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Soil Sorts Modify the Composition of Mediterranean Mandarin Essential Oils

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Abstract

Soil sorts vary in their physical and chemical properties, which in turn lead to diversity in essential oil constituents. Mediterranean mandarin (*Citrus reticulata* (Ten.) var. deliciosa) essential oil is high value as it is used in perfume and food industry. This research paper aimed to evaluate leaf, flower and peel essential oils of Mediterranean mandarin trees grown in fertile and arid soils. Various essential oils were isolated by hydro-distillation and analyzed by gas chromatography and gas chromatography/mass spectrometry. Various modifications have occurred in Mediterranean mandarin essential oils produced from trees grown in both fertile and arid soils. Linalool, dimethyl anthranilate, and limonene were the major components of leaf, flower and peel essential oils, respectively. Oxygenated monoterpenes was the major chemical group of leaf and flower essential oils, while it was monoterpene hydrocarbons of peel essential oil. Samples collected from trees planted in arid soil resulted in the maximum values of essential oils content (%), major components and main chemical groups. It may be concluded that Mediterranean mandarin essential oils and their components vary according to characteristics of growing soil, and this leads to diversity of its biological characteristics and effects. On the other hand, results current study indicates the possibility of cultivating mandarin in arid soils to increase the production of natural products.

Keywords: Soil sorts, Mediterranean mandarin, essential oil, linalool, dimethyl anthranilate, limonene.

Introduction

Due to the increase in human awareness regarding medicine and food with their relationship to health; there has been a revolution in pharmaceutical and food industries to search for natural products that are safe for human health to reduce chemically manufactured products that have bad effects on human health. It has recently been proven that essential oils have various biological controls that make them environmentally friendly (Chutia et al., 2006; Sokovic and Griensven, 2006). *Citrus* essential oils have several biological activities such as antioxidant, anti inflammatory, anxiolytic, antimicrobial and antifungal (Dosoky and Setzer, 2018), which made it of great importance in various fields such as food chemistry, pharmacology and pharmaceutics (Cristani et al., 2007). Mediterranean mandarin (*Citrus reticulata* (Ten.) var. deliciosa) is a member of *Citrus* family (Rutaceae) and its essential oil is high value as it is used mainly in perfume and food industry, for its prestigious scent (Dugo et al., 2011).

Soil sorts differ in their physical and chemical properties, which in turn affect the content of essential oils of aromatic plants (Melito et al., 2013; Rapposelli et al, 2015). Also, previous research papers reported that essential oil bearing plants differ with various soil properties and the production of essential oil requires suitable soil for greater yield and better quality (Khalid et al., 2020).

In Egypt, there are many economic crops that grow in old soils, which are characterized by its fertile properties with a small area; in this context, the Egyptian government is reclaiming large newly areas of arid soils located in the desert areas by planting some important crops, especially *Citrus* trees such as Mediterranean mandarin, in order to produce fruits. *Citrus* trees produce different wastes from their different parts such as leaves, flowers and fruit peels, which can be used in the production of essential oils as one of the sources of natural products. So, this investigation was aimed to evaluate the essential oil of Mediterranean mandarin trees that are cultivated in fertile and arid soils to find out the extent of opportunities for expanding their cultivation in arid soils as a natural source of essential oils.

Materials and methods

Plant materials

Experiments were carried out on Mediterranean mandarin trees (age: 10 years) cultivated at two *Citrus* farms located in different soils of Egypt (fertile and arid) in two consecutive years (2017 and 2018). Soil analyses are presented in Table (1).

Samples collection

In both years: during last week of February, three Kilograms of leaves were collected from the pruning trees; two kilograms of flowers produced from the precipitation were collected in June where many flowers fall during the month of June, as a result of the exposure of *Citrus* trees to the wind during that period; while, in last week of October, ten kilograms of fruits were harvested. Fresh leaves, flowers and peels of fruits that were collected from Mediterranean mandarin trees and divided into small pieces. Then, they were weighed to isolate essential oils.

Essential oil isolation

Fresh plant materials were collected and then 250 g from each replicate (three replicates) were subjected to hydro-distillation (HD) for 3h using a Clevenger-type apparatus (Clevenger, 1928). For HD, divided samples (250g) and 1000 ml of water were put into a 2000 ml round bottomed flask. Temperature was set at 100°C for the extraction of essential oil. The process in Clevenger-type apparatus was run for 3h (the time till no further essential oil could be extracted). Collected essential oil was treated with anhydrous sodium sulphate to remove the traces of water present and stored in a sealed tube at 4ºC until further use. Essential oil content was calculated as a relative percentage (v/w).

Gas chromatography (GC) and Gas chromatography/*mass spectrometry* (GC/MS) analysis

GC analyses were performed using a Shimadzu GC-9 gas chromatograph equipped with a DB-5 (dimethylsiloxane, 5% phenyl) fused silica column (J & W Scientific Corporation) (60 m x 0.25 mm i. d., film thickness 0.25µm). Oven temperature was held at 50 ºC for 5 min and then programmed to rise to 240 ºC at a rate of 3 ºC/ min. Diluted samples (1/100, v/v, in n-pentane) of 1 µl were injected. Flame ionization detector (FID) temperature was 265 ºC and injector temperature was 250 ºC. Helium was used as carrier gas with a linear velocity of 32 cm/s. Quantification was done by external standard method using the calibration curves generated from the GC analyses of representative compounds.

GC/MS analyses were carried out in a Varian 3400 GC-MS system equipped with a DB-5 fused silica column (60 m x 0.25 mm i. d., film thickness 0.25 µm); oven temperature was 50–240 ºC at a rate of 4 ºC/min, transfer line temperature 260 ºC, carrier gas, helium, with a linear velocity of 31.5 cm/s, split ratio 1:60, ionization energy 70 eV, scan time 1s, and mass range 45-600 amu.

Identification of volatile component

The components of essential oil were identified by comparison of their mass spectra with those of a computer library or with authentic compounds and confirmed by comparison of their retention indices, either with those of authentic compounds or with data published in the literature (Adams, 1995). Mass spectra from the literature were also compared (Adams, 1995). Identification of the individual The author thanks the National Research Center for providing it with all the means and equipment to complete this study components was accomplished by comparison of retention times with standard substances and by matching mass spectral data with MS libraries (NIST/NBS and Wiley 275.l) In addition to this, co-injection with the available authentic sample of identified compounds to GC or GC/MS is needed to confirm the assignment made. For the purpose of quantification was done by external standard method using calibration curves generated by running GC analysis of representative compounds. The retention indices (RI) were also determined for all constituents by injecting a homologous series of n-alkanes, C_8-C_{22} into the chromatographic column and they were then compared with the values given in the literature to confirm identification (Adams, 1995). Computer matching was against commercial (Wiley GC/MS Library, Mass Finder 3 Library) (Adams, 1995).

Statistical analysis

In this experiment, one factor was considered: soil sorts. For each soil there were 3 replicates, the experimental design followed a complete random design. Data averages of both years were statistically analyzed using one way analysis of variance (ANOVA-1) (Snedecor and Cochran, 1990). Significant values determined according to *p* values ($p<0.05$ = significant, $p<0.01$ = moderate significant and $p<0.001$ = highly significant). Applications of that technique were according to the STAT-ITCF program version 10 (Foucart, 1982).

Results

Leaf essential oil

Data in Table (2) present the content and components of leaf essential oil that was separated from Mediterranean mandarin trees grown in both fertile and arid soils. Soil sort led to several changes in the content of leaf essential oil and its components. Leaf essential oil content (%) isolated from trees cultivated in arid soil was higher (0.4%) than that isolated from trees cultivated in fertile soil (0.3%). Twenty-seven compounds were identified in leaf essential oil that belonged to five chemical groups of terpenes, namely: monoterpene hydrocarbons, oxygenated monoterpenes, sesquiterpene hydrocarbons, oxygenated sesquiterpenes and oxygenated diterpenes, where the oxygenated monoterpenes group appeared as the main group. Both linalool and thymol appear as the major compounds. Leaf essential oil was extracted from trees grown in arid soil resulted in the highest amounts of linalool (79.5%), thymol (13.8%), oxygenated monoterpenes (94.2%) and oxygenated sesquiterpenes (1.3%). The maximum values of monoterpene hydrocarbons (3%), sesquiterpene hydrocarbons (2.9%) and oxygenated diterpenes (0.7%) were recorded in leaf essential oil isolated from trees grown in fertile soil. Statistical changes in leaf essential oil content, its components and various chemical groups were presented in Table (2).

Flower essential oil

Effects of both fertile and arid soils on flower essential oil and their constituents are shown in Table (3), where nineteen compounds were identified and they are belonged under six chemical groups, namely: monoterpene hydrocarbons, oxygenated monoterpenes, sesquiterpene hydrocarbons, oxygenated sesquiterpenes, diterpenes hydrocarbons and alkyl aldehydes. Oxygenated monoterpenes was the major chemical group. The major components were dimethyl anthranilate, γ-terpinene, limonene and *p*-cymene. Flower essential oil content (%) was higher under arid soil (0.5%) than under fertile one (0.3%). Flower essential oil produced from trees cultivated in arid soil resulted in the maximum amounts of dimethyl anthranilate (53.9%), limonene (11.6%), *p*-cymene (6.4%), oxygenated monoterpenes (58.9), oxygenated sesquiterpenes (1.5%) and alkyl aldehydes (4.1%). Flower essential oil isolated from trees cultivated in fertile soil gave the greatest amounts of γ-terpinene (17.8%), monoterpene hydrocarbons (41%), sesquiterpene hydrocarbons (1.6%) and diterpenes hydrocarbons

(1.8%). All statistical changes that occurred in various flower essential oil contents, components and chemical groups are shown in Table (3).

Peel essential oil

Changes in peel essential oil composition in response to soil sort are shown in Table (4). Peel essential oil produced from trees planted in arid soil was higher in its content (1.1%) compared to that planted in fertile soil (0.8%). The number of compounds resulting from the analysis of peel essential oil was twenty-one. These twenty-one compounds belong to the following chemical groups: monoterpene hydrocarbons, oxygenated monoterpenes, sesquiterpene hydrocarbons, oxygenated sesquiterpenes and alkyl aldehydes; a monoterpene hydrocarbon was the major one. Limonene was the major component. Peel essential oil distilled from trees cultivated in arid soil resulted in the greatest values of limonene (84.3%), monoterpene hydrocarbons (88.1%), sesquiterpene hydrocarbons (3.4%) and alkyl aldehydes (1.4%). The greatest values of oxygenated monoterpenes (7.3%) and oxygenated sesquiterpenes (1.3%) were recorded in peel essential oil isolated from trees planted in fertile soil. Statistical changes occurring in peel essential oil content and its components are referred to in Table (4).

Discussion

Obtained results in this trial showed that Mediterranean mandarin essential oil was affected by arid and fertile soils. The contents of leaf, flower and peel essential oils and their major components were higher in samples collected from trees cultivated in arid soil than those harvested from trees planted in fertile one. Occurrence of these results is due to the fact that arid soil has many characteristics of stress such as high levels of salinity, drought, acidity and lack of nutrients, which in turn leads to a high production rate of by-products, especially essential oils that help plants to resist these stressful conditions (Jeshni et al., 2017; Bhatla and Lal, 2018). Note that stress conditions increase the activity of enzymes responsible for essential oil production (Burbott and Loomis, 1969). Acquired results are in accordance with those some previous studies which were conducted on the essential oils of *Citrus* crops such as lemon, grapefruit and shaddock (Khalid et al., 2020; Khalid et al., 2021). Passioura (1991) indicated that soil texture causes changes in the production of essential oil and its components. Soil texture also affects the ability of plant roots to grow, absorb nutrients, and transfer plant hormones to the vegetative system to help plants grow, and this in turn affects the composition and yield of essential oil (Mehalaine and Chenchouni, 2020).

Chemical properties of soil affect the production of plant secondary compounds. Soil pH affects the production of essential oils through its effect on the degree of availability of nutrients for absorption through plant roots and thus on its vital role within plants (Robson, 1989). Relationships were detected between essential oil composition and other chemical characters of soil such as total calcium carbonate and active calcium carbonate (Mehalaine and Chenchouni, 2020); the presence of calcium in soil affects the essential oil production because it acts as an activator for the enzymes involved in its formation (Hopkins, 2003). Significant changes were detected in rosemary essential isolated from plants gown in calcareous soil, while sandy soil resulted in non significant variations of marigold essential oil (Aboukhalid et al., 2017), the antagonistic effects on rosemary and marigold essential oils in response to both soils may be attributed to the physiological responses of each plant to the amount of calcium carbonate in both soils; this finding reported that aromatic plants produce higher composition of essential oil in calcareous soil than in sandy soil. Some aromatic plants are named according to the sort of soil that greatly affects the production of their essential oils, for example sage behaved as a calcicole species, whereas thyme behaved as a calcifuge species (Mehalaine and Chenchouni, 2020). Production of essential oil affected in some plants (rosemary and thyme) due to the ratio of carbon to nitrogen (C/N ratio) in soil (Hopkins, 2003). Carbon in soil affects essential oil production through the formation of carbohydrates during photosynthesis process, carbohydrate derivatives such as acetyl CoA are used in the synthesis of terpenes compounds via mevalonic acid pathway (Hopkins, 2003; Bhatla and Lal, 2018). Terpenes are the major components of essential oils (Chamorro et al., 2012). Soil content of macro and micro elements has positive effects on the essential oil and its components, because play a basic role in energy metabolism of cells (Hopkins, 2003). Degree of electrical conductivity of soil can modify the chemical content of essential oils (Hasani et al., 2017). Soil stress condition such as salinity, soil moisture, heavy metals induce variations in essential oils from aromatic plants (Khalid, 2011; Khalid, 2015; Khalid and Shedeed, 2016; Ahmed et al., 2017; Khalid and Ahmed, 2017). Current study indicates that Mediterranean mandarin essential oils and their components vary according to characteristics of growing soil, and this leads to diversity of its biological characteristics and effects.

Conclusion

This investigation reported that Mediterranean mandarin essential oils (contents and constituents) are related to soil sort. Leaf, flower and peel essential oils contents (%) and their major components were higher in the samples collected from trees cultivated in arid soil than those obtained from trees planted in fertile soil. This attempt is recommended to cultivate Mediterranean mandarin trees in arid soils as a source of essential oils, in addition to planting them in fertile soils.

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Table 1. Properties of both soils

Items	Soil sorts	
	Fertile	Arid
Sand (%)	17.6	91.4
Silt (%)	22.8	5.9
Clay (%)	59.6	2.7
pH	7.5	8.5
EC (dS/m)	2.1	3.3
Organic matter (%)	2.1	0.2
$CaCO_3$ (%)	3.1	8.7
Total N (%)	67.3	0.4
P (mg)	22.1	0.6
Cations (mg /100g Soil)		
K	16.9	1.5
Ca	9.5	4.9
Mg	6.4	3.9
Na	11.9	19.8
Anions (mg/100 g Soil)		
HCO_3	15.9	11.9
Cl	14.9	11.4
SO_4	13.9	6.8

Table 2. Effect of soil sort on leaf essential oil.

No.	Constituents	Rt.	RI^L	RI^C	Soil sort Fertile	Arid	F values
Essential oil content (%)					0.3 ±0.1	0.4 ±0.1	1.5^{ns}
Monoterpene Hydrocarbons					**3.0 ±0.4**	**2.4 ±0.3**	$\mathbf{2.6^{ns}}$
1	α-Pinene	7.6	939	939	0.6 ±0.2	0.3 ±0.1	13.5^{*}
2	Camphene	8.7	953	954	0.1 ±0.0	0.2 ±0.0	3.1^{ns}
3	Sabinene	9.5	976	976	0.5 ±0.2	0.2 ±0.0	13.5^{*}
4	β-Pinene	10.6	980	981	0.3 ±0.1	0.1 ±0.0	12.1^{*}
5	Myrcene	12.6	991	990	0.5 ±0.2	0.1 ±0.0	48.2^{**}
6	ρ-Cymene	13.3	1026	1026	0.2 ±0.0	0.3 ±0.1	1.5^{ns}
7	Limonene	15.6	1031	1034	0.4 ±0.1	0.3 ±0.1	1.5^{ns}
8	*cis*-β-Ocimene	17.7	1040	1040	0.1 ±0.0	0.5 ±0.2	48.1^{**}
9	α-Terpinene	19.4	1062	1062	0.3 ±0.1	0.4 ±0.1	1.5^{ns}
Oxygenated Monoterpenes					**91.9 ±3.7**	**94.2 ±4.2**	$\mathbf{18.7^{**}}$
10	Linalool	20.9	1098	1099	77.6 ±2.6	79.5 ±3.1	17.8^{**}
11	Terpinen-4-ol	22.5	1177	1177	0.4 ±0.1	0.2 ±0.0	6.1^{*}
12	Myrtenol	23.7	1194	1194	0.2 ±0.0	0.1 ±0.0	3.1^{ns}
13	*cis*-Carveol	25.8	1229	1229	0.6 ±0.2	0.3 ±0.1	13.5^{*}
14	Geraniol	27.7	1255	1255	0.2 ±0.0	0.1 ±0.0	3.1^{ns}
15	*cis*-Anethole	29.6	1269	1269	0.3 ±0.1	0.2 ±0.0	1.5^{ns}
16	Thymol	30.8	1290	1292	12.6 ±0.9	13.8 ±1.2	21.6^{**}
Sesquiterpene Hydrocarbons					**2.9 ±0.3**	**1.6 ±0.3**	$\mathbf{253.5^{***}}$
17	β-Elemene	31.2	1375	1375	0.2 ±0.0	0.1 ±0.0	3.1^{ns}
18	β-Caryophyllene	31.9	1418	1418	0.7 ±0.2	0.3 ±0.1	9.6^{*}
19	β-Humulene	32.7	1440	1440	0.5 ±0.1	0.3 ±0.1	6.1^{*}
20	(*E*) β-Farnesene	33.8	1458	1458	0.7 ±0.2	0.3 ±0.1	9.6^{*}
21	Viridiflorene	35.1	1493	1493	0.1 ±0.0	0.2 ±0.0	3.1^{ns}
22	α-Muurolene	37.8	1499	1499	0.3 ±0.1	0.3 ±0.1	0.0^{ns}
23	γ-Cadinene	38.1	1513	1513	0.4 ±0.1	0.1 ±0.0	27.2^{**}
Oxygenated Sesquiterpenes					**1.2 ±0.4**	**1.3 ±0.3**	$\mathbf{0.2^{ns}}$
24	Caryophyllene oxide	40.2	1581	1681	0.7 ±0.2	0.5 ±0.2	2.4^{ns}
25	Nerolidol	41.4	1564	1565	0.4 ±0.1	0.5 ±0.2	1.5^{ns}
26	α-Sinensal	42.7	1752	1752	0.1 ±0.0	0.3 ±0.1	12.1^{**}
Oxygenated Diterpenes					**0.7 ±0.2**	**0.4 ±0.1**	$\mathbf{5.4^{*}}$
27	*E*-Phytol	46.9	1949	1951	0.7 ±0.2	0.4 ±0.1	5.4^{*}
Total Identified					99.7	99.9	

Not: Rt., retention time (min); RI^L, retention index from literature ; RI^C, retention Index determined on DB-5 gas chromatography column using *n-alkanes* series; F ratio, the ratio of two mean square values; *, $p < 0.05$ (significant); **, $p < 0.01$ (moderate significant); ***, $p < 0.001$ (highly significant); ns, non significant. All values are given as mean (%) ± SD.

Table 3. Effect of soil sort on flower essential oil.

No.	Components	Rt.	RI^L	RI^C	soil sort Fertile	Arid	F values
Essential oil content (%)					0.3 ±0.1	0.5 ±0.1	6.1^{ns}
Monoterpene Hydrocarbons					**41.0 ±2.7**	**32.6 ±1.9**	**182.5^{***}**
1	α-Pinene	7.6	939	939	1.6 ±0.5	0.9 ±0.2	8.6^{*}
2	Sabinene	8.3	976	976	0.8 ±0.3	1.9 ±0.4	72.6^{***}
3	β-Pinene	8.9	980	981	1.9 ±0.4	1.1 ±0.3	96.2^{***}
4	Myrcene	11.8	990	990	1.7 ±0.3	0.5 ±0.1	43.2^{**}
5	γ-Terpinene	12.7	1063	1063	0.5 ±0.1	1.0 ±0.3	15.2^{*}
6	*p*-Cymene	13.8	1026	1026	4.3 ±0.6	6.4 ±0.4	52.9^{**}
7	Limonene	15.5	1031	1034	9.4 ±0.7	11.6 ±0.9	45.4^{**}
8	*cis*-β-Ocimene	18.5	1040	1040	1.7 ±0.3	0.2 ±0.9	153.6^{***}
9	γ-Terpinene	20.6	1063	1063	17.8 ±1.2	7.9 ±0.8	5880.6^{***}
10	α-Terpinolene	22.2	1088	1088	1.3 ±0.4	1.1 ±0.3	1.2^{ns}
Oxygenated Monoterpenes					**52.1 ±3.8**	**58.9 ±4.6**	**6936.2^{***}**
11	Linalool	24.9	1098	1099	1.7 ±0.4	1.9 ±0.3	1.2^{ns}
12	Terpinene4-ol	26.1	1177	1177	1.2 ±0.3	1.6 ±0.2	2.4^{ns}
13	Thymol	27.9	1290	1292	1.7 ±0.4	1.5 ±0.3	0.4^{ns}
14	Dimethyl anthranilate	31.6	1402	1402	47.5 ±2.8	53.9 ±3.1	472.6^{***}
Sesquiterpene Hydrocarbons					**1.6 ±0.4**	**1.5 ±0.3**	**1.5^{ns}**
15	β-Caryophyllene	32.7	1481	1481	1.6 ±0.4	1.5 ±0.3	1.5^{ns}
Oxygenated Sesquiterpene					**1.2 ±0.3**	**1.5 ±0.3**	**5.4^{ns}**
16	E-E-Farnesol	33.9	1722	1722	1.2 ±0.3	1.5 ±0.3	5.4^{ns}
Diterpenes Hydrocarbons					**1.8 ±0.4**	**1.2 ±0.2**	**13.5^{*}**
17	Cembrene	35.9	1959	1959	1.8 ±0.4	1.2 ±0.2	13.5^{*}
Alkyl aldehydes					**2.0 ±0.3**	**4.1 ±0.4**	**661.5^{***}**
18	Benzeneacetaldehyde	16.8	1646	1646	0.7 ±0.2	1.6 ±0.3	14.3^{*}
19	Octadecenal	38.1	1989	1989	1.3 ±0.3	2.5 ±0.4	12.7^{*}
Total Identified					99.7	99.8	

Not: Rt., retention time (min); RI^L, retention index from literature ; RI^C, retention Index determined on DB-5 gas chromatography column using *n-alkanes* series; F ratio, the ratio of two mean square values; *, $p < 0.05$ (significant); **, $p < 0.01$ (moderate significant); ***, $p < 0.001$ (highly significant); ns, non significant. All values are given as mean (%) ± SD.

Table 4. Effect of soil sort on peel essential oil.

No.	Constituents	Rt.	RI^L	RI^C	Soil sort Fertile	Arid	F values
Essential oil content (%)					0.8 ±0.2	1.1 ±0.3	5.4^{*}
Monoterpene Hydrocarbons					**86.7 ±3.4**	**88.1 ±3.9**	**100.1^{***}**
1	α-Pinene	7.1	939	939	0.9 ±0.3	0.5 ±0.1	24.1^{**}
2	Sabinene	8.4	976	976	1.5 ±0.4	1.2 ±0.2	0.9^{ns}
3	β-Pinene	9.9	980	981	0.4 ±0.1	0.3 ±0.1	1.5^{ns}
4	Δ-3-Carene	11.7	1011	1011	0.7 ±0.2	0.9 ±0.2	2.4^{ns}
5	Limonene	12.9	1031	1034	81.7 ±3.8	84.3 ±4.2	97.5^{***}
6	γ-Terpinene	13.8	1063	1063	0.8 ±0.2	0.6 ±0.2	2.4^{ns}
7	α-Terpinolene	14.7	1088	1088	0.7 ±0.2	0.3 ±0.1	24.1^{**}
Oxygenated Monoterpenes					**7.3 ±0.5**	**6.2 ±0.4**	**27.9^{**}**
8	*cis*-Limonene oxide	16.8	1144	1144	0.6 ±0.2	0.4 ±0.1	1.5^{ns}
9	Terpinen-4-ol	17.8	1177	1177	0.5 ±0.1	0.6 ±0.2	2.7^{ns}
10	α-Terpineol	19.4	1189	1189	1.3 ±0.3	1.6 ±0.3	2.7^{ns}
11	*cis*-Carveol	22.7	1229	1229	0.8 ±0.2	0.6 ±0.2	2.4^{ns}
12	Citral	23.8	1240	1240	1.8 ±0.3	1.4 ±0.3	9.6^{*}
13	Carvone	25.9	1242	1242	1.5 ±0.3	1.2 ±0.2	0.9^{ns}
14	Thymol	29.5	1290	1292	0.9 ±0.2	0.4 ±0.1	37.5^{**}
Sesquiterpene Hydrocarbons					**3.2 ±0.5**	**3.4 ±0.3**	**0.6^{ns}**
15	β-Caryophyllene	32.8	1418	1418	1.4 ±0.3	1.7 ±0.3	1.4^{ns}
16	β-Bisabolene	34.7	1509	1509	1.1 ±0.3	1.2 ±0.3	0.6^{ns}
17	Germacrene D	36.9	1560	1560	0.7 ±0.2	0.5 ±0.2	2.4^{ns}
Oxygenated Sesquiterpene					**1.3 ±0.3**	**0.7 ±0.2**	**8.3^{*}**
18	Nerolidol	38.4	1564	1565	0.6 ±0.2	0.3 ±0.1	13.5^{*}
19	Caryophyllene oxide	40.8	1582	1582	0.7 ±0.2	0.4 ±0.1	5.4^{*}
Alkyl aldehydes					**1.3 ±0.4**	**1.4 ±0.4**	**0.1^{ns}**
20	Decanal	20.6	1204	1204	0.8 ±0.2	0.6 ±0.2	2.4^{ns}
21	Neryl acetate	31.1	1365	1367	0.5 ±0.2	0.8 ±0.3	2.7^{ns}
Total Identified					99.8	99.8	

Not: Rt., retention time (min); RI^L, retention index from literature ; RI^C, retention Index determined on DB-5 gas chromatography column using *n-alkanes* series; F ratio, the ratio of two mean square values; *, $p < 0.05$ (significant); **, $p < 0.01$ (moderate significant); ***, $p < 0.001$ (highly significant); ns, non significant. All values are given as mean (%) ± SD.