ORIGINAL ARTICLE

Chemical Composition and Synergistic Repellent Activity of Jasminum officinale and Anthemis nobilis Essential Oils Against Aedes aegypti Mosquitoes

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ABSTRACT

Introduction: Mosquitoes are important vectors responsible for transmission of many pathogens that cause major human morbidity and mortality. Natural repellents such as essential oils may provide a means of protection from mosquito bites that are safe and more pleasant to use. **Methods:** In this study, essential oils from the flowers of *Jasminum officinale* and *Anthemis nobilis* were characterised by gas chromatography-mass spectrometry and were further tested for their repellent efficacy, individually and in combinations, against *Aedes aegypti* using a rat model. **Results:** Forty-two compounds accounting for 77.50% of *J. officinale* essential oil and fifty-one compounds representing 87.96% of *A. nobilis* essential oil were identified. Oxygenated monoterpenes and diterpenes constituted 31.14% and 21.20% of *J. officinale* essential oil, respectively. For *A. nobilis* essential oil, oxygenated monoterpenes accounted for 84.79% of the compounds identified. The essential oils of *J. officinale* and *A. nobilis* at 20% concentration provided repellency of 68.45% and 73.15%, respectively, against *Ae. aegypti* for 120 min. The mixture of essential oils in a 1:1 ratio (JC1) exhibited 88.20% repellency for 120 min, which was significantly higher than the repellency of 20% concentration of the individual oils. **Conclusion:** The synergistic interactions among the varied constituents of *J. officinale* and *A. nobilis* essential oils enhanced the mosquito repellent activity.

Keywords: Essential oil, *Jasminum officinale*, *Anthemis nobilis*, Repellent efficacy, *Aedes aegypti*

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INTRODUCTION

Essential oils are complex mixtures of volatile organic compounds that contribute to the flavour and fragrance of a plant. They also act as a repellent/deterrent against phytophagous insects (1,2). Therefore, plants have been used for protection against insects or other pests. For instance, plant materials are burnt to produce smoke or bruised plants are placed in homes, and also plant oils or oil mixtures are applied onto the skin for protection from biting insects, particularly mosquitoes (3–5).

Dengue fever is an arboviral infection caused by a flavivirus transmitted by *Aedes aegypti* or *Aedes albopictus* mosquitoes (6). The World Health Organisation (WHO) reported the global occurrence of dengue has expanded over the last 50 years and estimated about 50 to 100 million new infections happen every year, with around 20,000 deaths (7). A more recent study has revealed an estimation of 390 million dengue infections to occur each year using cartographic approaches (8). Personal protection plays a crucial role in preventing mosquito bites and thereby reducing the risk of infection. DEET (N,N-diethyl-3-methylbenzamide)-based insect repellents have been used globally for more than 60 years. Despite its wide usage for protection against mosquito bites and its proven effectiveness, DEET comes with several drawbacks. For example, it emits an unpleasant odour and it was found to damage synthetic fabrics, plastics and painted surfaces which prevents its use in bed nets and in many urban locations (9,10). Besides that, instances of DEET resistance have been shown in mosquitoes (11,12), as well as in flies (13). DEET also blocks sodium and potassium ion channels of mammals, which may contribute to lip numbness (14).

In recent decades, plant-based insect repellents have gained increasing popularity among consumers. For example, lemon eucalyptus (*Corymbia citriodora*) is an effective natural repellent against several species of mosquitoes and ticks (15). The essential oil from *C. citriodora* contains p-menthane-3,8-diol (PMD), citronella, citronellol, geraniol, isopulegol, and delta pinene (16). Protection against Anopheles by PMD was found to be comparable to that of DEET. Therefore, its use is advocated by the Centers for Disease Control (CDC) for use in malaria endemic areas (17,18). However, blends of pure compounds were shown to be less effective at repelling mosquitoes compared to their corresponding essential oil (19–21).

The flowers and leaves of jasmine are well-known for their multiusage. For instance, the flowers were used conventionally for the treatment of various ailments such as diarrhoea, fever, conjunctivitis, abdominal pain, dermatitis, eye diseases etc. (22,23). Chamomile is commonly used in herbal teas to relieve spasms or inflammatory disorders related to the gastrointestinal tract (24), and it also is used to relieve sleeping disorders, diarrhoea, colic, wounds, mucositis, and eczema (25,26). The mosquito repellent studies of jasmine were reported concerning the essential oil derived from *Jasminum grandiflorum* L. (27–29). The chemical composition of Roman chamomile essential oil has been determined previously (30–32), and only three studies have evaluated its mosquito repellent activity to date (27–29).

To our knowledge, this study reports for the first time the chemical composition and mosquito repellent activity of *Jasminum officinale*. Moreover, it is interesting to evaluate the repellent efficacy of *J. officinale* in combination with chamomile, the plant renowned for its enormous medicinal properties (33). Furthermore, chemical compounds such as champene, α-pinene, β-pinene and 1,8-cineole found in *A. nobilis* have been shown to repel mosquitoes (19,34,35). Thus, *A. nobilis* can be a good candidate for mosquito repellent study. Therefore, the goal of this study was to determine the chemical composition of jasmine (*Jasminum officinale* L.) and Roman chamomile (*Anthemis nobilis* L.) essential oils and to evaluate their repellent efficacy, individually and in combinations, against *Ae. aegypti* using a rat model.

MATERIALS AND METHODS

Test Materials

Essential oils extracted from the flowers of *J. officinale* and *A. nobilis* were purchased from Renaissance of Switzerland (Singapore). Fractionated coconut oil was purchased from doTerra (Pleasant Grove, Utah, USA), and DEET was obtained from the Vector Control Research Unit at Universiti Sains Malaysia (USM, Penang, Malaysia).

Essential Oil Analysis

The essential oils were analysed by gas chromatography-mass spectrometry (GC-MS) on an Agilent 7890 GC instrument coupled with an Agilent 5975c mass specific detector (Santa Clara, CA, USA). Separation was performed on a 5% phenyl methyl silicon capillary column HP-5MS (30 m x 0.25 mm ID and 0.25 μm film thickness). The initial oven temperature was set at 40 °C for 10 min and it was raised to 230 °C at 5 °C/min and to 300 °C at 20 °C/min. This temperature was held for 10 min. Helium was used as the carrier gas at a constant flow rate of 1 mL/min. Electron impact mass spectra were recorded in the 30–800 amu range at 71 eV ionisation energy, and ion source temperature was 230 °C. Essential oils were diluted (1:10, v/v) with methanol and filtered through a 0.22 μm membrane. The injector was set at 230 °C, and the samples were run in split mode (50:1) with an injection volume of 2 μL. Retention indices of the components were determined by a series of n-alkanes C_7-C_{30} on the HP-5MS column. The compounds were identified by comparison of their mass spectra with those from the NIST 2005 Mass Spectral Library and their retention indices with those available in the literature (36,37).

Repellent Test

Experimental animals

Twenty-four female Sprague Dawley rats (8 weeks old) were obtained in batches from the Animal Research Section, Advanced Medical and Dental Institute of USM. The animals were acclimatised for 1 week to the new environment, and they were kept three per ventilated cage with a 12:12 (light: dark) cycle and a continuous supply of food and water. The rats were shaved on the ventral surface prior to the experiment. All procedures involving the use of animals were in compliance with the global and national rules and guidelines, and they were approved by the Institutional Animal Care and Use Committee at our institution (Approval/2013/(90)(508)).

Mosquitoes

Adult female *Ae. aegypti* mosquitoes (5–7 days old) were acquired from the Vector Control Research Unit at USM. Mosquitoes were reared in the laboratory at 27 ± 2°C and 75–80% relative humidity in a 12:12 (light: dark) photoperiod. Adult mosquitoes were maintained in cages measuring 30 cm x 30 cm x 30 cm and fed with 10% sucrose solution in water. The sucrose solution was removed from the cage at least 12 h before testing.

Preparation of the oils

Fractionated coconut oil served as the negative control, and 10% DEET prepared in absolute ethanol was used as the positive control. The essential oils were diluted to the concentrations of 5%, 10%, and 20% (v/v) in fractionated coconut oil to assess their repellent properties. The concentration that showed good repellent activity was used in the preparation of oil mixtures. The oil mixtures JC1, JC2, and JC3 were prepared with 20% *J. officinale* and *A. nobilis* at the ratio of 1:1, 2:1, and 1:2, respectively.

Mosquito repellent efficacy of essential oils using a rat model

The evaluation method used was adapted from previous study with some modifications (38). Briefly, the rat was confined in a customised wire mesh cage. Subsequently, 0.5 mL of test substance was applied onto a 2 x 6 cm hairless area of the rat abdomen using a camel hairbrush. The application was left to dry for 30 min. Thereafter, the rat was placed on top of the mosquito cage (30 cm x 30 cm x 30 cm) containing 50 nulliparous female *Ae. aegypti* mosquitoes for the first 5 min of every half-hour interval. Before the test was conducted, the mosquitoes were tested for their readiness to feed. An untreated rat was placed on top of the mosquito cage, and the test was conducted if at least two mosquitoes bit the untreated rat. The number of mosquitoes biting at the marked area was recorded at each interval for a duration of 2 h. The experiment was performed in triplicate with a new batch of mosquitoes on alternate days. The percentage of repellency was determined using the number of mosquitoes biting at the control and treated areas using the following formula:

$$\text{Percentage of repellency (\%)} = \left[\frac{C - T}{C}\right] \times 100$$

where C is the total number of mosquitoes biting at the control area and T is the total number of mosquitoes biting at the treated area.

Statistical Analysis

All experiments were repeated three times, and the data were expressed as mean ± standard error of the mean (SEM). Differences between the treatment means were analysed using analysis of variance (ANOVA) with Tukey's multiple comparison test. Differences were considered significant at $p < 0.05$. All statistical analyses were performed using IBM SPSS Statistics, Version 24.0 (Armonk, NY, USA).

RESULTS

Chemical composition of J. officinale and A. nobilis essential oils

The chemical compositions of *J. officinale* and *A. nobilis* essential oils were characterised by GC-MS. Forty-two compounds representing 77.50% of jasmine oil and fifty-one compounds representing 87.96% of chamomile oil were identified (Table I and Table II). The chemical constituents of *J. officinale* varied entirely from those present in *A. nobilis*. Oxygenated monoterpenes were the major constituents (84.79%) of *A. nobilis*, whereas they accounted for only 31.14% of the total found in *J. officinale*. Fig. 1 and Fig. 2 show the total ion chromatogram of *J. officinale* and A. nobilis, respectively.

Synergistic mosquito repellent activity of combined essential oils

Table III shows the repellency of the essential oils, individually and in combinations, at every half-hour interval. The *J. officinale* essential oil provided repellency of 13.69%, 27.38%, and 68.45% against *Ae. aegypti* at concentrations of 5%, 10%, and 20%, respectively, at

Table I: Chemical composition of the essential oil of *J. officinale*

No.	RT (min)	Compound[a]	RI[b]	Composition (%)
1	17.44	4-Hexenyl acetate	1017	0.03
2	18.53	Benzyl alcohol	1044	2.38
3	20.12	Benzyl formate	1084	0.05
4	20.30	p-Cresol	1089	0.25
5	20.73	Methyl benzoate	1099	0.07
6	21.05	Linalool	1109	2.76
7	23.31	Benzyl acetate	1177	11.50
8	24.10	Methyl salicylate	1201	0.04
9	27.05	Indole	1302	0.60
10	28.40	Methyl anthranilate	1352	0.07
11	28.84	Eugenol	1369	1.96
12	29.95	*cis*-Jasmone	1411	1.80
13	31.27	Geranyl acetone	1463	0.05
14	31.49	2,6,10,14-Tetramethylheptadecane	1472	0.03
15	32.67	α-Farnasene	1520	0.56
16	34.00	(±)-*trans*-Nerolidol	1576	0.11
17	34.17	*cis*-3-Hexenyl benzoate	1584	0.85
18	34.83	2-Diacetylaminobenzoic acid, methylester	1612	0.46
19	35.94	Methyl jasmonate	1662	0.63
20	38.61	Benzyl benzoate	1786	8.14
21	40.11	Hexahydrofarnesyl acetone	1859	0.45
22	40.63	Benzyl salicylate	1885	0.10
23	41.73	Methyl hexadecanoate	1941	1.20
24	42.22	Isophytol	1966	5.39
25	42.56	α-Springene	1984	0.03
26	43.03	Ethyl hexadecanoate	2008	0.05
27	43.75	Geranyl linalool	2048	2.72
28	44.92	Methyl linoleate	2111	0.27
29	45.05	Methyl linolenate	2119	3.01
30	45.27	Phytol	2131	2.46
31	45.49	Methyl octadecanoate	2144	0.18
32	46.11	Ethyl 9,12-octadecadienoate	2179	0.07
33	46.23	Ethyl linolenate	2186	0.10
34	47.23	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	2244	12.31
35	48.48	Methyl *cis*-11-eicosenoate	2325	0.16
36	49.12	4,8,12,16-Tetramethylheptadecan-4-olide	2378	0.23
37	49.29	2,6,10,14,18-Pentamethyl-2,6,10,14,18-eicosapentaene	2392	0.15
38	50.77	Diisooctyl phthalate	2576	0.48
39	52.07	Benzyl linolenate	2803	1.69
40	52.44	*trans*-Squalene	2868	5.76
41	53.12	2,3-Oxidosqualene	2979	6.80
42	53.67	1,6,10,14,18,22-Tetracosahexaen-3-ol, 2,6,10,15,19,23-hexamethyl-, (all-E)-	3068	1.55
		Total identified (%)		77.50
		Grouped components (%)		
		Monoterpene hydrocarbons (9)		0.60
		Oxygenated monoterpenes (1-8, 10-13, 17-20, 22)		31.14
		Sesquiterpene hydrocarbons (15)		0.56
		Oxygenated sesquiterpenes (16, 21, 23, 26, 28-29, 31)		7.99
		Diterpene hydrocarbons (14, 25)		0.06
		Oxygenated diterpenes (24, 27, 30, 32-36, 38)		21.20
		Other constituents (37, 39, 40-42)		15.95

Notes: RT, retention time. [a]Compounds are listed in order of their elution time from a HP-5MS column. [b]RI, retention indices are determined on HP-5MS column using a series of *n*-alkanes (C_7-C_{30}).

Table II: Chemical composition of the essential oil of *A. nobilis*

No.	RT (min)	Compound[a]	RI[b]	Composition (%)
1	7.96	3-Methyl-1-pentanol	843	0.41
2	10.18	Isopentyl acetate	881	0.13
3	12.71	Isobutyl isobutyrate	926	0.40
4	13.44	1R-α-pinene	939	1.44
5	13.71	2-Bromo-2-methylbutane	944	0.31
6	14.18	Camphene	953	0.51
7	14.70	Butyl propyl oxalate	962	0.04
8	15.50	Allyl acetone	977	0.12
9	15.65	β-pinene	979	0.17
10	16.83	Isopropyl 3-methyl-2-butenoate	1001	0.47
11	17.20	Isobutyl 2-methylbutanoate	1011	0.06
12	17.66	Isopentyl isobutyrate	1022	1.09
13	17.81	2-Methylbutyl isobutyrate	1026	1.45
14	18.20	1,8-Cineole	1036	0.30
15	18.80	3-Methylbutyl cyclopropanecarboxylate	1051	0.99
16	19.26	Ethyl 2-methyl cyclopropanecarboxylate	1062	3.10
17	19.49	*cis*-2-pentenyl butyrate	1068	0.23
18	19.92	Cyclohexyl propyl oxalate	1079	11.22
19	20.12	Hexyl propionate	1084	0.38
20	21.03	Isopentyl 2-methylbutanoate	1108	0.27
21	21.15	2-Methylbutyl 2-methylbutanoate	1112	0.22
22	21.67	Hexyl isobutyrate	1128	9.43
23	21.87	α-Campholenal	1134	0.15
24	22.31	*trans*-Pinocarveol	1147	3.93
25	22.43	(-)-Camphor	1151	0.04
26	22.60	Hexyl methacrylate	1156	2.39
27	22.78	Isopentyl 3-methyl-2-butenoate	1161	7.76
28	22.92	Pentyl 3-methyl-2-butenoate	1165	5.96
29	23.09	Pinocarvone	1170	3.25
30	23.24	(-)-Borneol	1175	0.33
31	23.46	*cis*-Pinocamphone	1182	0.19
32	23.85	p-Cymen-8-ol	1193	0.15
33	24.15	Myrtenol	1203	0.55
34	24.45	Hexyl 3-methylbutanoate	1213	1.68
35	24.54	Verbenone	1216	0.23
36	24.91	*cis*-Carveol	1229	0.21
37	25.50	(-)-*trans*-Pinocarvyl acetate	1249	0.47
38	25.74	2-Hydroxy-2-methyl-but-3-enyl 2-methyl-2(Z)-butenoate	1257	0.17
39	26.07	Hexyl 3-methyl-2-butenoate	1268	26.02
40	26.75	Isopentyl 3-hydroxy-2-methylenebutanoate	1291	0.40
41	29.09	(+)-Cycloisosativene	1378	0.15
42	29.35	Copaene	1387	0.21
43	31.13	*cis*-Pinonic acid	1458	0.06
44	31.39	β-Farnesene	1468	0.09
45	31.61	(-)-Myrtenyl acetate	1477	0.12
46	32.05	α-Curcumene	1494	0.09
47	32.16	β-Selinene	1499	0.27
48	34.51	Caryophyllene oxide	1598	0.17
49	35.13	Humulene epoxide II	1626	0.25
50	36.87	4-(1,5-Dimethyl-4-hexenyl)-2-cyclohexen-1-one	1704	0.06
51	40.11	Hexahydrofarnesyl acetone	1859	0.14
		Total identified (%)		87.96
		Grouped components (%)		
		Monoterpene hydrocarbons (4-6)		1.95
		Oxygenated monoterpenes (1-3, 7-40, 45, 50)		84.79
		Sesquiterpene hydrocarbons (41-42, 44, 46-47)		0.51
		Oxygenated sesquiterpenes (48-49, 51)		0.65
		Other constituents (43)		0.06

Notes: RT, retention time. [a]Compounds are listed in order of their elution time from a HP-5MS column. [b]RI, retention indices are determined on HP-5MS column using a series of *n*-alkanes (C_7-C_{30})

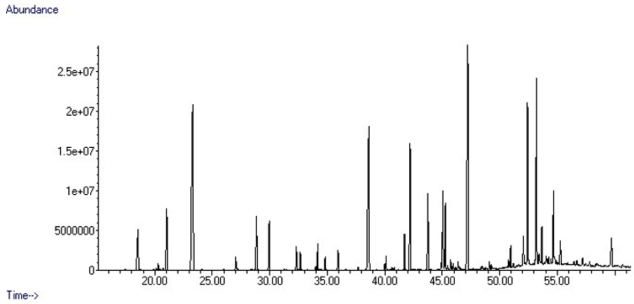

Figure 1: Total ion chromatogram of Jasminum officinale

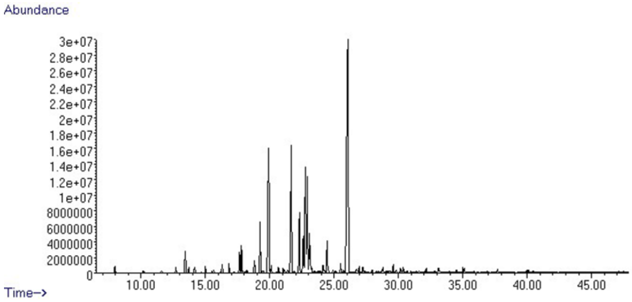

Figure 2: Total ion chromatogram of Anthemis nobilis

Table III: Repellent efficacy of *J. officinale* and *A. nobilis* essential oils tested individually and in combinations against *Ae. aegypti* mosquitoes.

Treatment	Repellency (%) ± SEM at half-hour interval (min)			
	30	60	90	120
J. officinale essential oil				
5%	51.52 ± 1.52^{a}	41.48 ± 1.48^{a}	35.56 ± 2.22^{a}	13.69 ± 0.60^{a}
10%	71.46 ± 2.49^{b}	55.19 ± 2.89^{b}	39.26 ± 3.23^{a}	27.38 ± 1.19^{b}
20%	100^{c}	86.30 ± 3.16^{c}	80.37 ± 1.61^{b}	68.45 ± 2.98^{c}
A. nobilis essential oil				
5%	65.48 ± 2.98^{a}	52.80 ± 5.14^{a}	46.33 ± 4.30^{a}	34.26 ± 5.63^{a}
10%	86.90 ± 0.60^{b}	81.16 ± 2.36^{b}	69.80 ± 1.75^{b}	53.70 ± 1.85^{b}
20%	100^{c}	88.20 ± 1.28^{b}	83.57 ± 2.71^{c}	73.15 ± 3.34^{c}
Essential oil mixtures				
JC1	100^{a}	100^{a}	90.19 ± 0.81^{a}	88.20 ± 1.28^{a}
JC2	100^{a}	87.41 ± 2.06^{b}	80.37 ± 1.61^{b}	73.07 ± 2.39^{b}
JC3	100^{a}	100^{a}	84.07 ± 2.59^{a}	76.40 ± 2.57^{b}
Negative control				
Fractionated coconut oil	17.17 ± 0.51	16.56 ± 1.75	17.42 ± 4.61	18.24 ± 2.94
Positive Control				
10% DEET	100	100	100	100

Notes: Data shown are the mean values ± SEM of three independent experiments. Statistical analysis was performed using ANOVA with Tukey multiple comparisons test. Mean values in each treatment group within a column followed by a different letter are significantly different at $p < 0.05$. JC1, JC2 and JC3 are composed of 20% of *J. officinale* and *A. nobilis* at the ratio of 1:1, 2:1 and 1:2 respectively.

120 min. Values for *A. nobilis* were 34.26%, 53.70%, and 73.15% at 5%, 10%, and 20%, respectively. The percentage of repellency was significantly ($p < 0.05$) higher at 20% essential oil compared to 5% and 10% at all exposure times. Therefore, the essential oil mixtures JC1, JC2, and JC3 were prepared with 20% of *J. officinale* and *A. nobilis* at the ratio of 1:1, 2:1, and 1:2 respectively. JC1, JC2, and JC3 provided 88.20%, 73.07%, and 76.40% repellency, respectively, at 120 min (Table III). The repellency provided by JC1 was significantly ($p < 0.05$) higher compared to JC2 and JC3. JC1 also exhibited significantly ($p < 0.05$) greater repellency than 20% jasmine and 20% chamomile individually at 120 min post-treatment (Fig. 3). The positive control (10% DEET) provided 100% repellency against *Ae. aegypti* for the duration of 120 min.

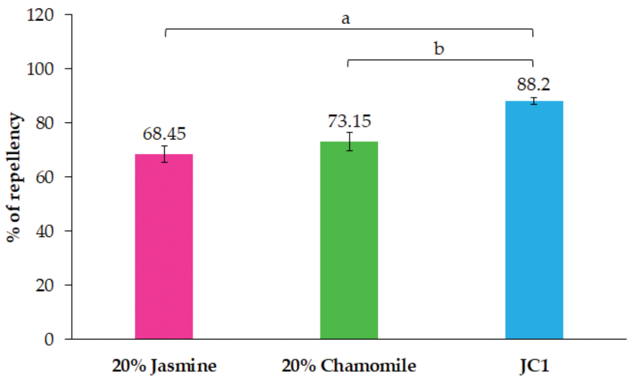

Figure 3: Comparison of repellent efficacy of individual essential oils and oil mixture, JC1 at 120 min post-treatment. Data shown are the mean values ± SEM for three independent experiments. Statistical analysis was performed using ANOVA with Tukey multiple comparisons test. Values marked with the same alphabet are significantly different at $p < 0.05$. Error bars represent SEM. JC1 is composed of 20% of *J. officinale* and *A. nobilis* at the ratio of 1:1.

DISCUSSION

The essential oils of *J. officinale* and *A. nobilis* were screened to identify their chemical constituents using GC-MS and their repellent activities against *Ae. aegypti* were studied using a rat model. GC-MS is a commonly used method for the analysis and profiling of volatile compounds. It provides reproducible and accurate measurements of the retention time, m/z and abundance of volatile compounds together with their fragmentation patterns (39). For safety concern, we have used the rat model to assess the mosquito repellent potential of the test substance before it is being applied on human skin. Mosquito repellent studies have been conducted previously using different animal models such as mouse, rabbit and bird (38,40–42).

Eugenol (1.96%), linalool (2.76%), and phytol (2.46%) were present in *J. officinale*, and champene (0.51%) was found in *A. nobilis*. These compounds have been previously reported to show repellent activity against *A. gambiae* (19,20). Methyl jasmonate (0.63%), the oxygenated monoterpene found in *J. officinale* was shown to act as a repellent against *C. quinquefasciatus* (43). Sesquiterpenes and their oxygenated derivatives constituted 8.55% and 1.16% of jasmine and chamomile essential oils, respectively. The oxygenated sesquiterpene, *trans*-Nerolidol present in *J. officinale* exhibited 67% spatial repellency for 180 min against *Ae. aegypti* (44). The *A. nobilis* essential oil contained the monoterpenes α-pinene (1.44%) and β-pinene (0.17%), and it was reported that 3% α-pinene and β-pinene diluted in olive oil provided 56 and 39 min of protection against *Culex pipiens molestus*, respectively (34). The oxygenated monoterpene 1,8-cineole (0.30%) present in *A. nobilis* was previously reported to provide 80% repellency (1.4 mg/cm^2) against *Ae. aegypti* (35). The essential oils analysed in this study had some similarities in composition with previous studies (39,45). For instance, several compounds which were identified in this study such as isobutyl isobutyrate, 1R-α-pinene, isopropyl 3-methyl-2-butenoate, isobutyl 2-methylbutanoate, *trans*-Pinocarveol and pinocarvone have been reported previously as major marker compounds of Roman chamomile essential oil (39). As for jasmine, compounds such as nerolidol, geranyl linalool, phytol and squalene were identified in *Jasminum grandiflorum* L. essential oil (45).

A. nobilis essential oil at 20% was shown to provide complete protection up to 30 min against *Ae. aegypti* (28). Besides that, the essential oil from the leaves of *A. nobilis* at 1000 ppm exhibited repellency of 40.73% against *Ae. aegypti* (27). We have demonstrated that 20% *A. nobilis* gave 100% repellency for 30 min and decreased to 73.15% at 120 min post-treatment. In addition, the combination of *J. officinale* and *A. nobilis* showed enhanced repellency against *Ae. aegypti* with 100% repellency for 60 min. The greater repellent activity of the essential oil mixture compared to the individual oils can be attributed to the synergistic action between diverse components present as either minor or major constituents of the essential oils. This indicates that the chemical complexity of the two different essential oils provided additional strength to the repellent activity. A mixture of *Litsea cubeba* (LC) and *Litsea salicifolia* (LS) essential oils at 0.075% showed higher non-contact repellency (62.7%) against *Ae. aegypti* than LC (20%) or LS (20.3%) tested alone (46). In another study, blend of ylang-ylang and citronella essential oils on corn starch-based thixogel significantly enhanced repellent activity against *Ae. aegypti* compared to the oil independently (47). Moreover, synergist actions were claimed to be functioning in 8% of essential oil containing patents (48). For instance, essential oil of lippia in combination with geranium, lemon eucalyptus or basil essential oils formulated into a gradually vaporising hydrocarbon soluble composition was thought to alter neuronal action in adult mosquitoes and exhibit repellent activity comparable to commercial pyrethroids (49).

CONCLUSION

The essential oils of *J. officinale* and *A. nobilis* studied here are primarily comprised of monoterpenes, the compounds frequently associated with repellent activity (50). This indicates the potential of the essential oils to be a good source of repellent. The combination of *J. officinale* and *A. nobilis* essential oils was shown to repel the *Ae. aegypti* mosquitoes effectively for 1 h. Nevertheless, a decrease in repellency was noted over time due to high volatility of the essential oils. This property of essential oil could be improved through the formulation of nanoemulsion which will be addressed in our future studies.

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