

Original Article

Effect of an essential oil blend of citronella, lemongrass, and patchouli on acne-causing bacteria

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Abstract

Currently there is increasing interest in antibacterial effects of blended essential oils. Our study aimed to determine a suitable ratio of citronella (C), lemongrass (L), and patchouli (P) essential oils, which could be used to combat the acne causing bacteria *Propionibacterium acnes* and *Staphylococcus epidermidis*. MIC values of the oil blends were estimated by broth dilution method. Effects of blended oil against *P. acnes* and *S. epidermidis* were assessed from the fraction inhibitory concentration index (FICI). Patchouli oil exhibited the strongest activities against both microorganisms, but excessive patchouli oil in the blend was antagonistic to effects against both microorganisms. A suitable blend ratio was C:L:P (1:2:1) by weight, which showed the strongest antibacterial activities against *P. acnes* and *S. epidermidis* (MIC = 0.3125 and 0.625 mg/ml, respectively), with synergistic effects (FICI = 0.09 and 0.20, respectively). The major constituents in the oil blend were citronellal, citral, and patchouli alcohol, provided by each type of oil.

Keywords: citronella oil, lemongrass oil, patchouli oil, *Propionibacterium acnes*, *Staphylococcus epidermidis*

1. Introduction

Propionibacterium acnes and *Staphylococcus epidermidis* are important pathogens causing acne vulgaris that affects almost every person at least once during their lifetime. *P. acnes* is an anaerobic bacterium that resides underneath the skin surface and inhabits the androgen stimulated sebaceous follicles. In contrast, *S. epidermidis* is an aerobic organism involved in the superficial infection within the sebaceous unit. The hyperactivity of resident microflora populations is one stage of acne pathology, and both of the microorganisms are targets for anti-acne drugs (Dawson & Dellavalle, 2013; Kurokawa *et al.*, 2009; Thiboutot *et al.*, 2009). Increased bacterial resistances due to long-term and frequent uses of antibacterial

drugs drive the search for novel compounds from natural sources (Sheetal & Singh, 2011; Sinha, Srivastava, Mishra, & Yadav, 2014).

Several reports have indicated activities of essential oils against various kinds of bacteria, including *P. acnes* and *S. epidermidis* (Andradea, Barbosaa, Probst & Júnior, 2014; Chouhan, Sharma, & Guleria, 2017; Sinha *et al.*, 2014;). Recently, effects of combining essential oils have been studied. Mixtures of essential oils have produced various effects on microorganism, with synergistic, additive, or antagonistic effects (Bassolé *et al.*, 2010; Chouhan *et al.*, 2017; Fu *et al.*, 2007; Goñi *et al.*, 2009; Gutierrez, Barry-Ryan, & Bourke, 2008; Tadtong *et al.*, 2012; Wagnera & Ulrich-Merzenich, 2009;). These effects might be caused by interactions between the constituents in the essential oils, or among different essential oils (Sinha *et al.*, 2014; Wagnera & Ulrich-Merzenich, 2009). The interactions in each combination of essential oils are estimated with a checkerboard assay, which

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is used to explore the potency of combinations of antibacterial components in comparison to their individual activities. This comparison is then represented in terms of the fractional inhibitory concentration index (FICI), which is the sum of fraction inhibitory concentrations (FICs). The FIC is the ratio of essential oil concentration to its own MIC. The effect is categorized as synergistic, indifferent (or no interaction), or antagonist, according as the FICI is 0.5 or less, more than 0.5-4.0, or more than 4, respectively (Odds, 2003).

Citronella (*Cymbopogon nardus*), lemongrass (*Cymbopogon citratus*), and patchouli (*Pogostemon cablin*) oils were obtained from hydrodistillation. The most abundant compounds of citronella oil are citronellal, citronellol, and geraniol (Ganjewala, 2009; Nakahara, Alzoreky, Yoshihashi, Nguyen, & Trakoontivakorn, 2013; Wei & Wee, 2013). Lemongrass oil is primarily composed of citral and β -myrcene (Hamad, Nuritasari, & Hartanti, 2017; Tajidin, Ahmad, Rose nani, Azimah, & Munirah, 2012). Patchouli oil consisted of patchouli alcohol, α -bulnesene, and α -guaiene (Bunrathep, Lockwood, Songsak, & Ruangrunsi, 2006). These essential oils and their active constituents have previously been reported as active against microorganisms, including *P. acnes* and *S. epidermidis* (Chouhan *et al.*, 2017; Luangnarumitchai, Lamlertton, & Tiyaboonchai, 2007; Onawunmi, 1989; Onawunmi, Yisak, & Ogunlana, 1984; Singh, Singh, Singh, & Ebibeni, 2011; Wan *et al.*, 2016; Yang, Zhang, Yang, & Lui, 2013). However, effects of blends of these oil preparations have not been reported.

This study aimed to evaluate the effects of these essential oils in various blend ratios, and to determine suitable mixture proportions with synergistic antibacterial effects against *P. acnes* and *S. epidermidis*. The results could then be applied in cosmetic or pharmaceutical products.

2. Materials and Methods

2.1 Essential oils and chemicals

Pure essential oils of *Cymbopogon nardus* leaf (citronella oil), *Cymbopogon citratus* leaf (lemongrass oil), and *Pogostemon cablin* (patchouli oil) were obtained by steam distillation of known constituents purchased from Thai-China Flavors and Fragrances Industry Co. Thailand. Standard citronellal, citral, *cis*-caryophyllene and patchouli alcohol were purchased from Sigma-Aldrich, Inc. (St. Louis, Mo, USA). All the components were kept in amber glass bottles with tightly fitting lids, which were stored at 4°C prior to use.

2.2 Bacterial strains

The test microorganisms used in this experiment were *Propionibacterium acnes* DMST 14916 and *Staphylococcus epidermidis* TISTR 518. These bacteria were obtained from the Drug and Herbal Product Research and Development Center, College of Pharmacy, Rangsit University. *P. acnes* was incubated at 37°C in Brain Heart Infusion (BHI) agar for 48-72 h under anaerobic conditions, while *S. epidermidis* was cultured at 37°C in Mueller Hinton agar (MHA) for 24 h.

2.3 Blended oil preparation

Various ratios (by weight) of citronella oil, lemongrass oil, and patchouli oil were mixed. Briefly, the largest amount of an essential oil was pipetted into the vial first, followed by adding the minor components into the same vial. The oil blend was then homogenized and stored at 4°C prior to use.

2.4 Disc diffusion method

The antibacterial activity was evaluated with traditional antibiotic susceptibility testing, using the disc diffusion method as described by Bauer, Kirby, Sherris, & Turck (1966). The activity was determined by measuring the diameter of the zone of inhibition against the test microorganisms. Three independent experiments were performed and each experiment was run in triplicate. Sterile 6 mm paper discs were impregnated with 15 μ l undiluted essential oil and deposited on the agar surface, with 1% clindamycin serving as the positive control.

2.5 Broth dilution method

The antibacterial effects of essential oils were evaluated by determining the minimum inhibitory concentration (MIC) with the broth dilution method, according to the modified protocol described by Gutierrez *et al.* (2008). Briefly, essential oils were dissolved in 5% DMSO, then diluted in media and tested over a range of concentrations from 0.3125-10,000 μ g/ml against overnight broth cultures of bacteria grown to a concentration of 5×10^5 CFU/ml in liquid culture media. Clindamycin was used as a positive control. Microbial growth was determined by observable turbidity. The lowest concentration of essential oil that completely inhibited growth of a particular microorganism is the MIC. Three independent experiments were performed and each experiment was run in triplicate. To study the effects of essential oils in a blend, the MIC for blended oils was compared to the MICs of single oils.

2.6 FIC index analysis

Checkerboard assay was used to estimate the interactions of essential oils in blends. Fraction inhibitory concentration index (FICI) was derived from MIC of essential oil combination, with no visible growth of the test microorganism. This demonstrated that the combinations of agents could exert inhibitory effects that exceeded the sum of their effects singly. FICI was calculated as follows.

$$FIC \text{ index} = \sum \left(\frac{MIC \text{ of each essential oil combination}}{MIC \text{ of essential oil alone}} \right)$$

The interpretation was considered synergistic for FIC index of 0.5 or less (FICI \leq 0.5), indifferent (or no interaction) for FICI $>$ 0.5-4.0, and antagonistic for FIC index $>$ 4.0 (Odds, 2003).

2.7 GC-MS analysis

Essential oil constituents were identified by use of 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 μ M); oven temperature 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 μ l; transfer temperature 290°C for 5 min, and the carrier gas was He (2 ml/min). MS parameters were as follows: EI mode, ionization voltage 70eV, ion source temperature 230°C, and scan range 40-650 amu. *Cis-caryophyllene* was the internal standard.

Compounds were analyzed by comparing the Kovats gas chromatographic retention indices of the peaks on the HP-5MS column with literature values, computer matching against the NIST 2011 database, and comparison of the fragmentation patterns of mass spectra with those reported in the literature (Adams, 2007; Davies, 1990).

3. Results and Discussion

3.1 Antibacterial activity

Antibacterial activities of individual oils and their major constituents against acne-causing bacteria were screened by the disc diffusion method. All of the oils exhibited promising inhibition zones against *Propionibacterium acnes* DMST 14916 and *Staphylococcus epidermidis* TISTR 518 with the diameters of inhibition zones given in Table 1. The strongest antibacterial activities against *P. acnes* and *S. epidermidis* belonged to lemongrass oil and patchouli oil, respectively, these having the widest inhibitory clear zones.

The minimum inhibitory concentrations (MIC) of essential oils and their major constituents were determined by broth dilution method, with results given in Table 2. Among the single oils, patchouli oil exhibited the highest efficacy against both *P. acnes* and *S. epidermidis* with the lowest MICs in the range 1.25-2.5 mg/ml, followed by lemongrass oil and then citronella oil. Even though the inhibition zone of lemongrass oil was larger than that of patchouli oil, its MIC value was greater than that of patchouli oil. This might be due to its ability to diffuse from the paper disc and solubilize in agar. Citral, the major constituent in lemongrass oil, gave the highest efficacy against both *P. acnes* and *S. epidermidis* with MIC in the range 0.125-0.25 mg/ml, followed by patchouli alcohol and citronellal.

Table 1. Antibacterial activities of the selected essential oils by disc diffusion method.

Microorganism	Inhibition zone \pm SD (mm)		
	Citronella	Lemongrass	Patchouli
<i>Propionibacterium acne</i>	17.20 \pm 0.00	21.47 \pm 0.81	19.97 \pm 0.06
<i>Staphylococcus epidermidis</i>	15.50 \pm 0.70	17.80 \pm 1.13	25.00 \pm 0.00

1% Clindamycin was used as a positive control. The tests were done in triplicate

Previous reports have assessed the effects of combinations of essential oils on several microorganisms. The various ratios of the same constituents in essential oil blends tend to interact with each other, acting in additive, synergistic, and in a few cases antagonistic fashion (Bassolé *et al.*, 2010; Fu *et al.*, 2007; Goñi *et al.*, 2009; Gutierrez *et al.*, 2008; Tadtong *et al.*, 2012). Various mixtures (w/w) of blended oils were prepared (as shown in Table 2) in order to study the effects of blended oil preparations and find a suitable ratio against both *P. acnes* and *S. epidermidis*. The oil blends exhibited antibacterial activity against *P. acnes* and *S. epidermidis* in the agar disc diffusion test, and their MIC values as determined by broth dilution method are summarized in Table 2.

The results indicate that the blend with citronella (C), lemongrass (L) and patchouli (P) oils in (1:2:1) proportions exhibited the strongest activity against *P. acnes*, followed by the blends C:L (1:1) and L:P (1:1) in this order. Surprisingly, all three blends had a dominant fraction of lemongrass oil, with citral as its active constituent. This suggests citral as apparently the most active constituent against *P. acnes*. On the other hand, the blends C:L:P (2:1:2) and (1:2:2) gave the lowest activities against *P. acnes*, but they showed the strongest activities against *S. epidermidis*.

In this experiment, all these oil blends exhibited synergy against *S. epidermidis*. The blends C:L:P (1:1:1), (2:1:1), (1:1:2), and (2:2:1) displayed stronger activity against *S. epidermidis* than against *P. acnes*. Considering patchouli oil, although it exhibited the strongest activity against *P. acnes* with the lowest MIC value, unfortunately increasing the fraction of patchouli oil as in C:L:P (1:1:2) lead to lower activity than that of C:L:P (1:1:1).

According to previous reports, MIC values above 1000 μ g/ml denote inactivity, so the blends C:P (1:1), C:L:P (1:1:1) and (2:1:1) would not be appropriate for acne treatment, since their MICs were >1000 μ g/ml against both *P. acnes* and *S. epidermidis*.

3.2 FIC index analysis

The fractional inhibitory concentration index (FICI) of each blended oil was calculated from its MICs against *P. acnes* & *S. epidermidis*. The results shown in Table 2 were interpreted as synergistic (FIC \leq 0.5), no interaction (FICI > 0.5-4.0), or antagonistic (FICI > 4.0) (Odds, 2003).

All oil blends showed synergy against *S. Epidermidis* with the FICI values ranging from 0.07 to 0.35. The FICI of blends C:L:P (1:1:1), (2:1:1), and (1:2:1) against *P. acnes* were 4.33, 3.50, and 0.09, respectively, so the antibacterial effect against *P. acnes* would decrease with fraction of citronella oil, and this effect got stronger with larger fractions of lemongrass oil.

The synergistic effects against both *P. acnes* and *S. epidermidis* were present only for the mixtures C:L (1:1), L:P (1:1), and C:L:P (1:2:1). Considering the MIC values, although the blends C:L (1:1) and L:P (1:1) had similar MIC values against both *P. acnes* and *S. epidermidis*, the C:L (1:1) had more synergy than L:P (1:1) as the FICI value for C:L (1:1) is lower than that for L:P (1:1). Increasing the fraction of citronella oil or of lemongrass oil from the mixture of C:L:P (1:1:1) to (2:1:1), (1:2:1) or (2:2:1), showed increased synergy

Table 2. MICs of the essential oils and their major constituents against *P. acnes* and *S. epidermidis*, and FIC indexes of oil blends.

Micro-organism	Blend oil* (w/w)	MIC (mg/ml)												FICI (ΣFIC)**
		Citronella oil				Lemongrass oil				Patchouli oil				
		Citronellal	Alone	Combined	FIC	Citral	Alone	Combined	FIC	Pat-chouli alcohol	Alone	Combined	FIC	
<i>P. acnes</i>	C:L (1:1)	1.25	12.5	0.625	0.025	0.125	6.25	0.625	0.050	0.625	1.25	NA	NA	0.075
	C:P (1:1)			1.25	0.050			NA	NA			1.25	0.500	0.550
	L:P (1:1)			NA	NA			0.625	0.050			0.625	0.250	0.300
	C:L:P (1:1:1)			12.5	0.333			12.5	0.667			12.5	3.333	4.333
	(2:1:1)			12.5	0.500			12.5	0.500			12.5	2.500	3.500
	(1:2:1)			0.3125	0.006			0.3125	0.025			0.3125	0.063	0.094
	(1:1:2)			25	0.500			25	1.000			25	10.000	11.500
	(2:2:1)			12.5	0.400			12.5	0.800			12.5	2.000	3.200
	(2:1:2)			>25	>0.8			>25	>0.80			>25	>8.00	>9.60
(1:2:2)			>25	>0.4			>25	>1.60			>25	>8.00	>10.00	
<i>S. epidermidis</i>	C:L (1:1)	1.25	25	1.25	0.025	0.25	6.25	1.25	0.100	1.25	2.5	NA	NA	0.125
	C:P (1:1)			1.25	0.025			NA	NA			1.25	0.250	0.025
	L:P (1:1)			NA	NA			0.625	0.050			0.625	0.125	0.050
	C:L:P (1:1:1)			1.25	0.017			1.25	0.067			1.25	0.167	0.250
	(2:1:1)			1.25	0.025			1.25	0.050			1.25	0.125	0.200
	(1:2:1)			0.625	0.006			0.625	0.050			0.625	0.063	0.119
	(1:1:2)			0.625	0.006			0.625	0.025			0.625	0.125	0.156
	(2:2:1)			0.625	0.010			0.625	0.040			0.625	0.050	0.100
	(2:1:2)			0.3125	0.005			0.3125	0.01			0.3125	0.05	0.065
	(1:2:2)			0.3125	0.0025			0.3125	0.02			0.3125	0.05	0.073

*C= citronella oil, L= lemongrass oil, P= patchouli oil, **Result interpreted as synergy (FICI ≤ 0.5), antagonist (FICI > 4), or no interaction (FICI > 0.5-4). The tests were done in triplicate. NA = not available

effect against both *P. acnes* and *S. epidermidis*, whereas increasing the fraction of patchouli oil decreased synergy against *P. acnes*.

Most C:L:P blends exhibited synergistic antibacterial effects against *S. epidermidis* (FICI ≤ 0.5), but indifferent or antagonistic effects against *P. acnes*, except for the case C:L:P (1:2:1) that alone had synergy against both *P. acnes* and *S. epidermidis* with FICI values 0.09 and 0.20, respectively. This appears to be an appropriate ratio of lemongrass oil, citronella oil, and patchouli oil. Synergy can occur if the compounds affect different targets or interact with each other to increase solubility and therefore enhance bioavailability of one or several of the substances in an extract (Wagner & Ulrich-Merzenich, 2009).

A checkerboard assay gives comprehensive information to model drug interactions, but the FICI can also be prone to reproducibility problems as the replicate tests may give discordant interpretations. Since there is a widely accepted norm for MIC testing, that variation in a single result places the MIC in a three-dilution range, and the possibilities for reproducibility problems with MIC for checkerboard design are considerable (Odds, 2003).

Therefore, we suggest that the appropriate ratio of citronella, lemongrass, and patchouli oils is 1:2:1, which gave the lowest MIC with synergistic antibacterial effects against both *P. acnes* and *S. epidermidis*. Although this oil mixture offers moderate and weak activities against *P. acnes* and *S. epidermidis*, adding patchouli oil (classified as base note) to this mixture would help increase contact time with skin for acne treatment.

3.3 Chemical compound identification

The chemical constituents in the selected oil mixture C:L:P (1:2:1) were identified by GC-MS. Shown in Table 3, twenty seven compounds (84.28%) in the blend were identified as 3 monoterpenes, 10 oxygenated monoterpenes, 10 sesquiterpenes, 3 oxygenated sesquiterpenes, and 1 phenylpropanoid. Patchouli alcohol (23.38%) was the main constituent, followed by geranial (*trans*-citral) (11.44%), α -bulnesene (10.09%), and neral (*cis*-citral) (9.83%). The GC-MS chromatogram for the oil blend is exhibited in Figure 1.

Citronellal, citral (neral & geranial), and patchouli alcohol are the active components in citronella, lemongrass, & patchouli oils, respectively, and these dominated in all the oil mixtures. These three compounds have been reported to have antimicrobial activities. Citral exhibited antimicrobial activity against *P. acnes* (Onawunmi, 1989; Onawunmi *et al.*, 1984), similar to citronellal, geraniol, citronellol in citronella oil (Nakahara *et al.*, 2013; Tajidin *et al.*, 2012), and patchouli alcohol (Luangnarumitchai *et al.*, 2007; Wan *et al.*, 2016). Thus, antibacterial activities against *P. acnes* and *S. Epidermidis* of this blend may be due to the activities of citronellal, citral, and patchouli alcohol. Consequently, these three compounds were chosen as chemical markers for quality control because they are the dominant component in this oil blend.

4. Conclusions

Citronella, lemongrass, and patchouli oils can be combined to an oil blend, and a suitable blend ratio is 1:2:1. This blend has promising antibacterial effects against *Pro-*

Table 3. Chemical constituents of the selected oil blend compared to each single essential oil.

Chemical constituent	KI	RI	Area (%)			
			C	L	P	C:L:P (1:2:1)
Monoterpenes						
α -pinene	0939	0.25	-	-	0.09	-
β -pinene	0980	0.30	-	-	0.20	1.17
β -myrcene	0991	0.32	0.18	11.74	-	-
<i>d</i> -limonene	1031	0.38	4.58	-	-	0.34
<i>Z</i> - β -ocimene	1040	0.40	-	0.73	-	0.08
Oxygenated monoterpenes						
linalool	1098	0.49	1.00	0.97	-	0.12
citronellal	1153	0.59	30.94	1.84	-	5.72
α -thujenal	1181	0.62	-	3.19	-	0.26
citronellol	1228	0.71	12.49	-	-	1.91
neral (<i>cis</i> -citral)	1240	0.72	0.39	31.59	-	9.83
geraniol	1255	0.75	17.28	3.35	-	3.57
geranial (<i>trans</i> -citral)	1270	0.77	0.47	37.42	-	11.44
citronellyl acetate	1354	0.89	3.70	-	-	0.37
geranyl acetate	1383	0.94	3.60	1.74	-	0.07
Phenylpropanoids						
eugenol	1356	0.91	1.73	-	-	0.12
Sesquiterpenes						
α -copaene	1376	0.93	0.06	-	0.35	-
β -patchoulene	1380	0.94	-	-	3.95	0.93
β -elemene	1391	0.95	2.07	-	1.30	1.16
longifolene	1402	0.99	-	-	5.84	0.19
<i>cis</i> -caryophyllene (internal standard)	1404	1.00	0.14	0.48	4.00	1.37
α -guaiene	1439	1.03	-	-	14.86	6.54
α -humulene	1454	1.06	0.18	0.05	0.68	2.49
α -patchoulene	1456	1.08	-	-	1.12	0.30
γ -muurolene	1477	1.09	0.38	0.07	-	1.21
germacrene-D	1480	1.10	3.71	-	-	-
γ -selinene	1484	1.10	-	0.09	0.16	-
β -selinene	1485	1.11	-	-	0.46	-
α -muurolene	1499	1.12	1.10	-	-	-
α -bulnesene	1505	1.14	-	0.08	17.18	10.09
δ -cadinene	1524	1.15	3.24	0.15	0.28	1.13
cadina-1,4-diene	1532	1.16	0.09	-	-	-
α -cadinene	1538	1.20	-	-	0.16	-
Oxygenated sesquiterpene						
β -elemol	1549	1.25	3.40	-	0.06	-
γ -eudesmol	1630	1.32	0.46	-	-	-
α -muurolol	1645	1.34	0.63	-	-	0.05
α -cadinol	1653	1.37	1.63	0.16	2.37	-
patchouli alcohol	1659	1.39	-	-	25.13	23.38
farnesol (<i>Z,E</i>)	1697	1.44	0.06	-	3.01	0.40
Total Area (%)			94.32	93.65	81.86	84.28

K=Kovat's index, RI = retention index, C= citronella oil, L = lemongrass oil, & P = patchouli oil

pionibacterium acnes DMST 14916 and *Staphylococcus epidermidis* TISTR 518 with MIC values of 0.3125 and 0.625 mg/ml, respectively. This oil blend also had synergistic antibacterial effects against both of the acne-causing bacteria. The blend might be further developed to a pharmaceutical product for acne treatment. In quality control, the proper ratio should be maintained for efficacy, and the chemical markers for such quality control are citronellal, citral, and patchouli alcohol contents that should be routinely assessed.

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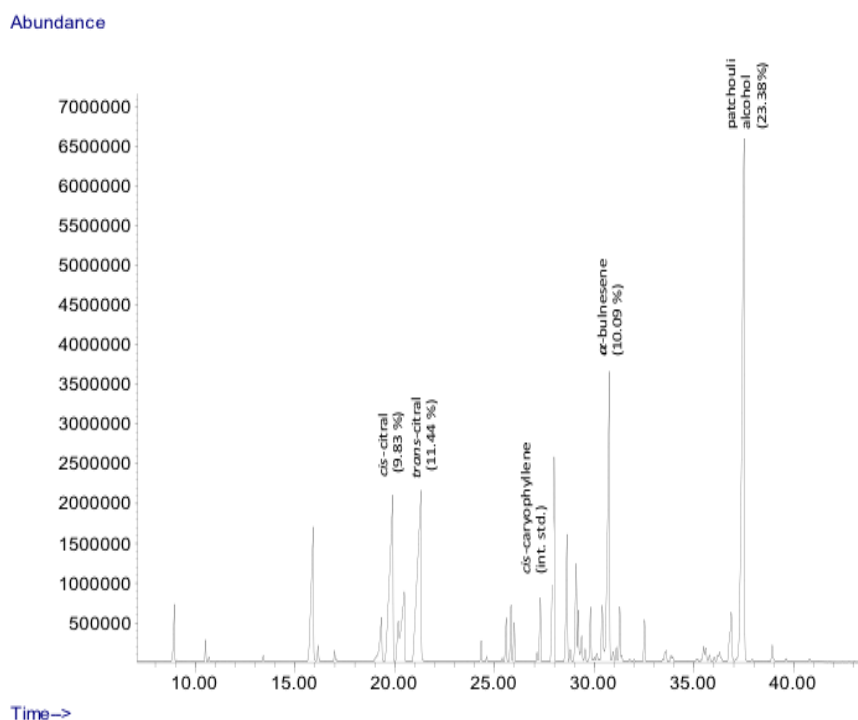


Figure 1. GC-MS chromatogram of the (1:2:1) essential oil blend of citronella: lemongrass: patchouli oils.

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