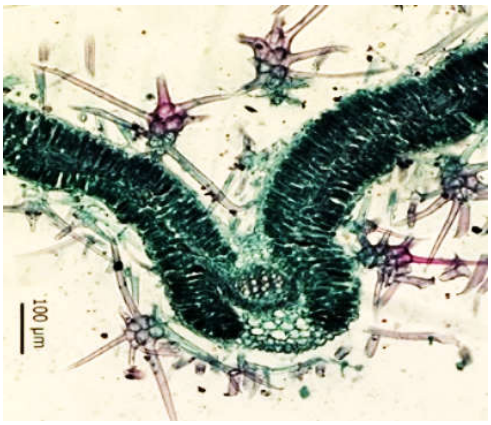
value of total soluble protein (61.45 mg/100 g FW). Extract of *Lavandula pubescen*s had the highest value of total antioxidants (72.4% inhibition of DPPH). The maximum percentage of essential oil (7.46%) was recorded in *Salvia multicaulis*. The high percentage of essential oil in *Salvia multicaulis* 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:

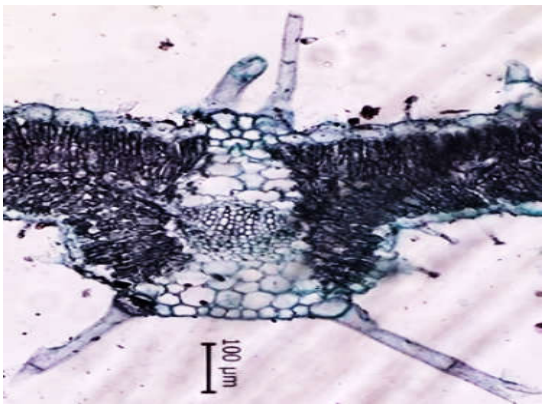
Rolling of leaves downward and sunken stomata as observed in *Rosmarinus officinalis,* multiseriate and isobilateral palisade tissue in all investigated species were obvious features of desert or xerophytes plants (Table 4 and Fig. 1). Previous features of modification of leaves in *Rosmarinus officinalis* 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).



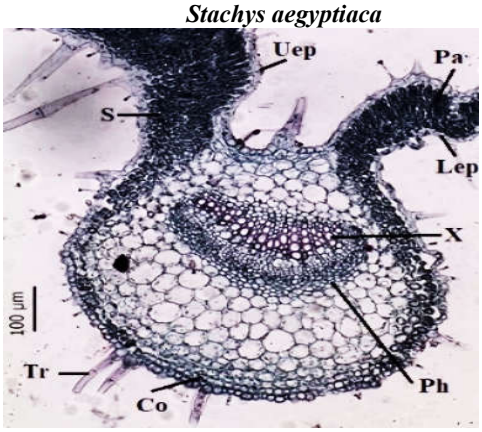
Ballota kaiseri



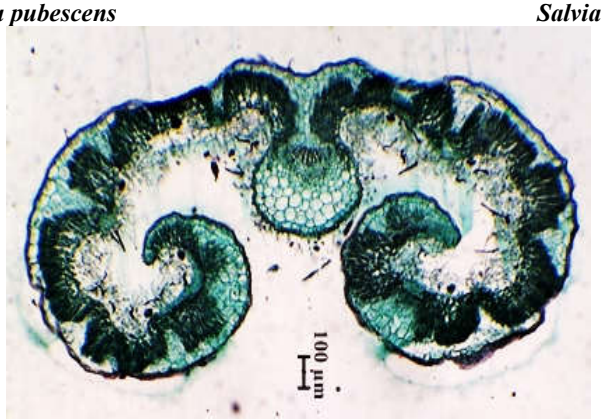
Stachys aegyptiaca



Lavandula pubescens



Salvia multicaulis



Rosmarinus officinalis

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

Table (3): Biochemical constituents of 3rd leaf of different species of *Lamiaceae* collected from different locations in Saint Catherine during April-2019

Species	mg /100 g FW				Total antioxidants (Inhibition % of DPPH)	Essential oil (%)
	Reducing sugars	Free amino acids	Total protein	Free Phenolic compounds		
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													Number of	
	midrib	lamina	epidermis with cuticle		collenchyma		parenchyma		Palisade tissue	Spongy tissue	Phloem tissue		Xylem tissue		xylem arms/bundle	xylem vessels/ arm
			upper	lower	upper	lower	Upper	lower			length	width	Length	width		
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	2 upper layers (60.0) and 2 lower layers (50.0)	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), phloem length and width (26.6 and 113 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/mm^2) 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*.

Table (5): Stomatal measurements of different *lamiaceae* species

Species	Guard cells (µm)		Stomatal aperture (µm)		Number of stomata (mm^2)
	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 ٨٧ 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 terpens (22) were found in *Lavandula pubscens*. Essential hydrocarbons compounds as undecane, dodecene, dodecane, tridecane, tetradecane, cetene, 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 jasmone 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 menthane, decane, pentadecane, pentadecanone, pentadecanol, eicosane, phytol, eicosanol and pentatriacontane were separated from all studied species. Terpenes as cadalene (0.03%), sclareol (0.02%) and á-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	**diterpenes (C20)**	1	1	2	1	1
	oxygenated diterpenes	3	6	8	5	3
	sesterterpenes (C25)	1	1	1	1	1
	oxygenated teriterpenes	-	1	-	-	-
	sesquarterpenes (C35)	1	1	1	1	1
Total terpenes		17	21	22	18	15
Total constituents		59	69	66	58	49

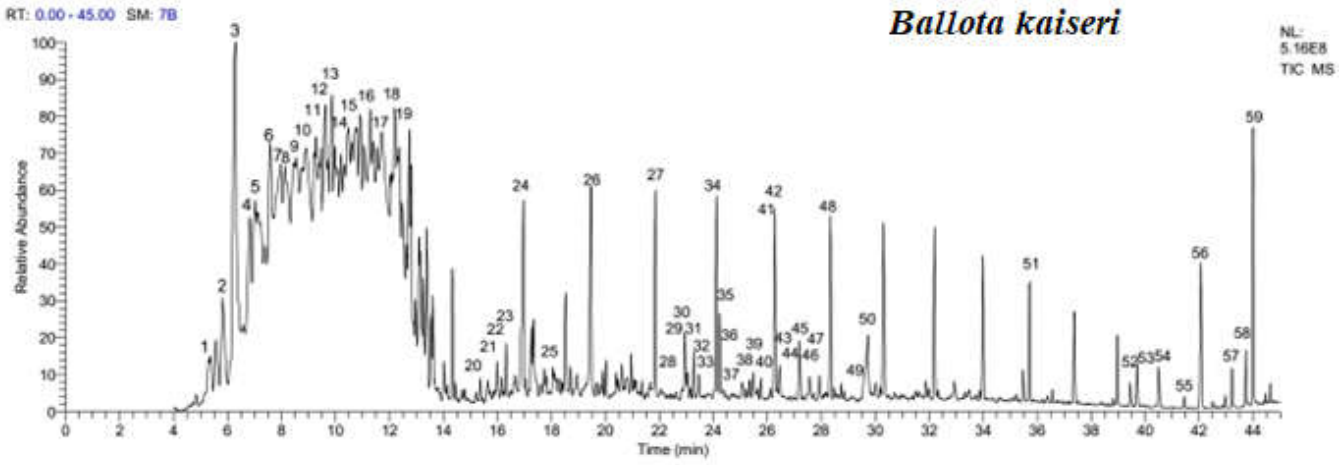


Fig. (2): Constituents of volatile oil of *Ballota kaiseri* separated by GC-MS. 1.menthane 2.decane 3.undecyne 4.undecane 5.decen-1-ol 6.dodecene 7.dodecane 8.methyl biphenyl 9.tridecene 10.tridecane 11.chamazulene 12.dodecanol (lauryl alcohol) 13.ionol 14.tetradecene 15.tridecanal 16.tetradecane 17.tridecanol 18.á-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.pentadecanone 27.pentadecanol 28.limonen-6-ol 29.platambin (geranyl isovalerate) 30.heptadecane 31.menthol isovalerate 32.cetanol (hexadecanol) 33.octadecene 34.cyclohexadecanolide 35.octadecane 36.1-nonadecene 37.norphytane (pristane) 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.

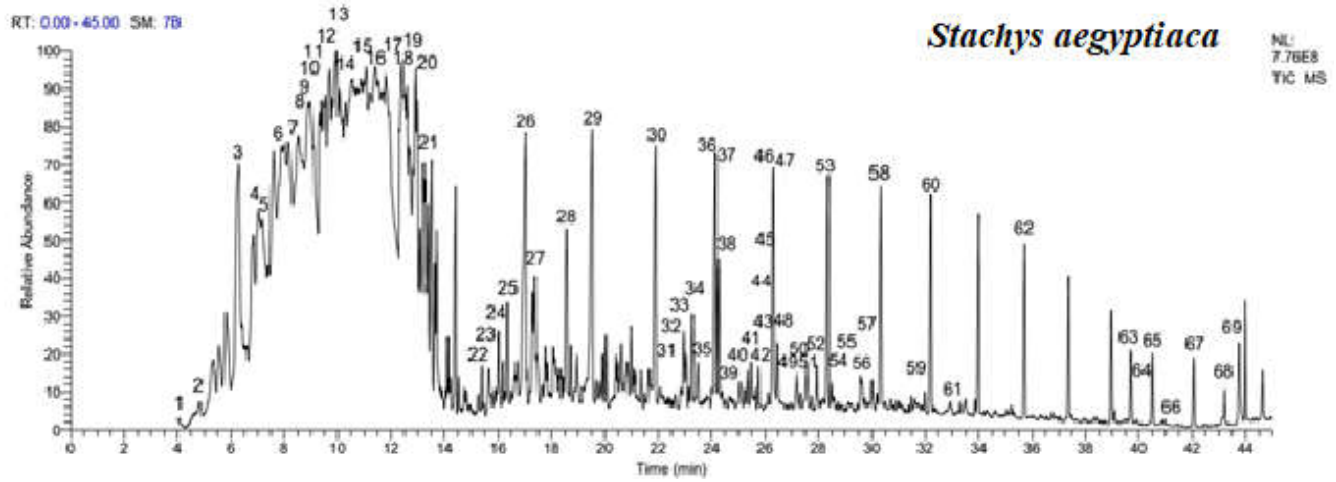


Fig. (3): Constituents of volatile oil of *Stachys aegyptiaca* separated by GC-MS

1.nonane 2.menthane 3.decane 4.undecane 5.decen-1-ol 6.dodecene 7.dodecane 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 isobutanoate 22.tetradecanal (myrist aldehyde) 23.pentadecane 24.tetradecanol (myristyl alcohol) 25.(bisabolene) caryophyllene oxide spathulenol 26.cetene (hexadecene) 27.methyl jasmonate 28.cetane (hexadecane) 29.pentadecanone 30.pentadecanol 31.limonen-6-ol 32.heptadecane 33.menthol isovalerate 34.cetanol (hexadecanol) 35.octadecene 36.cyclohexadecanolide 37.octadecane 38.1-nonadecene 39.norphytane (pristane) 40.juvabione 41.ethyl hexadecanoate 42.stearol (octadecanol) 43.eicosane 44.nonadecanol 45.retinal 46.manool 47.heneicosane 48.phytol 49.eicosanol 50.cinnamodial, agandencidial 51.sclareol 52.docosane 53.ethyl octadecanoate 54.methyl dehydroabietate dronabinol 55.methyl eperuate 56.tricosane 57.1-docosanol 58.retinol acetate 59.phytol acetate (erucic acid) 60.idebenone 61.tetracosane 62.pentacosane 63.hexacosane 64.heptacosane 65.octacosane 66.á-amyrin 67.dotriacontane 68.pentatriacontane 69.hexatriacontane.

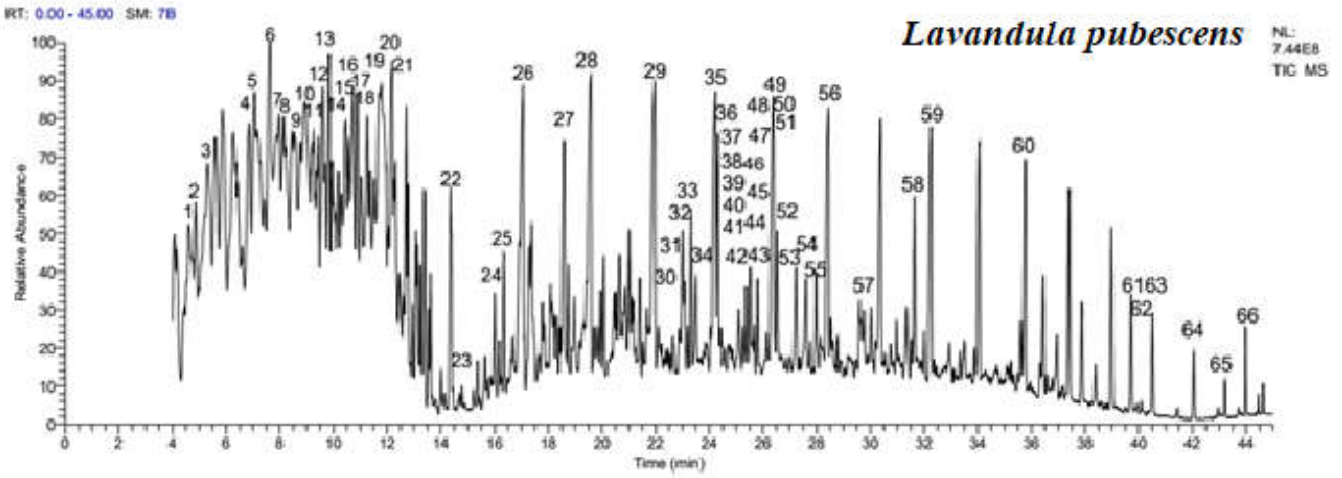


Fig. (4): Constituents of volatile oil of *Lavandula pubescens* separated by GC-MS

1.menthene 2.menthane 3.decane 4.undecyne 5.undecane 6.dodecene 7.dodecane 8.methyl biphenyl 9.tridecene 10.dodecadienal 11.tridecane 12.jasmone lactone 13.chamazulene 14.dodecanal 15.dodecanol (lauryl alcohol) 16.ionol 17.tetradecene 18.tridecanal 19.tetradecane 20.á-longipinene 21.ionone 22.ionol 23.pentadecane 24.tetradecanol (myristyl alcohol) 25.(bisabolene) caryophyllene oxide spathulenol 26.cetene (hexadecene) 27.cetane (hexadecane) 28.pentadecanone 29.pentadecanol 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.stearol (octadecanol) 41.gerany-p-cymene 42.eicosane 43.nonadecanol 44.retinal 45.retinol 46.abietol 47.manool 48.methyl linoleate 49.heneicosane 50.phytol 51.totarol 52.eicosanol 53.4,8,13-duvatriene-1,3-dio 54.docosene 55.docosane 56.tricosane 57.methyl nidoresedate 58.phytol acetate (erucic acid) 59.idebenone 60.pentacosane 61.hexacosane 62.heptacosane 63.octacosane 64.dotriacontane 65.pentatriacontane 66.hexatriacontane.

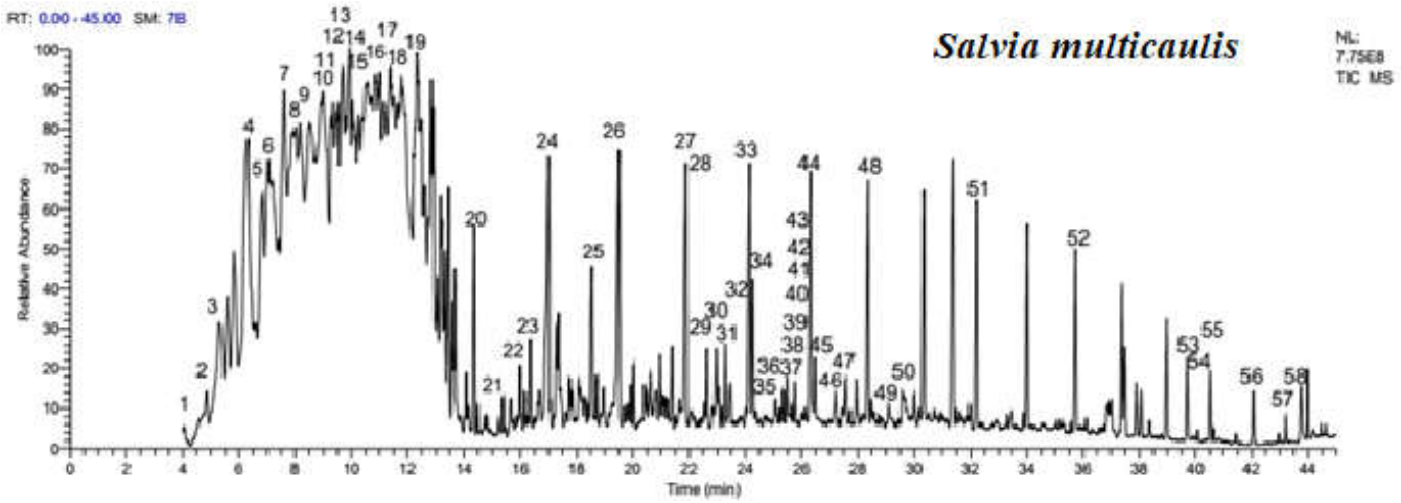


Fig. (5): Constituents of volatile oil of *Salvia multicaulis* separated by GC-MS

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.tridecanal 17.tetradecane 18.tridecanol 19.á-longipinene 20.tetradecanal (myrist aldehyde) 21.pentadecane 22.tetradecanol (myristyl alcohol) 23.(bisabolene) caryophyllene oxide spathulenol 24.cetene (hexadecene) 25.cetane (hexadecane) 26.pentadecanone 27.pentadecanol 28.limonen-6-ol 29.platambin (geranyl isovalerate) 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 dronabinol 49.tricosane 50.1-docosanol 51.phytol acetate (erucic acid) 52.pentacosane 53.hexacosane 54.heptacosane 55.octacosane 56.dotriacontane 57.pentatriacontane 58.hexatriacontane.

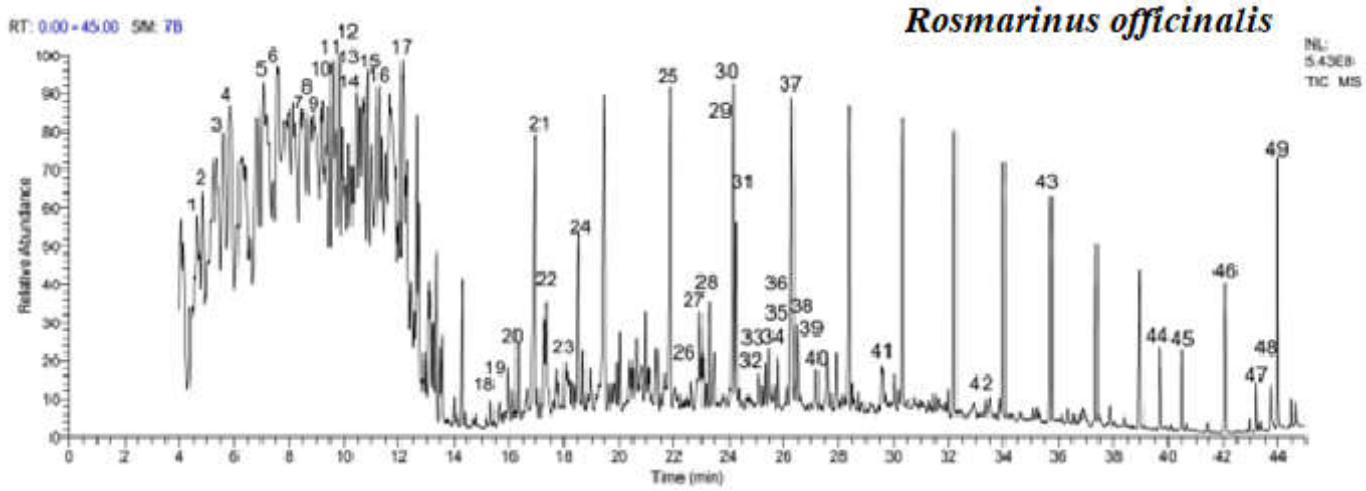


Fig. (6): Constituents of volatile oil of *Rosmarinus officinalis* separated by GC-MS

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.tridecanal 16.tetradecane 17.á-longipinene 18.tetradecanal (myrist aldehyde) 19.pentadecane 20.tetradecanol (myristyl alcohol) 21.(bisabolene) caryophyllene oxide spathulenol 22.cetene (hexadecene) 23.cetane (hexadecane) 24.pentadecanone 25.pentadecanol 26.platambin (geranyl isovalerate) 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.tetracosane 43.pentacosane 44.heptacosane 45.octacosane 46.dotriacontane 47.pentatriacontane 48.hexatriacontane 49.tetratetracontane

Antimicrobial activity of essential oils:

Essential oils from all studied species had anti-microbial 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%), sclareol (0.02%) and á-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 monoterpens, oxygenated monoterpens, 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 microbe. 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

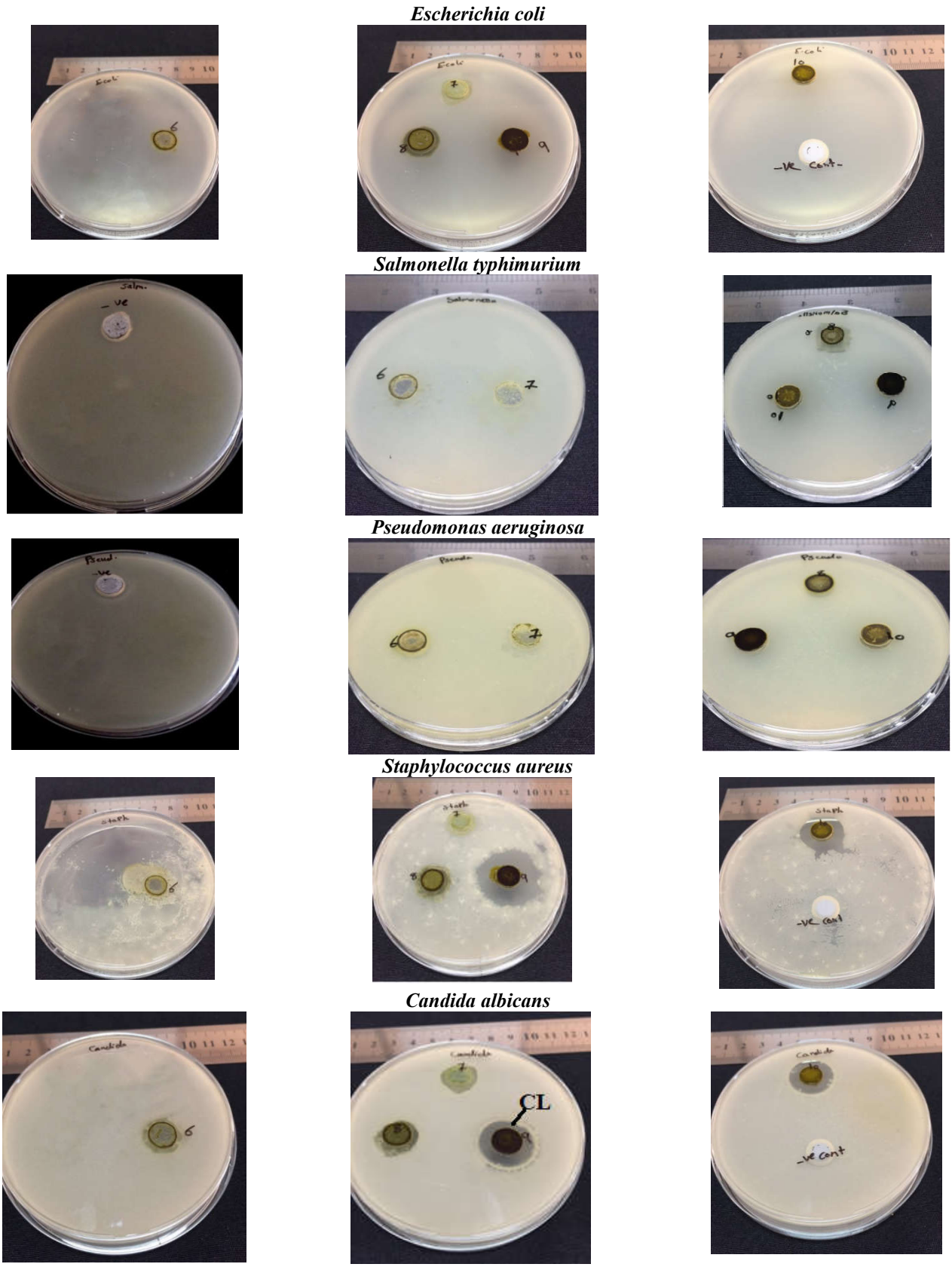


Fig. (7): Diameter of inhibitory clear zone (mm) of different pathogenic microbes after application of essential oils of *Lamiaceae* species. -ve control, negative control (Hexane); CL, Clear zone meaning the lethal effect of essential oil. *6- Ballota undulata 7- Stachys aegyptiaca 8- Lavandula pubescens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

REFERENCES

Abdelmajeed, N. A., E. N. Danial and H. S. Ayad (2013). The effect of environmental stress on qualitative and quantitative essential oil of aromatic and medicinal plants. Archives des sciences, 66(4): 100-120.

Abdel-Rahman, M., T. H. Thomas, G. J. Doss and L. Howell (1975). Changes in endogenous plant hormones in cherry tomato fruits during development and maturation. Physiologia plantarum, 34(1): 39-43.

Akhlaghi, H., A. Shafaghat, F. Salimi and M. Mohammadhoseini (2011). GC-MS analysis of the essential oil from wild Stachys pubescens Ten. leaves from Northwest Iran. Analytical chemistry letters, 1(4): 325-327.

Al-Badani, R. N., J. K. R. Da Silva, W. N. Setzer, N. A. Awadh Ali, B. A. Muharam and A. J. A. Al-Fahad (2017). Variations in essential oil compositions of *Lavandula pubescens (lamiaceae*) aerial parts growing wild in Yemen. Chemistry & biodiversity, 14(3). e1600286.

Ali, N. A., M. Wurster, A. G. Alkaf, I. Fadail, I. Sharaf and U. Lindequist (2011). *Lavandula pubescens* Decine-A potential source of carvacrol-rich essential oil in Yemen. The University of Aden Journal of Natural and Applied Sciences. p 1-13.

Alipour, M. and M. J. Saharkhiz (2016). Phytotoxic activity and variation in essential oil content and composition of Rosemary (*Rosmarinus officinalis* L.) during different phenological growth stages. Biocatalysis and agricultural biotechnology, 7: 271-278.

Allison, L. E. and L. A. Richards (1954). Diagnosis and improvement of saline and alkali soils: Soil and Water Conservative Research Branch, Agricultural Research Service.

Arcila-Lozano, C. C., G. Loarca-Piña, S. Lecona-Uribe and E. González de Mejía (2004). Oregano: properties, composition and biological activity. Archivos Latinoamericanos de Nutricion, 54(1): 100-111.

Arnon, D. I. (1949). Copper enzymes in isolated chloroplasts. Polyphenoloxidase in Beta vulgaris. Plant physiology, 24(1): 1-15.

Atlas, R. M. and R. Unterman (1999). Bioremediation. Manual of Industrial Microbiology and Biotechnology. In: ASM Press. Washington DC.

Baser, K. H. C. (1993). Essential oils of Anatolian *Labiatae*: a profile. Acta Horticulturae, 333: 217-238.

Bekut, M., S. Brkić, N. Kladar, G. Dragović, N. Gavarić and B. Božin (2018). Potential of selected *Lamiaceae* plants in anti (retro) viral therapy. Pharmacological research, 133: 301-314.

Boulos, L. (1995). Flora of Egypt. Checklist. Al-Hadara Publishing, Cairo.

Bozin, B., N. Mimica-Dukic, I. Samojlik and E. Jovin (2007). Antimicrobial and antioxidant properties of rosemary and sage (*Rosmarinus officinalis* L. and *Salvia officinalis* L., *Lamiaceae*) essential oils. Journal of agricultural and food chemistry, 55(19): 7879-7885.

Bradford, M. A. (1976). Rapid and sensitive method for the quantitation of protein utilizing the principle of protein-dye binding. Annu. Rev. Biochem., 72: 248–254.

Dadach, M., and Z. Mehdadi (2018). Germination responses of *Ballota hirsuta* seeds under conditions of temperature, salinity and water stress. Hellenic Plant Protection Journal, 11: 34-39.

Danh, L. T., N. D. A. Triet, J. Zhao, R. Mammucari and N. Foster (2013). Comparison of chemical composition, antioxidant and antimicrobial activity of lavender (*Lavandula angustifolia* L.) essential oils extracted by supercritical CO 2, hexane and hydrodistillation. Food and bioprocess technology, 6(12): 3481-3489.

El-Gazzar, A. and L. Watson (1968). *Labiatae*: Taxonomy and Susceptibility to *Puccinia menthae* Pers. New Phytologist, 67(3): 739-743.

Elless, M. P., M. J. Blaylock, J. W. Huang and C. D. Gussman (2000). Plants as a natural source of concentrated mineral nutritional supplements. Food Chemistry, 71(2):181-188.

Fu, Z., H. Wang, X. Hu, Z. Sun and C. Han (2013). The pharmacological properties of *salvia* essential oils. Journal of applied pharmaceutical science, 3(7): 122-127.

Ghomi, J. S., R. Masoomi, F. J. Kashi and H. Batooli (2012). *In vitro* bioactivity of essential oils and methanol extracts of *Salvia Reuterana* from Iran. Natural Product Communications, 7(5): 651-4.

Hassanein, H. D., A. E. N. G. El-Gendy, I. A. Saleh, S. F. Hendawy, M. M. Elmissiry and E. A. Omer (2020). Profiling of essential oil chemical composition of some *Lamiaceae* species extracted using conventional and

microwave-assisted hydrodistillation extraction methods via chemometrics tools. Flavour and Fragrance Journal, 35(3): 329-340.

Hatano, T., H. Kagawa, T. Yasuhara and T. Okuda (1988). Two new flavonoids and other constituents in licorice root: their relative astringency and radical scavenging effects. Chemical and pharmaceutical bulletin, 36(6):2090-2097.

Horwitz, W., P. Chichilo and H. Reynolds (1970). Official Methods of Analysis of the Association of Official Analytical Chemists. Washington, DC, USA. Association of official agricultural chemists. 85: 647589.

Hyldgaard, M., T. Mygind and R. L. Meyer (2012). Essential oils in food preservation: mode of action, synergies, and interactions with food matrix components. Frontiers in microbiology, 3:12.

Jackson, M. L. (1967). Soil chemical analysis. Englewood cliffs: Printica Hall. Inc., NJ 498p.

Jassbi, A. R., M. Asadollahi, M. Masroor, M. C. Schuman, Z. Mehdizadeh, M. Soleimani and R. Miri (2012). Chemical classification of the essential oils of the Iranian *Salvia* species in comparison with their botanical taxonomy. Chemistry and biodiversity, 9(7): 1254-1271.

Khanavi, M., M. Hajimahmoodi, M. Cheraghi-Niroomand, Z. Kargar, Y. Ajani, A. Hadjiakhoondi and M. R. Oveisi (2009). Comparison of the antioxidant activity and total phenolic contents in some *Stachys* species. African Journal of Biotechnology, 8(6): 1143-1147.

Kochhar, S. L. and K. G. Sukhbir (2020). Plant Physiology: Theory and Applications. Cambridge University Press. pp 894.

Lambert R. J., P. N. Skandamis P. J. Coote and G. J. Nychas (2001). A study of the minimum inhibitory concentration and mode of action of oregano essential oil, thymol and carvacrol. Journal of applied microbiology, 91(3):453-62.

Moore, T. C. (2012). Research experiences in plant physiology: a laboratory manual: Springer Science & Business Media. pp 462.

Morteza-Semnani, K., M. Akbarzadeh and S. Changizi (2006). Essential oils composition of *Stachys byzantina*, *S. inflata*, *S. lavandulifolia* and *S. laxa* from Iran. Flavour and fragrance journal, 21(2): 300-303.

Moustafa, A. A. (2001). Impact of grazing intensity and human disturbance on the population dynamics of Alkana orientalis growing in Saint Catherine, South Sinai, Egypt. Pakistan Journal of Biological Science, 4(8): 1020-1025.

Mstat-C, A. (1990). A microcomputer program for the design, management, and analysis of agronomic research experiments. Plant and Soil Sciences, Michigan State University.

Ohtsuka, T., H. Ito and A. Tanaka (1997). Conversion of chlorophyll b to chlorophyll a and the assembly of chlorophyll with apoproteins by isolated chloroplasts. Plant Physiology, 113(1): 137-147.

Page, A. L., R. H. Miller and D. R. Keeney (1982). Methods of soil analysis. Part 2. Chemical and Microbiological Properties. 2nd. Am. Soc. Agron. Inc. Publisher Madison, Wisconsin, USA.

Pharande, A. L. and K. R. Sonar (1997). Clay Mineralogy of Some Vertisol Soil Series of Mabarashtra. Journal of the Indian Society of Soil Science, 45(2): 373-377.

Rosen, H. (1957). A modified ninhydrin colorimetric analysis for amino acids. Archives of biochemistry and biophysics, 67(1): 10-15.

Salem, M. Z. M., M. Z. Zayed, H. M. Ali and M. S. M. Abd El-Kareem (2016). Chemical composition, antioxidant and antibacterial activities of extracts from *Schinus molle* wood branch growing in Egypt. Journal of wood science, 62(6): 548-561.

Sass, J. (1961). Botanical microtechnique. 3rd: Ames. In: IA: The Iowa State University Press.

Shafaghat, A. and K. Oji (2010). Nepetalactone content and antibacterial activity of the essential oils from different parts of *Nepeta persica*. Natural product communications, 5(4), 625-8.

Steele, R. G. D., J. H. Torrey and D. A. Dickeys (1997). Multiple Comparisons. Principles and Procedures of Statistics–A Biometrical Approach. In: McGraw Hill, New York.

Suryanti, V., T. Kusumaningsih, S. D. Marliyana, H. A. Setyono and E. W. Trisnawati (2020). Identification of active compounds and antioxidant activity of teak (*Tectona grandis*) leaves. Biodiversitas Journal of Biological Diversity, 21(3): 946-952.

Täckholm, V. (1974). Students' Flora of Egypt. 2nd Edition, Cairo University Publishing, Beirut, 888.

Taiz, L. and E. Zeiger (2004). Plant physiology, 2nd Ed. Sinauer, Sunderland, MA, p 700.

Tributh, H. (1970). Importance of extended clay-fractionation for the more precise characterization of soil minerals and their properties. Zeitschrift fur Pflanzenernahrung, Dungung und Bodenkunde, 126 (2): 117-134.

Willey, R. L. (1971). Microtechniques: a laboratory guide: Macmillan New York.

دراسات فسيولوجية لبعض الانواع البرية الطبية للعائلة الشفوية النامية فى محمية سانت كاترين

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أجريت دراسة مقارنة فسيولوجية على خمسة أنواع برية طبية من العائلة الشفوية هي: Stachys *aegyptiaca* Pers., *Lavandula pubescens* L., *Rosmarinus officinalis* L., *Ballota kaiseri* Tackh. and *Salvia multicaulis* Vahl. أوضحت النتائج أن عشب نبات المرمرية *Salvia multicaulis* يحتوى على أعلى نسبة من الزيت العطري (٧.٤٦٪) والذي ارتبط بأقصى سمك للعرق الوسطي والنصل وطول وعرض كلا من نسيج الخشب واللحاء وعدد اذرع الخشب لكل حزمة في الورقة الثالثة للنبات. سجل أعلى قيمة للأحماض الأمينية الحرة والمركبات الفينولية (٨.٧٦ و ١٢.١٦ ملجم/١٠٠ جم وزن طازج، على التوالي) في نبات القرطم Stachys *aegyptiaca*. سجل أعلى عدد للثغور (٦٥٠ ثغر لكل ملليتر مربع) في *Ballota kaiseri*. بينما أقصى طول وعرض لفتحة الثغر (٢٢.٨٥ و ٢.٨٥ ميكروميتر) في *Lavandula pubescens*. أظهر تحليل كروماتوجرافيا الغاز-الطيف الكتلى (GC-MS) للزيت العطري للأنواع المختلفة وجود ٨٧ مركبً طيار. كما اظهر الزيت العطري لهذه الأنواع كفاءة عالية كمضاد للميكروبات على بعض مسببات الأمراض البكتيرية والفطرية قيد الدراسة. حيث اظهر الزيت العطري للمرمرية كفاءة عالية ضد بكتريا الاستيفالوكوكس وبكتريا السالمونيلا بظهور أقصى قطر للمنطقة الخالية من الميكروب (٢٢ و ٣٠ ملم، على التوالي)، في حين ظهر للعطان *Lavandula pubescens* كفاءة عالية ضد بكتيريا الايكولاى مع تكوين أقصى قطر للمنطقة الخالية من الميكروب (١٣ ملم) بسبب ارتفاع قيمة مضادات الأكسدة إلى ٧٢.٤%. أما نبات اكليل الجبل *Rosmarinus officinalis* تميز بكفاءة عالية ضد بكتيريا السيدوموناس و فطر الكانديدا مع ظهور أقصى قطر للمنطقة الخالية من الميكروب (٢٥ و ٢٢ ملم، على التوالي). ارتبطت الكفاءة العالية للزيوت العطرية كمضادات للمسببات المرضية تحت الدراسة للأنواع البرية تحت ظروف المعمل بوجود مركبات فعالة.