GC-MS Analysis of the Compositions and Antimicrobial Activities of Essential Oils From Five Species of Lamiaceae in Iran

mansureh ghavam (✉ mansurehghavam@gmail.com)

University of Kashan

Research Article

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Abstract

Essential oils (EOs) separated from *Lamiaceae* species attract more attention due to their abundant use in the preservation of natural foods and pharmaceuticals and have gained considerable interest in research and industry. The aim of this study was to evaluate composition and antimicrobial activity of EOs obtained from five species *Lamiaceae* in Iran. After extraction of EOs by Clevenger, their composition was evaluated by gas chromatography-mass spectrometry (GC-MS). Antimicrobial properties were assayed by measuring inhibition zones, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). The results showed that the effects of species, on yield and predominant compounds amount of EOs of five species were significant with a probability of error of 1 %. The yield of oils were varied from ~ 0.02 to ~ 1.88%. About 66 components were identified by gas chromatography-mass spectrometry (GC-MS), and the dominant compounds were included thymol (67.71%), oleic acid (0.49–62.09%), (-)-caryophyllene oxide (0.41–24.81%), α -pinene (1.09–19.41%), 1,8-cineole (0.22–15.40%), palmitic acid (0.32–13.28%), (+)-spathulenol (11.16%), and germacrene D (0.30–10.26%) in different species. The results of analysis of variance showed that there was a significant difference between the mean of the inhibition zone obtained treating the different microorganisms with the essential oil of five species ($P \leq 0.01$). The highest inhibition zone belonged to TDEO (39.33 ± 0.58 and 25.00 ± 0.00 mm) against Gram-positive *S. aureus* and *A. brasiliensis*. The Gram-negative *P. aeruginosa* showed the lowest inhibitory resistance to HIEO, SIEO, and ROEO (with a MIC value of $31.25 \mu\text{g/mL}$), which was very significant compared to rifampin. Therefore, EOs of five species have potential applications in the control of various bacteria and fungi and can be a natural alternative to some antibiotics.

Introduction

Due to the uncontrolled use of antibiotics, we have seen the emergence of multidrug-resistant bacterial and fungal strains that have become increasingly popular in recent decades to find new and safe antimicrobial molecules of natural origin, especially plant metabolites (Stojanović-Radić et al., 2018 and Mitić et al., 2018). Against this worrying situation, other options must be developed to make natural, safe and effective antimicrobial drugs. Natural products, such as essential oils (EOs), have historically proven their value as sources of antimicrobial potential molecules and may have the potential for subsequent topical use or systemic administration in bacterial and fungal infections. Indeed, the synergistic interaction of EOs with antimicrobials has previously been reported to prevent multidrug resistance by restoring antimicrobial activity and preventing adverse antimicrobial side effects. (Fadli et al., 2012; Kasrati et al., 2014 and Ait sidi Brahim, 2015).

Lamiaceae EOs because of their interesting physicochemical characteristics of substantial value, have garnered research and industrial interests for use as natural products. These EOs mainly contain various phenolic compounds, such as monoterpene perillaldehyde, polyphenols, coumarins, tannins, iridoids, diterpenoids, triterpenoids, quinones, saponins, and pyridine and pyrrolidine alkaloids (El Asbahani et al., 2015). Studies have shown that many mint family plants such as thyme, peppermint, lavender, rosemary, peppermint, savory, marjoram have antimicrobial effects (Zargari, 2012 and Sekman et al., 2004).

Thymus daenensis Celak. is an Iranian herbaceous, that is distributed in most parts of Iran, especially throughout the Zagros and some parts of the Alborz Mountains (Jamzad, 2012; Zarshenas and Kern, 2015). Thymol, carvacrol, and p-cymene have been reported as the most important constituents in the EO of this plant (Sajjadi and Khatamsaz, 2003; Ghasemi Pirbalouti et al., 2013; Mehran et al., 2016; Nikavar and Mojab 2005; Askari and Sefidkon, 2003; Weisany et al., 2019 and Tohidi et al., 2017). In some studies, Thymol and β -caryophyllene were the dominant essential oil composition (Behbahani Hosseini et al., 2013). Antifungal and antibacterial effects of this plant (Ghasemi Pirbalouti et al., 2014; Safarpour et al., 2018; Nematollahi et al., 2011; Hosseini Behbahani et al., 2013; Teimouri, 2012 and Moghimi et al., 2016) and therapeutic effects It has been proven for the treatment of asthma, recurrent dry cough and bronchitis (Nikavar and Mojab, 2004). It is also used as a food ingredient, herbal tea, and a medicinal plant because of its renowned therapeutic properties (Alizadeh et al. 2013).

Nepeta sessilifolia Bung is one of the exclusive species in Iran. It is a perennial shrubby plant a vital form of hemicryptophyte, often growing on the slopes of semi-arid mountains and rocky and rocky terrain (Batuli, 2002). The predominant essential oil constituents of this species are spathulenol (Talebi et al., 2019 and Safaei Ghomi et al., 2011) and some studies have reported linalool acetate and linalool (Jamzad et al., 2008). The antimicrobial activity of some *Nepeta* genera has been studied and proven (Formisano et al., 2011; Alim et al., 2009 and Ezzatzadeh et al., 2014).

Hymenocrater incanus Bunge is one of the exclusive species of this genus in Iran. The herbaceous plant is perennial with a wooden base 30 to 50 cm high (Jamzad, 2012). The major constituents of the EO of this plant have been reported as β -caryophyllene and 1,8-cineole, α -pinene, β -pinene, trans- β -ocimene, germacrene-D, and caryophyllene oxide (Mirza et al., 2001). Recent reports confirm the drug potential of the genus *Hymenocrater* for the development of new drugs. Plants of this genus have antibiotic, antifungal, antiparasitic, anti-cancer and

anti-diabetic compounds that may be effective in the treatment of diseases (Alan et al., 2014, 2015 and Hooshyar et al., 2015). The antimicrobial properties of some species of this genus have been confirmed (Morteza-Semnani et al., 2010 and 2012 and Ahmadi et al., 2010).

Stachys inflata Benth. is a perennial plant at the base of a tree at a height of 15 to 40 cm (Khanvai et al., 2009). The aerial parts of this plant have been used as a herbal medicine in the treatment of infection, asthma, rheumatism and other inflammatory diseases (Alibakhshi et al., 2014 and Garjani et al., 2004). In most studies, germacrene-D, bicyclogermacrene, and α -pinene were the major constituents of the EO of this plant (Sajjadi and Somae, 2004; Morteza-Semnani et al., 2006; Meshkatsadat et al., 2007 and Alibakhshi et al., 2014). The antimicrobial effects of the extract (Saeedi et al., 2008 and Rajabi et al., 2014) and the EO of this plant (Ebrahimabadi et al., 2009) have been reported.

Rosmarinus officinalis L. The plant is in the form of durable small shrubs with aromatic leaves and small blue flowers (Inouye et al., 2001). Rosemary EO is one of the compounds that have antimicrobial and antioxidant properties in many cases and antimicrobial compounds such as phenolic compounds are found in abundance in it. Predominantly 1,8-cineole, camphor, (Pereira et al., 2017) α -pinene, borneol, and camphene (Ben Abada et al., 2020, Takayama et al., 2016; Tawfeeq et al., 2016 and Bajalan et al., 2017), have been reported as the major constituents of the EO of this plant. Antimicrobial effect of the extract (Oluwatuyi et al., 2004 and Yesil Celik et al., 2005) and EO (Bajalan et al., 2017; Messaoudi Moussi et al., 2019 and Risaliti et al., 2019), recorded. Rosemary EO is used in the treatment of indigestion and milder forms of gastrointestinal disorders, circulatory disorders, as a supplement in the treatment of musculoskeletal pain and inflammation (Rašković et al., 2014).

Studies on these species indicate the importance of these plants in terms of their essential oil composition and antimicrobial properties. However, no comprehensive study of these species has been performed simultaneously using Twelve strains of microorganisms. Since these plants have not been studied in the habitats of Isfahan province in Iran, this study is of particular importance. In particular, most of the species studied are native to Iran. The aims of this study were 1) to determine the chemical composition of EOs of five species, and 2) to compare chemical compositions, yield variations and antimicrobial activities of EOs of 5 species.

Materials And Methods

Plant material

To select the sampling region, at first, habitats of plants (*T. daenensis*, *N. sessilifolia*, *H. incanus*, *S. inflata*, and *R. officinalis*) were identified through field surveys. Then, Daran region, located in Isfahan, Iran was selected (longitude: E 46° 49' 02" and latitude: N 36° 54' 17"). To sample of each plant, in June 2018, coinciding with the flowering, three points were selected randomly from Daran region. In each point aerial parts of each plant randomly from different plants (50-100 plants in each point) were collected. The specimens were transferred to the laboratory after being harvested and then exposed to free air to dry. One sample of the whole plant was also collected and pressed. The specimens was identified and recorded in the herbarium of the University of Kashan

Extraction of EOs

After complete drying, the samples were grinded by using a small electric mill. Then from each sample of plant was weighed (100 g) and subjected to the extraction process by means of water distillation by using a Clevenger apparatus (Made in Germany) for five hours. The essential oil was dried by anhydrous sodium sulfate and after filtration, were stored in dark bottles at 4°C until their use for further studies. Essential oil yield was calculated based on weight percent (w/w). This process was repeated three times for each plant.

Gas chromatography-mass spectrometry (GC-MS) analysis

The main bioactives contained in the essential oils have been determined by means of GC-MS, using a chromatograph (Model 6890 Chromatography) coupled with an Agilent Mass Spectrometer (Model N-5973). A capillary column (HP-5MS) with 5% Methylphenylsiloxane Static Phase (Length 30 m, Internal Diameter 0.25 mm, Layer Static Thickness 0.25 μ m) and ionization energy of 70 eV was used. The temperature for the analyses was first set at 60°C and then it was increased, at a rate of 3°C up to 246°C. The injector and detector temperatures were maintained at 250°C, the volume of the injected sample was 1 μ l and the helium carrier gas was maintained at a flow rate of 1.5 ml/min. The identification of chemical components was based on the analysis of the chromatograms obtained for each oil, by means of the retention indices (RI) evaluation in comparison with standards of n-alkane mixtures (C8-C20) and mass spectral data of each peak by using a computer library (Wiley-14 and NIST-14 Mass Spectral Library), and comparison of the obtained results with those contained in the literature (Adams, 2007).

Antimicrobial activity

Tested microorganisms

Twelve microorganisms, provided by the Iranian Research Organization for Science and Technology (IROST), have been used to evaluate the antimicrobial activity of the essential oils. Three Gram-positive bacteria *Staphylococcus epidermidis* (ATCC 12228), *Staphylococcus aureus* (ATCC 29737) and *Bacillus subtilis* (ATCC 6633) and nine Gram-negative bacteria *Klebsiella pneumonia* (ATCC 10031), *Shigella dysenteriae* (PTCC 1188), *Pseudomonas aeruginosa* (ATCC 27853), *Salmonella paratyphi-A serotype* (ATCC 5702), *Proteus vulgaris* (PTCC 1182) and *Escherichia coli* (ATCC 10536). Fungal strains were used as well *Aspergillus niger* (ATCC 16404), *Aspergillus brasiliensis* (PTCC 5011) and *Candida albicans* (ATCC 10231). Bacterial strains were cultured overnight at 37°C in nutrient agar and fungi were cultured overnight at 30°C in sabouraud dextrose agar.

2.4.2. Agar diffusion method of Well diffusion

This procedure was performed according to CLSI standards. 6.0 mm in diameter well plates containing Müller Hinton agar were prepared and 100 µl of bacterial suspensions with a half-McFarland turbidity equivalent in culture medium were cultured. The essential oils were dissolved in dimethyl-sulfoxide (DMSO) at a concentration of 30 mg/mL. 10 µL (equivalent to 300 µg) of each oil was poured into the wells. The plates were incubated at 37°C for 24 h for bacterial strains and 48 h and 72 h at 30°C for yeast and its antimicrobial activity was evaluated for each microorganism by measuring the diameter of the inhibition halo (in millimeters), according to the antibiogram ruler. To evaluate the repeatability of the results, three replicates for each essential oils and each strain were performed. DMSO was used as negative control. Gentamicin (10 lg/disk), and rifampin (5 lg/disk) for bacteria and nystatin (100 I.U.) for yeast were used as standard drugs for positive control in the same conditions to tested oils.

Determine the Minimum inhibitory concentration (MIC)

The minimum concentration able to inhibit the growth of bacteria was calculated by means of microdilution method. Essential oils (2000 µg/ml), were dissolved in a mixture of tryptic soy broth medium and DMSO and then were opportunely diluted, by using the same mixture, to reach different concentrations (1000, 500, 250, 125, 62.5, 31.25 and 15.63 mg/ml).

Sterile 96-well microplates were filled with 95 µl of culture medium, 5 µl of bacterial suspension with 0.5 McFarland dilution, and 100 µL of the essential oils at different concentrations, then plates were incubated at 37°C for 24 h for bacterial strains and 48 h and at 30°C for yeast. The MIC was determined by means of the improvement of opacity or the change in color. The MIC was the lowest concentration of an antimicrobial that inhibited the visible growth (absence of turbidity).

Determination of Minimum bactericide concentration (MBC)

To determine the minimum concentration able to kill the bacteria the same microdilution method described above was used. After 24 h of incubation with both bacteria and oils at different concentrations, 5 µl of the content of each well were inoculated with neutrin agar medium and incubated at 37°C for 24 h for bacterial strains. After incubation, the colony-forming units were enumerated. The MBC was the lowest concentration able to effectively reduce the growth of microorganisms (99.5%).

Statistical analysis

The statistical analysis was performed using SPSS software. First, the normality of the statistical variables was investigated using a Kolmogorov-Smirnov test, and after ensuring the normality of the data, the variance of the data (essential oil and Antimicrobial activity) was analyzed using an F-test and a comparison of the means using a Duncan test with a probability level was 1 % error was performed.

Results

EOs content

The EOs color varied from pale yellow to dark yellow with yields ranging from 1.88% -0.02% (w/w). Based on the results of the analysis of variance, the yield of essential oil of five species was significantly different. The effect of the species on the yield of essential oil was significant ($P \leq 0.01$) (Table 1), which is consistent with the findings of Luo et al., (2019) for Lamiaceae, Silva et al. (2018) for Myrtaceae, and Ngahang Kamte et al., (2018) for Apiaceae. The highest essential oil yield belonged to TDEO (~1.88%) and the lowest to HIEO (~0.02%) (Table 2). Essential oil yields can be greatly affected by environmental factors and plant species (Llorens et al., 2014). Compared to previous studies, the highest TDEO yields were 2.09% (Tohidi et al., 2017), NSEO 0.56% (Safaei Ghomi et al., 2011), HIEO 0.6% (Mirza et

al., 2001), SIEO. 2.9% (Alibakhshi et al., 2014) and ROEO 2.35% (Bajalan et al., 2017), that were larger than the present study. The synthesis of secondary compounds in plants is one of the most important defense mechanisms against pathogens, and its quantity varies depending on habitat, organ, species. These differences are most likely due to the differences in chemotyp, which are themselves due to the environmental conditions and climate (Yavari et al., 2010).

GC–MS analysis

The chemical constituents of EOs collected from five *Lamiaceae* species were investigated using GC-MS. About 68 components were identified and the relative composition of these five EOs, including TDEO (100%), NSEO (84.56%), HIEO (100%) SIEO, (99.35%) and ROEO (100%) is presented in Table 3. Most of the compounds were monoterpenes hydrocarbons belonging to ROEO (39.55%), oxygenated monoterpenes belonging to TDEO (74.9%), sesquiterpenes hydrocarbons belonging to SIEO (26.25%) and oxygenated sesquiterpenes belonging to HIEO (41.22%). NSEO was mainly composed of non-terpene and acidic compounds (77.18%).

Most of the compounds in TDEO were from oxygenated monoterpenes (74.9%) and monoterpenes hydrocarbons (18.3%). The predominant components in TDEO were thymol (67.71%), γ -terpinene (6.20%), p-cymene (5.16%) and borneol (3.67%) and 1,8-cineole (15%). (Table 3).

In NSEO, most of the compounds belonged to acids and mainly to non-terpenes (Table 3). The predominant components in the EO of this plant were oleic Acid (62.09%), Stearic acid (8.16%) and linoleic acid (6.06%). These three compounds were first observed in this plant and so far no reports of these compounds have been reported even to a minor extent.

In HIEO, most of the compounds were acidic (50.65%) and oxygenated sesquiterpenes (41.22%). Predominant components in HIEO were (-)-caryophyllene oxide (24.81%), oleic acid (23.53%), palmitic acid (28.23%), phytol (9.22%), α -cadinol (7.66%), and caryophyllenol-II (5.06%), respectively (Table 3).

In SIEO, other compounds, especially acids (42.22%) and sesquiterpenes hydrocarbons (26.25%), were the major constituents of it, which is different from previous reports of this plant. Oleic acid (20.75%), palmitic acid (12.12%), (+)spathulenol (11.16%), germacrene D (12.26%) and bicyclogermacrene (9.9%) are the main constituents of this EO (Table 3). In none of the previous reports has oleic acid been recorded so far.

In ROEO, mainly oxygenated monoterpenes (50.04%) and monoterpenes hydrocarbons (39.55%) comprised the major part of the essential oils, with findings by Pereira et al., (2017) and Takayama et al.(2016) corresponds. α -pinene (19.41%), 1,8-cineole (15.40%), camphor (8.87%), borneol (8.48%) and verbenone (8.8%) were the major constituents of this EO (Table 3).

Based on the results of analysis of variance the effect of the species on the amount of 1,8-cineole, oleic acid, (-)-caryophyllene oxide, trans-caryophyllene and linalool was significant ($P \leq 0.01$) (Table 1). The data presented in Table 1 show that 1,8-cineole and oleic Acid were found in the essential oils of 5 plant species. The highest amount of 1,8-Cineole was observed in ROEO with 15.40% and the lowest with 0.22% in HIEO (Figure 1). The highest and lowest amount of oleic acid was in NSEO (62.09%) and TDEO (0.49%), respectively (Figure 2). Linalool was found in all essential oils except HIEO, with the highest amount being 2.97% in ROEO. Also, trans-caryophyllene and (-)-caryophyllene oxide were found in all essential oils except SIEO with the highest amount being 3.68% and 24.81% in HIEO, respectively. Some high-value compounds such as thymol (67.71) and p-cymene (5.16), only in TDEO, linoleic acid (6.06) only in NSEO, caryophyllenol-II (5.06) only in HIEO, (+) spathulenol (11.16) only in SIEO and β -myrcene (5.38) existed only in ROEO.

Antimicrobial activity

Inhibition-zone

The results of analysis of variance showed that there was a significant difference between the mean of the inhibition halo obtained treating the different microorganisms with the essential oil of five *Lamiaceae* species ($P \leq 0.01$) (Table 4). Similar trend was observed by Luo et al., (2019) for six other species of *lamiaceae*. The highest inhibition zone was belonged to TDEO (39.33 ± 0.58 mm) against Gram-positive *S. aureus* compared to the inhibition zone rifampin (~ 21 mm) and gentamicin (~ 27 mm) is a significant activity (Table 5). Other essential oils such as NSEO (9.67 ± 0.58 mm), SIEO (8.50 ± 0.50 mm) and ROEO (9.00 ± 0.00 mm) had little activity on this bacterium and HIEO did not produce any diameter. Reduction of monoterpenes in HIEO seems to be one of the factors affecting the lack of inhibition halo against this *S. aureus*. In addition, this antibacterial activity of TDEO, NSEO, SIEO, and ROEO can be attributed to other oxygenated compounds such as linalool.

Secondly, was the highest inhibition zone (25.00 ± 0.00 mm), belonging to TDEO against *A. brasiliensis*, which is a significant activity compared to the Nystatin (~ 30 mm) control antibiotic growth zone against *A. brasiliensis* (Table 5). On the other hand, the effect of TDEO

on the fungi *A. niger* and *C. albicans* also caused an inhibition zone diameter of 12.00 ± 0.00 mm and 11.67 ± 0.58 mm, respectively, in comparison with the inhibition zone of Nystatin control antibiotic (~27 and ~33 mm), had low activity on these fungi.

On the other hand, TDEO with inhibition zone diameter of 17.67 ± 0.58 mm and 16.33 ± 0.58 mm against two Gram-negative bacteria *K. pneumonia* and *Sh. dysenteriae* showed good activity in comparison with inhibition zone of antibiotic rifampin (~8 mm and ~9 mm) and antibiotic control gentamicin (~17 mm). so far no report of the effect of TDEO on these two bacteria has been reported and therefore, the present study is important. Also TDEO against *B. subtilis* and *P. vulgaris* had a low activity, with inhibition zone diameter of 14.00 ± 0.50 mm compared to inhibition zone rifampin (~19 and ~8 mm) and gentamicin (~30 and ~24 mm).

TDEO also had little effect against two Gram-negative bacteria *E. coli* and *S. paratyphi-A* with inhibition zone 11.33 ± 0.58 and 11.67 ± 0.58 mm, respectively, compared to inhibition zone diameter rifampin (~10 and ~8 mm) and gentamicin (~23 and ~18 mm) low effect is quite obvious.

Inhibition zone of NSEO was only present against three Gram-positive *B. subtilis*, *S. epidermidis* (9.00 ± 0.00 mm), and *S. aureus* (9.67 ± 0.58 mm), which had little activity compared to rifampin and gentamicin. The NSEO did not create inhibition zone against other microorganisms.

Inhibition zone of HIEO was created only against two Gram-positive bacteria, *B. subtilis* (10.00 ± 0.00 mm) and *S. epidermidis* (11.00 ± 0.00 mm), which had little activity compared to rifampin and gentamicin. HIEO did not create inhibition zone against other microorganisms. The SIEO was exhibited little activity against Gram-positive bacteria *B. subtilis* (inhibition zone diameter 9.00 ± 0.00 mm) and *S. aureus* (inhibition zone diameter 8.50 ± 0.50 mm). The present results are remarkable when compared to the report of Ebrahimabadi et al., (2009), for the lack of SIEO inhibition zone diameter against these micro-organisms. Inhibition zone diameter of ROEO against three gram-positive bacteria *B. subtilis*, *S. aureus* (9.00 ± 0.00 mm) and *S. epidermidis* (9.33 ± 0.58 mm), which had little activity compared to control antibiotics rifampin and gentamicin.

Minimal inhibitory concentrations (MIC)

The values of MIC varied from $15.63 > \mu\text{g/mL}$ to >2000 as a function of the organism tested and the used oil (Table 6). The lowest inhibitory concentration was found against *P. aeruginosa* (MIC = $31.25 \mu\text{g/mL}$) by HIEO, SIEO, and ROEO, which was very significant compared to rifampin ($\sim 31.25 \mu\text{g/mL}$) and gentamicin ($\sim 7.81 \mu\text{g/mL}$). It has been previously reported that *P. aeruginosa* is highly sensitive to EOs (De Martino et al., 2009). Other EOs with a MIC value of $125 \mu\text{g/mL}$ had a much weaker inhibitory effect against *P. aeruginosa*. All EOs (except TDEO) with MIC value of $2000 \mu\text{g/mL}$ showed the highest resistance to *A. brasiliensis* and *A. niger*. On the other hand, SIEO with a MIC value of $>1000 \mu\text{g/mL}$ showed the highest resistance to *S. aureus*, which was two to four times weaker than other EOs studied.

The MIC value of TDEO against all the different bacteria varied between 125 and $500 \mu\text{g/mL}$, with its weakest inhibitory effect against *Sh. dysenteriae* in compared with rifampin (MIC = $15.63 \mu\text{g/mL}$) and gentamicin (MIC = $3.90 \mu\text{g/mL}$), which contradicts the results of Golkar et al. (2020) for TDEO. The MIC value of TDEO against all fungi varied from 31.25 to $250 \mu\text{g/mL}$. *C. albicans* showed the least inhibitory resistance to TDEO among all microorganisms, which is significantly more potent and three times more potent than nystatin ($\sim 125 \mu\text{g/mL}$). *C. albicans* is one of the most common pathogenic fungi in nature that causes serial infectious diseases and has become a serious threat to human health. The MBC value of TDEO also varied from $62.50 \mu\text{g/mL}$ to $500 \mu\text{g/mL}$ (Table 4). The results indicate that the MBC value of TDEO on all microorganisms (except *S. aureus*, *K. pneumonia*, and *C. albicans*) was always equal to the MIC value (Table 6 and 7), indicating that growth and lethal inhibitory power of TDEO are the same.

The MIC value of NSEO against all microorganisms varied between 125 and $2000 \mu\text{g/mL}$. Its strongest inhibitory effect was against Gram-negative bacteria *Sh. dysenteriae*, *K. pneumonia* and *P. aeruginosa*, that it is three to four times weaker than control antibiotics. The weakest inhibitory effect of this essential oil was against *A. niger* and *A. brasiliensis*. The results showed that the MBC value of NSEO was the same as MIC value of NSEO in all microorganisms (except *S. aureus*, *B. subtilis*, *Sh. dysenteriae*, and *P. vulgaris*). This indicates that NSEO's growth and lethal inhibitory potency against many of microorganisms is the same. The lowest MBC value of NSEO belonged to *Sh. dysenteriae* and *K. pneumonia*, which was equivalent to MIC value of NSEO, confirming the importance of this EO in inhibiting and killing *Sh. dysenteriae* and *K. pneumonia*. The absence of terpenes predominantly in the EO of this plant may be due to its poor antimicrobial effect, although the relatively weak effect of NSEO on some bacteria may be due to the presence of fatty acids (Oleic acid $\sim 62.09\%$). However, the mechanism of the antibacterial activity of fatty acids is not yet known. But it is believed that their functional nature is related to the permeability and membrane disruption and fatal alterations in the cytoplasmic membrane of the bacterium, thereby disrupting the membrane-dependent conduction systems.

The MIC value of HIEO was against all microorganisms varied between 15.63 > - >2000 µg/mL. The strongest inhibitory effect was against Gram-negative *P. aeruginosa* (MIC 15.63 > µg/mL), which is very significant in comparison with rifampin (MIC = 31.25 µg/mL). HIEO had a good effect against *Sh. dysenteriae*, *K. pneumonia*, and *S. paratyphi-A* with MIC value of HIEO 62.50 µg/mL (compared with rifampin MIC value of 15.63 µg/mL against *Sh. dysenteriae*, *K. pneumonia*, and *S. paratyphi-A*), that was consistent with results of Fazly Bazzaz and Haririzadeh, (2003) for *H. calycinus*. Also the MIC value of HIEO against *C. albicans* was 62.50 µg/mL, which was weaker than nystatin (MIC =125 µg/mL). This is evident in the results of the MBC value of HIEO and the lethality of this against *C. albicans* has been increased to 250 µg/mL.

Also the MIC value of SIEO was against all microorganisms varied between 15.63 > - >2000 µg /mL, that the strongest inhibitory effect was against the Gram-negative bacterium *P. aeruginosa*. ROEO had the strongest inhibitory effect against *P. aeruginosa* (with MIC value 15.63 > µg/mL), which was twice as potent as the MIC value of rifampin.

Discussion

Similarly, a difference in the relative composition of the essential oils of different species of the *Lamiaceae* family by Luo et al., (2019) and species of the *Apiaceae* family by Ngahang Kamte et al., (2018) has been observed. The production and accumulation of metabolites in individuals with a similar species that are growing in different regions may be influenced by environmental factors, such substances appear to play a chemical interface between the plant and the environment (Gutbrodt et al., 2012). Environmental factors (eg, average temperature, precipitation, and longitude) are likely to play a role in the selection of specialized genotypes/chemotypes for monoterpenes that lead to different chemistry (Taft et al., 2015).

In almost all studies on this plant, thymol had the highest percentage of essential oil with different amount, that the highest amount of thymol by Weisany et al., (2019) from Kurdistan region of Iran (91.15%) observed. Similarly, differences in the proportion of constituent components of essential oils between samples of one plant from different geographical areas, particularly the predominant composition, have been observed by Lukas et al., (2009) for *Origanum syriacum* L., and by Sampaio and Batista (2017) for *Tithonia diversifolia* (Hemsl.) A.Gray species. These data from literature indicate that the surrounding environment seems to significantly influence the production and accumulation of volatile constituents in aerial parts of *T. daenensis*. Due to the high percentage of thymol, this species can be considered as the main base of this valuable compound. Numerous studies have shown that thymol is one of the phenolic compounds that have been shown to have very effective antimicrobial and antimicrobial properties in EOs (Villanueva-Bermejo et al., 2015; Gavaric et al., 2015 and Ultee et al., 1999).

As reported in Safaei Ghomi et al., (2019) and Talebi et al., (2011), oxygenated sesquiterpene (35.3% and 33.14%), and in the study of Jamzad et al. (2008), oxygenated monoterpene (49%) contained the highest percentage of EO compounds of this species, which did not correspond to the present results.

The yields and effects of medicinal plants vary depending on the locations of growth, climatic and ecological conditions, field operations, growth stages, and genetic traits (Millauskas et al., 2004). Oleic acid (9-octadecenoic acid) is a component of omega-9 fatty acids. This fatty acid is effective in treating cancer and cardiovascular disease, autoimmune diseases, Parkinson's, Alzheimer's, inflammatory diseases and high blood pressure. Its derivatives have a regulatory role on the cell membrane and are used as an anticancer drug that induces cell apoptosis and cell division (Sales-Campos et al., 2013; Choque et al., 2014 and Gonçalves et al., 2014). Linoleic acid is one of the most abundant unsaturated fatty acids in the human diet, which is one of the omega-6 fatty acids and plays an active role in the growth and general health of the human body (Sales-Campos et al., 2013).

Although some common compounds in the present study were similar to the results of Mirza et al., (2001); however, there was a significant difference between the two studies. In particular, none of the major constituents of the essential oil present, was recorded in Mirza et al., (2001). These differences are most likely due to the chemotypic differences that result from the environmental and climate conditions of the studied habitats (Yavari et al., 2010). Caryophyllene oxide inhibits abnormal accumulation of fluid in the intercellular space of body tissues and antitumor (Jaimand and Rezaei, 2006).

Palmitic acid as the predominant compound with amount 9.1% have recorded by Morteza-Semnani et al., (2006). Germacrene D and bicyclgermacrene by Morteza-Semnani et al., (2006) 8.9% and 5.1%, by Sajjadi and Somae, (2004) 16.9% and 16.6%, and by Meshkatsadat et al., (2007) 32.9% and 7.3% was reported. Differences in the types and percentages of constituents may be due to genetic or non-genetic variations in response to environmental differences such as soil chemical composition and physiographic factors and or quantity of plant material (Zuzarte and Salgueiro, 2015) or various growth stages of the collected species (Ghasemi Pirbalouti et al., 2013),

and or different methods for the extraction of EOs (Hashemi et al., 2010). Germacrene are a class of volatile organic hydrocarbons, in particular terpenes. Germacrene in some plant species possess antimicrobial and insecticidal properties (Telascrea et al., 2007).

Basically in all previous reports, 1,8-cineole with a content of 48.72% (Risaliti et al., 2019), 37.06% (Moussi et al., 2019), 32.90% (Pereira et al., 2017), 57.88% (BenAbada et al., 2020), and 26.89% (Bajalan et al., 2017) had the highest percentage of EO of this species and also Camphor with 11.72% (Risaliti et al., 2019), 11.81% (Pereira et al., 2017), 18.99% (BenAbada et al., 2020), and 24.82% (Bajalan et al., 2017) was the second main compound in previous reports. α -pinene, which accounted for the most in the present study, in previous reports with a content of 9.86% (Risaliti et al., 2019), 10.71% (Pereira et al., 2017), 9.18% (BenAbada et al., 2020), and 20.81% (Bajalan 2017), was the third major constituent of essential oils. These results indicate that the essential oils of these five Lamiaceae species can be considered as economically viable natural sources for this various high-value compounds. Similar trend was observed by Luo et al., (2019) for six species of lamiaceae.

Thymol is a highly effective phenolic and antimicrobial compound in TDEO. Thymol, as the most important phenol present in plant essential oils, is an inexpensive antibacterial source and can be used in pathogenic systems (Nejad Ebrahimi et al., 2008). The antimicrobial effect of thiol is due to permeability of cell membranes, which can be clotted with membrane surface cations and disrupt vital activities (Ultee et al., 1999). Similar results have been obtained by Wetwitayaklung et al. (2009) for *Aquilaria crassna* L. extract against *S. aureus* due to the presence of some compounds. The antimicrobial effect of essential oils is not limited to their major ingredients. In fact, it is possible that compounds with a lower percentage are likely to have a synergistic effect with other active compounds (Khajehie et al., 2017 and Naeini et al., 2009).

TDEO contained high Thymol, and extensive research has shown that Thymol exhibits good antifungal activity against a wide range of plant pathogenic fungi and food contaminants (Villanueva-Bermejo et al., 2015 and Gavaric et al., 2015). The mechanism of action of these compounds has not been fully elucidated, but it is believed that can interfere with or damage the cell wall of the fungi, or cause their cell wall to decay (Isman and Machial, 2006). Which is inconsistent with findings Hosseini Behbahani et al., (2013) for effect of TDEO against *A. sniger* and *C. albicans* (~53.7 and ~37.7 mm) and Dadashpour et al., (2011) for effect of TDEO against *C. albicans* (~63 mm). While no one of the EOs of the other four species did not produced any inhibition zone (ND) on *A. brasiliensis*, *A. sniger*, and *C. albicans* (Table 5).

But Hosseini Behbahani et al., (2013) reported a remarkable effect of TDEO (43 mm) against *B. subtilis*, which contradicts the present study. This effect may be related to monoterpene and phenolic compounds that are able to break down the outer membrane of Gram-negative strains (Callo et al., 2015).

In previous reports, the effect of TDEO against *E. coli* was ~7 mm (GhasemiPirbalouti et al., 2010), ~7.1 mm (Golkar et al. 2020), ~27.7 mm (Hosseini Behbahani et al., 2013), and ~43.67mm (Dadashpour et al., 2011). Inhibition zone TDEO was recorded against Gram-positive *S. epidermidis* with 9.00 ± 0.001 . However, there is no reported effect of this EO on *S. paratyphi-A* and *S. epidermidis*. The difference of antimicrobial activities between species from the same genus might be due to the difference of their major compounds and also to the complexity of EOs chemical profiles. In the present study EOs against *P. aeruginosa* did not produce any inhibition zone. The reverse process has been developed by Luo et al., (2019) for six species of lamiaceae against *P. aeruginosa*, so that ROEO has created the highest inhibition zone against this bacterium. This difference in antibacterial activity can be due to the difference in the composition of the essential oil of this species in different regions. The biological activities of the essential oils depend on chemical constituents (Popović-Djordjević et al., 2019).

These results are inconsistent with the findings of Ezzatzadeh et al., (2014) for *Nepeta asterotricha*. Since the major constituents of NSEO were, oleic acid, stearic acid, and linoleic acid, one might argue that the microbial effect is due to these fatty acids. Fatty acids have antifungal and antibacterial activities against many microorganisms whose spectrum of activity varies based on the degree of saturation, the length of the carbon chain and the orientation of the double bond (Mattanna et al., 2014). Studies have shown that Lauric, palmitic and linolenic have potential antibacterial activity against *S. aureus* (McGaw et al., 2002 and Agoramoorthy et al., 2007). There are no reports of antimicrobial activity of this plant so far and these results are important.

It may be explained by different composition and percentage of active compounds in EOs, variation in subtypes of genotypes, drying and extraction methods that appear to have a significant effect on the capacity to slow down or stop the growth of bacteria or kill them. (Mohsenipour and Hassanshahaian, 2015). ROEO did not create a diameter against other microorganisms, which is in some cases consistent with the results of Risaliti et al., (2019).

Essential oils of different specie due to the large number of chemical compounds, which work simultaneously, have unparalleled antibacterial potentials and prevent bacterial resistance mechanisms. In addition, synergistic interactions between EOs can enhance their natural antimicrobial effect. Therefore, antimicrobial potential cannot be associated with a single component or mechanism of action

(Candy et al., 2018). Meanwhile, antibiotic abuse exacerbates the harm of *C. albicans* (Delcours, 2009). So TDEO as a natural alternative against this fungus has significant and promising potential. The presence of phenolic monoterpenes, and especially p-cymene and Thymol, is a major cause of the strong antifungal activity of TDEO. Phenolic compounds have high antimicrobial properties and in fact, phenolic compounds in plant essential oils have the most effect on the development of the antimicrobial activity (Lemos et al., 2017; Mahboubi et al., 2017). These compounds both penetrate the cell membrane and can contribute to the clotting of cell contents (Burl & Coote 1999 and Dorman & Dean 2000). Also, although α -pinene is present in small amounts in TDEO (~1.09%), it can be another factor influencing this antifungal activity. Research shows the antifungal effect of this compound (Dorman, 2000). In general, the synergistic effects of the diversity between the main and minor components of essential oils in their biological and antimicrobial activity should be considered (Raut and Karuppayil, 2014). The lowest MBC value of TDEO has belonged to *C. albicans*, that was consistent with results of Dadashpour et al., (2011).

Similar findings were observed by Morteza-Semnani et al. (2010) for *H. elegans* and Ahmadi et al. (2010) for *H. longiflorus* is consistent with. But MBC value of HIEO in other microorganisms was always equal to MIC value of HIEO. This result demonstrates the remarkable inhibitory and lethal potency of this EO against most bacteria. The higher amount of oxygenated sesquiterpenes compounds such as (-)-Caryophyllene oxide (~24.81%) and α -Cadinol (~7.66%) in HIEO compared to other essential oils can be one of the factors affecting this antimicrobial activity. Similarly, Ali et al., (2017) observed that essential oil of *Teucrium spicatum* Hedge & A.G. Mill. (Lamiaceae) characterized by the abundance of γ -selinene presented important antimicrobial activities against several microorganisms.

In the study of Ebrahimabadi et al., (2009), SIEO had no inhibitory effect on any of the microorganisms, so the plant sample of this study is valuable. Differences in habitat conditions are among the most important causes of these biological traits in the plant, which has led to the dominance of acidic compounds and Sesquiterpenes, followed by the ability of this essential oil to control some bacteria. The HIEO MBC value against various bacteria (except *S. epidermidis* and *S. aureus*) was always higher than its MIC value, indicating that the lethal potency of this essential oil was lower than its inhibitory potency. Another report showed that aqueous extract from *Aquilaria crassna* (Wetwityaklung et al., 2009) rich of the some sesquiterpene compound was active against some microorganisms.

Some differences in the amount of antimicrobial effects observed in this study and similar studies could be due to differences in the growth locations of the EO producing plants, the use of different methods for extraction, harvesting time and even the concentration of EOs (Has-Szymanczuk et al., 2011; Lopez et al., 2005 and Toyoshi et al., 2006). Differences in antimicrobial effects indicated differences in essential oil composition. The ROEO also showed a good inhibitory effect (31.25 μ g/mL) against *Sh. dysenteriae*, *E. coli* and *K. pneumonia*, which was weaker than control antibiotics. The lowest MIC value of ROEO was recorded against fungi. The MBC value against the associated microorganisms was also higher than the MIC value, indicating a lower lethal force than the inhibitory power, which is consistent with the results of Messaoudi Moussi et al. (2019). The major compounds that have been attributed to the antimicrobial properties of ROEO include α -pinene, 1,8-cineole, verbenone, and linalool (Okoh, 2010 and Oluwatuyi et al., 2004), among these compounds, three conditions may occur: Synergistic, Antagonist, and Additive. Occurrence of any of these conditions affects the antimicrobial properties of essential oil (Marzouk et al., 2006). There is ample evidence that the essential compounds of essential oils also play an important role in antimicrobial properties by creating a synergistic effect between the main compounds and their easier transfer into the bacterial cell (Burt, 2004 and Tavassoli et al., 2011).

Essential oils (EOs) produced by plants as secondary metabolites, usually composed of different compounds with different ratios. The present study was conducted for the first time on five important species of the *Lamiaceae* family in Iran which after analysis by chromatography included a variety of compounds such as thymol, oleic acid, (-)-caryophyllene oxide, α -pinene, 1,8-cineole, palmitic acid, (+)-spathulenol, germacrene D, bicyclgermacrene, phytol, camphor, and borneol. 1,8-cineole and oleic acid were found in the EOs of five plant species. The diversity of compounds of different species resulted in a variety of antibacterial and antifungal activity. TDEO had the highest inhibitory zone against various microorganisms compared to other essential oils, which had a particularly significant effect against *S. aureus* and *A. brasiliensis*. Based on the MIC value results, essential oils of five species of *Lamiaceae* had a relatively good effect against *Sh. dysenteriae*, *P. aeruginosa*, *E. coli*, *K. pneumonia*, *C. albicans*. Overall, the MIC value of ROEO against all bacteria was significantly more effective than other EOs. These essential oils from five species of *Lamiaceae* can have potential applications in the food and pharmaceutical industries as natural food preservatives, natural fungicides and a potential natural alternative to some antibiotics. In future research, it is important to identify and separate the main components or general compounds of these essential oils to confirm the usefulness of these compounds.

Declarations

Ethical Approval

Not applicable.

Consent to Participate

Not applicable.

Consent to Publish

Not applicable.

Authors Contributions

Mansureh Ghavam was the supervisor, designer of the hypotheses, and responsible and functor for all the steps and wrote the text of the article.

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Tables

Table 1. Analysis of variance of species effects on yield and some predominant compounds of essential oils of five Lamiaceae species

Source of variation	df	MS					
		Yield of	essential oil				
			1,8-Cineole	Oleic acid	(-)-Caryophyllene oxide	trans-Caryophyllene	Linalool
species	4	1.790**	191.383**	1887.685**	348.869**	7.305**	3.691**
Error	10	0.000015	0.000021	0.000033	0.000003	0.000027	0.000060

ns: Not significant, **: 1 % level of probability is significant

Table 2. Comparison of the mean of species on the yield of essential oil of five Lamiaceae species

species	Mean (%) $\pm$ SD
<i>T. daenensis</i>	1.88 $\pm$ 0.01 ^a
<i>N. sessilifolia</i>	0.20 $\pm$ 0.01 ^c
<i>H. incanus</i>	0.02 $\pm$ 0.00 ^e
<i>S. inflata</i>	0.14 $\pm$ 0.00 ^d
<i>R. officinalis</i>	0.83 $\pm$ 0.00 ^b

The different letters indicate a significant difference based on Duncan's multiple range test at the 1 % level.

Table 3. The chemical composition of EOs from five Lamiaceae species.

No.	Compound (%)	RI	TDEO	NSEO	HIEO	SIEO	ROEO	Molecular formula
1	Tricyclene	860.095	-	-	-	-	0.63± 0.00 ^a	C ₁₀ H ₁₆
1	α-Thujene	863.546	0.85± 0.01 ^a	0.39± 0.01 ^b	-	-	-	C ₁₀ H ₁₆
2	α-pinene	871.428	1.09± 0.02 ^c	-	-	1.52± 0.01 ^b	19.41± 0.01 ^a	C ₁₀ H ₁₆
3	Camphene	888.177	0.82± 0.01 ^b	-	-	0.55± 0.00 ^c	7.83± 0.01 ^a	C ₁₀ H ₁₆
4	Sabinene	908.276	-	0.33± 0.01 ^a	-	-	-	C ₁₀ H ₁₆
5	β-pinene	912.251	0.43± 0.01 ^b	-	-	-	2.56± 0.00 ^a	C ₁₀ H ₁₆
6	β-Myrcene	920.529	1.50± 0.01 ^b	-	-	-	5.38± 0.01 ^a	C ₁₀ H ₁₆
7	α-Phellandrene	932.781	0.28± 0.00 ^b	-	-	-	0.64± 0.00 ^a	C ₁₀ H ₁₆
8	α-Terpinene	942.715	1.71± 0.00 ^a	-	-	-	0.38± 0.01 ^b	C ₁₀ H ₁₆
9	p-Cymene	952.649	5.16± 0.00 ^a	-	-	-	-	C ₁₀ H ₁₄
10	1,8-Cineole	957.284	3.52± 0.00 ^b	0.91± 0.00 ^d	0.22± 0.00 ^e	1.65± 0.00 ^c	15.40± 0.01 ^a	C ₁₀ H ₁₈ O
11	γ-Terpinene	979.801	6.20± 0.01 ^a	-	-	-	0.42± 0.01 ^b	C ₁₀ H ₁₆
12	α-Terpinolene	1002.375	0.26± 0.01 ^b	-	-	-	0.87± 0.00 ^a	C ₁₀ H ₁₆
13	Linalool	1012.962	0.67± 0.01 ^d	1.45± 0.01 ^b	-	1.20± 0.01 ^c	2.97± 0.01 ^a	C ₁₀ H ₁₈ O
14	Chrysanthenone	1029.894		-	-	-	1.27± 0.01 ^a	C ₁₀ H ₁₄ O
15	Camphor	1043.915		-	-	1.67± 0.00 ^b	8.87± 0.00 ^a	C ₁₀ H ₁₆ O
16	trans-Pinocamphone	1050.118		-	-	-	0.87± 0.01 ^a	C ₁₀ H ₁₆ O
17	Silane, tetraethenyl-	1060.846		-	-	-	0.31± 0.01 ^a	C ₈ H ₁₂ Si
18	Borneol	1064.021	3.67± 0.01 ^b	-		1.17± 0.01 ^c	8.48± 0.00 ^a	C ₁₀ H ₁₈ O
19	Terpinen-4-ol	1070.105		-		-	1.62± 0.01 ^a	C ₁₀ H ₁₈ O
20	α-Terpineol	1080.158	-	0.50± 0.01 ^c	-	0.91± 0.00 ^b	1.95± 0.01 ^a	C ₁₀ H ₁₈ O
21	1,2-Cyclononadiene	1087.830	1.19± 0.02 ^a	-	-	-	-	C ₉ H ₁₄
22	Myrtenol	1088.095	-	-	-	-	0.33± 0.01 ^a	C ₁₀ H ₁₆ O

23	Verbenone	1092.592	-	-	-	2.20± 0.01 ^b	8.28± 0.00 ^a	C ₁₀ H ₁₄ O
24	Thymol	1169.951	67.71± 0.00 ^a	-	-	-	-	C ₁₀ H ₁₄ O
25	Urea, phenyl-	1113.942	-	-	-	-	1.02± 0.01 ^a	C ₇ H ₈ N ₂ O
26	Tricyclo[4.2.1.1(2,5)]decane	1117.788	-	-	-	-	1.43± 0.01 ^a	C ₁₀ H ₁₆
27	Acetic acid, bornyl ester	1128.846	-	-	-	1.29± 0.010 ^a	-	C ₁₂ H ₂₀ O ₂
28	trans-Bornyl acetate	1137.019	-	-	-	-	4.77± 0.01 ^a	C ₁₂ H ₂₀ O ₂
29	1,5,5-Trimethyl-6-methylene-cyclohexene	1166.105	-	-	-	0.83± 0.01 ^a	-	C ₁₀ H ₁₆
30	α-Copaene	1190.865	-	-	-	0.69± 0.01 ^a	-	C ₁₅ H ₂₄
31	β- Bourbonene	1197.115	-	1.03± 0.02 ^a	-	-	-	C ₁₅ H ₂₄
32	β-Elemene	1200.947		-	-	1.12± 0.01 ^a	-	C ₁₅ H ₂₄
33	trans-Caryophyllene	1224.489	2.99± 0.01 ^b	0.67± 0.01 ^d	3.68± 0.01 ^a	-	1.24± 0.01 ^c	C ₁₅ H ₂₄
34	(+)-Aromadendrene	1232.464	0.15± 0.00 ^a	-	-	-	0.24± 0.01 ^a	C ₁₅ H ₂₄
35	α-Humulene	1238.862	-	-	1.50± 0.01 ^a	-	-	C ₁₅ H ₂₄
36	Alloaromadendrene	1242.417	-	-	0.29± 0.01 ^a	-	-	C ₁₅ H ₂₄
37	α-Amorphene	1250.947	-	-	-	0.92± 0.00 ^a	-	C ₁₅ H ₂₄
38	β-Cubebene	1254.028	-	0.26± 0.01 ^a	-	-	-	C ₁₅ H ₂₄
39	Germacrene D	1254.502	-	-	0.30± 0.01 ^b	10.26± 0.01 ^a	-	C ₁₅ H ₂₄
40	Bicyclogermacrene	1263.033	-	-	0.57± 0.00 ^b	9.19± 0.01 ^a	-	C ₁₅ H ₂₄
41	β-Bisabolene	1268.009	0.50± 0.01 ^a	-	-	-	-	C ₁₅ H ₂₄
42	δ-cadinene	1276.540	-	0.34± 0.00 ^b	-	4.7± 0.01 ^a	-	C ₁₅ H ₂₄
43	cis-α-Bisabolene	1286.255	0.40± 0.00 ^a	-	-	-	-	C ₁₅ H ₂₄
44	Phenol, 2,4-bis(1,1-dimethylethyl)-	1294.163	-	-	0.95± 0.01 ^a	-	-	C ₁₄ H ₂₂ O
45	Elemol	1296.208	-	0.24± 0.00 ^a	-	-	-	C ₁₅ H ₂₆ O
46	4,7-Methano-5H-inden-5-one,	1296.445	-	-	0.41±	-	-	C ₁₀ H ₁₄ O

	octahydro-				0.00 ^a			
47	Furan, 2-(2-furanylmethyl)-5-methyl-	1310.169	-	-	-	0.71± 0.00 ^a	-	C ₁₀ H ₁₀ O ₂
48	(+) spathulenol	1314.043	-	-	-	11.16± 0.00 ^a	-	C ₁₅ H ₂₄ O
49	(-)-Caryophyllene oxide	1315.254	0.41± 0.01 ^d	1.26± 0.00 ^b	24.81± 0.00 ^a	-	1.24± 0.00 ^c	C ₁₅ H ₂₄ O
50	Viridiflorol	1321.065	-	-	-	2.26± 0.01 ^a	-	C ₁₅ H ₂₆ O
51	(-)-Humulene epoxide II	1329.782	-	-	3.69± 0.01 ^a	-	-	C ₁₅ H ₂₄ O
52	α-Chamigrene	1347.215	-	-	2.37± 0.01 ^a	-	-	C ₁₅ H ₂₄
53	α-Cadinol	1355.690	-	-	7.66± 0.00 ^a	3.25± 0.01 ^b	-	C ₁₅ H ₂₆ O
54	Caryophylla-3,8(13)-dien-5β-ol	1358.592	-	-	-	-	0.34± 0.01 ^a	C ₁₅ H ₂₄ O
55	Caryophyllenol-II	1367.312	-	-	5.06± 0.01 ^a	-	-	C ₁₅ H ₂₄ O
56	(3S,4R,5S,6R,7S)-aristol-9-en-3-ol	1374.334	-	-	-	1.51± 0.02 ^a	-	C ₁₅ H ₂₄ O
57	Myristic acid	1414.357	-		1.31± 0.01 ^a	-	-	C ₁₄ H ₂₈ O ₂
58	2-Pentadecanone, 6,10,14-trimethyl-	1444.584	-		1.39± 0.01 ^a	0.89± 0.01 ^b	-	C ₁₈ H ₃₆ O
59	Phthalic acid	1462.216	-		0.55± 0.01 ^a	-	-	C ₈ H ₆ O ₄
60	Tridecane	1477.581	-		0.42± 0.01 ^b	0.45± 0.01 ^a	-	C ₁₃ H ₂₈
61	Hexadecanoic acid= Palmitic acid	1515.263	-		13.28± 0.01 ^a	12.10± 0.00 ^b	0.32± 0.01 ^c	C ₁₆ H ₃₂ O ₂
62	Phytol	1580.263	-		9.22± 0.00 ^a	2.14± 0.00 ^b	-	C ₂₀ H ₄₀ O
63	Oleic acid	1600.277	0.49± 0.01 ^e	62.09± 0.01 ^a	23.53± 0.01 ^b	20.75± 0.01 ^a _c	0.94± 0.01 ^d	C ₁₈ H ₃₄ O ₂
64	Stearic acid	1626.869	-	8.16± 0.00 ^a	-	3.89± 0.01 ^a	-	C ₁₈ H ₃₆ O ₂
65	Linoleic acid	1632.963	-	6.06± 0.00 ^a	-	-	-	C ₁₈ H ₃₂ O ₂
66	Lauric acid	1692.337	-	0.87± 0.00 ^a	-	-	-	C ₁₂ H ₂₄ O ₂
	Total		100	84.56	100	99.35	100	
	Monoterpenes hydrocarbons		18.3	0.72	0	3.9	39.55	
	Oxygenated monoterpenes		74.9	2.86	0.63	8.8	50.04	
	Sesquiterpenes hydrocarbons		4.04	2.3	8.71	26.25	1.48	
	Oxygenated sesquiterpenes		0.41	1.5	41.22	18.18	1.58	

Others

1.68

77.18

50.65

42.22

7.36

#Retention indices (RIs) relative to n-alkanes (C6–C40) on the same methyl silicone capillary column. Values with different letters are statistically different (Duncan, $p \leq 0.01$).

Table 4. Analysis of variance of species effect on Inhibition zone of essential oil of five Lamiaceae species against some microorganisms

Source of variation	df	MS		
		<i>B. subtilis</i>	<i>S. epidermidis</i>	<i>S. aureus</i>
species	4	16.067**	9.767**	583.917**
Error	10	0.067	0.067	0.183

** : 1 % level of probability is significant

Table 5. Inhibition-zone diameters of EOs from five Lamiaceae species and referent antibiotics

Test microorganisms	IZ (mm)							
	EOs					Antibiotics		
	TDEO	NSEO	HIEO	SIEO	ROEO	Rifampin	Gentamicin	Nystatin
<i>Sh. dysenteriae</i>	16.33±0.58 ^a	ND	ND	ND	ND	9	17	NA
<i>P. aeruginosa</i>	ND	ND	ND	ND	ND	ND	20	NA
<i>B. subtilis</i>	14.00±0.50 ^a	9.00±0.00 ^c	10.00±0.00 ^b	9.00±0.00 ^c	9.00±0.00 ^c	19	30	NA
<i>S. epidermidis</i>	9.00±0.00 ^b	9.00±0.00 ^b	11.00±0.00 ^a	ND	9.33±0.58 ^b	44	39	NA
<i>E. coli</i>	11.33±0.58 ^a	ND	ND	ND	ND	10	23	NA
<i>S. aureus</i>	39.33±0.58 ^a	9.67±0.58 ^b	ND	8.50±0.50 ^c	9.00±0.00 ^{bc}	21	27	NA
<i>K. pneumonia</i>	17.67±0.58 ^a	ND	ND	ND	ND	8	17	NA
<i>P. vulgaris</i>	14.00±0.50 ^a	ND	ND	ND	ND	8	24	NA
<i>S. paratyphi-A</i>	11.67±0.58 ^a	ND	ND	ND	ND	8	18	NA
<i>C. albicans</i>	11.67±0.58 ^a	ND	ND	ND	ND	NA	NA	33
<i>A. niger</i>	12.00±0.00 ^a	ND	ND	ND	ND	NA	NA	27
<i>A. brasiliensis</i>	25.00±0.00 ^a	ND	ND	ND	ND	NA	NA	30

IZ: The diameters of the inhibition zones includes the diameters of disks (6 mm). Values are the means ± SD of three cultures. Results are expressed as means ± SD of triplicate values.. NA: no activity, ND: not determined. Values with different letters are statistically different (Duncan, $p \leq 0.01$).

Table 6. Minimal inhibitory concentrations (MIC) of EOs from five Lamiaceae species and referent antibiotics.

Test microorganisms	MIC (µg/mL)							
	EOs					Antibiotics		
	TDEO	NSEO	HIEO	SIEO	ROEO	Rifampin	Gentamicin	Nystatin
<i>Sh. dysenteriae</i>	500	125	62.50	125	31.25	15.63	3.90	NA
<i>P. aeruginosa</i>	125	125	15.63>	15.63>	15.63>	31.25	7.81	NA
<i>B. subtilis</i>	125	250	500	125	62.50	31.25	3.90	NA
<i>S. epidermidis</i>	125	250	500	500	62.50	1.95	1.95	NA
<i>E. coli</i>	125	500	250	125	31.25	15.63	31.25	NA
<i>S. aureus</i>	125	500	500	>1000	125	31.25	1.95	NA
<i>K. pneumonia</i>	125	125	62.50	125	31.25	15.63	3.90	NA
<i>P. vulgaris</i>	250	250	500	250	62.50	15.63	15.63	NA
<i>S. paratyphi-A</i>	125	250	62.50	125	62.50	15.63	3.90	NA
<i>C. albicans</i>	31.25	250	62.50	500	500	NA	NA	125
<i>A. niger</i>	250	2000	>2000	>2000	2000	NA	NA	31.2
<i>A. brasiliensis</i>	250	2000	>2000	>2000	2000	NA	NA	31.2

Table 7. Minimal bactericidal/fungicidal (MBC) concentrations of EOs from five Lamiaceae species

Test microorganisms	MBC (µg/mL)				
	EOs				
	TDEO	NSEO	HIEO	SIEO	ROEO
<i>Sh. dysenteriae</i>	500	1000	62.50	1000	500
<i>P. aeruginosa</i>	125	125	15.63>	250	62.50
<i>B. subtilis</i>	125	>1000	500	250	500
<i>S. epidermidis</i>	125	250	500	500	125
<i>E. coli</i>	125	500	250	500	125
<i>S. aureus</i>	500	>1000	500	>1000	500
<i>K. pneumonia</i>	250	125	62.50	1000	125
<i>P. vulgaris</i>	250	500	500	500	125
<i>S. paratyphi-A</i>	125	250	62.50	500	125
<i>C. albicans</i>	62.50	250	250	1000	1000
<i>A. niger</i>	250	2000	>2000	>2000	2000
<i>A. brasiliensis</i>	250	2000	>2000	>2000	2000

Figures

Figure 1

Comparison of the mean effect of species on 1,8-Cineole content of essential oil of five Lamiaceae species

Figure 2

Comparison of the mean effect of species on Oleic acid content of essential oil of five Lamiaceae species