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Chemical analysis of Eucalyptus and Rosemary essential oils using gas chromatography-mass spectrometry (GC-MS) and evaluation of their toxicity against the most common urban pest

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ABSTRACT

Using plant essential oils (EOs) as a pesticide alternative has gained increasing interest as a promising strategy to reduce the harmful effects of chemical pesticides. This study aims to investigate the chemical composition of *Eucalyptus globulus* Labill and *Rosmarinus officinalis* L. essential oils and evaluate their impacts against *Blattella germanica* L. under laboratory conditions. The essential oils were prepared from dried leaves using hydro-distillation (HD) as a chemical extraction method. The gas chromatography-mass spectrometry (GC-MS) was employed to analyze and identify their chemical compounds. Bioassays were conducted using the standard contact method recommended by the World Health Organization, and the data were analyzed using the probit regression model. By GC-MS analysis, the major components included 1,8-cineole (50.67%), alpha-pinene (17.48%), limonene (4.26%) for eucalyptus and alpha-pinene (20.67%), camphor (10.69%), 1,8-cineole (9.38%), Borneol (9.02%), comphene (7.15%), and limonene (4.88%) for rosemary. The LD_{50} values were 9. 27, 10.54, and 3.23 %, and LD_{95} values increased to 27.2, 22.3 %, and 14.3% for rosemary, eucalyptus, and their mixture. The EOs mixture had a higher repellent effect with a repellency rate of 98.9% at a concentration of 3% compared to 93.3% and 90% at a concentration of 5% for rosemary and eucalyptus alone. The mixture of eucalyptus and rosemary EOs can be a promising alternative for controlling the German cockroach.

1. Introduction

Essential oils are a liquid that is chemically extracted from plants by different solvents. Essential oils are liquefied versions of plants such as *Eucalyptus globulus* Labill. and *Rosmarinus officinalis.* The extraction methods include steam distillation (SD), solvent extraction (SE), enfleurage, cold extraction (CE), and water distillation (WD). Also, pressure and temperatures affect the extraction procedure or sample preparation of essential oil.

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Steam distillation is a common method used for extracting essential oils from plants by chemistry methods b and analysis by UV-Vis, GC-FID, GC-MS, HPLC, LC-MS, solvent-microwave, and FTIR techniques. Solvent microwave extraction was also used to prepare essential oil [1,2]. The German cockroach (*Blattella germanica* L.) is one of the most important and common urban pests that can significantly threaten public health. This cockroach species exists in human environments, including restaurants, hotels, hospitals, and food storage areas. It can mechanically transmit disease-causing agents such as bacteria, viruses, fungi, protozoa, and parasite eggs to humans through their digestive system. Therefore, controlling this pest is of great importance. This cockroach species can also cause allergic reactions, including severe respiratory distress [3, 4]. The overuse of synthetic pesticides has led to the development of resistance in many populations of German cockroaches, highlighting the need for alternative pest control strategies [5]. On the other hand, insect resistance is one of the major consequences of pesticides in recent decades, resulting from excessive and indiscriminate chemical pesticides. Excessive use of pesticide compounds has led to resistance in various populations of health-importance pests and insect vectors. It has caused the emergence of insects resistant to various insecticides [6]. The high toxicity of chemical insecticides to humans and wildlife and environmental contamination has led to extensive research in recent years to introduce low-risk compounds for pest control [7-8]. Given the public concerns about chemical pesticides in residential areas, finding safe alternatives for urban pest control is a serious necessity [9]. GC–MS was used for essential oil analysis, and extracting, separating, and identifying essential oils (EOs) from a natural plant was studied. The chemical compounds in EOs were separated and analyzed by gas-chromatography-mass–spectrometry (GC–MS). The different chemical components are compared with two extraction methods [10]. An analytical chemistry method was used based on the identification and determination of linalool in essential oils. The internal standard method was used based on headspace solid phase microextraction (SPME) and gas chromatography–mass spectrometry (GC-MS). The experiments showed the effectiveness of the internal standard method based on the regression analysis of the calibration curve with perfect linearity (R2 ≥ 0.98) [11]. Over 20 plant essential oils and their components have been listed as low-risk pesticides by environmental protection agencies. They are exempt from registration requirements [12]. Rosemary and eucalyptus are two plants that have been studied in recent years for their insect toxicity and repellent effects against pests. Rosemary (*Rosmarinus officinalis* L.) from Lamiaceae is a perennial herb that grows as small evergreen shrubs in countries around the western Mediterranean Sea. The yield of rosemary essential oil by steam distillation is high, and it has a wide range of applications in the pharmaceutical, cosmetic, and food industries [1]. *Eucalyptus* spp., a genus belonging to the Myrtaceae family, has more than 800 different species, and although it originates from the continent of Australia, it has been grown in many tropical and subtropical regions due to its high adaptability and rapid growth for obtaining wood, gum, cellulose, and essential oils. Essential oils and extracts isolated from the aerial parts of this plant have been used in traditional medicine [13]. The use of essential oils as plant-based pesticides has numerous advantages. Firstly, complex mixtures of active compounds present in essential oils can often exhibit synergistic effects that can slow down pesticide-resistant growth in pest populations. Additionally, mixtures of two or more essential oil components have been shown to exhibit synergistic, additive, or antagonistic effects on various insects, including spiders, nematodes, house flies, plant mites, and moths. Synergistic toxic interactions are relevant to pest management and the development of effective natural product formulations, and they enable us to achieve higher mortality rates with lower amounts of active ingredients [14-15]. There are many plants' essential oil components that have insecticidal effects. Some essential oils contain compounds such as carvacrol, thymol, and eugenol, which have insecticidal properties. Other essential oils contain compounds like citronellal, geraniol, and limonene, known for their insect-repellent properties. In addition to these compounds, many essential oils contain other volatile organic compounds that can have insecticidal or insect-repellent properties. These include terpenes, aldehydes, ketones, and esters. The other components in the essential oils that contribute to their insecticidal properties depend on the specific plant from which the essential oil is extracted. Each plant has a unique combination of essential oil components contributing to its insecticidal properties. It's worth noting that the precise mechanisms by which these essential oil components exert their insecticidal effects are not always fully understood. Some compounds may interfere with the insect's nervous system, while others may disrupt its feeding or reproductive systems [16-19]. Although the insecticidal activity of plant extracts against numerous agricultural and storage pests and some medical pests has been demonstrated, few studies have been conducted on the German cockroach [1]. This study investigates the chemical composition of essential oils extracted from *R. officinalis* and *E. globulus* using gas chromatography-mass spectrometry (GC-MS). Furthermore, this study evaluates these essential oils' toxicity and repellent activity alone and in combination as an environmentally friendly method against the German cockroach (*B. germanica*). Essential oils as a pesticide alternative have become increasingly popular due to their environmentally friendly nature. By conducting this research, we aim to contribute to the development of safer and more efficient pest control methods using plant-based essential oils.

2. Materials and Methods

2.1. Instrument

A gas-chromatography-mass spectrometer (GC-MS) was used to analyze and identify rosemary and eucalyptus essential oil compounds (Hewlett-Packard 6890, Agilent Technology, Santa Clara, California, USA). The Clevenger-type apparatus was used for sample preparation of necessary oil extraction by two chemistry methods (Fig.1).

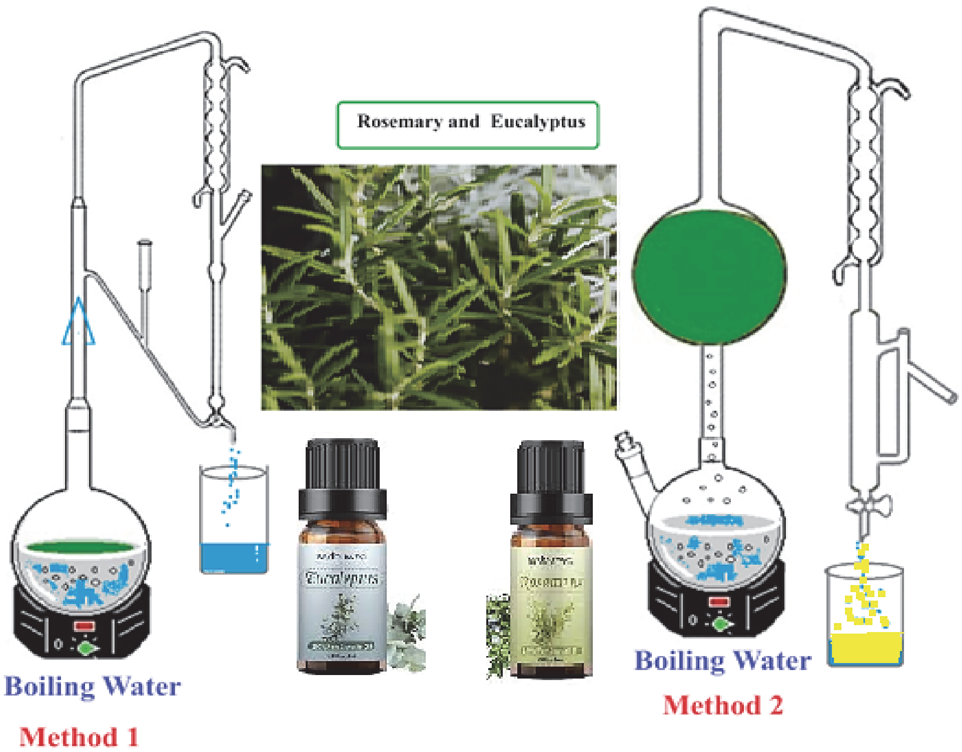

Fig.1. The Clevenger-type apparatus was used for sample preparation of essential oil by two extraction methods.

2.2. Plant collection

The fresh leaves of the *E. globulus* and *R. officinalis* plants were collected from the herbal garden of Chamran University in Ahvaz, Iran. Collected rosemary and eucalyptus specimens were identified by the Department of Horticultural Sciences at Chamran University in Ahvaz.

2.3. Extraction procedure of essential oil

Dry samples (100 g) of rosemary and eucalyptus were poured into a 1L flask. Then, 600 cc of deionized water was added to the flask and subjected to hydro-distillation in a Clevenger-type apparatus. In the Clevenger apparatus, rosemary and eucalyptus are mixed with water and boiled at 100^{0}C. After boiling, volatile components evaporated (in a position of steam distillation). In addition, the steam moved to a bed of rosemary and eucalyptus plants. In both methods, two layers of liquid and oil-rich phases were achieved. The oil phase was separated by separating funnels. Also, compounds in water are frequently distilled, water saturated with mixtures of rosemary and eucalyptus plants and their combinations are collected in the oil phase. The essential oil extraction process lasted approximately four h to 100 °C (Fig. 2). Then, the extracts were exsiccated by anhydrous sodium sulphate and stored in a dark glass vial at 4°C in a refrigerator for further experiment.

2.4. Rearing of the German cockroaches

The study was conducted on a susceptible strain of German cockroach kept in the Medical Entomology and Vector Control laboratory of Jundishapur University of Medical Sciences for one year. The cockroaches were reared in plastic containers with open mouths (Boucal) and fed on breadcrumbs, biscuits, and baguette crumbs. The containers were kept in a germinator at 27±3°C and 55±5% humidity under a 12:12 L: D.

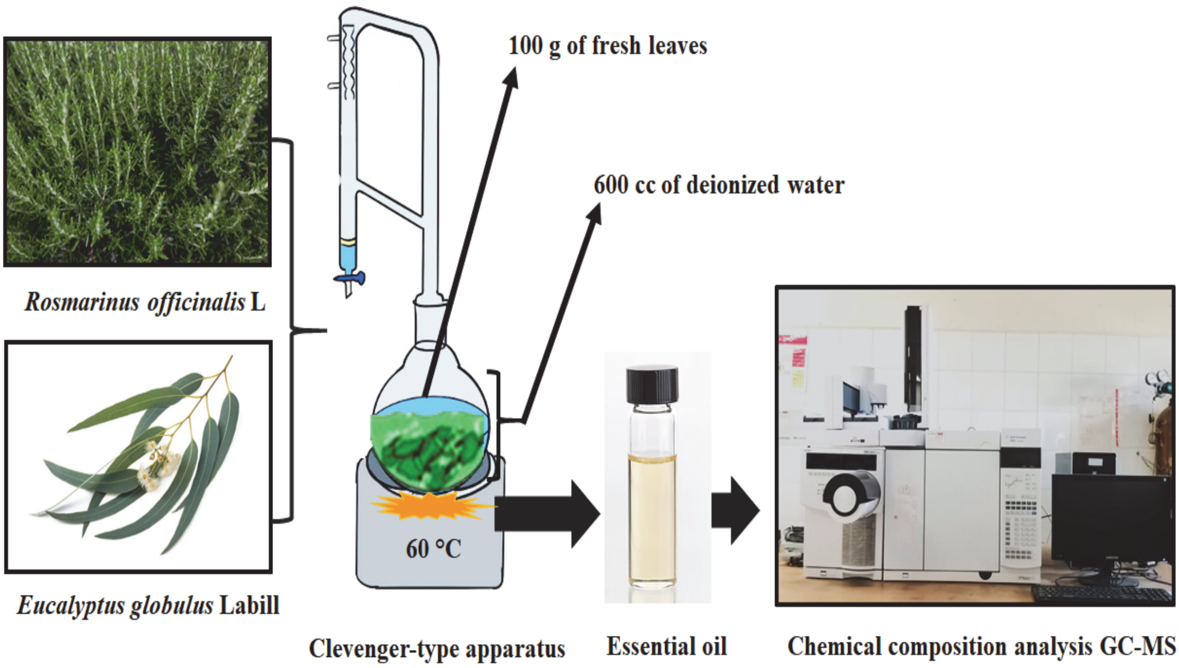

Fig. 2. The procedure of collecting plants, extracting, sample preparation, and GC-MS analysis of essential oils

2.5. Plant Analysis by Gas-chromatography-mass spectrometer (GC-MS)

GC-MS was used for EOs analysis (Fig. 2). It is equipped with HP–5MS column (30 m× 0.25 mm× 0.25 µm). The initial temperature used was 40 °C for 1 min and was later raised to 220 °C at a rate of 3 °C/min and finally raised to 270 °C for 5 min at a rate of 20 °C/min. Other parameters of the GC-MC machine included carrier gas Helium (99/999%), injector temperature (260 °C), detector temperature (FID, 270 °C), split-less mode, ionization potential of 70eV, scan rate of 1 scan/sec, the scan range of m/z 40–48 was used for all analysis. The essential oil constituents were identified by comparing their retention indices mass spectra fragmentation with those in a stored Wiley 7n.1 mass computer library and those of the National Institute of Standards and Technology (NIST).

2.6. Toxicity and Repellency assay

To assess the sensitivity of German cockroaches to the essential oils, the tarsal contact bioassay method recommended by the World Health Organization was used [20]. Different concentrations of eucalyptus and rosemary essential oils (5%, 7.7%, 10%, 14.1%, and 20%) were prepared using acetone as a solvent before analysis by GC-MS. Then, 2 mL of each concentration was applied to the inner surface of 500 mL glass jars. After the solvent evaporated, ten males or high-instar nymphs were released into each jar, and the jars were covered with mesh lids. The cockroaches were exposed to the treated surface for 30 minutes, transferred to clean jars, and kept at 27±2°C, 55±5% RH, and a 12:12 L: D. The control group was treated with acetone. The number of dead cockroaches was recorded after 24 hours. The bioassay was performed in four replicates. To evaluate the repellency of eucalyptus and rosemary essential oils against German cockroaches, plastic containers (17 × 10 × 5 cm) were used. The inner side of the container`s walls was lined with Vaseline to prevent the escape of cockroaches. The bottom of each container was covered with filter paper and divided into two equal parts by a line. Different concentrations (1%, 3%, 5%, and 7%) of each essential oil and their mixture in a 1:1 ratio was prepared with acetone [$(CH3)_2CO$] as solvent. One side of the filter papers was treated with 2 mL of each concentration. Ten adult cockroaches were released in the center of each container. They were kept under 27±2°C, 55±5% RH, and 12:12 L: D conditions. The containers were checked after 24 and 48 hours, and the number of dead and alive cockroaches in each half of the filter paper was recorded. The control group was treated with 2 mL of acetone. The test was performed in four replicates. The repellency percentage was calculated using Equation 1.

$$\text{Repellency}\ (\%) = 100 - (T \times 100)/N \qquad \text{(Eq.1)}$$

T: The number of cockroaches in the treated half of the filter paper.
N: The total number of released cockroaches.

2.7. Combined Insecticidal Effect

A proportion of eucalyptus (1:1) and rosemary essential oils was combined, and different concentrations (5%, 7.7%, 10%, 14.1%, and 20%) were prepared using acetone as a solvent. The contact bioassay method, as described above, was used, and the mortality of the cockroaches was recorded after 24 hours. The test was performed in four replicates.

2.8. Statistical GC-MS Analysis

Data were first corrected using the Abbott formula and mortality of the control group. Then, a probit regression analysis (dose-response analysis) was performed. Furthermore, the data were analyzed through ANOVA with mean comparisons and SPSS 24 software for statistical analysis.

3. Results and Discussion

3.1. GC-Mass Analysis

The GC-Mass analysis of eucalyptus and rosemary essential oils is represented in Table 1. The significant components of eucalyptus essential oil typically include 1,8-cineole (Eucalyptol, 1,3,3-Trimethyl-2-oxabicyclo[2.2.2]octane, 1,8-Cineole, 1,8-Epoxyp-menthane; 50.67%), α-pinene ((1S,5S)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene(−)-α-Pinene); 17.48%), Aromadendrene (1,1,7-Trimethyl-4-methylenedecahydro-1H-cyclopropa[e]azulene; 4.27%) and limonene (1-Methyl-4-(prop-1-en-2-yl)cyclohex-1-ene; 4.26%), while the major components of rosemary essential oil typically include alpha-pinen ((1S,5S)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene (−)-α-Pinene); 20.67%), verbenone (rel-(1R,5R)-Pin-2-en-4-one; 11.8%), camphor (1,7,7-Trimethylbicyclo[2.2.1]heptan-2-one; 10.69%), 1,8-cineole (9.38%), Borneol ((1R,2S,4R)-1,7,7-trimethylbicyclo[2.2.1]heptan-2-ol; 9.02%) and comphene (7.15%). Limonene in both essential oils has been almost the same (4.26 and 4.28%). In addition, chemical analysis of rosemary (*officinalis*) and eucalyptus (*globulus*) is shown in Figure 3.

3.2. Toxicity assay

The probit analysis also showed that the insecticidal effect or lethal doses of the mixture of rosemary and eucalyptus essential oils in the mean of LD_{50} and LD_{95} values decreased to nearly one-third compared to essential oils used separately. In other words, the results indicated the synergistic effect of combining these two essential oils (Table 2). The lethal dose values of 50% and 95% of rosemary and eucalyptus essential oils do not significantly differ due to the overlap of the confidence interval ranges. However, each has a significant difference with a mixture of EOs due to the lack of overlap in confidence intervals.

Table1. Constituents of *Rosmarinus officinalis* and *Eucalyptus globulus* essential oils by GC-MS analyses.

Rosmarinus officinalis (GC-MS)			Eucalyptus globulus (GC-MS)		
RT	***Major Constituents (%)***	***Components***	***RT***	***Major Constituents (%)***	***Components***
7.825	20.67	α-Pinene	7.819	17.48	α-Pinene
8.208	7.15	Camphene	9.015	0.79	β-Pinene
9.003	0.71	β-Pinene	9.461	1.05	β-Myrcene
9.318	2.39	3-Octanone	9.833	0.2	1-Phellandrene
9.456	2.45	β-Myrcene	10.565	4.26	Limonene
10.182	0.32	α-Terpinene	10.731	50.67	1,8-Cineole
10.423	1.82	p-Cymene	11.452	0.68	γ-Terpinene
10.554	4.88	Limonene	12.328	2.48	α-Terpinolene
10.634	9.35	1,8-Cineole	14.92	1.26	4-Terpineol
11.435	0.26	γ-Terpinene	15.309	1.99	α-Terpinolene
12.316	0.6	α-Terpinolene	20.31	0.17	δ-Cadinene
12.666	1.98	Linalool	21.265	1.29	α-Gurjunene
13.987	10.69	Camphor	21.838	0.2	Calarene
14.622	9.02	Borneol	22.015	4.27	Aromadendrene
14.834	0.93	Pinocamphone	23.394	1.47	Ledene
14.92	1.22	4-Terpineol	23.84	0.15	Naphthalene
15.326	2.13	β-Fenchyl	24.046	0.25	δ-Cadinene
15.893	11.8	Verbenone	25.122	0.39	Azulene
17.97	3	Bornyl acetate	26.387	0.61	γ-Gurjunene
19.44	0.23	3-Terpinolenone			
21.512	0.95	t-Caryophyllene			
22.364	0.18	α-Humulene			
25.483	0.29	Isocaryophillene			

RT: Retention time

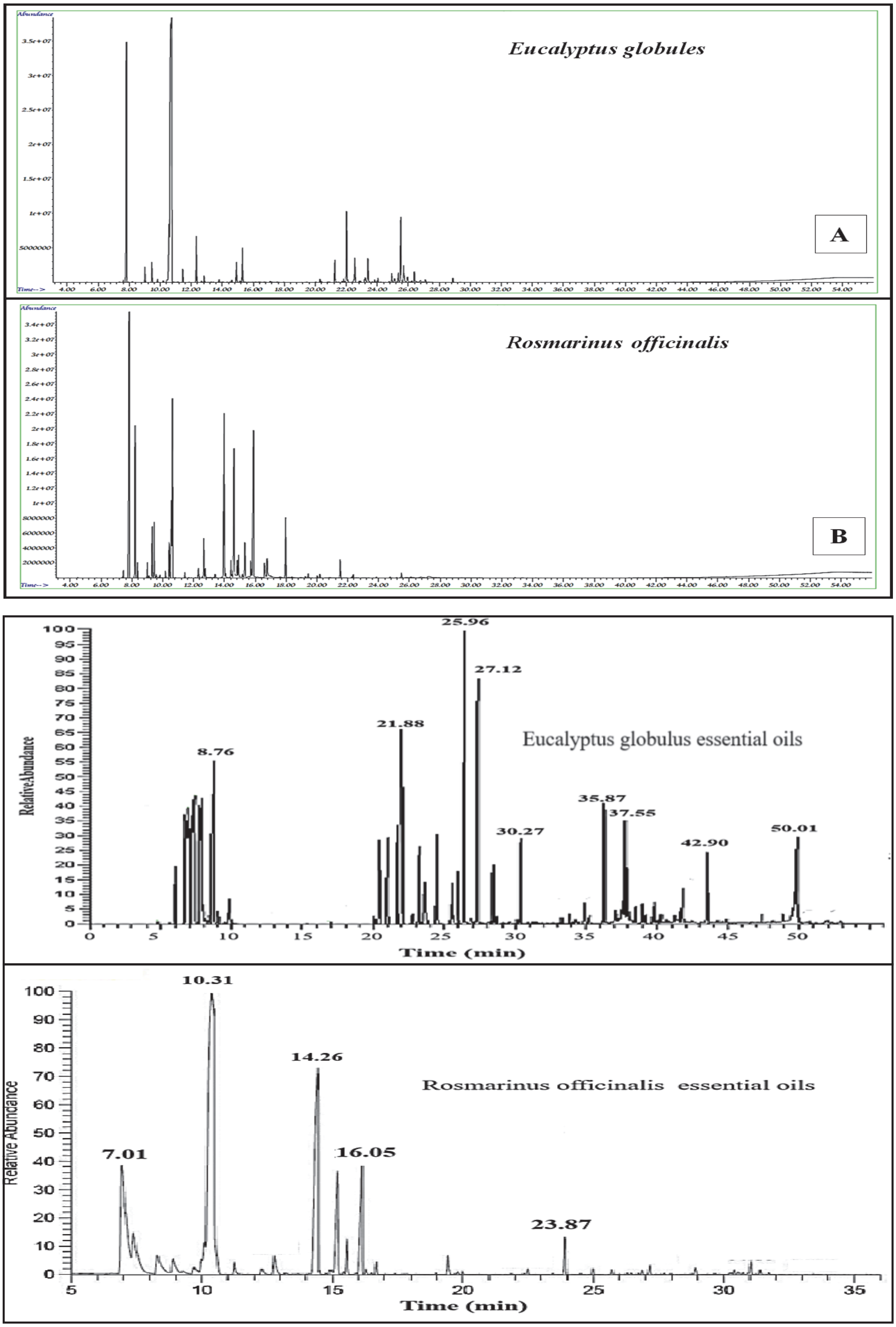

Fig. 3. GC-MS chromatogram based on chemical analysis for essential rosemary oil (Rosmarinus officinalis L.) and eucalyptus (Eucalyptus globulus Labill).

Table 2. Lethal doses of rosemary and eucalyptus essential oils and their mixture against *B. germanica* under laboratory conditions.

Essential oil	LD_{50} (Cl)	LD_{95} (Cl)	Slop (±SE)	N^2 (df)
Eucalyptus	10.54 (9.2 – 12.1)	27.2 (21 – 42.4)	3.9 ± 0.63	12.85 (13)
Rosemary	9.27 (8.11 - 10.51)	22.31 (17.9 – 32.2)	4.3 ± 0.63	6.41 (13)
Eucalyptus+ Rosemary	3.23 (0.83 – 4.85)	14.3 (10.3 – 31.14)	2.26 ± 0.67	8.73 (13)

The results of this insecticidal activity showed that the cockroach mortality rate ranged from 10% to 93% for rosemary and 8.6% to 90% for eucalyptus, depending on the concentration used in the study on susceptible German cockroach males by the contact surface method. The highest mortality effect for both essential oils was observed at a concentration of 20%. However, no significant difference in the percentage of cockroach mortality was observed between 14.1% and 20% concentrations of rosemary. Eucalyptus essential oil showed the highest effect at a concentration of 20%, with a mortality rate of 90%, while the lowest was at a concentration of 5%, with a mortality rate of 6.6%. The average mortality rate in the mixture of two essential oils ranged from 70% to 96.6%, indicating that the insecticidal effect of the mix of two essential oils at the same concentrations was much more significant than that of essential oils alone. The comparison of mortality means among different treatments revealed that significant differences were observed in the percentage of mortality means when using rosemary and eucalyptus essential oils individually. However, there was no significant difference in the mortality rate observed at different concentrations of the two essential oils when combined (Fig. 4).

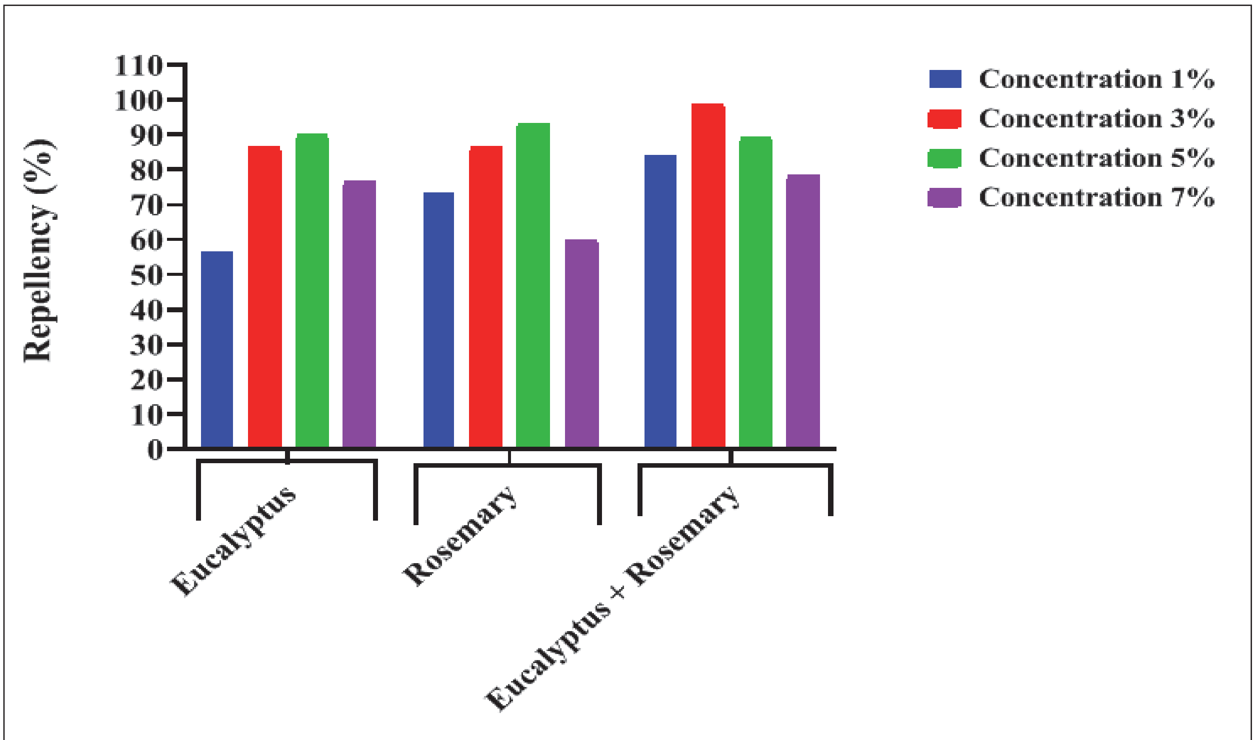

Fig. 4. Toxicity percentages of concentrations of eucalyptus, rosemary, and their mixture against B. germanica after GC MS analysis

As observed, there is a significant difference in the repellency percentages among eucalyptus, rosemary, and their mixture against *B. germanica* at concentrations of 1%, 3%, 5%, and 7% after 24 hours (P_{value}<0.0001). In the single essential oil treatments, the repellency rate increases from 1% to 5% concentration, with the highest repellency rate observed at 5% concentration (90 and 93.3% for eucalyptus and rosemary), followed by a decreasing trend at 7% concentration. However, in the mixed essential oil treatment, the repellency rate increases from 1% to 3% concentration. Then, it decreases to 7%, with the highest repellency rate at 3% concentration (98.9%). Therefore, it can be concluded that at equal concentrations of the three treatments, the mixed essential oils had a higher efficacy compared to the single essential oils. Additionally, the effective concentration for the mixed treatment was 3%. In contrast, the highest repellency rate for the single essential oil treatments was observed at a concentration of 5% (Fig. 5).

3.3. Discussion

Water distillation (WD) was used for extracting essential oils from plants as a chemistry procedure and analyzed by different methods such as UV-Vis, GC-FID, GC-MS, HPLC, LC-MS, and solvent-microwave. Solvent microwave extraction was also used to prepare essential oil [1,2]. Many extractions were obtained from different samples such as plants, human matrix, water samples by water distillation (WD), SPME, DSPME, LLE, DLLME. IL-SPE, and ultrasound assisted-dispersive ionic liquid-suspension SPE [21-27]. The German cockroach is a common household pest that can cause significant health problems for humans. Essential oils, as a natural alternative to synthetic insecticides, have gained popularity recently. In this study, we investigated the toxicity and repellency of eucalyptus and rosemary essential oils and their mixture against the German cockroach under laboratory conditions. The results showed that essential oils of rosemary and eucalyptus, grown in the climatic conditions of

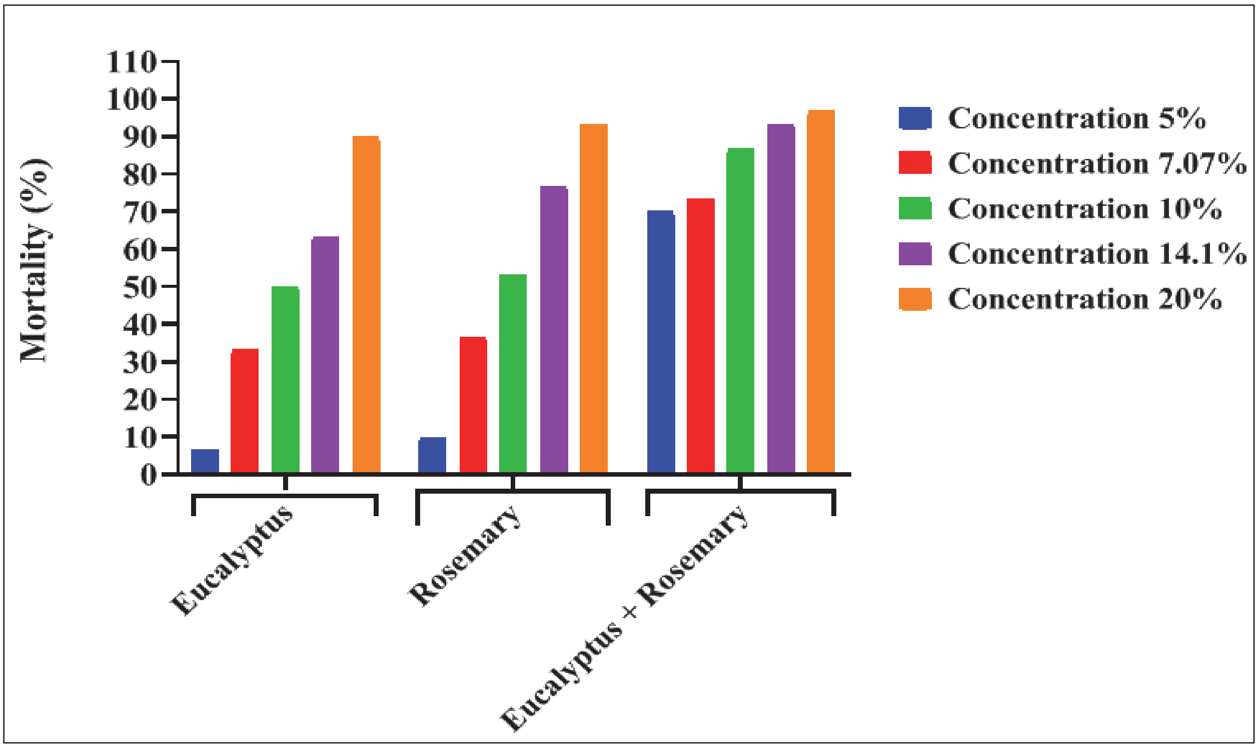

Fig 5. Repellency percentages of concentrations of eucalyptus, rosemary, and their mixture against B. germanica after GC MS analysis

Khuzestan province, have a suitable potential for controlling the German cockroach, especially when used in combination. The main components of eucalyptus essential oil detected by *GC-MS* were 1,8-cineole, α-pinene, and limonene. In contrast, the major components of rosemary essential oil include α -pinen, camphor, 1,8-cineole, borneol comphene and limonene. Limonene in both plant essential oils has been almost the same (4.26% and 4.28%). Gas chromatography-mass spectrometry (*GC-MS*) is a commonly used technique for analyzing plant essential oils. It can also detect the presence of adulterants or contaminants in essential oils, which can help ensure their purity and quality. 1,8-cineole, α-pinene, and limonene are three types of terpenes commonly found in various plants' essential oils. Each of these compounds has insecticidal or repellent properties to varying degrees1,8- .cineole, or eucalyptol, is a colourless terpene oxide commonly found in eucalyptus oil and other essential oils such as rosemary. It has been found to have insecticidal properties against various insects, including mosquitoes, flies, and cockroaches. Its mode of action is thought to be disrupting the insects' nervous system, leading to paralysis and death [12-20, 28]. α-pinene is a monoterpene that is commonly found in essential oils of pine trees, as well as in other plants such as rosemary and eucalyptus. It has been found to have repellent properties against several insect species, including mosquitoes and flies. Its mode of action is thought to be through blocking the olfactory receptors of the insects, making it difficult for them to locate their targets [29, 30]. Limonene is a monoterpene that is commonly found in essential oils of citrus fruits, as well as in other plants such as rosemary and peppermint. It has been found to have insecticidal properties against several insect species, including mosquitoes, fruit flies, and cockroaches. Its mode of action is thought to be disrupting the insects' cell membranes, leading to cell death [31]. Overall, these terpenes have shown promise as natural insecticides or repellents. However, their effectiveness may vary depending on the specific insect species and the concentration and formulation of the terpenes used. Further research is needed to understand their potential as alternatives to synthetic pesticides. Many studies have investigated the effectiveness of different essential oils against this cockroach species, all of which have indicated the high potential of these natural compounds for killing and repelling the German cockroach. The insecticidal and repellent properties of eucalyptus and rosemary essential oils against the American cockroach were also demonstrated, with a dose of 32.54 µg cm^{-2} and 35.97 µg cm^{-2} resulting in 50% mortality, which is comparable to the doses of chemical insecticides [1]. The repellent and insecticidal effects of eucalyptus and rosemary essential oils against the brown-banded cockroach, *Supella longipalpa,* were demonstrated, with rosemary essential oil causing 100% mortality and 94.5% repellency at concentrations of 2.5% to 30%. Eucalyptus essential oil causes 100% mortality with 27.7% to 51.7% repellency [9]. Clove oil, thyme oil, and peppermint oil had potent insecticidal activity against German cockroaches, with mortality rates ranging from 82% to 100% [32]. Another study found that essential oils from lavender, peppermint, and tea trees had insecticidal activity against German cockroaches, with mortality rates ranging from 70% to 100% [33]. In addition to their insecticidal properties, essential oils have also been found to have repellent effects against German cockroaches. Essential oils from catnip and Osage orange had strong repellent effects against German cockroaches, with repellency rates ranging from 82% to 100% [34]. Other essential oils that have insecticidal or repellent effects against German cockroaches include cinnamon, lemongrass, and eucalyptus [35, 36]. However, the effectiveness of these oils may vary depending on factors such as the concentration and formulation of the oil, as well as the specific strain of cockroaches being targeted. The combination of eucalyptus and rosemary essential oils had a synergistic effect on the German cockroach, with the LC_{50} and LC_{95} values decreasing significantly to one their compared to individual essential oils, with an LC50 value of 3.23% and 14.3%. Some essential oils have shown a synergistic effect when combined with other compounds, which can increase their insecticidal activity against German cockroaches. One study investigated the synergistic effect of thymol, a compound found in thyme essential oil, and piperonyl butoxide, a common insecticide synergist, against German cockroaches. The combination of thymol and piperonyl butoxide had a greater insecticidal effect than either compound alone, with mortality rates of up to 100% [37]. Another study investigated the synergistic effect of eucalyptus oil and limonene, a compound found in citrus fruits, against German cockroaches. The combination of eucalyptus oil and limonene had a synergistic effect on the fumigant toxicity of the oils against German cockroaches, increasing their mortality rates [38]. In addition, the synergistic effect of essential oils from five different plants, including peppermint, cinnamon, lemongrass, and piperonyl butoxide, against German cockroaches had a synergistic effect on the insecticidal activity against German cockroaches, with mortality rates ranging from 81% to 100% [39, 40]. The use of essential oils in combination with synergists has shown promise as an effective approach to control German cockroaches. However, further research is needed to fully understand the mechanism of action and optimize the formulation and application of these compounds [39, 40].

4. Conclusion

Overall, plant essential oils analyzed with GC-MS have shown promise as natural insecticides and repellents against German cockroaches. Our findings suggest that the combination of eucalyptus and rosemary essential oils has a potent insecticidal effect against the German cockroach and can be an effective natural alternative to synthetic insecticides. The synergistic effect of the two essential oils can be attributed to their different chemical compositions, determined by GC-MS after sample preparation method and act on various targets in the insect's body. Further studies are needed to evaluate the effectiveness of the essential oil combination against other insect pests and to investigate the safety and practicality of using essential oils as insecticides on the field scale.

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6. Conflicts of Interest

The authors declare that there is no conflicts of interest.

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