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CONSTITUENTS OF ESSENTIAL OIL IN INSECT REPELLENT RECIPE

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Abstract: Note is evaporation time and characteristic of individual essential oil. The base note essential oil is the heaviest and will last longest time than that of middle note and top note, respectively. In order to increase evaporation time of desired scent, base note oil which has fixative property could be added into blend oil. The objectives of this study were to 1) perform blend oil temp to have longer evaporation time, 2) compare the chemical constituents of blend oil and single oils, and 3) select the chemical maker suitable for quality control this insect repellent recipe. Blending of 4 essential oils which had been previously reported their insect repellent activities i.e. kaffir lime oil (top note), citronella grass oil (top note), lemongrass oil (middle note), together with patchouli oil (base note) in various ratio were performed. The blend oil which had the best scent and appearance with the ratio of 1:3:1:10 was further chosen for applied in insect repellents in order to increase the evaporation time of the scent and insect repellent activity. The chemical compositions of blend oil and single oils were analyzed by GC-MS. The result exhibited that citronellal, citral, and patchouli alcohol which shown insect repellent activities in each oils were still remain in the blend oil. Patchouli alcohol (20.16%) and citronellal (9.60%) were also the most abundant together with α-bulnesene (12.95%) and α-guaiene (10.86%) in the blend oil. Compared with single oil, patchouli alcohol was the main compound in patchouli oil (23.13%), and citronellal was also the major compound in kaffir lime oil (70.98%), and citronella oil (30.94%). Even though citral which was exhibited the most abundant in lemongrass oil (69.01%), but it was less than that of the single oil. This finding was assumed that the blend oil could be able to further applying in insect repellent formulation; and patchouli alcohol and citronellal were selected to be the chemical markers for quality control. However the evaporation time of blend oil compared with individual single oil was further studied

Keywords: Insect repellent recipe, Kaffir lime oil, Citronella oil, Lemongrass oil, Patchouli oil

บทคัดย่อ: ระยะเวลาในการระเหยของน้ำมันหอมระเหยแต่ละชนิดเป็นลักษณะเฉพาะตัว น้ำมันหอมระเหยชนิด base note จะมีความหนักและให้กลิ่นที่ติดทนนานกว่าชนิด middle note และ top note ตามลำดับ ในการที่จะใช้กลิ่นที่ต้องการติดตรึงอยู่นานขึ้นนั้น สามารถเติมน้ำมันหอมระเหยชนิด base note ที่มีคุณสมบัติช่วยตรึงกลิ่นลงในน้ำมันผสมได้ วัตถุประสงค์ของงานวิจัยครั้งนี้ เพื่อ 1) เตรียมน้ำมันผสมที่มีระยะเวลาในการระเหยที่ยาวนานขึ้น 2) เปรียบเทียบองค์ประกอบทางเคมีของน้ำมันผสมกับน้ำมันเดี่ยวแต่ละชนิด และ 3) คัดเลือกองค์ประกอบทางเคมีที่เหมาะสมเพื่อใช้ในการควบคุมคุณภาพของตำรับไล่แมลงต่อไป น้ำมันหอมระเหยจำนวน 4 ชนิดซึ่งเคยมีรายงานว่ามีฤทธิ์ไล่แมลงได้ ได้แก่ น้ำมันมะกรูด (top note) น้ำมันตะไคร้หอม (top note) น้ำมันตะไคร้ (middle note) และน้ำมันพิมเสนต้น (base note) ถูกนำมาผสมกันในอัตราส่วนต่างๆ น้ำมันผสมที่มีสัดส่วน 1:3:1:10 ให้มีกลิ่นและลักษณะดีที่สุดจึงถูกเลือกเพื่อใช้ในการศึกษาและเตรียมเป็นตำรับไล่แมลงต่อไป เมื่อศึกษาองค์ประกอบทางเคมีของน้ำมันผสมและน้ำมันเดี่ยวโดยวิธี GC-MS พบว่า citronellal, citral, and patchouli alcohol ซึ่งเป็นสารที่มีฤทธิ์ไล่แมลงในน้ำมันเดี่ยวแต่ละชนิดนั้นยังคงมีอยู่ในน้ำมันผสม patchouli alcohol (20.16%) และ citronellal (9.60%) ยังเป็นองค์ประกอบเคมีหลักร่วมกับ α-bulnesene (12.95%) และ α-guaiene (10.86%) ในน้ำมันผสมด้วย และเมื่อเปรียบเทียบกับน้ำมันเดี่ยวพบว่า patchouli alcohol เป็นองค์ประกอบหลักในน้ำมันพิมเสนต้น (23.13%) และ citronellal เป็นองค์ประกอบหลักในน้ำมันมะกรูด (70.98%) และน้ำมันตะไคร้หอม (30.94%) ถึงแม้ว่า citral ซึ่งเป็นองค์ประกอบเคมีหลักในน้ำมันตะไคร้จะยังพบบ้างในน้ำมันผสม แต่มีปริมาณน้อยมากเมื่อเปรียบเทียบกับน้ำมันเดี่ยว จึงสรุปว่าน้ำมันผสมชนิดนี้สามารถใช้เพื่อเตรียมเป็นตำรับไล่แมลง และควรเลือกใช้ patchouli alcohol และ citronellal เป็นสารควบคุมคุณภาพของตำรับต่อไป

คำสำคัญ: สูตรตำรับไล่แมลง น้ำมันมะกรูด น้ำมันตะไคร้หอม น้ำมันตะไคร้ น้ำมันพิมเสนต้น

INTRODUCTION

Mosquito is a terrible insect to public health, which transmits several tropical diseases including malaria, dangue, filariasis, yellow fever and Japanese encephalitis. At least 500 million people in the world encounter from mosquito-borne diseases every year. (Phasomkusolsil and Soonwera, 2010). Thus mosquito control and personal protection from mosquito bites are currently the most important measures to control mosquito transmitted diseases.

Synthetic insect repellent, DEET (N,N-diethyl-meta-toluamide) provides long-lasting protection against mosquitoes and biting flies. However, DEET is concerned with its human toxicity which has been reported symptoms varying from mild to severe. It may be unsafe for children possibly causing encephalopathy (Briassoulis *et al.*, 2001), skin irritation, hot sensation rashes or allergy (Das *et al.*, 2003).

Researches on insect repellents derived from plant extracts are required to find alternatives that are safer but still effective. Plant essential oils are potential natural repellents that are expected to replace synthetic compounds (Choochote *et al*., 2007). They have been accepted to be the insecticides and insect repellents. Several of them have been reported their acute toxic effects against insects, including mosquitoes (Nerio *et al*., 2010, Kalita *et al*., 2013, Soonwera and Phasomkusolsil, 2014). Essential oil of *C. hystrix* leaves exhibited insecticidal properties against *Spodoptera litura* fabricius (Loh *et al*., 2011). Various concentrations (2.4%, 2.1%, 1.8%, 1.5% and 1.2%) of *Citrus hystrix* leaves ethanol extract also caused 99.5%, 85.5%, 62.5%, 26.5% and 2% mortality of *Aedes aegypti* larvae in 24 hrs. respectively (Mya *et al*., 2015). Repellent activities of *Cymbopogon* essential oils have been review (Ganjewala, 2009). Essential oil of *C. citratus* at 0.33 $\mu L/cm^2$ stated excellent mosquito repellent activity against *A. aegypti* and *Culex quinquefasciatus* (Soonwera and Phasomkusolsil, 2014). Citronellal which was the main component in citronella oil and kaffir lime leaf oil had shown insect repellent activity against Drosophila TRPA1 (Du *et al*., 2015). Patchouli oil has exhibited repellent and pupicidal activities. The major compound, patchouli alcohol found to be the most effective for repellent activity and $2mg/cm^2$ concentration provided 100% protection up to 280 min against selected important vector mosquitoes, viz., *A.aegypti*, *Anopheles stephensi* and *C. quinquefasciatus*. (Gokulakrishnan *et al*., 2013).

Although essential oils are effective when freshly applied, their effects usually lavished relatively quickly. The mosquito repellent effect will be limited due to their volatility. Some fixative materials have been applied for increasing repellency duration (Nerio *et al*., 2010). The combination of citronella oil and vanillin is likely to have a longer protection time compared with citronella oil alone (Kongkaew *et al.*, 2011). The combinations from eucalyptus oil and sweet basil oil were effective as repellents and feeding deterrents against *A. aegypti*. The combination of essential oil showed better protection time against two mosquito species than each essential oil. The combinations of eucalyptus oil plus sweet basil oil, and peppermint oil plus orange oil were effective and the protection time was more than 120 minutes and biting rate was less than 1.0% (Sritabutra *et al.*, 2011).

Essential oils are assigned notes as top, middle, and base. Notes of essential oils are evaporation rate of individual oil. The top note essential oils are usually light and airy usually lasting 1-2 hours, meanwhile the base note essential oils are heavy and will last for long time (24-48 hours). The blend essential oil which is combination of top, middle, and base notes of essential oils will be last for longer time (Maia and Moore, 2011).

The objective of this study were to improve repellency duration of essential oils by mixing 4 essential oils which were kaffir lime oil and citronella oil (top note), lemongrass oil (middle note) and patchouli oil (base note) together, and find out chemical markers for quality control this blend oil.

MATERIALS AND METHODS

Young and fresh leaves of kaffir lime (*Citrus hystrix* DC.) were purchased from Rangsit fresh food market, Pathum Thani, Thailand. After cleaning and drying at room temperature (28-30°C), they were cut into small pieces and then hydrodistilled by Clevenger apparatus as described in Thai Herbal Pharmacopoiea, 2016.

Citronella oil (batch no. 6010117-1) and lemongrass oil (batch no. 6010117-2) obtained from their leaves and pseudo stem, and patchouli oil (batch no. 6010117-3) obtained from leaf hydrodistillation were purchased from Thai-China Flavours and Fragrances Industry Co., Ltd. (TCFF). These oils were authenticated by comparison their chemical constituents with Thai Herbal Pharmacopoiea or previous reports (Bunrathep *et al*, 2006, Ganjewala, 2009, Loh *et al*., 2011, Tajidin *et al*, 2012, Gokulakrishnan *et al*., 2013, Othman *et al*., 2016, Thai Herbal Pharmacopoiea, 2016, Toledo *et al*., 2016, Hamad *et al*., 2017 and Shah *et al*., 2017).

All these oils were was kept in amber glass with tight-fitting lid bottle, and stored at 4°C in refrigerator prior to use.

Blend oil preparation

Based on their insect repellent activities and toxicities (Phasomkusolsil and Soonwera, 2010, Maia and Moore, 2011, Kalita *et al.*, 2013), various ratios of essential oils were blended (as shown in Table 1). Each of blended oils was organoleptic tested for the best appearances which were clear, fine color and redolence scent. The selected ratio would not be irritated sensitive skin after applying.

GC-MS Analysis

The blended oil was analyzed for its chemical constituents compared with each singlet oils by Gas Chromatography-Mass Spectrometry (GC-MS) 7890A, 5975C MSD (Agilent Technologies. The column was Mega-5MS (5% phenyl 95% methyl polysiloxane) (30 m. X 0.25 mm. i.d; 0.25 μM); oven temperature programming was 60 °C for 1 min., then increased to 240 °C at a rate of 3°C/min; injector temperature, 180 °C; injection volume 1 μl; transfer temperature, 290 °C for 5 min. and the carrier gas was He (2 ml/min). MS parameters were as follows: EI mode, with ionization voltage 70eV, ion source temperature, 230°C; scan range, 40-650 amu.

Compound Identification

Compounds were identified by comparing the Kovats gas chromatographic retention indices of the peaks on the HP-5MS column with literature values, computer matching using the NIST 2011 database, and comparison of the fragmentation patterns of the mass spectra with those reported in the literature (Adam, 1995 and Davies 1990).

RESULTS AND DISCUSSION

Four essential oils were mixed together by different ratios as shown in Table 1. The appearances of each blend oil which were color, odor, and taste were organoleptic tested. Blend oil no.5 (1:3:1:10) was shown best appearance with clear & yellowish color, earthy green & sweet scent. After applying on sensitive skin, this oil was not irritated. This blend oil was chosen for further study.

Table 1. Various ratios of essential oils in individual blend oils

Blend oil no.	Essential oil ratio			
	Kaffir lime leaf oil *Top note*	**Citronella oil** *Top note*	**Lemongrass oil** *Middle note*	**Patchouli oil** *Base note*
1	1	1	1	1
2	1	1	1	5
3	1	1	1	10
4	1	1	3	10
5	1	3	1	10
6	3	1	1	10
7	1	3	3	10
8	3	3	1	10
9	3	1	3	10
10	3	3	3	10

Chemical constituents of kaffir lime leaf oil

The essential oils obtained by hydrodistillation on *Citrus hystrix* fresh leaves yielded 1.09±0.30%. It was exhibited clear, yellowish, and slightly bitter with the characteristic odor. Essential oil constituents were listed in Table 2. A total of 28 compounds representing 97.81% of the total oil contents in *C. hystrix* leaf oil were identified as 7 monoterpenes, 13 oxygenated monoterpenes, 1 phenylpropanoid, 5 sesquiterpenes, and 2 oxygenated sesquiterpenes. Citronellal (70.98%) was the main component, followed by linalool (6.12%), citronellol (5.41%) and d-limonene (3.10%). Previous reports showed essential oil obtained from leaves of *C. hystrix* contained citronellal as main compound following by other components (Loh *et al*., 2011, Othman *et al*., 2016).

Table 2. Chemical constituents of *Citrus hystrix* leaf oil

Chemical constituents	Chemical formula	Molecular weight	Kovat's Index	% in total oil
Monoterpenes				
α-pinene	$C_{10}H_{16}$	136	0939	0.17
β-pinene	$C_{10}H_{16}$	136	0980	0.34
β-myrcene	$C_{10}H_{16}$	136	0991	1.25
δ-2-carene	$C_{10}H_{16}$	136	1001	0.20
d-limonene	$C_{10}H_{16}$	136	1031	3.10
Z–β-ocimene	$C_{10}H_{16}$	136	1040	0.59
γ-terpinene	$C_{10}H_{16}$	136	1062	0.32
Oxygenated monoterpenes				
cis-linalool oxide	$C_{10}H_{18}O_2$	170	1074	0.26
trans-linalool oxide	$C_{10}H_{18}O_2$	170	1088	0.63
linalool	$C_{10}H_{18}O$	154	1098	6.12
isopulegol	$C_{10}H_{18}O$	154	1146	0.23
citronellal	$C_{10}H_{18}O$	154	1153	70.98
terpinen-4-ol	$C_{10}H_{18}O$	154	1177	0.63
α-terpineol	$C_{10}H_{18}O$	154	1189	0.14
pulegol	$C_{10}H_{18}O$	154	1213	0.80
citronellol	$C_{10}H_{20}O$	156	1228	5.41
geraniol	$C_{10}H_{18}O$	154	1255	0.93
geranial (*trans*-citral)	$C_{10}H_{16}O$	152	1270	0.11

Chemical constituents	Chemical formula	Molecular weight	Kovat's Index	% in total oil
citronellyl acetate	$C_{12}H_{22}O_2$	198	1354	1.06
geranyl acetate	$C_{12}H_{20}O_2$	196	1383	1.21
Phenylpropanoids				
eugenol	$C_{10}H_{12}O_2$	222	1356	0.18
Sesquiterpenes				
α-copaene	$C_{15}H_{24}$	204	1376	0.17
cis-caryophyllene	$C_{15}H_{24}$	204	1404	1.26
α-humulene	$C_{15}H_{24}$	204	1454	0.20
α-farnesene	$C_{15}H_{24}$	204	1508	0.16
δ-cadinene	$C_{15}H_{24}$	204	1524	0.34
Oxygenated sesquiterpenes				
trans-nerolidol	$C_{15}H_{26}O$	222	1564	0.87
α-cadinol	$C_{15}H_{26}O$	222	1653	0.15

Chemical constituents of citronella oil, lemongrass oil, and patchouli oil

Essential oils of citronella oil, lemongrass oil, and patchouli oil purchased from Thai-China Flavours and Fragrances Industry Co., Ltd. (TCFF) were identified by GC-MS. Chemical constituents of individual oils were listed in Table 3-5.

A total of 34 compounds representing 96.35% of the total oil contents in citronella oil were identified as 2 monoterpenes, 10 oxygenated monoterpenes, 1 phenylpropanoid, 15 sesquiterpenes, and 6 oxygenated sesquiterpenes. Citronellal (30.94%) followed by geraniol (17.28%) and citronellol (12.49%) were the most abundant compounds like citronella oil obtained by *Cymbopogon nardus* leaf hydrodistillation (Ganjewala, 2009)

Table 3. Chemical constituents of citronella oil obtained from TCFF

Chemical constituents	Chemical formula	Molecular weight	Kovat's Index	% in total oil
Monoterpenes				
β-myrcene	$C_{10}H_{16}$	136	0991	0.18
d-limonene	$C_{10}H_{16}$	136	1031	4.58
Oxygenated monoterpenes				
linalool	$C_{10}H_{18}O$	154	1098	1.00
isopulegol	$C_{10}H_{18}O$	154	1146	0.08
citronellal	$C_{10}H_{18}O$	154	1153	30.94
pulegol	$C_{10}H_{18}O$	154	1213	0.52
citronellol	$C_{10}H_{20}O$	156	1228	12.49
neral (*cis*-citral)	$C_{10}H_{16}O$	152	1240	0.39
geraniol	$C_{10}H_{18}O$	154	1255	17.28
geranial (*trans*-citral)	$C_{10}H_{16}O$	152	1270	0.47
citronellyl acetate	$C_{12}H_{22}O_2$	198	1354	3.70
geranyl acetate	$C_{12}H_{20}O_2$	196	1383	3.60
Phenylpropanoids				
eugenol	$C_{10}H_{12}O_2$	222	1356	1.73
Sesquiterpenes				
α-cubebene	$C_{15}H_{24}$	204	1351	0.11

Chemical constituents	Chemical formula	Molecular weight	Kovat's Index	% in total oil
α-copaene	$C_{15}H_{24}$	204	1376	0.06
β-cubebene	$C_{15}H_{24}$	204	1390	0.20
β-elemene	$C_{15}H_{24}$	204	1391	2.07
cis-caryophyllene	$C_{15}H_{24}$	204	1404	0.14
γ-elemene	$C_{15}H_{24}$	204	1433	0.14
α-humulene	$C_{15}H_{24}$	204	1454	0.18
γ-muurolene	$C_{15}H_{24}$	204	1477	0.38
germacrene-D	$C_{15}H_{24}$	204	1480	3.71
epizonarene	$C_{15}H_{24}$	204	1497	0.09
α-muurolene	$C_{15}H_{24}$	204	1499	1.10
γ-cadinene	$C_{15}H_{24}$	204	1513	0.08
7-epi−α-selinene	$C_{15}H_{24}$	204	1517	0.81
δ-cadinene	$C_{15}H_{24}$	204	1524	3.24
cadina-1,4-diene	$C_{15}H_{24}$	204	1532	0.09
Oxygenated sesquiterpenes				
β-elemol	$C_{15}H_{26}O$	222	1549	3.40
germacrene D-4-ol	$C_{15}H_{26}O$	222	1574	0.81
γ-eudesmol	$C_{15}H_{26}O$	222	1630	0.46
α-muurolol	$C_{15}H_{26}O$	222	1645	0.63
α-cadinol	$C_{15}H_{26}O$	222	1653	1.63
farnesol (*Z,E*)	$C_{15}H_{26}O$	222	1697	0.06

Lemongrass oil, 20 compounds representing 92.23% of the total oil contents were analysed as 3 monoterpenes, 9 oxygenated monoterpenes, 7 sesquiterpenes, and 1 oxygenated sesquiterpenes. Geranial and neral which represented for citral (69.01%), followed by β-myrcene (11.74%) were the major constituents like lemongrass oil obtained by *Cymbopogon citratus* leaf hydrodistillation (Ganjewala, 2009, Tajidin *et al*, 2012, Hamad *et al*., 2017).

Table 4. Chemical constituents of lemongrass oil obtained from TCFF

Chemical constituents	Chemical formula	Molecular weight	Kovat's Index	% in total oil
Monoterpenes				
β-myrcene	$C_{10}H_{16}$	136	0991	11.74
Z–β-ocimene	$C_{10}H_{16}$	136	1040	0.73
E-β-ocimene	$C_{10}H_{16}$	136	1050	1.42
Oxygenated monoterpenes				
linalool	$C_{10}H_{18}O$	154	1098	0.94
perillene	$C_{10}H_{14}O$	150	1099	0.12
citronellal	$C_{10}H_{18}O$	154	1153	0.61
α-thujenal	$C_{10}H_{14}O$	150	1181	0.17
nerol	$C_{10}H_{18}O$	154	1228	1.12
neral (*cis*-citral)	$C_{10}H_{16}O$	152	1240	31.59
geraniol	$C_{10}H_{18}O$	154	1255	3.35
geranial (*trans*-citral)	$C_{10}H_{16}O$	152	1270	37.42
geranyl acetate	$C_{12}H_{20}O_2$	196	1383	1.74

Chemical constituents	Chemical formula	Molecular weight	Kovat's Index	% in total oil
Sesquiterpenes				
cis-caryophyllene	$C_{15}H_{24}$	204	1404	0.48
trans–α-bergamotene	$C_{15}H_{24}$	204	1436	0.27
α-humulene	$C_{15}H_{24}$	204	1454	0.05
γ-muurolene	$C_{15}H_{24}$	204	1477	0.08
germacrene-D	$C_{15}H_{24}$	204	1480	0.07
γ-selinene	$C_{15}H_{24}$	204	1484	0.09
δ-cadinene	$C_{15}H_{24}$	204	1524	0.15
Oxygenated sesquiterpenes				
α-cadinol	$C_{15}H_{26}O$	222	1653	0.16

Together with patchouli oil, 26 compounds representing 87.95% of the total oil contents were listed as 2 monoterpenes, 1 oxygenated monoterpenes, 20 sesquiterpenes, and 3 oxygenated sesquiterpenes. The main compounds were identified as patchouli alcohol (23.13%), α-bulnesene (17.18%), and α-guaiene (14.86%) like patchouli oil obtained by Pogostemon cablin leaf hydrodistillation (Bunrathep *et al*, 2006 and Gokulakrishnan *et al*., 2013, Shah *et al*., 2017).

Table 5. Chemical constituents of patchouli oil

Chemical constituents	Chemical formula	Molecular weight	Kovat's Index	% in total oil
Monoterpenes				
α-pinene	$C_{10}H_{16}$	136	0939	0.09
β-pinene	$C_{10}H_{16}$	136	0980	0.20
Oxygenated monoterpenes				
trans-linalool oxide	$C_{10}H_{18}O_2$	170	1088	0.15
Sesquiterpenes				
α-copaene	$C_{15}H_{24}$	204	1376	0.35
β-patchoulene	$C_{15}H_{24}$	204	1380	3.95
β-elemene	$C_{15}H_{24}$	204	1391	1.30
longifolene	$C_{15}H_{24}$	204	1402	5.84
cis-caryophyllene	$C_{15}H_{24}$	204	1404	0.34
α-cedrene	$C_{15}H_{24}$	204	1409	3.09
trans-caryophyllene	$C_{15}H_{24}$	204	1418	4.00
α-guaiene	$C_{15}H_{24}$	204	1439	14.86
β-humulene	$C_{15}H_{24}$	204	1440	0.54
α-humulene	$C_{15}H_{24}$	204	1454	0.68
α-patchoulene	$C_{15}H_{24}$	204	1456	1.12
seychellene	$C_{15}H_{24}$	204	1460	7.23
allo-aromadendrene	$C_{15}H_{24}$	204	1461	2.13
γ-selinene	$C_{15}H_{24}$	204	1484	0.19
β-selinene	$C_{15}H_{24}$	204	1485	0.06
α-bulnesene	$C_{15}H_{24}$	204	1505	17.18
γ-cadinene	$C_{15}H_{24}$	204	1513	0.46
7-epi–α-selinene	$C_{15}H_{24}$	204	1517	0.28

Chemical constituents	Chemical formula	Molecular weight	Kovat's Index	% in total oil
δ-cadinene	$C_{15}H_{24}$	204	1524	0.09
α-cadinene	$C_{15}H_{24}$	204	1538	0.16
Oxygenated sesquiterpenes				
β-elemol	$C_{15}H_{26}O$	222	1549	0.06
(-)-spathulenol	$C_{15}H_{24}O$	220	1576	0.47
patchouli alcohol	$C_{15}H_{26}O$	222	1659	23.13

Chemical constituents of blend oil [kaffir lime : citronella : lemongrass : patchouli (1:3:1:10)]

The blend oil were identified their chemical constituents by GC-MS. Thirty six compounds representing 84.34% of total oil were stated in Table 6. The most abundant in blend oil were patchouli alcohol (20.16%), α-bulnesene (12.95%), α-guaiene (10.86%), and citronellal (9.60%) with representing 53.57% of total oil content. The major compounds in each individual oils which were patchouli oil, citronellal and citral (3.12%) were still existed in blend oil. Most components found in single oil were also existed in blend oil viz geraniol (3.24%), citronellol (2.46%), β-patchoulene (2.79%), *cis*-caryophyllene (2.94%), and seychellene (5.43%), however some were disappeared which were α-cedrene, nerol, *trans*-caryophyllene, β-elemol, and α-cadinol. However some compounds which were not identified in single oil were revealed e.g. δ-elemene (0.09%), β-gurjunene (0.36%), and (-)-dehydroaromadendrene (0.20%) in blend oil.

Table 6. Chemical constituents of blend oil

Chemical constituents	Chemical formula	Molecular weight	Kovat's Index	% in total oil
Monoterpenes				
α-pinene	$C_{10}H_{16}$	136	0939	0.07
β-pinene	$C_{10}H_{16}$	136	0980	0.15
β-myrcene	$C_{10}H_{16}$	136	0991	0.52
d-limonene	$C_{10}H_{16}$	136	1031	0.92
E-β-ocimene	$C_{10}H_{16}$	136	1050	0.08
Oxygenated monoterpenes				
linalool	$C_{10}H_{18}O$	154	1098	0.47
isopulegol	$C_{10}H_{18}O$	154	1146	0.23
citronellal	$C_{10}H_{18}O$	154	1153	9.60
citronellol	$C_{10}H_{20}O$	156	1228	2.47
neral (*cis*-citral)	$C_{10}H_{16}O$	152	1240	1.50
geraniol	$C_{10}H_{18}O$	154	1255	3.24
geranial (*trans*-citral)	$C_{10}H_{16}O$	152	1270	1.62
citronellyl acetate	$C_{12}H_{22}O_2$	198	1354	0.72
geranyl acetate	$C_{12}H_{20}O_2$	196	1383	0.71
Phenylpropanoids				
eugenol	$C_{10}H_{12}O_2$	222	1356	0.21
Sesquiterpenes				
δ-elemene	$C_{15}H_{24}$	204	1339	0.09
α-copaene	$C_{15}H_{24}$	204	1376	0.25

Chemical constituents	Chemical formula	Molecular weight	Kovat's Index	% in total oil
β-patchoulene	$C_{15}H_{24}$	204	1380	2.79
β-elemene	$C_{15}H_{24}$	204	1391	1.24
cis-caryophyllene	$C_{15}H_{24}$	204	1404	2.94
β-gurjunene	$C_{15}H_{24}$	204	1432	0.36
α-guaiene	$C_{15}H_{24}$	204	1439	10.86
β-humulene	$C_{15}H_{24}$	204	1440	0.14
α-humulene	$C_{15}H_{24}$	204	1454	0.55
α-patchoulene	$C_{15}H_{24}$	204	1456	0.91
(-)-dehydroaromadendrene	$C_{15}H_{22}$	202	1459	0.20
seychellene	$C_{15}H_{24}$	204	1460	5.43
allo-aromadendrene	$C_{15}H_{24}$	204	1461	0.69
γ-gurjunene	$C_{15}H_{24}$	204	1473	0.06
γ-muurolene	$C_{15}H_{24}$	204	1477	0.16
germacrene-D	$C_{15}H_{24}$	204	1480	0.68
α-bulnesene	$C_{15}H_{24}$	204	1505	12.95
7-epi–α-selinene	$C_{15}H_{24}$	204	1517	0.34
δ-cadinene	$C_{15}H_{24}$	204	1524	0.64
α-cadinene	$C_{15}H_{24}$	204	1538	0.39
Oxygenated sesquiterpenes				
patchouli alcohol	$C_{15}H_{26}O$	222	1659	20.16

Patchouli oil have been identified as base note, whereas kaffir lime oil and citronella oil were top note, meanwhile lemongrass oil was middle note and. Due to patchouli alcohol and citronellal which were the marker compounds in patchouli oil and citronella oil had shown insect repellent activity (Gokulakrishnan *et al*., 2013, Du *et al*, 2015). As the previous reports, combination of non–volatile oils or essential oils which were base note with middle note or top note had assisted to increase protection time of insect repellent products (Nerio *et al*., 2010, Maia and Moore, 2011, Kipland and Mwangi, 2014). This finding showed protection time of blend oil which consisted of high ratio of patchouli oil might be increased.

CONCLUSION

The main constituents of kaffir lime oil were citronellal (70.98%), linalool (6.12%), citronellol (5.41%) and *d*-limonene (3.10%); of citronella oil were citronellal (30.94%), geraniol (17.28%), and citronellol (12.49%); of lemongrass oil were citral (69.01%) and β-myrcene (11.74%); and of patchouli oil were patchouli alcohol (23.13%), α-bulnesene (17.18%), and α-guaiene (14.86%).

After 4 essential oils were blended, patchouli alcohol (20.16%) and citronellal (9.60%) still remained in this blend oil as major contents, together with α-bulnesene (12.95%) and α-guaiene (10.86%). Compared to single oils, some components were disappeared, e.g. nerol, α-cedrene, *trans*-caryophyllene, and β-elemol, while some compounds e.g. δ-elemene (0.09%), β-gurjunene (0.36%), and (-)-dehydroaromadendrene (0.20%) which were not identified in single oil were revealed in blend oil.

High content of patchouli oil (base note) in this blend oil might help to increase protection time of insect repellent products. Amongst main contents of blend oil, patchouli alcohol and citronellal had shown insect repellent activity in previous reports (Gokulakrishnan

et al., 2013 and Du *et al*., 2015). This finding was assumed that the blend oil could be able to further applying in insect repellent formulation and patchouli alcohol and citronellal were selected to be the chemical markers for quality control. However, the insect repellent activity and evaporation time of this blend oil was further studied.

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