Turkish Journal of Agriculture and Forestry

Volume 47 | Number 1 Article 8

1-1-2023

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Recommended Citation

ÇELEBİ, ÖZGÜR; FIDAN, HAFIZE; ILIEV, IVAN; PETKOVA, NADEZHDA; DINCHEVA, IVAYLA; GANDOVA, VANYA; STANKOV, STANKO; and STOYANOVA, ALBENA (2023) "Chemical composition, biological activities, and surface tension properties of Melissa officinalis L. essential oil," *Turkish Journal of Agriculture and Forestry*: Vol. 47: No. 1, Article 8. https://doi.org/10.55730/1300-011X.3065
Available at: https://journals.tubitak.gov.tr/agriculture/vol47/iss1/8

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Turkish Journal of Agriculture and Forestry

http://journals.tubitak.gov.tr/agriculture/

Research Article

Turk J Agric For
(2023) 47: 67-78
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doi:10.55730/1300-011X.3065

Chemical composition, biological activities, and surface tension properties of *Melissa officinalis* L. essential oil

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Received: 23.08.2022 • **Accepted/Published Online:** 06.12.2022 • **Final Version:** 08.02.2023

Abstract: Lemon balm (*Melissa officinalis* L.) is a plant of the family Lamiaceae. The present study aimed to determine the chemical composition of the lemon balm essential oil grown in Northeastern Bulgaria, to perform a comparative analysis of its antimicrobial activity with that of citral, and to determine its surface tension properties. The main compounds of the essential oil (concentration above 3%) were: β-caryophyllene (20.45%), geranial (19.74%), neral (15.81%), germacrene D (11.50%), (R)-(+)-citronellal (3.07%), and geranyl acetate (3.04%). The antibacterial activity of the essential oil was weak against the tested gram-negative and gram-positive bacteria. Exceptions were observed for *Listeria monocytogenes* and *Salmonella enterica* subsp. *Enterica serovar* Abony. Citral exhibited higher antimicrobial activity against investigated gram-positive bacteria compared to gram-negative. The surface tension of the essential oil was determined at six different temperatures (6, 10, 15, 20, 25, and 3 °C). The energy presented minimal differences from 124.52 to 125.57 mN/m at different temperatures. Surface heat capacity was between 10492.11 and 11394.17 N/(m.K).

Key words: Lemon balm, biological activity, essential oil, chemical composition, antibacterial activity, surface tension

1. Introduction

Lemon balm (*Melissa officinalis* L.) is a perennial herbaceous plant of the family Lamiaceae. The essential oil is deposited in glandular trichomes and labial glands on flowers, leaves, and stems (Chwil et al., 2016). Essential oil yield ranges from 0.01% to 0.30% and depends on the habitat, time of harvest and variety (Başer and Buchbauer, 2010; Stoyanova, 2022).

The essential oil is an easy mobile transparent, pale yellow to yellow-greenish liquid with a specific pleasant lemon scent and bitter-spicy taste (Başer and Buchbauer, 2010; Stoyanova, 2022).

More than 130 compounds have been identified in the essential oil composition, the amount of which varied depending on the habitat, phase of development, and plant variety. The main components of essential oil in different countries around the world were detected: from Algeria (Abdellatif et al., 2014): geranial (44.2%), neral (30.2%), citronellal (6.3%), β-caryophyllene (1.3%), and caryophyllene oxide (1.3%); from Brazil (Sodre et al., 2012): geranial (48.82%–50.45%), neral (31.97%–33.39%), citronellal (5.6%–7.5%), and caryophyllene oxide (1.77%–2.16%); from Iran (Adinee et al., 2008): *trans*-carveol (28.89%), citronellol (25.24%), δ-3-carene (5.26%), citronellal (4.90%), geraniol (2.20%), spatulenol (2.06%), and 1-octen-3-ol (2.03%); from Cuba (Pino et al., 1999): geranial (41.0%), neral (29.9%), and citronellal (0.2%); from Morocco (El Quadi et al., 2017): *p*-mentha-1,2,3-triol (13.1%), *p*-mentha-3-en-8-ol (8.8%), pulegone (8.8%), piperytinone oxide (8.4%), and 2-piperitone oxide (7.3%); from Slovakia (Holla et al., 1997): geranial (33.60%), neral (22.18%), citronellal (11.30%), caryophyllene oxide (8.35%), geranyl acetate (5.89%), and β-caryophyllene (4.20%); from Turkey (Cosge et al., 2009): citronellal (36.62%–43.78%), citral (10.10%–17.43%), thymol (0.40%–11.94%), and β-caryophyllene (5.91%–7.27%).

It was found that during the vegetative season, there is a decrease in the content of geraniol and caryophyllene

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oxide, and an increase of caryophyllene, carvacrol, and methyl citronelate; however, no changes in the amount of geranyl acetate were detected (Saeb and Gholamrezaee, 2012). In the 2nd year of the growing season, the number of citronellal decreased, geranyl acetate increased, and neral and geranial remained the same (Nurzyńska-Wierdak et al., 2016).

The method of drying has a significant impact on both the amount of essential oil and its composition. Oxidative, polymerization, and other processes are likely to occur during drying, leading to a change in the chemical composition of the oil (Khalid et al., 2008).

The essential oil possessed antimicrobial activity (Dastmalchi et al., 2008; Abdellatif et al., 2014; El Quadi et al., 2017; Chien et al., 2019) and exhibited an antiviral effect (Dastmalchi et al., 2008; Meftahizade et al., 2010; Dias et al., 2012; Abdellatif et al., 2014) and repellent action against *Anopheles stephensi* (Oshaghi et al., 2003).

The essential oil was used in perfumery and cosmetics (Moradkhani et al., 2010) for flavoring liqueurs, jams, marmalades (Başer and Buchbauer, 2010; Moradkhani et al. 2010; Marei et al., 2018; Hassan et al., 2019; Sani et al., 2020; Yoplac et al., 2021; Stoyanova, 2022) and in aromatherapy (Moradkhani et al., 2010).

In Bulgaria, lemon balm grows in the wild in many parts of the country, but it has been cultivated in recent years. The following main components have been identified in the composition of wild lemon balm essential oil: geranial (26.4%–37.3%), neral (19.6%–28.5%), (*E*)-*β*-caryophyllene (6.6%–17.5%), citronellal (3.2%–25.1%), linalool (up to 2.9%), geranyl acetate (1.9%), germacrene D (7.8%), etc. (Dogan et al., 2021).

There are data in the literature determining some physicochemical parameters of different vegetable and essential oils, for example, density, viscosity, refractive index, surface tension, etc. (Benkovskii et al., 1966; Markarian and Terzyan, 2007; Florido et al., 2014; Melo-Espinosa et al., 2014; Yerima et al., 2015). However, similar measurements for lemon balm oil were still missing.

Therefore, the aim of the present work was to study the chemical composition of essential oil of cultivated lemon balm (*Melissa officinalis* L.) to evaluate its biological activity (antimicrobial and antioxidant) and physicochemical properties in order to apply it in cosmetics and food products.

2. Materials and methods

2.1. Materials

The essential oil (the cultivation year: 2022) was provided by a manufacturer from Northeastern Bulgaria, while the citral was from PQ Extra, BBA Aroma Chemicals, London, UK.

2.2. Determination of physical parameters and chemical composition

The following properties of the essential oil were determined: appearance, color, and odor (BSS 9200, 1994), relative density (ISO 279, 1998), refractive index (ISO 280, 1998), and acid number (BSS ISO 1242, 2002).

The chemical composition of the essential oil was determined by GC/MS analysis. GC analysis was performed with an Agilent 7890A instrument (Agilent Technologies Inc., Santa Clara, CA, USA) under the following conditions: HP-5ms capillary column, 30 m ´ 250 mm ´ 0.25 μm; temperature 35 °C/3 min, 5 °C/min to 250 °C for 3 min, total 49 min; helium as a carrier at a constant rate of 1 mL/min; split 30:1. GC-MS analysis was performed on an Agilent 5975C under the same conditions as the GC analysis. Component identification was based on comparing retention indices with spectral databases (Adams, 2007).

2.3. Determination of antimicrobial activity

2.3.1. Test microorganisms

Strains of eight pathogenic bacteria reported as causing infections, toxic infections, and toxicosis, were used as test microorganisms. Test microorganisms strains were supplied by National Bank for Industrial Microorganisms and Cell Cultures. The following gram-positive bacteria: *Listeria monocytogenes* NCTC 11994, *Staphylococcus aureus* ATCC 25093, *Bacillus subtilis* ATCC 6633, *Bacillus cereus* (clinical isolate); and the following gram-negative bacteria: *Escherichia coli* ATCC 8739, *Salmonella enterica* subsp. *Enterica serovar abony* NCTC 6017, *Klebsiella* (clinical isolate) were used in this study.

Selective cultivation media were used for the microbiological assay, respectively: Listeria Oxford Agar Base with an additive containing cycloheximide /Biolife/; ENDO agar /Merck/; LEIFSON Agar /Merck/; Baird Parker Agar Base /Biolife/ with yolk-tellurite supplement and Plate Mount Agar /Merck/.

2.3.2. Determination of antibacterial activity

Antibacterial activity was determined by modifying the agar diffusion method by measuring the inhibition zones of pathogen growth around metal rings in which a certain amount of test material was imported. Selective media for test cultures were inoculated with suspensions (1.10^4 cfu/mL) of pathogens prepared from 24-h culture on PCA slant. From a suitable tenfold dilution of the suspension, the selective media melted and cooled to 45–50 °C were inoculated. The active concentration of the cells in the agar was equated to the concentration of the diluted suspension since 1 mL of suspension was inoculated into 99 mL of the medium. After solidifying the media, sterilized metal rings with a petri diameter of Ø = 6 mm were placed on their surface, in which 150 μL of summer savory essential oil

was imported, respectively. As a negative control, a 0.15-µL solution consisting of phosphate-buffered saline (PBS, Sigma, São Paulo, Brazil) with 0.5% (v/v) polysorbate 80 (Tween® 80) was used. The plates were incubated at 37 °C. The diameter (mm) of the growth inhibition zones of the tested cultures was measured at 24 and 48 h. A comparative review of their antibacterial activity was done.

2.4. Antioxidant activity by DPPH radical scavenging activity

The DPPH radical scavenging activity was evaluated as the essential oil (0.15 mL) properly diluted in methanol was added to 2.85 mL of freshly prepared 0.1 mM DPPH solution in methanol. The samples were incubated for 15 min at 37 °C in darkness. The reduction of the absorbance at 517 nm was measured by spectrophotometer in comparison to the blank containing methanol (Misharina, 2016). The radical scavenging activity of essential oil was expressed as mM Trolox® equivalent (TE) per g dw. The IC_{50} value was calculated.

2.5. Maximum bubble pressure method

The essence of the method is to determine the maximum pressure Pmax that needs to be exerted to blow a bubble from the end of the capillary (Yankova et al., 2019). An advantage of the method is that the surface tension is determined at a point when the value of the contact angle is zero.

2.5.1. Determination of density of liquids by pycnometer

The density of liquids was determined by a pycnometer (Yankova et al., 2019).

2.5.2. Determination of refractive index and molar refraction

Measurements of the refractive index (n_D) were performed at a wavelength equal to 589.3 nm using an Abbe type refractometer (Yankova et al., 2016). The measurements were used to calculate the molar refraction by equation (1):

$$R_m = \frac{(n_D^2-1)M}{(n_D^2+2)\rho} \quad (1)$$

where R_m is the molar refraction (m^3/mol), ρ is the density of the test liquid (kg/m^3), n_D is the refractive index, and M is the molar mass of the component (kg).

2.5.3. The dependence γ = f (T) is expressed by different empirical equations

For unassociated liquid phases and temperatures sufficiently distant from the critical temperature, the dependence can be represented by van der Waals and Guggenheim (Fujisawa et al., 1981) equation (2):

$$^{y} = {}^{y}{}_0 - k(T - T_0) \quad (2)$$

where k is coefficient, which can be assumed with sufficient accuracy to be equal to 1.10^{-4} N/(m.K), T_0 is the temperature at which the substance has a surface tension y_0, K, T is the temperature at which the substance has a surface tension y, K.

In addition to the surface tension relationships shown so far, there are a number functional dependencies expressing the participation of this quantity in the consideration of issues related to surface thermodynamics presented by equations (3) and (4):

$$E^s = \gamma - T\left(\frac{\partial\gamma}{\partial T}\right) \quad (3)$$

where E_s is the surface energy, N/m, y is the surface tension, N/m, $\left(\frac{\partial\gamma}{\partial T}\right)$ is the temperature gradient of the surface tension at constancy of the interfacial area at first order transition, N/(m.K), T - temperature at which the substance has a surface tension y, K.

$$C^s = -T\left(\frac{\partial^2\gamma}{\partial T^2}\right) \quad (4)$$

where C_s is the surface heat capacity, N/(m.K), $\left(\frac{\partial^2\gamma}{\partial T^2}\right)$ is the temperature gradient of the surface tension at constancy of the interfacial area at second order transition, N/(m.K), T is the temperature at which the substance has a surface tension y, K.

2.6. Statistical analysis

The measurements were performed in triplicate and the results were presented as the mean value of the individual measurements with the corresponding standard deviation (SD), using Microsoft Excel. The differences were considered statistically significant if $p < 0.05$.

3. Results and discussions

3.1. Physical and chemical characteristics and chemical composition

Some physical and chemical parameters of the essential oil are presented in Table 1. The physical and chemical parameters of the studied essential oil do not differ from the values reported in the literature (Stoyanova, 2022).

The chemical composition of the essential oil is presented in Table 2. The data showed that 48 compounds

Table 1. Physical and chemical characteristics of lemon balm essential oil.

Properties	
Appearance	Easily mobile transparent liquid
Color	Light yellow to pale yellow
Odor	Specific, lemon scent
Taste	Bitter-spicy
Relative density (d_{20}^{20})	0.919 ± 0.01
Refractive index (n_D^{20})	1.4916 ± 0.01
Acid value (mg KOH/g oil)	3.02 ± 0.01

Table 2. Chemical composition of lemon balm essential oil (% от TIC[a]).

R.T.[b]	RI[c]	RI[d]	Compounds	% of TIC
9.74	922	931	α-Thujene	0.09 ± 0.0
9.97	930	939	α-Pinene	0.08 ± 0.0
10.50	945	940	Camphene	0.06 ± 0.0
11.58	976	980	β-Pinene	0.25 ± 0.02
11.70	984	993	(3E)-Octen-2-ol	1.46 ± 0.13
11.86	990	992	6-Methyl-5-heptene-2-one	0.17 ± 0.01
13.15	1023	1031	Limonene	0.60 ± 0.05
13.37	1035	1040	*cis*-β-Ocimene	0.21 ± 0.02
13.73	1048	1050	*trans*-β-Ocimene	2.15 ± 0.20
15.41	1096	1098	β-Linalool	0.66 ± 0.06
15.56	1001	1095	α-Pinene oxide	0.20 ± 0.01
16.58	1118	1124	1-Octanol acetate	0.30 ± 0.03
16.90	1129	1134	1-Terpineol	0.59 ± 0.05
17.03	1147	1153	(*R*)-(+)-Citronellal	3.07 ± 0.29
17.27	1161	1167	(*Z*)-Isocitral	0.54 ± 0.05
17.83	1179	1180	(*E*)-Isocitral	0.82 ± 0.07
19.22	1222	1228	β-Citronellol	0.97 ± 0.09
19.31	1226	1228	Nerol	0.70 ± 0.06
19.68	1233	1240	Neral	15.81 ± 1.11
19.99	1248	1255	Geraniol	1.79 ± 0.16
20.08	1255	1257	Linalool acetate	0.67 ± 0.06
20.56	1261	1270	Geranial	19.74 ± 1.80
20.80	1264	1268	(S)-(-)-Citronellic acid, methyl ester	0.17 ± 0.01
21.84	1320	1323	Methyl geranate	0.71 ± 0.06
22.60	1344	1350	α-Terpinyl acetate	0.06 ± 0.0
22.83	1367	1365	Neryl acetate	0.15 ± 0.01
23.38	1375	1383	Geranyl acetate	3.04 ± 0.29
23.60	1384	1384	β-Bourbonene	0.43 ± 0.04
23.70	1395	1397	Ethyl geranate	0.45 ± 0.04
23.73	1408	1410	Citronellyl oxy-acetaldehyde	0.38 ± 0.03
24.63	1415	1418	β-Caryophyllene	20.45 ± 2.00
24.80	1432	1435	β-Copaene	0.19 ± 0.01
25.31	1441	1443	β-Farnesene	0.39 ± 0.03
25.45	1455	1456	α-Caryophyllene	1.53 ± 0.14
25.56	1467	1467	9-epi-(*E*)-Caryophyllene	0.27 ± 0.02
25.94	1476	1477	γ-Muurolene	0.31 ± 0.02
26.14	1488	1480	Germacrene D	11.50 ± 1.10
26.24	1487	1485	(*Z,E*)-α-Farnesene	0.78 ± 0.07
26.36	1495	1494	(*Z*)-Cadina-1,4-diene	0,16 ± 0.01
26.49	1499	1499	α-Muurolene	0.69 ± 0.06
26.58	1504	1508	(*E,E*)-α-Farnesene	0.53 ± 0.05
26.85	1515	1513	γ-Cadinene	0.44 ± 0.04

Table 2. (Continued).

26.97	1532	1538	*α*-Cadinene	1.92 ± 0.18
28.39	1571	1574	Germacrene D-4-ol	0.51 ± 0.05
28.53	1582	1581	Caryophyllene oxide	1.60 ± 0.15
29.91	1638	1640	*tau*-Muurolol	0.98 ± 0.08
30.18	1650	1653	*α*-Cadinol	0.84 ± 0.07
34.10	1841	1845	Hexahydrofarnesyl acetone	0.10 ± 0.01
		Oxygenated aliphatics, %		1.64
		Monoterpene hydrocarbons, %		3.46
		Oxygenated hydrocarbons, %		51.07
		Sesquiterpene hydrocarbons, %		39.78
		Oxygenated sesquiterpenes, %		4.05

[a]: total ion current, [b]: retention time, min, [c]: experimental retention (Kovat's) index,
[d]: Kovats retention index on DB-5 column with reference to *n*-alkanes.

have been identified, representing 99.51% of the total content. Twelve of them had a concentration over 1% and the remaining 36 were below 1%. The main compounds (concentration above 3%) were sesquiterpene hydrocarbons *β*-caryophyllene (20.45%) and germacrene D (11.50%), as well as monoterpene oxygen derivatives geranial (19.74%), neral (15.81%), (R)-(+)-citronellal (3.07%), and geranyl acetate (3.04%).

The chemical composition of the essential oil did not differ from the data in the literature on the identified components (Holla et al., 1997; Pino et al., 1999; Adinee et al., 2008; Khalid et al., 2008; Cosge et al., 2009; Başer and Buchbauer, 2010; Saeb et al., 2012; Sodre et al., 2012; Abdellatif et al., 2014; Nurzyńska-Wierdak et al., 2014; El Quadi et al., 2017; Stoyanova, 2022). Differences could be explained by the origin of the plant and the conditions of cultivation, as well as by the method of production and analytical methods of analysis.

The distribution of the main groups of compounds in the oil is presented in Table 2. The data showed that monoterpene oxygen derivatives predominated in the oil, followed by sesquiterpene hydrocarbons and three other groups which were in trace amounts compared to the first two.

The distribution of oxygen derivatives determined the oil's leading properties, antioxidant and antimicrobial activity. The functional groups showed that the aldehydes predominated in the oil (71.45%) with the monoterpenes: geranial and neral as their main representatives. Their presence was followed by alcohols (15.05%) with the monoterpene geraniol, esters (9.83%) with their main constituent the monoterpene geranyl acetate, oxides (3.19%) mainly comprised of the sesquiterpene caryophyllene, oxides and ketones (0.48%) mostly consisting of the oxygenated aliphatic 6-methyl-5-heptene-2-one. The detected monoterpene hydrocarbons, some of which determined the odor of the oil, acyclic predominated with *trans-β*-ocimene, as the main compound, followed by monocyclic, limonene and bicyclic, mainly *β*-pinene. Monoterpene oxygen derivatives, which are responsible for oil odor, were dominated and were presented by acyclic, mostly by the compound geraniol, followed by monocyclic, mainly 1-terpineol.

Results obtained in our study slightly differed from that reported in the literature, which could be explained by the geographical characteristics of the region where the plant material was obtained, the specificity of the method used, and the period of harvesting. According to Galgano et al. (2022), about thirty compounds were identified in the essential oil of *M. officinalis*, accounting for 91.64% of the total. They found that the oil was characterized by the presence of citral (43%), caryophyllene (25%), humulene (4.4%), limonene (4.3%), cariophyllene oxide (2.2%), geranyl acetate (1.95%), and eucalyptol (1.2%). On the other hand, Zineb et al. (2015) reported that thirty-three components were identified representing 89.30% of the total oil in leaves composition of lemon balm essential oil obtained from Morocco. Six predominant components in the essential oil composition were citronellal (14.40%), isogeraniol (6.40%), geraniol acetate (10.20%), nerol acetate (5.10%), caryophyllene (8.10%), and caryophyllene oxide (11.00%), representing 55.20% of the total oil.

According to the EU Directive (Sarkic and Stappen, 2018), the following nine allergens were identified in descending order, which are the monoterpene compounds: geranial (17.74%), neral (15.81%), (R)-(+)-citronellol (3.07%), geraniol (1.79%), *β*-citronellol (0.97%), *(E)*-isocitral (0.82%), *β*-linalool (0.66%), limonene (0.60%), and *(Z)* -isocitral (0.54%).

3.2. Antimicrobial activity

The diameter of the inhibition zones of the essential oil and its main component citral are presented in Table 3. The data show that the activity of the lemon balm oil was weak against the tested gram-negative and gram-positive bacteria. The exceptions were observed for gram-positive bacteria *L. monocytogenes* NCTC 11994 and gram-negative *S. enterica* NCTC 6017. Citral exhibited higher antimicrobial activity against gram-positive bacteria compared to gram-negative.

The differences in the antimicrobial activity of the essential oil compared to the literature data can be explained by the chemical composition and the test cultures used. Citral is a major component of the essential oil. However, other components have been found in its composition that has a synergistic or antagonistic effect when compared to each other. The inhibitory effect of citral (geranial and neral) against microorganisms could be explained by its structural characteristics and the ability to alter and penetrate the lipid and protein structure of bacterial cell walls (Wuryatmo et al., 2003; Guimarães et al., 2011; Adukwu et al., 2016; Shi et al., 2016; Lu et al., 2018).

Galgano et al. (2022) investigated the antimicrobial activity of *M. officinalis* essential oil against *Escherichia coli* and *Staphylococcus aureus*. They revealed the strength of the antimicrobial compounds having dose-effects correlation. The lemon balm essential oil had a bactericidal effect with a MIC and MBC of 1.25% (v/v) against *S. aureus* strains. Zineb et al. (2015) tested the antibacterial activity of *M. officinalis* essential oil with agar disc diffusion method against four bacteria strains (*P. aeruginosa*, *K. pneumonia*, *S. aureus*, and *C. koseri*). The results revealed that the essential oil of *M. officinalis* inhibited the growth of bacteria such as *P. aeruginosa* (16 mm), *K. pneumonia* (13 mm), *S. aureus* (20 mm), and *C. koseri* (14 mm).

3.3. Antioxidant activity

The inhibition percentages tested by DPPP assay for different concentrations of essential oil and citral (1, 10, and 100 µl/mL) are summarized in Table 4 and Figure 1.

The results showed that IC_{50} lemon balm essential oil was evaluated as 2.67 µl/mL (equation $y = 18.72x$; $R^2 = 0.8786$). The results obtained in the current study were close to those reported by Carvalho de Sousa et al. (2004), for the antioxidant activity of lemon balm essential oil measured by DPPH assay reduction. These authors demonstrated 60% to 80% inhibition in the 12–20 µL/mL concentration range, as IC_{50} was below 5 µL/mL. Kowalczyk et al. (2012) found that lemon balm essential oil from 20 to 100 µL showed EC_{50} 0.04 to 0.07 (mL/DPPH assay) and 23–35 µmol TE/mL. Most of the researchers presented the inhibition as µg/mL. According to Mimica-Dukic et al. (2004) the essential oil showed DPPH radical formation (IC_{50} = 7.58 µg/mL), while Fernández et al. (2020) found that the same oil in a concentration of 40 µg/mL demonstrated 48.24 ± 0.71% DPPH inhibition. However, Ehsani et al. (2017) found that 5 mg/mL of lemon balm essential oil showed inhibition of 49.36% (DPPH assay).

3.4. Surface tension, density, and refractive index

The experimental data of surface tension, density, and refractive index are presented in Table 5. They were measured at different temperatures between 6 °C and 30 °C.

3.4.1. Surface energy, surface heat capacity, and molar refraction

The surface energy and surface heat capacity were calculated based on the surface tension measurements. According to equation (3), the surface energy was calculated with dependence between surface tension and temperature as a first-order transition (Figures 2 and 3).

According to equation (4), the surface heat capacity was calculated with dependence between surface tension and temperature as a second-order transition. The results are presented in Figure 4.

The dependence between molar refraction and temperature is presented in Figure 5. The molar refraction decreased with the temperature increase, and linear dependence was observed.

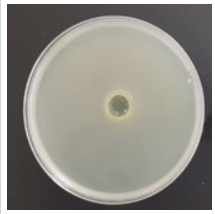
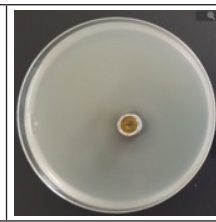
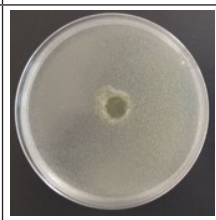
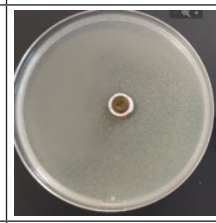
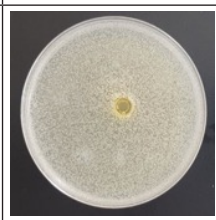
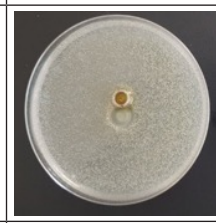
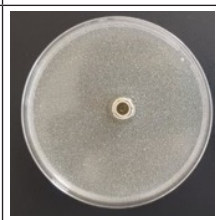
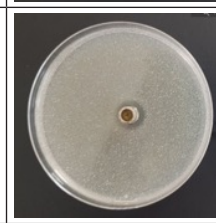
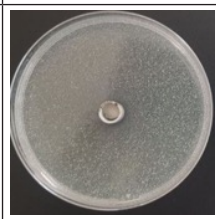
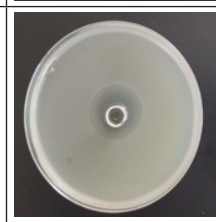
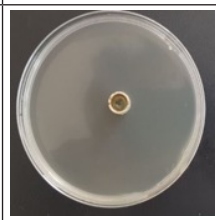
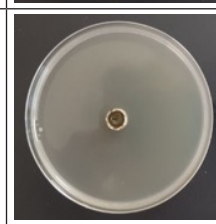
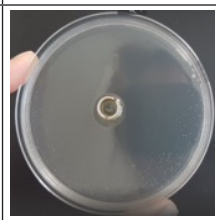
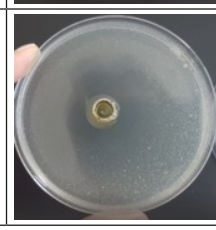
The data from Table 5 showed that the increase in the temperature is observed with a decrease in the surface tension. For many substances, this dependence is linear to near-critical temperatures. At the critical temperature, the surface tension becomes equal to zero, and the densities of the liquid and gas phases equalize (Raev, 2008).

The surface tensions of lemon balm essential oil were determined at different temperatures after providing the experiment. With an increase in the temperature, the surface tension decreased. The results were comparable with those of the authors' of previous studies (Yerima et al., 2015).

The energy values presented very small differences at different temperatures from 124.52 to 125.57 mN/m. Almost no temperature dependence was observed because the temperature gradient of surface tension and surface energy was calculated as a sum between them.

According to equation (4), the surface heat capacity was calculated with dependence between surface tension and temperature as a second-order transition. Surface heat capacity presented values between 10492.11 and 11394.17 N/(m.K). The system is determined as stable when the heat capacities are positive. In this case, the strong temperature dependence between surface capacity and temperature is observed: the small surface tension connected with small heat capacity.

Table 3. Antimicrobial activity of lemon balm essential oil and citral.

Test-microorganisms	**Lemon balm essential oil (diameter of zones of inhibition, mm)**		**Citral (diameter of zones of inhibition, mm)**	
E. coli ATCC 8739	-[a]		1.6 ± 0.01	
S. enterica NCTC 6017	1.3 ± 0.01		1.3 ± 0.01	
Klebsiella (clinical isolate)	-		-	
S. aureus ATCC 25093	-		1.5 ± 0.01	
B. subtilis ATCC 6633	-		4.1 ± 0.04	
*B. cereus (*clinical isolate*)*	-		-	
L.monocytogenes NCTC 11994	6.0 ± 0.05		2.1 ± 0.02	

[a]: no inhibitory activity was observed.

Table 4. Antioxidant activity of lemon balm essential oil.

Sample	Concentration, µL/mL	Inhibition, %	DPPH, mM TE/mL
Lemon balm essential oil	1	4.95	0.04
	5	45.05	0.43
	10	62.12	0.60
	20	70.05	0.68
	100	72.05	0.70

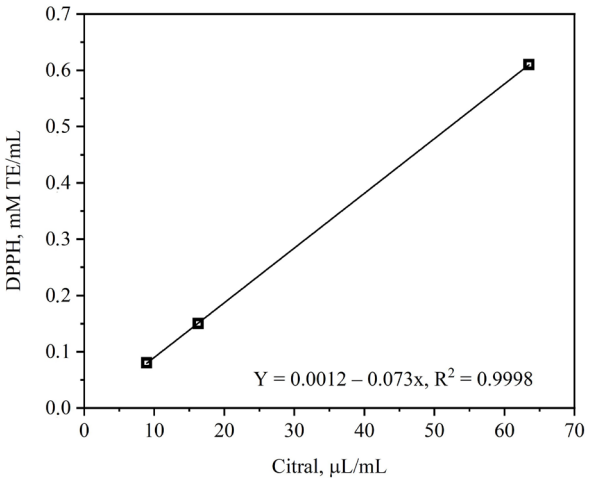


Figure 1. Antioxidant activity (DPPH radical scavenging ability) of citral.

Table 5. Surface tension, density, and refractive index for lemon balm essential oil.

Temperature, °C	Surface tension, γ, mN/m	Density, ρ, kg/m³	Refractive index, n_D
6	41.46 ± 0.19	0.933 ± 0.02	1.508 ± 0.01
10	40.27 ± 0.22	0.928 ± 0.01	1.501 ± 0.01
15	38.56 ± 0.16	0.921 ± 0.08	1.496 ± 0.01
20	37.84 ± 0.09	0.019 ± 0.01	1.492 ± 0.01
25	36.43 ± 0.13	0.907 ± 0.07	1.489 ± 0.01
30	34.27 ± 0.11	0.895 ± 0.09	1.486 ± 0.01

The molar refraction is expressed by the refractive index (n_D), the density (ρ), and the molar mass (M) of the oil. For this purpose, the experimental results presented in Table 3 were included in equation (2). The refractive indexes of the essential oil were measured and exhibited values between 1.486 and 1.508 at different temperatures.

The molar mass of lemon balm essential oil was determined experimentally using GC analysis. The value of molar mass is 172.01 kg/mol. After calculations, the obtained values of molar refractions were between 30.13×10^6 and 32.71×10^6 m³/mol.

The results were comparable with the values of molar refraction according to the literature data. The molar refraction of water-alcohol solutions with coriander essential oil (Gandova et al., 2020) was calculated and determined in 11.214×10^6 to 14.991×10^6 m³/mol. The molar refraction decreased with the temperature increase, and linear dependence was observed.

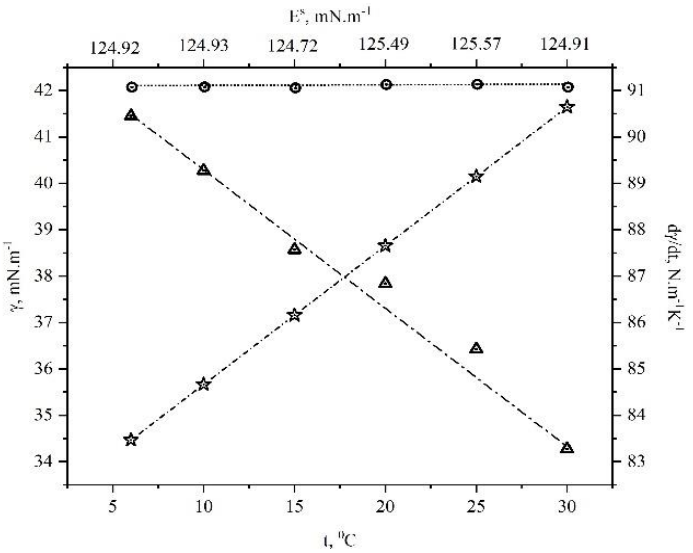


Figure 2. Temperature dependence between surface tension and temperature gradient dγ/dt.

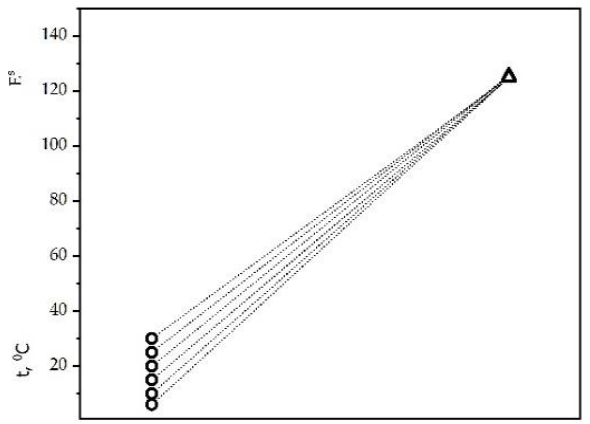


Figure 3. Surface energy values determined in the temperature range.

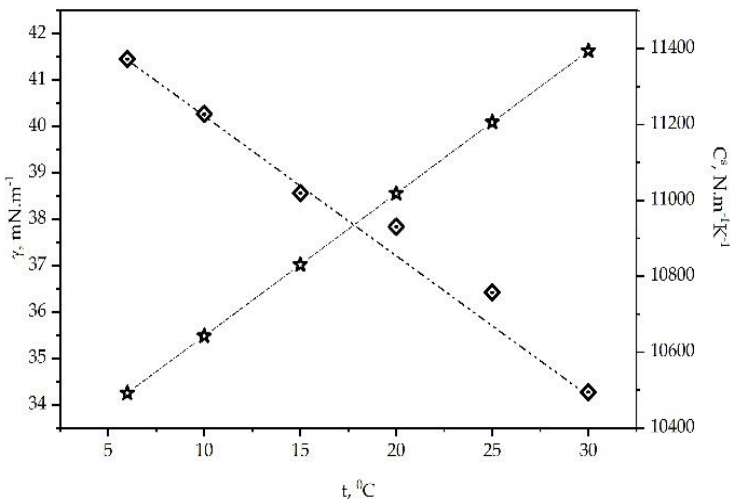


Figure 4. Dependence between surface tension, surface capacity, and temperature.

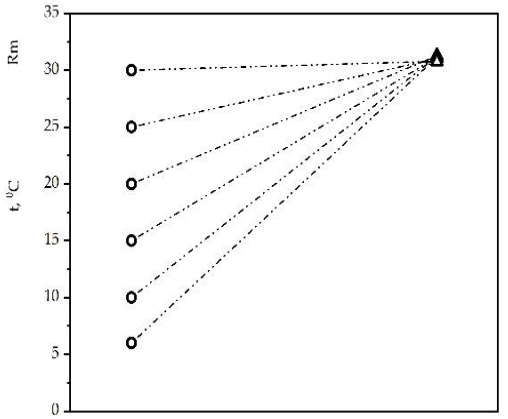


Figure 5. Dependence between molar refraction and temperature.

The temperature dependence of the density and refractive indexes of ethyl benzene, *o*- and *m*-xylenes from 273 K down to their crystallization temperatures was investigated and described (Benkovskii et al., 1966; Markarian and Terzyan, 2007). After investigations of surface tension and density of groundnut oil and palm oil in Yola, Nigeria with two methods 'catch overflow' and capillary rise methods temperature dependence of surface tension was observed (Yerima et al., 2015). According to Melo-Espinosa et al. (2014), a mathematical model was prepared to investigate the surface tensions of different vegetable oils. The molar refraction considers the polarization effects in molecules caused by field action in the optical range of the electromagnetic spectrum. Some empirical dependences between molar refraction and surface tension were presented by Raev (2008) when the temperature is sufficiently distant from the critical temperature.

4. Conclusion

The components in lemon balm essential oil were determined. The detected compounds with the highest content were *β*-caryophyllene (20.45%), geranial (19.74%), neral (15.81%), germacrene D (11.50%), (R)-(+)-citronellal (3.07%), and geranyl acetate (3.04%). A comparison was made between the antibacterial and antioxidant activity of the oil with its main component citral. It was established that the antibacterial activity of the essential oil was weak compared to the tested gram-negative and gram-positive bacteria; the exceptions were observed for *Listeria monocytogenes* and *Salmonella enterica* subsp. *Enterica serovar* Abony. The main constituent citral exhibits higher antimicrobial activity against investigated gram-positive bacteria compared to gram-negative. The surface tensions of essential oil were determined at different temperatures. The values of energy presented at different temperatures were calculated from 124.52 to 125.57 mN/m. Surface heat capacity showed values between 10492.11 and 11394.17 N/(m.K). The essential oil's specific biological and physicochemical properties are a prerequisite for application in cosmetic and food products, which are the subject of future research.

References

Abdellatif F, Boudjella H, Zitouni A, Hassani A (2014). Chemical composition and antimicrobial, activity of the essential oil from leaves of Algerian *Melissa officinalis* L. EXCLI Journal 13: 772–781.

Adams R. Identification of Essential Oil Components by Gas Chromatography /Mass Spectrometry, 4th ed. Allured Publishing Co.: Carol Stream, Illinois, 2007.

Adinee J, Piri K, Karami O (2008). Essential oil composition in flower of lemon (*Melissa officinalis* L.). American Journal of Biochemistry and Biotechnology 4: 277–278.

Adukwu EC, Bowles M, Edwards-Jones V, Bone H (2016). Antimicrobial activity, cytotoxicity and chemical analysis of lemongrass essential oil (*Cymbopogon flexuosus*) and pure citral. Applied Microbiology and Biotechnology 100: 9619–9627.

Başer HCK, Buchbauer G (2010). Handbook of Essential Oils: Science, Technology, and Applications, 1st ed.; Taylor and Francis Group, LLC CRC Press, 2010.

Benkovskii V, Bogoslovskaya T, Naurizov M (1966). Density, surface tension and refractive index of aromatic hydrocarbons at low temperatures. Chemistry and Technology of Fuels and Oils 2: 23–26.

Bulgarian State Standard 9200:1994. Aromatic products. Test methods.

Bulgarian State Standard ISO 1242:2002. Essential oils - Determination of acid value.

Carvalho de Sousa A, Alviano DS, Blank AF, Alves PB, Alviano CS et al. (2004). *Melissa officinalis* L. essential oil: antitumoral and antioxidant activities. Journal of Pharmacy and Pharmacology 56: 677–681.

Chien S-Y, Sheen S, Sommers C, Sheen L-Y (2019). Combination effect of high-pressure processing and essential oil (*Melissa officinalis* Extracts) or their constituents for the inactivation of *Escherichia coli* in ground beef. Food and Bioprocess Technology 12:359–370.

Chwil M, Nurzyńska-Wierdak R, Chwil S, Matraszek R, Neugebauerová J (2016). Histochemistry and micromorphological diversity of glandular trichomes in *Melissa officinalis* L. leaf epidermis. Acta Scientiarium Polonorum Hortorum Cultus 15: 153–172.

Cosge B, Ipek A, Gurbuz B (2009). GC/MS analysis of herbage essential oil from lemon balms (*Melissa officinalis* L.) grown in Turkey. Journal of Applied Biological Science 3: 149–152.

Dastmalchi K, Dorman HD, Oinonen PP, Darwis Y, Laakso I et al. (2008). Chemical composition and in vitro antioxidative activity of a lemon balm (*Melissa officinalis* L.) extract. LWT-Food Science Technology 41: 391–400.

Dias MI, Barrosa L, Sousa MJ, Ferreira ICFR (2012). Systematic comparison of nutraceuticals and antioxidant potential of cultivated, in vitro cultured and commercial *Melissa officinalis* samples. Food and Chemical Toxicology 50: 1866–1873.

Dogan H, Uskutoğlu T, Baş H, Stankov S, Fidan F et al. (2021). Phytochemical composition of wild lemon balm (*Melissa officinalis* L.) from the flora of Bulgaria. Anatolian Journal of Botany 5: 112–119.

Ehsani E, Alizadeh O, Hashemi M, Afshari A, Aminzare M (2017). Phytochemical, antioxidant and antibacterial properties of *Melissa officinalis* and Dracocephalum moldavica essential oils. Veterenary Research Forum 8: 223–229.

El Quadi Y, Manssouri M, Bouyanzer A, Majidi L, Bendaif H et al. (2017). Essential oil composition and antifungal activity of *Melissa officinalis* originating from north-Est Morocco, against postharvest phytopathogenic fungi in apples. Microbial Pathogenesis 107: 321–326.

Fernandez IM, Ribeiro PRE, Maldonado SAS, Ferraz VP, Aleman RS et al. (2020). Chemical composition of essential oil of *Melissa officinalis* L. and antioxidant activity from Boa Vista-RR. Brazil. African Journa of Pharmacy and Pharmacology 14: 41–45.

Florido F, Andrade I, Capellini M, Carvalho F, Aracava K et al. (2014). Viscosities and densities of systems involved in the deterpenation of essential oils by liquid-liquid extraction: New UNIFAC-VISCO parameters. The Journal of Chemical Thermodynamics 72: 152–160.

Fujisawa K, Shiomi T, Hamada F, Nakajima A (1981). Van der Waals-like equations of state. I. Application to pure liquids. Polymer Journal 13: 993–1002.

Galgano M, Capozza P, Pellegrini F, Cordisco M, A. Sposato A et al. (2022). Antimicrobial activity of essential oils evaluated in vitro against *Escherichia coli* and *Staphylococcus aureus*. Antibiotics 11: 979.

Gandova V, Tasheva S, Dobreva K, Prodanova-Stefanova V, Dincheva I et al. (2020). Density, surface tension, refractive index and ternary equilibria of coriander essential oil– ethanol–water system. Oxidation Communications 43: 220–233.

Guimarães LGL, Cardoso MG, de Sousa PE, Andrade J, Vieira SS (2011). Antioxidant and fungitoxic activities of the lemongrass essential oil and citral. Revista Ciência Agronomica 42: 464–472.

Hassan RA, Abotaleb ST, Hamed HB, M.S. Eldeen MS (2019). Antioxidant and antimicrobial activities of *Melissa officinalis* L. (Lemon Balm) extracts. Journal of Agricultural Chemistry and Biotechnology 10: 183–187.

Holla M, Svajdlenka E, Tekel J, Vaverkova S, Havranek E (1997). Composition of the essential oil from *Melissa officinalis* L. cultivated in Slovak Republic. Journal of Essential Oil Research 9: 481–484.

ISO 279:1998. Essential oils - Determination of relative density at 20 ^{O}C – Reference method

ISO 280:1998. Essential oils - Determination of refractive index.

Khalid K, Hu W, Cai W (2008). The effects of harvesting and different drying methods on the essential oil composition of lemon balm (*Melissa officinalis* L.). Journal of Essential Oil-Bearing Plants 11: 342–349.

Kowalczyk A, Biskup I, Fecka I (2012). Total phenolic content and antioxidative properties of commercial tinctures obtained from some Lamiaceae plants. Natural Product Commununications 7: 1631–1634.

Lu W-C, Huang D-H, Wang C-CR, Yeh C-H, J.-C. Tsai J-C et al. (2018). Preparation, characterization, and antimicrobial activity of nanoemulsions incorporating citral essential oil. Journal of Food and Drug Analisys 26: 82–89.

Marei GIK, Rabea EI, Badawy MEI (2018). Preparation and characterizations of chitosan/citral nanoemulsions and their antimicrobial activity. Applied Food Biotenology 5: 69–78.

Meftahizade H, Sargsyan E, Moradkhani H (2010). Investigation of antioxidant capacity of *Melissa officinalis* L. essential oils. Journal of Medicinal Plants Research 4: 1391–1395.

Markarian S, Terzyan A (2007). Surface tension and refractive Index of dialkylsulfoxide + water mixtures at several temperatures. Journal of Chemical and Engineering Data 52: 1704–1709.

Melo-Espinosa E-A, Sanchez-Borroto Y, Errasti M, Piloto-Rodriguez R, Sierens R et al. (2014). Surface tension prediction of vegetable oils using artificial neural networks and multiple linear regression. Energy Procedia 57: 886–895.

Mimica-Dukic N, Bozin B, Sokovic M, Simin N (2004). Antimicrobial and antioxidant activities of *Melissa officinalis* L. (Lamiaceae) essential oil. Journal of Agricultural and Food Chemistry 52: 2485–2489.

Misharina T (2016). Antiradical properties of essential oils and extracts from coriander, cardamom, white red, and black peppers. Applied Biochemistry and Microbiology 52: 79 – 86.

Moradkhani M, Sargsyan E, Bibak H, Naseri B, M. Sadat-Hosseini M et al. (2010). *Melissa officinalis* L., a valuable medicine plant: A review. Journal of Medicinal Plants Research 4: 2753–2759.

Nurzyńska-Wierdak R, Bogucka-Kocka A, Szymezak G (2014). Volatile constituents of *Melissa officinalis* leaves determined by plant age. Natural Product Communications 9: 703–706.

Oshaghi M, Ghalandari R, Vatandoosi H, Shayeghi M, Kamali-nejad M, Tourabi-Khaledi H, Abolhassani M, Hashemzadeh M (2003). Repellent effect of extracts and essential oils of *Citrus limon* (Rutaceae) and *Melissa officinalis* (Labiatae) against main malaria vector, *Anopheles stephensi* (Diptera: Culicidae). Iranian Journal of Public Health 32: 47–52.

Pino J, Rosado A, Fuentes V (1999). Composition of the essential oil of *Melissa officinalis* L. from Cuba. Journal of Essential Oil Research 11: 363–364.

Raev N. Matter – Nature, Properties and Events. UFT Academic Publishing House, Plovdiv, 2008.

Saeb K, Gholamrezaee S (2012). Variation of essential oil composition of *Melissa officinalis* L. leaves during different stages of plant growth. Asian Pacific Journal of Tropical Biomedicine 2: 547–549.

Sani I, Khaledabad M, Pirsa S, Kia E (2020). Physico-chemical, organoleptic, antioxidative and release characteristics of flavoured yoghurt enriched with microencapsulated *Melissa officinalis* essential oil. International Journal of Dairy Technology https://doi.org/10.1111/1471-0307.12691.

Sarkic A, Stappen I (2018). Essential oils and their single compounds in cosmetics – a critical review. Cosmetics 5: 11.

Shi C, Zhao X, Liu Z, Meng R, Chen X et al. (2016). Antimicrobial, antioxidant, and antitumor activity of epsilon-poly-L-lysine and citral, alone or in combination. Food and Nutrition Research 60: 1–8.

Sodre A, Luz J, Haber L, Marques M, Rodrigues C et al. (2012). Organic and mineral fertilization and chemical composition of lemon balm (*Melissa officinalis* L.) essential oil. Revista Brasileira Farmacognosia 22: 40–44.

Stoyanova A, A Guide for the Specialist in the Aromatic Industry *Cultivation*, Plovdiv, BNAEOPC, Plovdiv, Bulgaria, 2022.

Wuryatmo E, Klieber A, Scott ES (2003). Inhibition of citrus postharvest pathogens by vapor of citral and related compounds in culture. Journal of Agricultural and Food Chemistry 51: 2637–2640.

Yankova R, Gandova V, Dimov M, Dobreva K, Prodanova-Stefanova V et al. (2019). Studies on the structural, electronic and physical properties of linalool. Oxidation Communications 42: 293–306.

Yerima J, Solomon S, Barlow, Dikko A (2015). Temperature dependence of density and dynamic surface tension of groundnut oil and palm oil. International Journal of Engineering Science 4: 49–55.

Yoplac I, Vargas L, Robert P, Hidalgo A (2021). Characterization and antimicrobial activity of microencapsulated citral with dextrin by spray drying. Heliyon 7: e06737.

Zineb Jalal Z, El Atki J, Lyoussi B, Abdellaoui A (2015). Phytochemistry of the essential oil of *Melissa officinalis* L. growing wild in Morocco: Preventive approach against nosocomial infections. Asian Pacific Journal of Tropical Biomedicine 5: 458–461.