Biomedical & Pharmacology Journal, September 2020. Vol. **13**(3), p. 1339-1350

Chemistry in Aromatherapy – Extraction and Analysis of Essential Oils from Plants of *Chamomilla recutita*, *Cymbopogon nardus*, *Jasminum officinale* and *Pelargonium graveolens*

Chui-Man Lo*, Jie Han and Emily S. W. Wong

Department of Science, School of Science and Technology,
The Open University of Hong Kong, Ho Man Tin, Kowloon, Hong Kong SAR.
*Corresponding Author E-mail: cmlo@ouhk.edu.hk

https://dx.doi.org/10.13005/bpj/2003

(Received: 21 August 2020; accepted: 25 September 2020)

Aromatheraphy refers to the application of essential oils to treat diseases. Essential oils come from natural plants, with characteristic odor. Apart from aromatherapy, they had been used for thousands of years in many home products,such as cosmetic and mosquito repellents. Due to their different active ingredients, each essential oil has slightly difference in their functions. For example, Chamomile oil extracted from *Chamomilla recutita* can be used in anti-ulcer, anti-inflammatory, antimicrobial and has a function of sedation; Citronella oil extracted from *Cymbopogon nardus* is mainly used as mosquito repellent; Jasmine oil extracted from *Jasminum officinale* can be used for antidepressant and antiseptic; and Geranium oil extracted from *Pelargonium graveolens* can reduce inflammation, treat acne and alleviate anxiety. In our study, the essential oils were extracted from the corresponding plants by either steam distillation or Soxhlet extraction, and the chemical analysis of their active ingredients were performed by GC-MS. Most of the components in essential oils belong to monoterpenoids, sesquiterpenoids or oxygenated terpenes.

Keywords: Aromatheraphy, Essential oil, Steam distillation, Soxhlet extraction, GC-MS analysis.

Many essential oils were found to have the bioactivities such as antioxidant, antiviral, antibacterial, antifungal, anti-inflammatory, antidiabetic, antimutagenic and anticarcinogenic.[1-4] The term *aromatheraphy* describes the application of essential oils to treat diseases.[5,6] Aromatheraphy involves the massage of aromatic plant extracts into the skin[7] or/and inhalation of aromas[8] during the therapy,[9] and they can be applied on health care settings.[10] It has the function to alter human health, mood, mind and cognitive function.[8] For examples, anxiety disorders are usually treated pharmacologically and psychologically in conventional cases.[11] However, the medicine used in traditional pharmacological treatments are usually have many side-effects[12] and they may be addictive.[13] Although the effect in aromatherapy is not easy to be measured quantitatively in psychological, mental, spiritual,and social aspects, they are considered to be more safe and have less adverse effects when comparing with conventional drugs.[14] Hence, it is becoming popular as an alternative treatment in relieving anxiety symptoms, as aromatheraphy can produce a mild and transient anxiolytic effect.[15] For examples, Tsang *et al.* reported that aromatheraphy can be applied for quelling of anxiety symptoms without adverse effects, and it can be applied as complementary therapy.[16] Kutlu *et al.* reported the study on students with lavender inhalation to reduce examination anxiety.[17] Lehrner*et al.* reported on the use of odor in lavender and orange can alter emotional states and diminish patients' anxiety in dental office.[18] Hadfield reported the use of aromatheraphy massage can affect human autonomic nervous system, and it can reduce the anxiety from cancer patients having malignant brain tumour.[19] In addition, apart from reducing anxiety, some of the constituents in essential oils, such as β-caryophyllene,[20] carvacrol,[21] citral,[22] geraniols,[23,24] α-humulene,[25] D-limonene,[26] myrcene,[27,28] thymol[29,30] and perillyl alcohol,[31]were reported to have cytotoxic effect to cancer cell lines.[32-34]

Essential oils can be regarded as"*concentrated hydrophobic liquid containing volatile aroma compound distilled from plants*", as described according to International Organization for Standardization.[35] They are complex mixtures belong to the class of terpenes and terpenoids with characteristic aroma that originate from these condary metabolism of the plants.[36] Essential oils can be classified by their difference in extracted parts of plants, odor and functional groups. Extracted parts of plants include leaves, flowers, stems, barks, resins, fruits, roots and seeds.[2,37] The odor(also known as aroma or scent) can be classified into floral,citrus, woody, spicy, grassy, minty, pungent, *etc*.[38-40] Functional groups of essential oils can be categorized into monoterpenes, sesquiterpenes, monoterpenols, sesquiterpenols, diterpenols, phenol, phenyl methyl ether, aldehydes, ketones, esters, phenolic compounds and oxides.[3,32,41] Most of the components in essential oils belong to terpenoid which are built up from different orientations of isoprene

Table 1. Examples of terpenoids

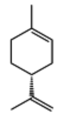
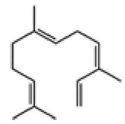
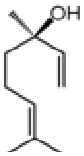
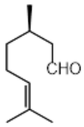
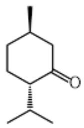
Types of terpenoid	Hemiterpenoids	Monoterpenoids	Sesquiterpenes
Chemical formula	C_5H_8	$C_{10}H_{16}$	$C_{15}H_{24}$
No. of isoprene units	1	2	3
No. of carbons	5	10	15
Example	 Isoprene	 D-Limonene	 α -Farnesene

Table 2. Examples of oxygenated terpenoids with different function groups

Functional group	Alcohol	Aldehyde	Ketone
Chemical formula	$C_{10}H_{18}O$	$C_{10}H_{18}O$	$C_{10}H_{18}O$
Example	 Linalool	 Citronellal	 Menthone

units with the structure CH_2=CH–C(CH_3)=CH_2. Different number of isoprene units can contribute the terpenoid with general formula of $(C_5H_8)_n$. If there is only one isoprene unit (n=1), it is called hemiterpenoid with the chemical formula of C_5H_8. When there are two isoprene units (n = 2), they belong tomonoterpenoids ($C_{10}H_{16}$). For n = 3, they are sesquiterpenes ($C_{15}H_{24}$). Some terpenoid appear as cyclic structures while some of them are acyclic. Some examples of terpenoids with their chemical structures were shown in Table 1.

Apart from the above terpenoid hydrocarbons in Table 1, some oxygenated terpenoids are resulted from additional rearrangements and oxidations. They contain functional groups such as alcohol, aldehyde, and ketone, *etc*. Some examples of their chemical structures were shown in Table 2.

Different essential oils have slightly difference in their functions, as their components contain different functional groups. For instance, Chamomile oil has anti-inflammatory and antimicrobial properties, and has a function of sedation.[42-46] Citronella oil can repel mosquitoes,[47-52]and it was also found to have antimicrobial activities against several oral pathogens.[53] Jasmine oil has the function of antidepressant and antiseptic.[54-58] Geraniumoil can treat acne, reduce inflammation and alleviate anxiety.[59-64]

There are various methods for extraction of essential oils from natural plants, including steam distillation,[65,66] solvent extraction,[67,68] Soxhlet extraction,[66,69] supercritical fluid extraction,[70,71] enfleurage[72,73] and cold pressing (expression).[74] Steam distillation is a common method which involves the evaporation of volatile components from the plant at a lower temperature with the presence of steam.[75] The essential oil can be collected after condensation of the vapour together with steam, and then isolated with water.[76]Alternatively, Soxhlet extraction is another common method for obtaining essential oil from plants. Solvent in the flask is heated up and vaporize, and then condense and drip back into the sample thimble. Repeated extraction can be proceeded with the use of the same batch of solvent.[69] In our study, we used steam distillation for the extraction of Chamomile, Jasmine and Geranium oils from *Chamomilla recutita*, *Jasminum officinale* and *Pelargonium graveolens* respectively, and Soxhlet extraction for isolation of Citronella oil from *Cymbopogon nardus*.

In the chemical identification of the extracted essential oils, the most common instrumentation is Gas Chromatography-Mass Spectrometry (GC-MS).[36] It is suitable for the volatile samples which can vaporized on heating. The gas chromatography part can separate the components in analyte by the partition between gaseous mobile phase and stationary phase in different retention times. All the components are finally eluted out and identify by the detector. The detector is a mass spectrometer which can breakdown the molecules into ionized fragments and detect these fragments in their characteristic mass-to-charge (*m/z*) ratio.[77]

MATERIAL AND METHODS

In our project, four types of common essential oils were chosen for our study. Chamomile, Jasmine and Geranium oils were extracted from dried flowers of *Chamomilla recutita*, dried flowers of *Jasminum officinale* and fresh leaves of *Pelargonium graveolens* respectively by steam distillation. Citronella oil was extracted from fresh leaves and stems of *Cymbopogon nardus* by Soxhlet extraction. Their chemical components were analyzed by Gas Chromatography-Mass Spectrometry (GC-MS).

Extraction of Essential Oils

General Procedure for Steam Distillation

Flowers or leaves from the plant material (~40 g) was cut and grinded into small pieces with a juice blender. The sample was placed inside a round-bottomed flask, which connected with the distillation apparatus. Water (200 mL) was added inside the round-bottomed flask to generate steam. The sample was heated to 200 ^{o}C and distilled for 6 hours. The oil-containing distillate was then collected and dried over anhydrous magnesium sulfate ($MgSO_4$) with further separation by centrifugingat 1300rpm for 30minutes. The essential oil was then transferred to an amber glass vialfor further analysis.

Steam Distillation of Chamomile: 38.52g dried flowers of *Chamomilla recutita* produced 0.23 g blue colour of Chamomile oil in 0.6% recovery.

Steam Distillation of Jasmine: 42.46 g dried flowers of *Jasminum officinale*produced 0.17g pale yellow colour of Jasmine oil in 0.4% recovery.

Steam Distillation of Geranium: 40.28 g dried leaves of *Pelargonium graveolens* produced 0.06 g pale yellow colour of Geranium oil in 0.15% recovery.

Procedure for Soxhlet Extraction of Citronella

Fresh leaves and stems of *Cymbopogon nardus* (7.94 g) was cut and grinded into small pieces with a juice blender, and then placed into Whatman cellulose extraction thimble (22 mm internal diameter × 80 mm external length). The sample containing thimble was then placed into a Soxhlet's apparatus. The solvent was*n*-hexane (250 mL) and the extraction was carried out at 150°C for 24 hours. The solvent was evaporated at reduced pressure using rotary evaporatory. The extracted essential oil was transferred to an amber glass vialfor further analysis. 1.35 g of pale yellow colour of citronella oil was obtained, with 17% recovery.

Analysis of Essential Oils

Preparation of Standard Solutions

Separated sets of standard solutions were prepared for each type of essential oil for GC-MS analysis. 10 mL of the extracted crude oils of Chamomile, Citronella, Jasmine and Geranium were diluted to prepare 1.00 mL of standard solutions with chloroform, ethanol, dichloromethane and *n*-hexane respectively.

General Procedure for GC-MS Analysis

GC-MS analysis was performed on Agilent 7890B GC system equipped with 5977B mass spectrometer and PAL RSI 85 auto-sampler. Agilent HP-5MS UI column (30m x 0.25mm) was used and Helium was used as the carrier gas. The sample injection volume is 1 μL, with flow rate of 1mL/min for Chamomile, Jasmine and Geranium, and 3 mL/min for Citronella. The selected *m/z* range is 30 to 550. For the temperature programming of Chamomile, it was hold at 45°C for 2 mins, then1.5°C/min ramp to 100°C, then 2°C/ min ramp to 200°C and finally 10°C/min

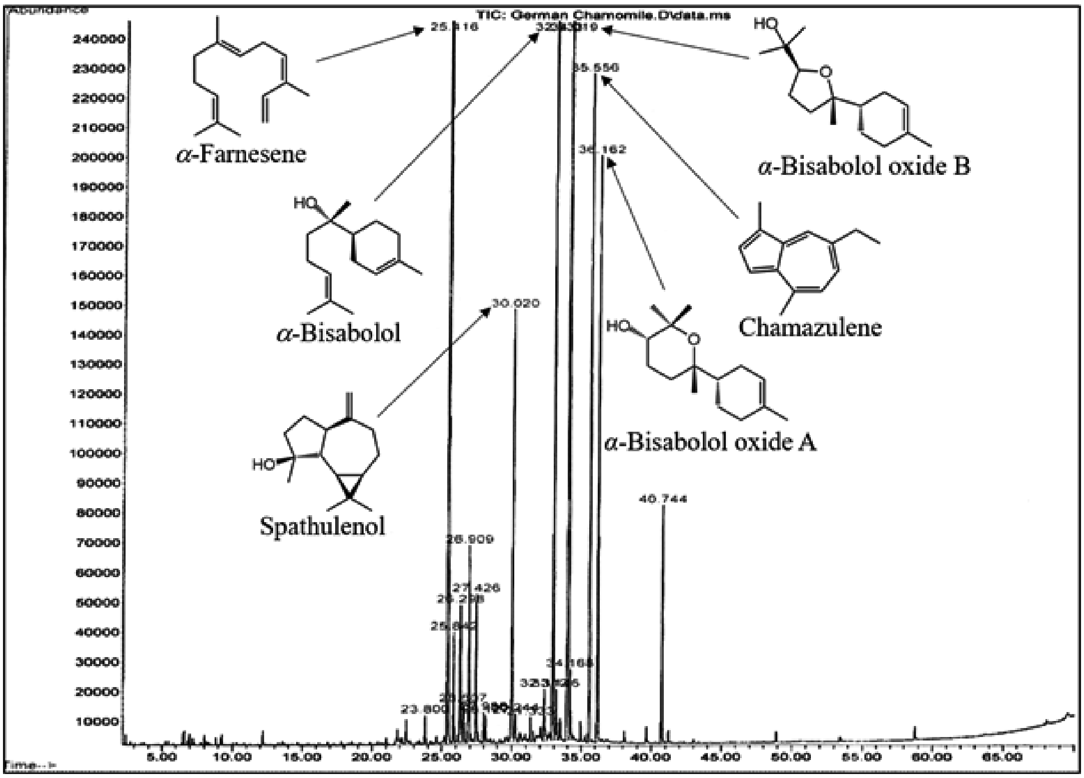


Fig. 1. GC-MS chromatogram for Chamomile oil

ramp to 250°C and hold for 30mins. For Citronella, it was hold at 50°C for 1 min, then rise 10°C/min to 100°C, 20°C/min to 220°C, and then hold at 220°C for 10 mins. For Jasmine and Geranium, it was programmed from 60°C, then rise 3°C/min to 240°C.

RESULTS AND DISCUSSION

Results indicated that most of the components in Chamomile, Citronella, Jasmine, and Geranium essential oils are all belong to the group of terpenoids, including monoterpenoids, sesquiterpenoids and oxygenated terpenes. The GC-MS analysis of each essential oil is shown below.

GC-MS Analysis for Chamomile (*Chamomilla Recutita*)

Figure 1 is the GC-MS Chromatogram for chamomile oil (extracted from *Chamomilla Recutita*), with the major signals assigned with the names and chemical structures of the corresponding components. Their retention times and IUPAC names were shown in Table 3. Chamomile oil mainly contains α-farnesene ($C_{15}H_{24}$), spathulenol ($C_{15}H_{24}O$), α-bisabolol ($C_{15}H_{26}O$), α-bisabolol oxide B ($C_{15}H_{26}O_2$), chamazulene ($C_{14}H_{16}$) and *a*-bisabolol oxide A ($C_{15}H_{26}O_2$). α-Farnesene has the chemical formula of $C_{15}H_{24}$, which belongs to sesquiterpenoids. The other components $C_{15}H_{24}O$, $C_{15}H_{26}O$ and $C_{15}H_{26}O_2$ are oxygenated terpenes.

Table 3. Main components in Chamomile oil by GC-MS analysis

Retention Time (min)	Common name	Component IUPAC name	Chemical Formula
27.426	α-Farnesene	3,7,11-trimethyl-1,3,6,10-dodecatetraene	$C_{15}H_{24}$
30.020	Spathulenol	(1a*R*,4a*R*,7*S*,7a*R*,7b*R*)-1,1,7-Trimethyl-4-methylidene-1a, 2,3,4a,5,6,7a,7b-octahydrocyclopropa[h]azulen-7-ol	$C_{15}H_{24}O$
32.309	α *a*-Bisabolol	6-methyl-2-(4-methylcyclohex-3-en-1-yl)hept-5-en-2-ol	$C_{15}H_{26}O$
32.919	*a*-Bisabolol oxide B	2-[5-Methyl-5-(4-methylcyclo-hex-3-en-1-yl) tetrahydrofuran-2-yl]propan-2-ol	$C_{15}H_{26}O_2$
35.556	Chamazulene	7-Ethyl-1,4-dimethylazulene	$C_{14}H_{16}$
36.162	α-Bisabolol oxide A	(3*R*)-2,2,6-Trimethyl-6-(4-methylcyclohex-3-en-1-yl)oxan-3-ol	$C_{15}H_{26}O_2$

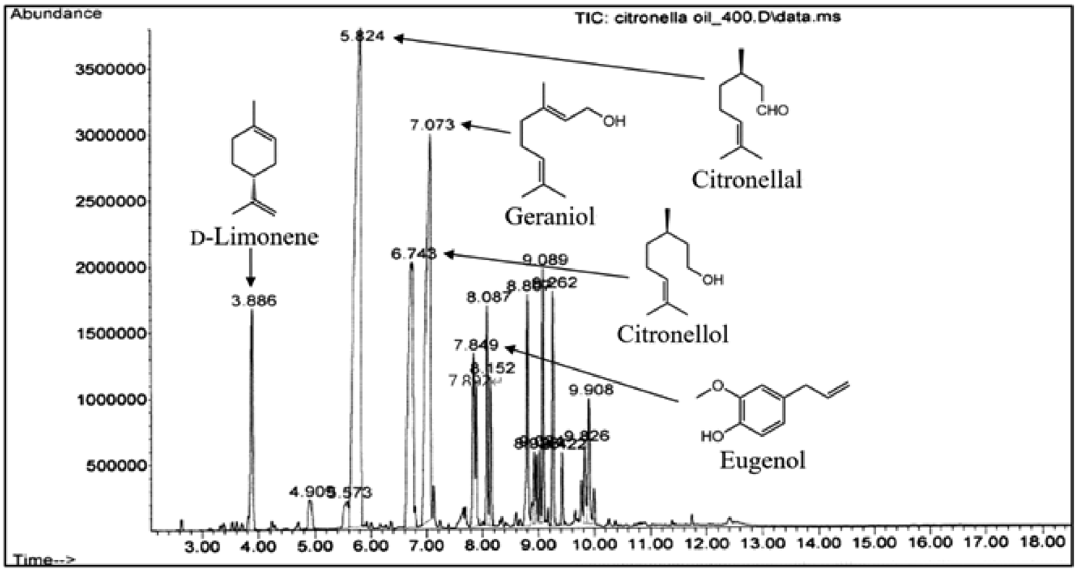


Fig. 2. GC-MS chromatogram for Citronella oil

GC-MS Analysis for Citronella (*Cymbopogon nardus*)

GC-MS Chromatogram for Citronellaoil (extracted from *Cymbopogon nardus*) was shown in Figure 2, with their retention times and IUPAC names shown in Table 4. The major components in Citronella oil are D-limonene ($C_{10}H_{16}$), citronellal ($C_{10}H_{18}O$), citronellol ($C_{10}H_{20}O$), geraniol ($C_{10}H_{18}O$) and eugenol ($C_{10}H_{12}O_2$). D-Limonene has a chemical formula of $C_{10}H_{16}$, which belongs to the class of monoterpenoids. The other components citronellal, citronellol, geraniol and eugenol are oxygenated terpenes.

GC-MS Analysis for Jasmine (*Jasminum officinale*)

GC-MS Chromatogram for Jasmine oil(extracted from *Jasminum officinale*) was shown in Figure 3, with their retention times and IUPAC names shown in Table 5. In Jasmine oil, the major components are benzyl alcohol (C_7H_8O), linalool ($C_{10}H_{18}O$), benzyl acetate ($C_9H_{10}O_2$), indole (C_8H_7N), methyl anthranilate

Table 4. Main components in Citronella oil by GC-MS analysis

Retention Time (min)	Component Common name	IUPAC name	Chemical Formula
3.886	D-Limonene	1-Methyl-4-(prop-1-en-2-yl)cyclohex-1-ene	$C_{10}H_{16}$
5.824	Citronellal	3,7-Dimethyl-oct-6-en-1-al	$C_{10}H_{18}O$
6.743	Citronellol	3,7-Dimethyloct-6-en-1-ol	$C_{10}H_{20}O$
7.073	Geraniol	(2*E*)-3,7-Dimethyl-2,6-octadien-1-ol	$C_{10}H_{18}O$
7.892	Eugenol	2-Methoxy-4-(prop-2-en-1-yl)phenol	$C_{10}H_{12}O_2$

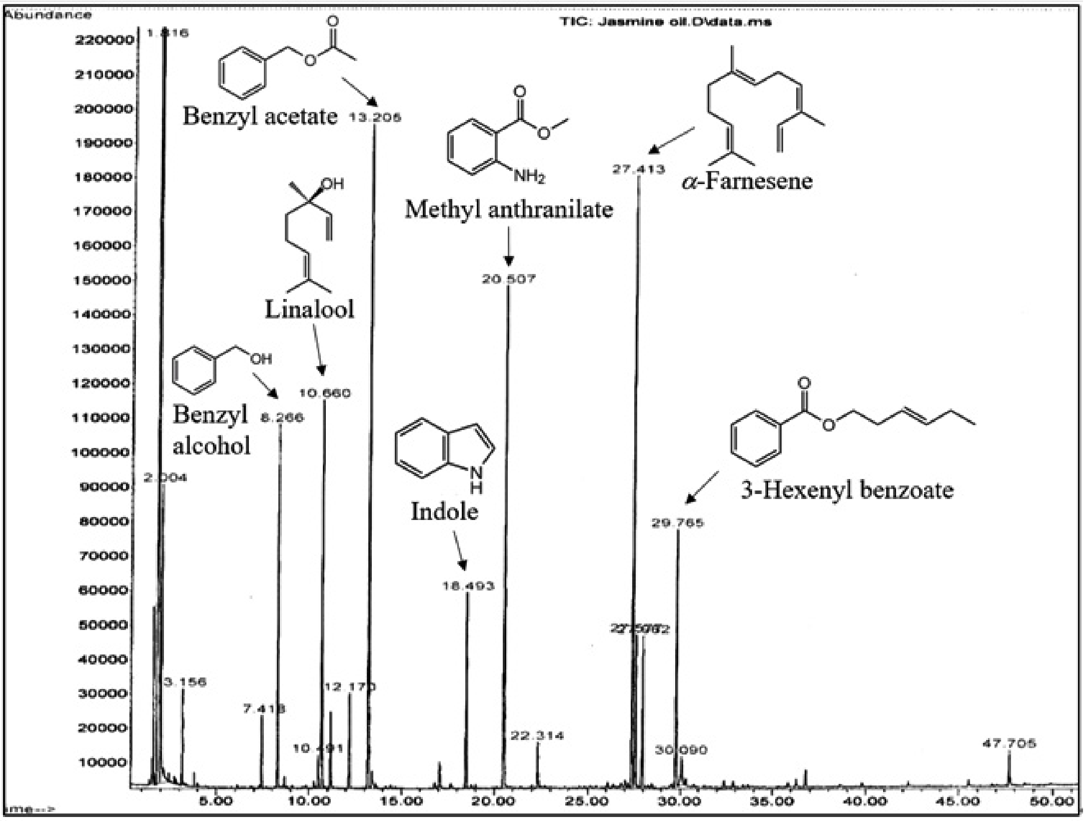


Fig. 3. GC-MS chromatogram for Jasmine oil

($C_8H_9NO_2$), α-farnesene ($C_{15}H_{24}$) and 3-hexenyl benzoate ($C_{13}H_{16}O_2$). α-Farnesene belongs to the class of sesquiterpenoids, while linalool belongs tooxygenated terpenes. Apart from the terpene family, Jasmine oil also contains some other non-terpene aromatic compounds, and some of them are also contain the ester functional group, such as benzyl acetate, methyl anthranilate and 3-hexenyl benzoate. These compounds have pleasant odour for antidepressant and antiseptic function.

GC-MS Analysis for Geranium (*Pelargonium graveolens*)

GC-MS Chromatogram for Geranium oil (extracted from *Pelargonium graveolens*) was shown in Figure 4, with their retention times and IUPAC names shown in Table 6. In Geranium oil, the major components are linalool ($C_{10}H_{18}O$), isomenthone ($C_{10}H_{18}O$), α-Terpineol ($C_{10}H_{18}O$), β-citral($C_{10}H_{16}O$), geraniol ($C_{10}H_{18}O$), α-citral($C_{10}H_{16}O$), β-bourbonene ($C_{15}H_{24}$),

Table 5. Main components in Jasmine oil by GC-MS analysis

Retention Time (min)	Component Common name	IUPAC name	Chemical Formula
8.266	Benzyl alcohol	Phenylmethanol	C_7H_8O
10.660	Linalool	3,7-Dimethylocta-1,6-dien-3-ol	$C_{10}H_{18}O$
13.205	Benzyl acetate	Benzyl ethanoate	$C_9H_{10}O_2$
18.493	Indole	1*H*-indole	C_8H_7N
20.507	Methyl anthranilate	Methyl 2-aminobenzoate	$C_8H_9NO_2$
27.413	α-Farnesene	3,7,11-Trimethyl-1,3,6,10-dodecatetraene	$C_{15}H_{24}$
29.765	3-Hexenyl benzoate	(*E*)-Hex-3-enyl benzoate	$C_{13}H_{16}O_2$

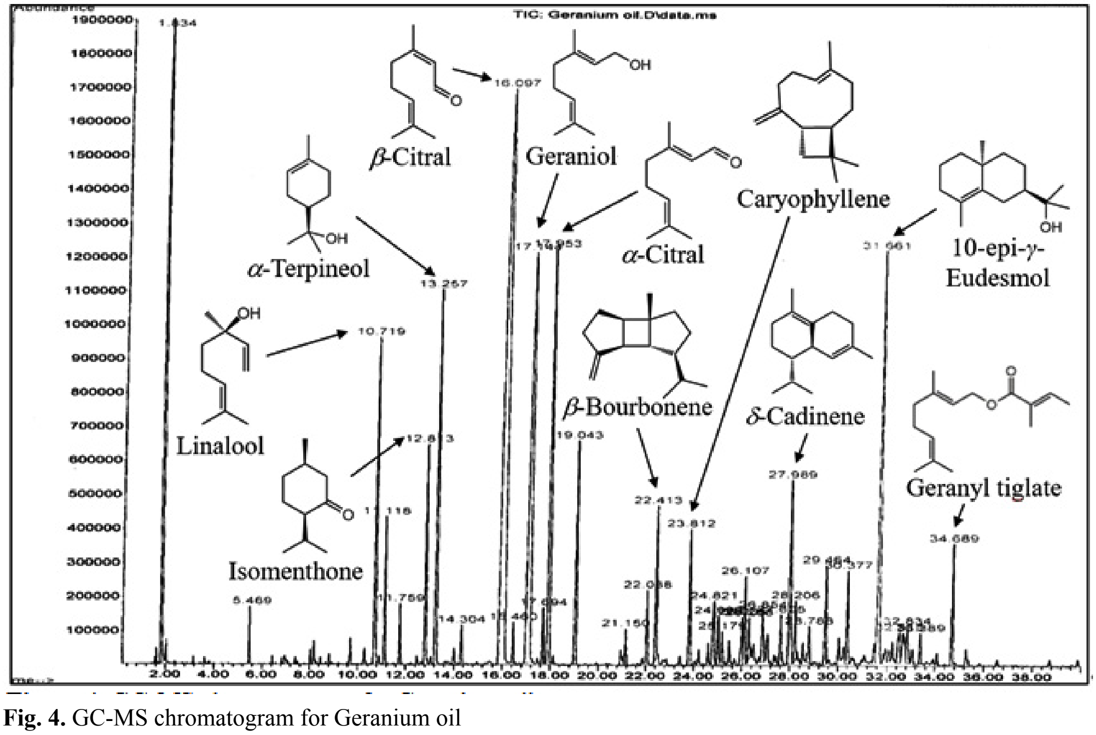


Fig. 4. GC-MS chromatogram for Geranium oil

Table 6. Main components in Geranium oil by GC-MS analysis

Retention Time (min)	Component Common name	IUPAC name	Chemical Formula
10.719	Linalool	3,7-Dimethyl-octa-1,6-dien-3-ol	$C_{10}H_{18}O$
12.813	Isomenthone	(2*S*,5*S*)-5-Methyl-2-propan-2-ylcyclohexan-1-one	$C_{10}H_{18}O$
13.257	α-Terpineol	2-(4-methylcyclohex-3-en-1-yl)propan-2-ol	$C_{10}H_{18}O$
16.097	β-Citral	3,7-Dimethylocta-2,6-dienal	$C_{10}H_{16}O$
17.144	Geraniol	3,7-Dimethyl-2,6-octadien-1-ol	$C_{10}H_{18}O$
17.953	α-Citral	3,7-Dimethyl-2,6-octadien-1-al	$C_{10}H_{16}O$
22.413	β-Bourbonene	(1*S*,2*R*,6*S*,7*R*,8*S*)-1-methyl-5-methylidene-8-propan-2-yltricyclo[5.3.0.0]decane	$C_{15}H_{24}$
23.812	Caryophyllene	(1*R*,4*E*,9*S*)-4,11,11-Trimethyl-8-methylidenebicyclo [7.2.0]undec-4-ene	$C_{15}H_{24}$
27.989	δ-Cadinene	(1*S*,8*aR*)-4,7-dimethyl-1-propan-2-yl-1,2,3,5,6, 8*a*-hexahydronaphthalene	$C_{15}H_{24}$
31.661	10-epi-γ-Eudesmol	2-[(2*R*,4a*S*)-4a,8-Dimethyl-1,2,3,4,4a,5,6,7-octahydro-2-naphtalenyl]-2-propanol	$C_{15}H_{26}O$
34.689	Geranyl tiglate	[(2*E*)-3,7-dimethylocta-2,6-dienyl] (*E*)-	$C_{15}H_{24}O_2$

caryophyllene ($C_{15}H_{24}$), δ-cadinene ($C_{15}H_{24}$), 10-epi-γ-eudesmol ($C_{15}H_{26}O_2$) and geranyl tiglate ($C_{15}H_{24}O_2$). There are many isomers of $C_{10}H_{18}O$ and $C_{15}H_{24}$. β-Bourbonene, caryophyllene and δ-cadinenehave the chemical formula of $C_{15}H_{24}$, which belongs to the class of sesquiterpenoids. The other components belong tooxygenated terpenes.

CONCLUSION

Essential oils of Chamomile, Citronella, Jasmine, and Geranium were extracted from the plants of *Chamomilla recutita*, *Cymbopogon nardus*, *Jasminum officinale* and *Pelargonium graveolens* respectively, using either steam distillation or Soxhlet extraction. Their chemical components were identified by GC-MS. They were found to contain different types of terpenoids (*e.g.*monoterpenoids, sesquiterpenoids and oxygenated terpenes) as the active ingredients. Due to the presence of these terpenoids with different structures, each type of essential oil has slightly difference in their functions.

ACKNOWLEDGEMENT

We would like to express our gratitude to The Open University of Hong Kong for the financial support in this project(R6265 and R6315).

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