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Chromatography-mass spectrometry and chemical characteristics of *Thymus zygis and Cymbopogon winterianus* essential oils: Possible insect repellents☆

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ABSTRACT

Background: Plant essential oils present a broad spectrum of activity against pests as possible insecticides, repellents, or deterrents. These oils also have a long tradition of use in the protection of stored products. Some chemical constituents of these oils interfere with the octopaminergic nervous system in insects. This target site is not shared with mammals; most essential oil chemicals are relatively non-toxic to mammals, including humans and domesticated animals, and meet the criteria for reduced-risk pesticides.
Materials and methods: The chemical composition of the two studied essential oils were analyzed using gas chromatography-mass spectrometry (GC-MS). Citronellal, α-Terpineol, 3,5,5-Trimethylhexanol, p-Thymol and p-Cymene were the main compounds focused on the study due to their known insect repellent properties.
Results: A total of 53 compounds were found in red thyme; p-Thymol (46.393%) and p-Cymene (22.154%) were the main constituents of the oil. Furthermore, Java citronella presented with 24 compounds, with 3,5,5-Trimethylhexanol (14.61%), Citronellal (15.94%), α-Terpineol (23.22%) being the major constituents of the oil. Red thyme had a much lower percentage of α-Terpineol of 0.290, which plays a role in how effectively the oil can act as an insect repellent. The difference in percentages shows that Java citronella is more potent as a possible repellent over red thyme.
Conclusion: The study further provides evidence that plant essential oils can be explored as insect repellents for future pest management programmes as they comprise compounds that are known to be effective in preventing pest infestations.

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Introduction

Cymbopogon winterianus is a robust, aromatic, evergreen, clump-forming grass with numerous erect culms that can be up to 2.5 metres tall [26,27]. Citronella is a medicinal plant that belongs to the Poaceae family generally known for insect repellent properites [7,25], it is mainly cultivated in tropical and subtropical regions of America, Asia and Africa [7,11]. Studies have shown that the oil possesses a mixture of bioactive compounds such as monoterpenes and sesqueterpenes (Lorenzo et al., 2000) with antiinflammatory and antioxidant properties (Leite et al., 2010; [7]). *Thymus zygis* is a blossoming plant in the family Lamiaceae, the family is reportedly used as spices and medicinal herbs [23,29]. The high content of phenolic compounds such as thymol and carvacrol [3,28] is linked to medicinal properties such as antioxidnat, antimicrobial and insecticidal properties [16,31]. Essential oils have been observed in both *Thymus zygis* and *Cymbopogon winterianus.* Essential oils are commonly extracted through steam distillation, but other methods include expression, solvent extraction and absolute oil extraction ([8,19]).

Steam distillation is applied by extracting essential oils at temperatures near 100 °C, followed by condensation to form an immiscible liquid from which the essential oil can be separated in a clarifier [6]. Solvent extraction is the partial removal of a constituent from a solution or mixture by dissolving it in another immiscible solvent in which it is more soluble [18]. The plant is put into a bath of solvents during solvent extraction, which melts the plant material, forming the essential oils. Alcohol is then used to help separate the essential oils. This method of extracting essential oils is widely used in the perfume industry [18]. The expression method is used for extracting essential oils from the peels of citrus fruits because citrus oils do not hold up well when heat is used [18]. In this process, a machine is used to grind the seeds or fruits to squeeze out the oil or pierce the rind and peel of the fruit while it is rotating to extract the oil [30].

Lastly, absolute oil extraction is a method used in perfumery and aromatherapy. Absolutes are very similar to essential oils; they are concentrated, highly aromatic, oily mixtures extracted from plants. In this process, raw plant material is soaked under vacuum into a solvent. After this, the solvent is removed through evaporation [20]. Even though essential oils are less toxic than conventional chemicals, improper use of essential oils may cause harm, including allergic reactions and skin irritation, and children may be particularly susceptible to the toxic effects of improper use [5].

An essential oil consists of different compounds that make up the whole oil and to analyse this, gas chromatography-mass spectrometry (GC-MS) is used [2]. The primary purpose of any chromatographic process is to separate compounds to be studied independently from the others due to their complex composition. The process combines the features of gas chromatography (GC) and mass spectrometry (MS) to identify different substances in a test sample [32]. GC is another type of chromatographic method used in analytical chemistry for separating and analyzing compounds that can be vaporized without decomposition [2].

GC is limited because its operation relies on gases, so the molecules being separated must be gaseous [2]. In plants, most compounds, for example, sugars and pigments, are not volatile. Their boiling point is so high that they usually degrade due to high temperatures before becoming gases. However, essential oils are, by nature, composed of volatile molecules and so readily shift to a gas phase. This type of chromatography is thus the soundest choice to study essential oils. MS is an analytical method that measures the mass ratio of ions [2].

The results are presented as a mass spectrum applied to pure samples and complex mixtures [2]. GC-MS is used in essential oils to identify those compounds responsible for the characteristic, pleasant floral or aroma odor or taste of some valuable oils [2].

Other methods used to analyse constituents of essential oils include gas chromatography flame-ionization detection (GC-FID), which is the traditional method for essential oils quantification, while GC-MS is the most common analytical method for qualitative analysis. GC-FID is widely used for lipid analysis. An alternative to GC analysis is high-performance liquid chromatography (HPLC), a technique in analytical chemistry used to separate, identify, and quantify each component in a mixture. The main difference between the two methods is that HPLC uses a liquid mobile phase and GC uses a gas as the carrier [4].

In this study, GC-MS is used to identify the composition of red thyme (*Thymus zygis)* and Java citronella *(Cymbopogon winterianus)* essential oils responsible for the insecticide and pesticide effects on insects [27]. Since this kind of testing allows us to identify many volatile constituents, GC-MS can be used to detail constituents of an essential oil with their respective proportions. This, in turn, can be used to confirm a stated botanical origin. *Cymbopogon winterianus* is a robust, aromatic, evergreen, clump-forming grass with numerous erect culms that can be up to 2.5 m tall [27]. *Thymus zygis* is a blossoming plant in the family Lamiaceae. Examples include sage and mint [10]. Its leaves are thin and about 8 mm in length, and its mainly used as a herb [10].

Materials and methods

Gas chromatography-mass spectrometry (GC-MS)

Two essential oils were used, *Cymbopogon winterianus* (Java citronella) and *Thymus zygis* (red thyme). The plants were bought at Thitapoho farm (the geographical coordinates of the farm are 28°56′S; 26°19′E), and the essential oils were extracted through the steam distillation method. The essential oils were then taken to the University of South Africa for GC-MS. The essential oils were kept at low temperature in the dark during transportation to the laboratory and analyzed on arrival.

Table 1
Essential oil composition of Java citronella and Thymus zygis (red thyme).

Java citronella			Red thyme		
Retention time (min)	Compound name	% Area	Retention time (min)	Compound name	% Area
5.94	α-Pinene	10.31	7,76	α-Thujene	0,813
6.35	Camphene	0.20	8,00	α-Pinene	1149
6.91	3,5,5-Trimethylhexanal	7.73	8,62	Camphene	0,395
7.13	β-Pinene	2.54	10,23	β-Pinene	0,417
8.29	1,4-Cineole	0.22	11,81	β-Myrcene	2958
8.6	p-Cymene	0.34	12,86	α-Terpinene	0,736
8.75	D-Limonene	0.72	14,44	p-Cymene	22,154
8.83	1,8-Cineole (Eucalyptol)	0.38	15,98	γ-Terpinene	8429
9.33	3,5,5-Trimethylhexanol	14.61	16,14	*trans*-β-Ocimene	0,145
9.8	γ-Terpinene	0.13	16,95	α-Terpinolene	0,327
10.91	Terpinolene	1.89	18,62	S-(+)-Linalool	6192
13.45	Citronellal	15.94	20,14	Iso-Propyl–2-furan acetaldehyde	0,048
13.59	Isopulegol	1.77	20,89	2-Ethyl-5-methylfuran	0,144
13.96	Isoborneol	0.90	21,46	Terpinen-4-ol	0,243
14.45	Terpinen-4-ol	0.75	24,82	Thymol methyl ether	0,569
15.03	α-Terpineol	23.22	25,92	Carvacrol methyl ether	0,232
15.28	γ-Terpineol	4.03	26,37	α-Terpineol	0,290
16.6	Citronellol	1.92	29,82	p-Thymol	46,393
17.12	β-Citral (neral)	0.11	30,02	Carvacrol	2657
18.37	α-Citral (geranial)	0.10	30,93	Thymol acetate	0,107
21.7	α-Terpinyl acetate	0.44	32,54	Caryophyllene	1781
21.89	Citronellyl acetate	0.40	33,24	Alloaromadendrene	0,194
24.01	Longifolene	0.48	33,86	Humulene	0,084
24.61	(E)-β-Caryophyllene	0.37	34,58	p-Tert-butyl catechol	1037
			35,57	Ledene	0,174
			36,39	γ-Cadinene	0,095
			36,85	δ-Cadinene	0,260
Total		89.50	Total		98.03

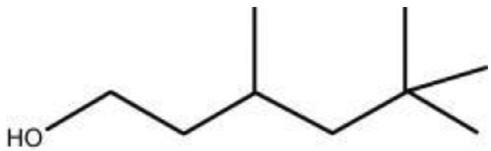

Fig. 1. Chemical structure of 3,5,5-Trimethyl-1-hexanol. ([22]. *Food and Chemical Toxicology).*

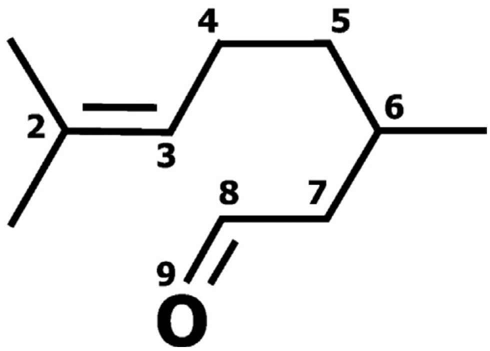

Fig. 2. Chemical structure of Citronellal. ([12]. *Physical Chemistry*).

No special safety measures were required. The two oils were selected based on their high affinity as potential repellents over other essential oils.

In order to characterize essential oils that were going to be used in the current study, GC-MS was carried out. Briefly, the oils were analyzed on an Agilent 7890B GC system coupled directly to a 5977 A mass spectrometer. A volume of 0.2 μL sample was diluted with n-hexane (2% v/v) and injected using a split ratio (100:1) with an autosampler at 24.79 psi and an inlet temperature of 220 °C. The GC system was equipped with an Agilent 19091S 433 UI: HP5-MS UI (30 m × 250 μm × 0.25 μm) column. The oven temperature was ramped from 60 °C to 280 °C at various rate increments.

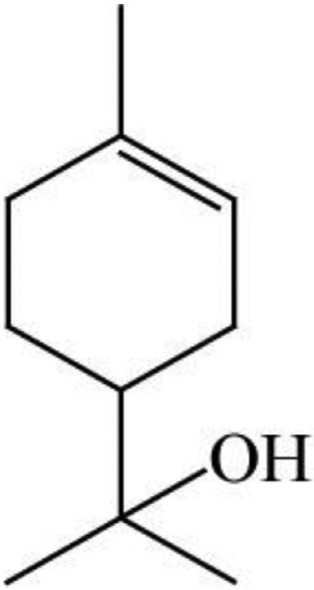

Fig. 3. Chemical structure of *a*-Terpineol. ([21]. *Open Chemistry*).

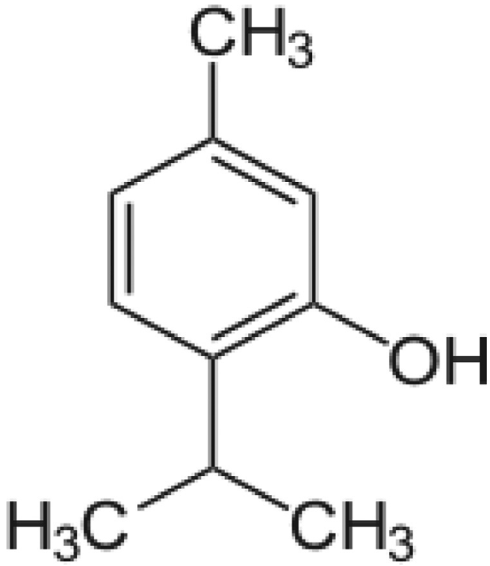

Fig. 4. Chemical structure of *p*-Thymol. ([9,17,24]).

Helium was used as carrier gas at a constant flow of 30 m/s. Spectra were obtained by electron impact at 70 eV, scanning from 40.5 to 415 *m/z*. The peak areas of the selected GC constituents were individually expressed as percentages of the total of all the total ion count (TIC) peak areas as determined by mass spectrometry detection (MSD, transfer line 240 °C; source 230 °C; MS Quad 150 °C) without using correction factors. The compounds were identified using the NIST11 and Agilent Flavor2 mass spectra libraries and by comparing peak retention times and mass spectra with data in the Adams' database [n-Pentadecane used as reference]. The use of this method and comparing them to the standards will reveal the purity of the oil.

Results and discussion

Table 1 of GC chromatogram results show different compounds of the two tested essential oils. Three compounds were found (Figs. 1 to 3) to be the significant constituents, accounting for 53.77% of the Java citronella essential oil, namely; 3,5,5-Trimethylhexanol ($C_9H_{20}O$) at 14.61%, Citronellal ($C_{10}H_{18}O$) at 15.94% and a-Terpineol ($C_{10}H_{18}O$) at 23.22%. Furthermore, for

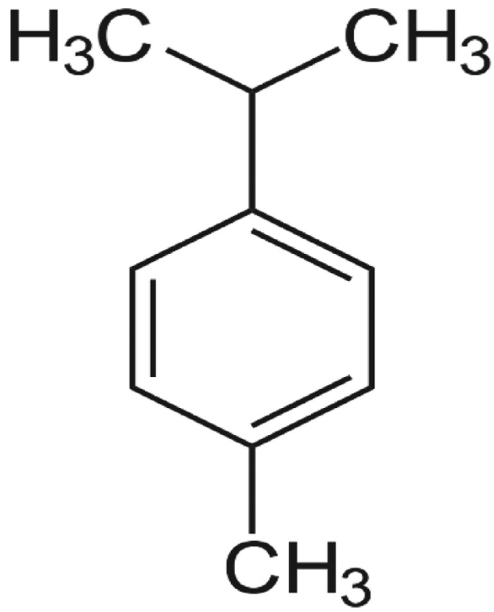

Fig. 5. Chemical structure of *p-Cymene*. ([9,24], pp.139, 597).

Table 2
Comparison with the international market (Java citronella).

ISO 3848:2016 Standard Market specifications (Chinese/Sri-Lankan oil)				
Compound name	Java type (commonly used) *Cymbobogon winterianus Jowitt*		Ceylon type *Cymopogon nardus rendle*	CUT EO-19–10–1
α-Pinene				10.31
3,5,5-Trimethylhexanal				7.73
β-Pinene				2.54
D-Limonene	2.0–5.0	1–10 (1–4)	9–11	0.72
3,5,5-Trimethylhexanol	14.61			14.61
Terpinolene				1.89
Linalool	0.5–1.5			
Citronellal	31.0–40.0	25–50 (32–45)	5–15	15.94
Isopulegol	0.5–1.7	1–10		1.77
α-Terpineol				23.22
Citronellol	8.5–14.0	10–25	6–8	1.92
β-Citral (neral)				0.11
Geraniol	20.0–25.0	10–25 (11–13)	18–20	
α-Citral (geranial)	0.3–1.0	Total Citral: 0.1–1		0.10
Citronellyl acetate	2.0–4.0	1–10	0.40	0.40
Eugenol	0.5–1.0	1–10		
Methyl isoeugenol			7–11	
Germacrene-D	1.5–3.0			
β-Elemene	0.7–2.5			
δ-Cadinene	1.5–2.5			
(E)-Geranyl acetate	2.5–5.5	1–10 (3–8)		
Elemol	1.3–4.8			

red thyme, two main compounds (Figs. 4 and 5) accounted for 68,45% of the oil: p-Cymene ($C_{10}H_{14}$) at 22.154% and p-Thymol ($C_{10}H_{14}O$) at 46.393%.

3,5,5-Trimethyl-1-hexanol is a fragrance ingredient used in cosmetics for fragrances, shampoos, soaps and household cleaners, and detergents. It is a colorless oily liquid with an oily-herbaceous texture [22]. This compound is responsible for giving the oil its unique fragrance [13]. Moreover, when this essential oil is inhaled it can cause lethargy, coma, ataxia, diarrhoea, piloerection, ptosis, chromorhinorrhea, infections in the kidneys, liver, intestines and eventually death [22].

The essential oil is of good quality due to its higher Citronellal concentration. Citronellal is a *monoterpenoid* aldehyde (Fig. 2). Aldehydes are usually irritating to the skin, and in the citronella oil, they are responsible for side effects related to

Table 3
Comparison with the international market (red thyme).

Compound name	ISO standard: 19,817:2017(E)	Market specifications: Spanish standard	CUT-21–1-1
α-Thujene	0.5–1.5		0,813
α-Pinene	0.5–2.5	1.0–3.0	1149
β-Myrcene	1–2.8		2958
α-Terpinene	0.9–2.6	15–28	0,736
p-Cymene	14.0–28.0	5–10	22,154
γ-Terpinene	4.0–13.0	4–6,5	8429
S-(+)-Linalool	0.5–6.5	0,2–2,5	6192
Terpinen-4-ol	0.1–2.5		0,243
Carvacrol methyl ether	0.1–1.5	36–55	0,232
p-Thymol	35.0–55.0	1–4	46,393
Carvacrol	0.5–5.5		2657
Caryophyllene	0.5–4.0		1781

skin inflammation when the pure oil comes in contact with the skin. Monoterpene aldehydes are Citronellal, and citral has a lemon-like smell that is very identifiable [1,14]. Citronellal is a primary isolate in distilled oils from the plants *Cymbopogon*, lemon-scented gum, and lemon-scented tea tree and has insect repellent properties against mosquitoes [15]. *α-Terpineol* is a terpene alcohol that is found in natural oils. Moreover, with other biological properties they present with (Fig. 4), *terpenoids* have shown promising insecticidal activities against anthropods.

In addition, Khaleel and colleagues [21] reported that a-Terpineol attracts a good interest as it has a wide range of biological applications as an antioxidant, anticancer, anticonvulsant, antiulcer, antihypertensive, anti-nociceptive compound. It is also used to improve skin penetration, and has remarkable insecticidal properties [21]. The study by Khaleel and colleagues [21] demonstrated a high percentage of a-Terpineol and this was tested to assess their repellent and insecticidal properties against *Aedes aegypti.* The authors found that a-Terpineol demonstrated repellent properties at a minimum effective dosage (MED) of 0.039 ± 0.008 mg/cm^2 [21]. Similar results relating to the presence of a-Terpineol were observed in the current study. Table 2 compares the same types of essential oils commonly used which are *Cymbobogon winterianus Jowitt* and Ceylon type *Cymopogon nardus rendle* with CUT EO-19-10-1essential oil presented in this table.

The results show that CUT-19-10-1 (*Cymbobogon winterianus*) had the highest percentage of a-Terpineol when compared to similar essential oils available in the international market, the compound is responsible for insecticide and repellent activity of an essential oil. This demonstrates that the oil can be used in future studies to assess insecticidal properties using contact toxicity, fumigant toxicity and repellence. Compared with red thyme (Tables 1 and 3), Java citronella presents a higher percentage of α-Terpineol of 23.22%, while red thyme is at 0,290%. This further indicates that Java citronella can be a better insect repellent than red thyme.

Conclusion and future directions

The application of GC-MS plays a vital role in analytical chemistry, particularly when it comes to natural-based products such as essential oils. With the increasing need of the world to “go green”, the application of essential oils, when properly grown, extracted and bottled, plays an important role in scientific research as some of the oils present profound healing properties that are antimicrobial, antifungal and antibacterial.

In this study, Java citronella and red thyme were used to illustrate some of the complex interactions that essential oils collectively can contribute to the repellent activity against insects. Many essential oil compounds have different modes of action such as insecticidal, feeding deterrence, repellence, growth inhibition and even attractant activities. When used in combination, they either reduce the concentration needed to achieve the desired effect, or the synergistic phenomenon can increase their effectiveness. Based on the results found in the study, the two essential oils (Java citronella and red thyme) had compounds that may have insecticidal properties. However, this will have to be assessed further to get evidence. It is recommended that the two essential oils should be further used to assess insecticidal properties using contact toxicity, fumigant toxicity and repellence in the future.

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Data availability statement

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

Disclaimer

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Declaration of Competing Interest

The authors declare that they have no financial or personal relationship(s) that may have inappropriately influenced them in writing this article.

CRediT authorship contribution statement

G.K. Sedikelo: Conceptualization, Data curation, Writing – original draft, Funding acquisition, Writing – review & editing. **G.G. Lenetha:** Conceptualization, Data curation, Writing – original draft, Funding acquisition, Writing – review & editing. **N.J. Malebo:** Conceptualization, Data curation, Writing – original draft, Funding acquisition, Writing – review & editing.

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