

Original Research Article

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## Comparison of Some Medicinal Plants and Macrofungi Essential Oil Components for Antimicrobial Activity against the Human and Fish Pathogens

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### ABSTRACT

In this study, essential oils obtained with water/steam distillation methods from plants of natural flora of Libya and two macrofungi from natural flora of Turkey were tested antimicrobial activity in vitro for isolated from humans and pathogenic *Enterococcus faecium*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* bacteria and for isolated from fish and pathogenic *Citrobacter freundii*, *Edwardsiella tarda*, *Lactococcus garvieae* and *Yersinia ruckeri* bacteria. There were found statistical similarity essential oils but it was not affected antimicrobial activity. However, there are a lot of effected factors for essential oil components such as plantation, texture, and structure of grown soil, climate, the moisture of the air, vegetation time, collection period, collected part of plants...etc. For these reasons, we must apply a machine learning application for the best sample collection time and preferred compounds.

#### Keywords

Medicinal plants,  
Macrofungi,  
Antimicrobial  
activity, Essential  
oil similarity

#### Article Info

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### Introduction

Microorganisms and their metabolic, physiological and genetic differences are the

most important factor affecting the health of vertebrate. In addition to normal infectious diseases, the number of infections due to nosocomial and opportunistic pathogens is

increasing day by day. Also, there is an increase in infections due to diseases caused by drug-resistant pathogens and immunological failure. Side effects such as immune suppression, hypersensitivity and allergic reactions are seen in antibiotic treatments (Chebaibi and Filali, 2013). Besides, antibiotics affect not only target pathogens but also non-target microbial populations in the treatment process. In some cases, antibiotic-induced diarrhea arises as a result of imbalance in the intestinal microbiota (Jernberg *et al.*, 2010). The search for novel antimicrobials of plant origin has gained importance due to the increase of resistance to the drugs in pathogenic microorganisms and the undesirable side effects of some antibiotics (Alviano and Alviano, 2009).

Medical plants include a wide variety of components that can be used in the treatment of chronic diseases as well as infectious diseases. Today, research on developed and developing countries; have concentrated on the medical values of the chemicals in the structures of medicinal and aromatic plants used in the field of traditional medicine, which have definite physiological effect on the human body. The most important of these bioactive compounds in the construction of medical and aromatic plants are alkaloids, flavonoids, tannins and phenolic compounds.

The components of natural products obtained from plants are effective in various ways on microorganisms. The most common mechanism of action of antimicrobial compounds obtained from plants; degradation of cytoplasmic membrane, inhibition of degradation and synthesis of DNA / RNA function, initiation of clotting of cytoplasmic components and inhibition of normal cell communication (Radulovic *et al.*, 2013). Some of the compounds obtained from plants, which are an important source in the treatment of diseases, also called plant derivatives, are

derived from essential oils (Mekonnen *et al.*, 2016). Essential oils are aromatic oleaginous liquids obtained from various parts of the plant (flowers, leaves, buds, seeds, weeds, trees, bark, fruits and roots) and the ratio may vary between different parts of the plant. Generally, water/steam distillation method is used when obtaining essential oils (Gültepe 2018).

Essential oils obtained from many medical and aromatic sources; antifungal, antiviral, antioxidant, antidiabetic, anti-inflammatory, anticancer activity, as well as treatment of many other conditions related to treatment fires, insect bites and skin (Holetz *et al.*, 2002; Prabuseenivasan *et al.*, 2006; Alviano and Alviano, 2009; Kazemi *et al.*, 2012; Mekonnen *et al.*, 2016). Qualitative, quantitative variability and effects of essential oil obtained from plants when assessed individually; the genetic character of the plant depends on its age and on the environment it is growing.

In this study, essential oils obtained with water/steam distillation methods from plants of *Artemisia herba alba*, *Capparis spinosa*, *Globularia alypum*, *Matricaria chamomilla*, *Ocimum basilicum*, *Origanum majorana*, *Peganum harmala*, *Phagnalon rupestre*, *Punica granatum* peel and *Thymus vulgaris* collected from natural flora of Libya and *Lactarius deliciosus* and *Pleurotus ostreatus* collected from natural flora of Turkey were tested antimicrobial activity *in vitro* for isolated from humans and pathogenic *Enterococcus faecium*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* bacteria and for isolated from fish and pathogenic *Citrobacter freundii*, *Edwardsiella tarda*, *Lactococcus garvieae*, and *Yersinia ruckeri* bacteria. The aim of this study is to evaluate the statistical similarity of components of essential oils of these plants

and macrofungi, and also its effects on antimicrobial activity.

## Materials and Methods

### Plants and macrofungi

More than 100 plant species in Libya are widely used by the Bedouin in medical treatment. These plants collected from four main centers; first area is the El-Jabal El-Akhdar which has about 50% of the total endemic species, second area is the coastal belt, third area the central part of Sahara and fourth area is southern part of Libya involving Jabal Al Awaynat, Tibesti, and Plateau of Ghat (El-Darier and El-Mogaspi, 2009; Bufrag *et al.*, 2017). *Artemisia herba alba*, *Capparis spinosa*, *Globularia alypum*, *Matricaria chamomilla*, *Ocimum basilicum*, *Origanum majorana*, *Peganum harmala*, *Phagnalon rupestre*, *Punica granatum* peel, and *Thymus vulgaris* were obtained from a commercial company in Libya.

There are 2422 macrofungi species in Turkey (Solak *et al.*, 2015). Most of them grow naturally in Kastamonu forests in the Black Sea Region and are sold commercially. *Lactarius deliciosus* and *Pleurotus ostreatus* were purchased from locally commercial company.

### Bacteria

From human pathogens; *Enterococcus faecium*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* bacteria used, which were obtained from Gazi University Research and Application Hospital in Ankara-Turkey.

From fish pathogens; *Citrobacter freundii* (KX388233.1), *Edwardsiella tarda* (KX388234.1), *Lactococcus garvieae*

(KY118086.1) and *Yersinia ruckeri* (KX388238.1) bacteria used, which were isolated from a fish farm in Turkey, sequenced at National Center for Biotechnology Information Laboratory and registered at GenBank.

### Experimental procedure

The essential oil of plants was obtained from dry plants and fresh macrofungi by hydro-distillation, using a Clevenger system with 150 g dry plant material and 1500 ml water. The oil was obtained after 3 h of distillation at boiling temperature and stored at  $4 \pm 1$  °C in airtight glass vials covered with aluminum foil. The gas chromatography-mass spectrophotometry (GC-MS) analysis of the obtained essential oil was conducted at the Kastamonu University Center Research Laboratory Application and Research Center by using a Shimadzu GCMS QP 2010 ULTRA (Bufrag *et al.*, 2017).

Bacteria were cultured on enriched tryptic soy (TS) agar. Incubation carried out at 37°C for 48 h to human pathogens and 25°C for 48 h to fish pathogens. Individual colonies grown on these re-streaked plates were used in the antimicrobial sensitivity tests. Each pure culture of bacteria was added to sterile phosphate buffer solutions and their concentrations were adjusted with the use of a spectrometer as a 30% transmittance (525 nm) according to McFarland 1. *In vitro* assays were conducted to determine the antimicrobial activity of essential oils. For the essential oil dilutions 250, 500, 1000 and 1250 µl in test tubes containing 5 ml phosphate buffer solution was prepared. Aliquots (0.1 ml) of the standardized bacterial isolates were added to each tube and left to stand at room temperature for 1 h, after which a loopful material from each tube was inoculated onto plates. Human pathogens at the 37°C for 48 h, fish pathogens at the 25°C for 48 h were

incubated. Control tubes containing sterile phosphate buffer were each inoculated with aliquots of standardized bacteria and cultured onto plates at the same conditions. At the bacteriological study, Merck (Merck, Germany) media were used (Cipriano *et al.*, 1996; Aydın *et al.*, 2000; Gültepe and Aydın, 2009).

### Statistical analysis

Data were analyzed by one-way analysis of variance (ANOVA), followed by Tukey multiple range test to determine significant differences ( $p < 0.05$ ).

### Results and Discussion

GS-MS, three compounds with the greatest amount were given in Table 1. Methyl stearate was found at both plants and macrofungi essential oils on FAMES analyses but not found at macrofungi on aroma analyses. Metilox and methyl palmitate were found at both plants and *Lactarius deliciosus* but not found at *Pleurotus ostreatus* on FAMES analyses and also they were not found on aroma analyses of macrofungi. Statistical analyses result of the essential oils of the plants both FAMES and aroma were given in Figure 1 and 2.

There is extensive use of *Artemisia herba alba* as an aromatic and medicinal plant in folk medicine and also different studies were made by scientists. Some studies reported that the chemical composition of the essential oil of the *A. herba alba* was dominated by  $\alpha$ - and  $\beta$ -thujone (Mighri *et al.*, 2009; Dahmani-Hamzaoui and Baaliouamer, 2015).  $\alpha$ - and  $\beta$ -thujone were not found, but eucalyptol (39.67%), methyl stearate (15.17%) and 1(S)-camphor (6.65%) were dominated in this research. Similarly, thujone was not found in Spain's population of the *A. herba alba* (Feuerstein *et al.*, 1988). Furthermore, Eastern

Morocco population of these plants included eucalyptol (Imelouane *et al.*, 2010). Minimum inhibitory concentration (MIC) of the essential oil of *Artemisia herba alba* has a for *Edwardsiella tarda* (KX388234.1) at 1000  $\mu$ l dose and for *Enterococcus faecium*, *Escherichia coli*, *Klebsiella pneumonia*, *Proteus vulgaris*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* at 1250  $\mu$ l dose. Morocco population of *A. herba alba* has a microbial activity for *S. aureus*, *K. pneumonia*, and *E. coli* but not effect *S. aureus* and *P. aeruginosa* (Imelouane *et al.*, 2010). Besides, the Tunisian population of this plant has a positive effect at the *S. aureus* and *E. coli* (Mighri *et al.*, 2010). Also, an extract of the Libyan population of *A. herba alba* has an antimicrobial activity for *E. coli*, *P. aeruginosa*, *S. aureus* (Bogdadi *et al.*, 2007). This result shows that the essential oil of *Artemisia herba alba* of Libyan population is not suitable for use as antimicrobial at this condition because it should be used in high doses.

Some studies reported (Tlili *et al.*, 2011; Kulisic-Bilusic *et al.*, 2012; Muhaidat *et al.*, 2013), *Capparis* species have therapeutic properties with contained compounds. The main component of the *C. spinosa* essential oil is kaempferol derivatives and isopropyl isothiocyanate (Inocencio *et al.*, 2000; Siracusa *et al.*, 2011). In this study, methyl stearate (38.83%), metilox (14.68%) and methyl palmitate (12.40%) were found as the main components.

Although the effect of caper extracts obtained by different extraction methods on various microorganisms has been reported (Tlili *et al.*, 2011; Muhaidat *et al.*, 2013), *C. spinosa* essential oil derived from Libyan population has only affected *Escherichia coli* at 250  $\mu$ l MIC dose as a consequence it can be used as an antimicrobial against *E. coli*. *Globularia* species have been medically used for many

years in both African and European countries (Cero *et al.*, 2014; Jamila and Mostafa, 2014; Ramdani *et al.*, 2014). Chemical composition of the essential oil of the *Globularia alypum* was dominated by methyl palmitate at the Boutaleb (14.64%) and Khenchela (29.52%) populations (Ramdani *et al.*, 2014). Methyl palmitate rate was found 7.23% and also methyl stearate (44.61%), metilox (17.15%) and tetradecane (11.39%) were dominated in this research.

*Origanum majorana* is known for its medicinal properties and usage in the food industry. The essential oil of the Venezuelan Andes population of *O. majorana* was contained *cis*-sabinene hydrate (30.2%), terpinen-4-ol (28.8%),  $\gamma$ -terpinene (7.2%),  $\alpha$ -terpineol (6.9%), *trans*-sabinene hydrate (4.4%), linalyl acetate (3.8%) and  $\alpha$ -terpinene (3.6%) (Ramos *et al.*, 2011). Similarly, oil of the Tunisian population of *O. majorana* was dominated by terpinen-4-ol (32.8%),  $\gamma$ -terpinene (9.9%), *cis*-sabinene hydrate (8.6%), and  $\alpha$ -terpineol (5.6%) (Ezzeddine *et al.*, 2001). In this study, essential oil of the Libyan population of *O. majorana* was dominated by  $\gamma$ -terpinene (17.61%), methyl stearate (16.04%) and  $\alpha$ -terpineol (5.6%).

At the end of the twentieth century, a lot of studies were conducted about the different part of pomegranate (*Punica granatum*) related to essential oil components, antioxidant and antimicrobial effects. Camphor (60.32%), benzaldehyde (20.98%) and (4)-borneol (4.75%) were dominated essential oil of the Tunisian population of pomegranate peel (Hadrich *et al.*, 2014). Methyl stearate (43.21%), metilox (16.82%) and methyl palmitate (7.90%) were dominated essential oil of the Libyan population of pomegranate peel in this research.

*Globularia alypum* essential oil has significant inhibitory action on *Acinetobacter baumannii*,

*Citrobacter freundii*, *Escherichia coli*, *Salmonella typhimurium*, *Bacillus subtilis*, *Enterococcus faecalis*, *Listeria monocytogenes*, but has not got effect on *Klebsiella pneumoniae*, *Proteus mirabilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus cereus* (Ramdani *et al.*, 2014), and also Boussoualim *et al.*, (2014), reported that methanolic extract of the *Globularia alypum* inhibits *Pseudomonas aeruginosa*, *Escherichia coli*, *Salmonella typhimurium*, *Acinetobacter baumannii*, *Citrobacter freundii*, *Proteus mirabilis*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Bacillus cereus*, and *Enterococcus faecalis*. The essential oil of the *Origanum majorana* has an inhibitory effect to *Escherichia coli*, *Klebsiella pneumoniae*, and *Salmonella enteritidis* but no any effect for *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Streptococcus A*, *Enterococcus faecalis*, *Proteus mirabilis*, *Shigella dysenteria* and *Staphylococcus epidermitis* by agar diffusion method (Ezzeddine *et al.*, 2001). Extracts of different parts of *P. granatum* have an inhibitory effect to methicillin-sensitive *Staphylococcus aureus*, methicillin-resistant *Staphylococcus aureus*, *Micrococcus luteus*, *Enterobacter aerogenes*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Salmonella typhi*, *Salmonella enteric* and some streptococci strains (Prakash and Prakash, 2011; Kaur *et al.*, 2016). This study result show that 1250  $\mu$ l dose of essential oils of *Globularia alypum*, *Origanum majorana*, and *Punica granatum* peel has a weak antimicrobial activity for *Enterococcus faecium*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*.

Studies with the essential oil of *Ocimum basilicum* are given different results to us for example: Iranian population methyl chavicol (47.0%), geranial (19.0%) and neural (15.0%) (Kavoosi and Amirghofran, 2017); different

varieties (*O. basilicum* var. *purpurascens* and *O. basilicum* var. *dianatnejadii*) of another Iranian population methyl chavicol (43.0% and 37.6%), linalool (28.9% and 33.4%),  $\alpha$ -Cadinol (1.0% and 5.7%) and  $\alpha$ -Eudesmol (4.7% and 0.9%) respectively (Dolatabad *et al.*, 2014); different varieties (*Ocimum basilicum* var. *basilicum*, *Ocimum basilicum* var. *difforme* and *Ocimum basilicum* var. *purpurascens*) of Croatia population linalool (66.40%, 20.82% and 0%), eugenol (8.26%, 0% and 0.43%),  $\alpha$ -bergamotene (7.96%, 6.84% and 0%), estragole (0%, 47.52% and 94.57%), methyl eugenol (0.31%, 0.13% and 1.23%), 1,8-cineole (7.23%, 6.17% and 0%) and  $\gamma$ -bisabolene (0%, 0% and 1.12%) respectively (Carović-Stanko *et al.*, 2013). Essential oil of the Libyan population of *O. basilicum* was dominated by methyl stearate (29.16%), metilox (10.57%) and docosane (9.87%) in this study.

Although there are a lot of studies on the presence of n-paraffins, fatty substances, terpenoids, flavonoids, glycosides of *P. rupestre* (Senatore *et al.*, 2005), there is no find study with the chemical composition of essential oil of the *P. rupestre*. According to study results, *P. rupestre* essential oil was dominated by methyl stearate (53.83%), metilox (16.84%) and methyl palmitate (9.35%).

There were a lot of studies with the antimicrobial and antifungal activity of *O. basilicum* essential oil. Data on the antibacterial activity of *O. basilicum* were higher than data on antifungal activity (Suppakul *et al.*, 2003). Suppakul *et al.*, (2003) based on different studies reported that it was more effective against Gram-positive than against Gram-negative bacteria, and also has potential use in food preservation.

Extract of the *Phagnalon rupestre* has antimicrobial activity against *Staphylococcus*

*aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Pseudomonas aeruginosa* and *Candida albicans* (Ali-Shtayeh *et al.*, 1998), and also a positive effect for contact hypersensitivity (Olmos *et al.*, 2007; Giner *et al.*, 2011). This study results showed *Ocimum basilicum* and *Phagnalon rupestre* essential oils MIC value at 1000  $\mu$ l dose for *Edwardsiella tarda* and also they have weak antimicrobial activity at 1250  $\mu$ l dose for *Enterococcus faecium*, *Escherichia coli*, *Klebsiella pneumonia*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*.

*Matricaria chamomilla* is a historical plant as a medicinal usage. Currently, it is cultivated due to different specialization such as medicinal, aromatic and flavoring properties. Essential oil of the Turkish population of *Matricaria chamomilla* was dominated by bisabolone oxide (47.6%), bisabolol oxide A (27.4%), en-yn-dicycloether (5.6%) and bisabolol oxide A (75.4%), followed by en-yn-dicycloether (4.4%) and bisabolol oxide B (4.1%) at 2011 and 2012, respectively (Rezaeih *et al.*, 2015). Bosnia and Herzegovina population of its was dominated by (E)- $\beta$ -farnesene (29.8%), (E,E)- $\alpha$ -farnesene (9.3%) and  $\alpha$ -bisabolol oxide A (7.0%) (Stanojevic *et al.*, 2016) and also Ethiopian population was dominated by  $\alpha$ -bisabolol oxide B (51.428%), chamazulene/azulene (17.688%) and en-in-dicycloether (10.841%) (Mekonnen *et al.*, 2016). The essential oil of the Libyan population of *M. chamomilla* was dominated by methyl stearate (26.02%), metilox (10.17%) and undecane (9.18%) in this study.

Interestingly, essential oil of *M. chamomilla* was not shown antimicrobial activity against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, *Shigella flexneri*, *Klebsiella pneumoniae*, *Salmonella typhi*, *Serratia marcescens*, and

*Escherichia coli* for Ethiopian population (Mekonnen *et al.*, 2016). Bosnia and Herzegovina's population of its give similar results against *Pseudomonas aeruginosa*, in spite of, it has antimicrobial activity *Listeria monocytogenes*, *Escherichia coli*, *Salmonella enterica* and *Staphylococcus aureus* (Stanojevic *et al.*, 2016). Study results show that essential oil of *Matricaria chamomilla* has weak antimicrobial activity against *Edwardsiella tarda*, *Enterococcus faecium*, *Escherichia coli*, *Klebsiella pneumonia*, *Proteus vulgaris*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* at 1250 µl dose.

*Peganum harmala*, which treats asthma, hiccup, colic, neuralgia, hysteria, and dysmenorrhea, is widely used owing to its medicinal properties in the Middle East and Asia countries (Sînâ 1998). Researchers were found different results related to the essential oil components of *P. harmala* populations. While Iranian population dominated by  $\alpha$ -pinene (72.6%), *trans*-verbenole (3.9%) and sabinene (2.6%) (Faridi *et al.*, 2013), Pakistan population was dominated by alcanfor (28.24%), capillin (13.176%) and eugenyl methyl (9.674%) (Dastagir *et al.*, 2014).

Another study reported that, eugenol (17.5%), thymol (7.0%) and  $\alpha$ -isomethyl-(E)-ionol (7.0%); eugenol (17.2%), n-tetradecanol (12.3%) and dodecanoic acid (5.9%); eugenol (17.8%), n-tetradecanol (11.3%) and  $\beta$ -acorenolo (7.4%); eugenol (13.2%), n-tetradecanol (11.1%) and bakerol (7.5%); and eugenol (69.2%), eugenol acetate (9.0%) and (E)-anethol (6.9%) were dominated to essential oil of the Algeria, Egypt, Libya, Morocco and Tunisia populations, respectively (Apostolico *et al.*, 2016). In this study, results differ from all of them, in such a way that, it was dominated by methyl stearate (29.33%), eicosane (11.31%) and metilox

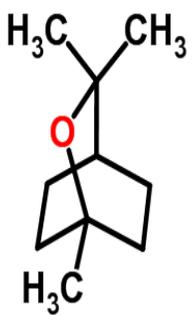
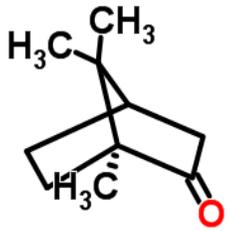
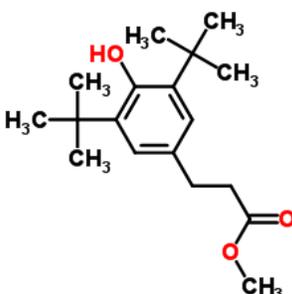
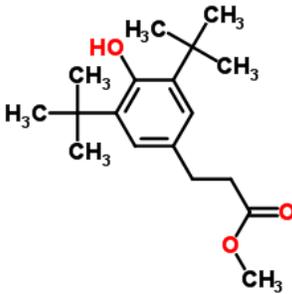
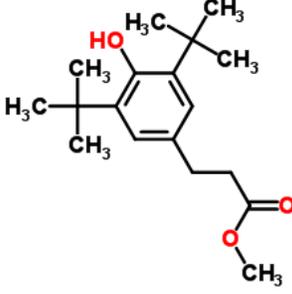
(7.06%). Apostolico *et al.*, (2016) reported that all samples were capable of inhibiting the growth of the *B. cereus* strains, *E. coli*, *P. aeruginosa* and *S. aureus* but they cannot explain different antimicrobial activity with the chemical composition of the essential oils.

MIC values of the *Peganum harmala* essential oil at 250 µl were found against to *Lactococcus garvieae* but no any antimicrobial activity to other test bacteria.

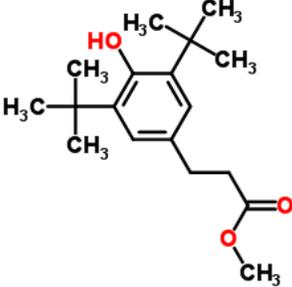
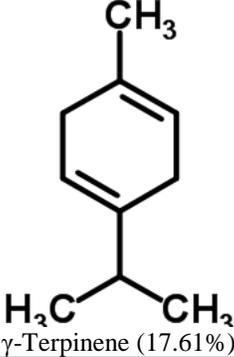
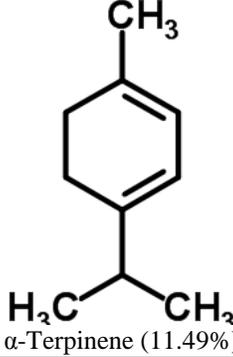
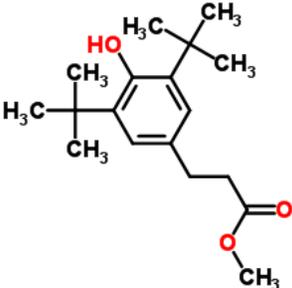
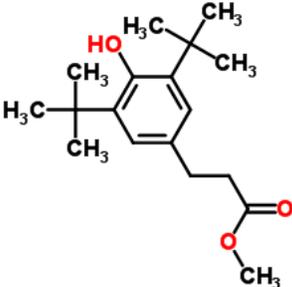
For many years, *Thymus vulgaris* has been extensively used for expectorant, antitussive, antispasmodic, antibroncholytic, carminative, diuretic and anthelmintic properties as a folkloric. *T. vulgaris* essential oils, which are collected from seven different areas at Italy and France, were rich in thymol (from 22.11% to 38.45%) accompanied by its biogenetic precursors  $\rho$ -cymene (from 21.5% to 44.9%) and  $\gamma$ -terpinene (from 0% to 17.3%) (Zambonelli *et al.*, 2004). Besides, essential oil of *T. vulgaris* from collected six different areas of Albania were dominated by thymol (from 32.02% to 53.29%),  $\rho$ -cymene (from 14.76% to 20.07%) and  $\gamma$ -terpinene (from 9.53% to 13.18%) (Asllani and Toska, 2003). Also, the Campania Region in Southern Italy population of *T. vulgaris* essential oil was dominated by thymol (33.02±0.3%),  $\rho$ -cymene (24.3±0.3%) and carvacrol (8.6±0.1%) (Catauro *et al.*, 2017).

Camphor (38.54%), camphene (17.19%) and  $\alpha$ -pinene (9.35%) were obtained from the essential oil of *T. vulgaris* Morocco population (Imelouane *et al.*, 2009). Similar to some Italy and France population,  $\rho$ -cymene (54.23%) was highly percentage of the essential oil composition. However, methyl stearate (23.26%) and  $\gamma$ -terpinene (4.90%) were dominated by *T. vulgaris* essential oil in this study.

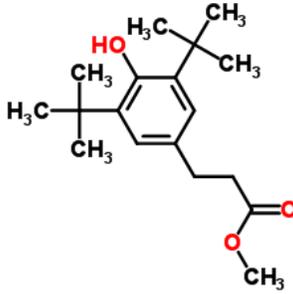
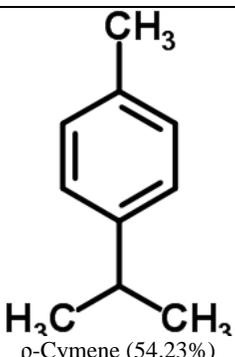
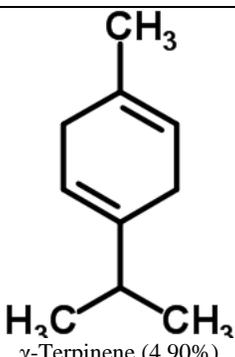
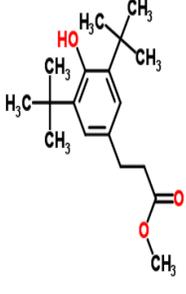
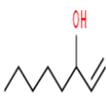
**Table.1** Quantity and structure of the first three compounds of essential oils

FAMES	Artemisia herba alba	
		
Eucalyptol (39.67%)	Methyl stearate (15.17%)	(1S)-camphor (6.65%)
Capparis spinosa		
		
Methyl stearate (38.83%)	Metilox (14.68%)	Methyl palmitate (12.40%)
Globularia alypum		
		
Methyl stearate (44.61%)	Metilox (17.15%)	Tetradecane (11.39%)
Matricaria chamomilla		
		
Methyl stearate (26.02%)	Metilox (10.17%)	Undecane (9.18%)

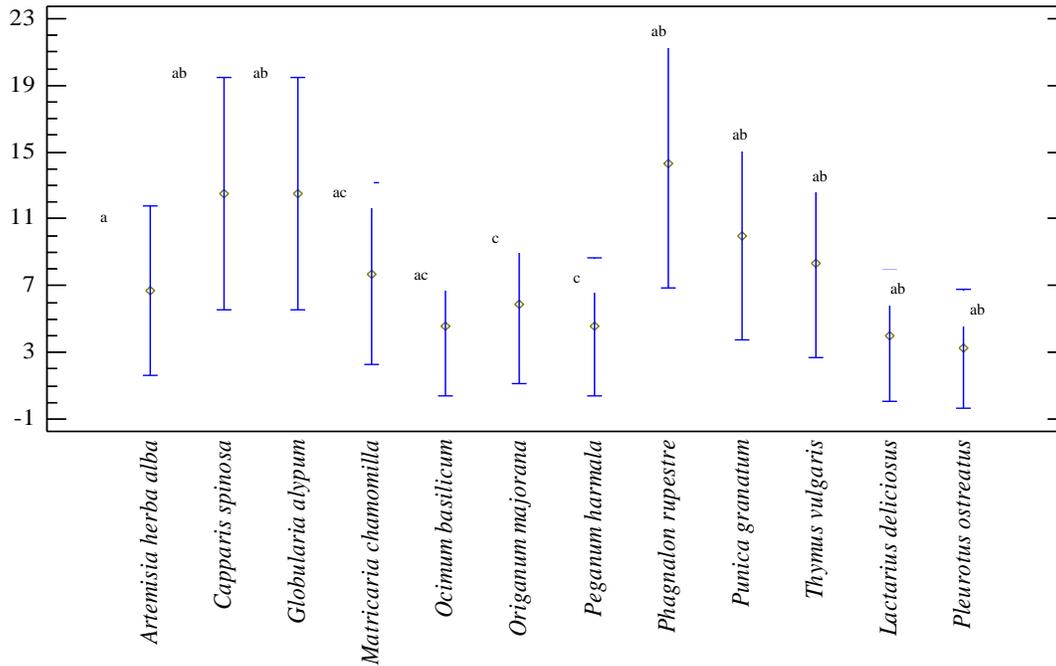
**Table.1** Quantity and structure of the first three compounds of essential oils (continued)

FAMES	Ocimum basilicum	
		
Methyl stearate (29.16%)	Metilox (10.57%)	Docosane (9.87%)
Origanum majorana		
		
$\gamma$ -Terpinene (17.61%)	Methyl stearate (16.04%)	$\alpha$ -Terpinene (11.49%)
Peganum harmala		
		
Methyl stearate (29.33%)	Eicosane (11.31%)	Metilox (7.06%)
Phagnalon rupestre		
		
Methyl stearate (53.83%)	Metilox (16.84%)	Methyl palmitate (9.35%)

**Table.1** Quantity and structure of the first three compounds of essential oils (continued)

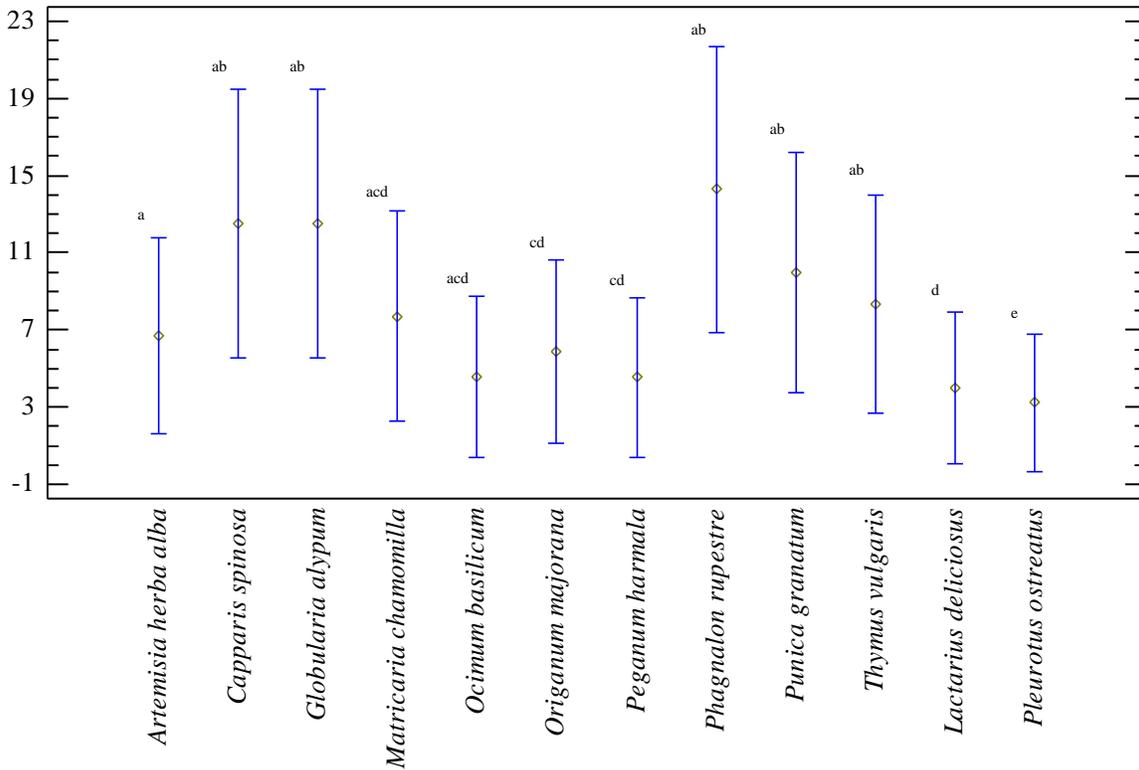
FAMEs			Aroma		
<i>Punica granatum</i>					
					
Methyl stearate (43.21%)	Metilox (16.82%)	Methyl palmitate (7.90%)			
<i>Thymus vulgaris</i>					
					
p-Cymene (54.23%)	Methyl stearate (23.26%)	γ-Terpinene (4.90%)			
<i>Lactarius deliciosus</i>					
					
Methyl stearate (59.46%)	Metilox (5.71%)	Octadecane (5.22%)	1-Octen-3-ol (22.58%)	Eicosane (7.45%)	Docosane (6.32%)
<i>Pleurotus ostreatus</i>					
					
Methyl stearate (51.24%)	Methyl 14-methyl pentadecanoate (12.55%)	4-Methyl pentadecane (9.56%)	Eicosane (13.47%)	Docosane (10.85%)	Cetane (6.94%)

**Fig.1** The statistical similarity of the FAMES analyses results of essential oils



Within the graph, means with different superscripts are significantly different ( $P < 0.05$ ).

**Fig.2** The statistical similarity of the aroma analyses results of essential oils



Within the graph, means with different superscripts are significantly different ( $P < 0.05$ ).

*T. vulgaris* essential oil has fungicidal activity against *Fusarium solani*, *Rhizoctonia solani* and *Colletotrichum lindemuthianum* and also the fungicidal activity of the oils was correlated with their thymol content (Zambonelli *et al.*, 2004). MIC values of essential oil of *T. vulgaris* Morocco population were found 0.66 mg/ml for *Pantoea* sp.; 1.33 mg/ml for *S. aureus*, *S. epidermidis* and *E. coli*; and 2.67 mg/ml for *Streptococcus* sp. (Imelouane *et al.*, 2009). MIC values of *Thymus vulgaris* essential oil was found 250 µl for *Edwardsiella tarda*, 1000 µl for *Lactococcus garvieae* and also 1250 µl dose for *Enterococcus faecium*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* in this study.

In addition to the use of macrofungi as nutrients, researchers deal with its some compounds to select fragrant components and enter the bioconversion process. Researchers were generally working with extract of *Lactarius deliciosus*. Its methanolic extract dominated by *o*-coumaric (497.45 mg/kg), quercetin (450.87 mg/kg) and syringic (78.64 mg/kg) (Dundar *et al.*, 2016). There is no find study with the chemical composition of essential oil of the *L. deliciosus*. The essential oil of *Pleurotus ostreatus* was dominated by octen-3-ol (59.3), 3-octanol (5.8%) and octan-3-one (5.3%) (Nyegue *et al.*, 2003). FAMES and aroma analyses of the essential oil of both *L. deliciosus* and *P. ostreatus* were found differently from each other. Methyl stearate (59.46%), Methyl (5.71%), methyl palmitate (5.15%) and 1-octen-3-ol (22.58%), eicosane (7.45%), docosane (6.32%) were dominated essential oil of *L. deliciosus* at the FAMES and aroma analyses. FAMES result shows that methyl stearate (51.24%), methyl 14-methyl pentadecanoate (12.55%) and 4-methyl pentadecane (9.56%) dominated *Pleurotus ostreatus* essential oil. According to aroma results, eicosane (13.47%), docosane (10.85%) and Cetane (6.94%) were dominated it.

The methanol extract of *L. deliciosus* has antimicrobial activity against *S. aureus*, *E. coli*, *Enterococcus hirae* and *P. aeruginosa* but no antimicrobial activity against *Micrococcus luteus* and *Bacillus subtilis* (Nyegue *et al.*, 2003). Extract of *L. deliciosus*, which is extracted with solvent cocktail (distilled water (1), ethanol (2.5), methanol (2.5), acetone (2), methylene chloride (2)), has an antimicrobial activity against *P. aeruginosa* and *Shigella flexneri* but has not got antimicrobial activity against *Escherichia coli*, *Salmonella enterica* and *Candida albicans* (Altuner and Akata, 2010). The liquid filtrate of *P. ostreatus* has inhibitory activity *P. aeruginosa*, *E. faecalis*, *C. parapsilosis* but not show inhibition against *E. coli* and *S. aureus* (Owaid *et al.*, 2015). The crude extract of *P. ostreatus* has an inhibition *C. albicans*, *P. aeruginosa* and *S. aureus* but no inhibitory effects for *E. faecalis*, *E. coli* and *Klebsiella pneumoniae* (Bawadekji *et al.*, 2017). In this research, essential oils of the *L. deliciosus* and *P. ostreatus* have not any antimicrobial effects for these bacteria.

Also, the essential oils of these plants did not show any antimicrobial activity against *Citrobacter freundii* and *Yersinia ruckeri* bacteria.

In conclusion, there were find similarity between essential oils components of both these plants and macrofungi, it was not affected antimicrobial activity against researched bacteria. Essential oils of plants may have been included similar compounds but this condition is insufficient for similar antimicrobial activity. However, there are a lot of affected factors for essential oil components such as plantation, texture, and structure of grown soil, climate, the moisture of the air, vegetation time, collection period, collected part of plants...etc. For these reasons, it will not be appropriate to use essential oils alone. To eliminate this condition, we must apply a

machine learning application for best sample collection time and preferred compounds.

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