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# Antibacterial oral sprays from kaffir lime (*Citrus hystrix* DC.) fruit peel oil and leaf oil and their activities against respiratory tract pathogens

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## ABSTRACT

**Background and aim:** Kaffir lime fruit peel oil and Kaffir lime leaf oil have been reported for their activities against respiratory tract pathogens. The purpose of the study was to develop clear oral sprays to be used as a first-defense oral spray.

**Experimental procedure:** Clear antibacterial oral sprays were prepared and analyzed for their respective active major compounds, using GC-MS. The sprays were tested against a Gr. A streptococcal clinical isolate and 3 standard respiratory tract pathogens, using Broth microdilution method. A 4-month stability test was carried out as well.

**Results and conclusion:** Six clear oral sprays, three formulae composed of Kaffir lime fruit peel oil (6, 10, 13%v/v KLO) and the other three formulae containing Kaffir lime leaf oil (4, 8, 12%v/v KLO), were developed. The active compounds in KLO were  $\alpha$ -terpineol and terpinene-4-ol whereas that in KLO was citronellal. All oral sprays exhibited antibacterial activity against one Group A streptococcal clinical isolate and three respiratory pathogenic pathogens, *Staphylococcus aureus* ATCC 29213, *Streptococcus pneumoniae* ATCC 49619, and *Haemophilus influenzae* ATCC 49247, among which the strongest activity was against *H. influenzae* ATCC 49247. The antibacterial activity of all oral sprays remained unchanged in an accelerated stability test, at 4, 30, and 45 °C under 75% relative humidity, throughout the 4-month storage.

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## 1. Introduction

Kaffir lime is a member of the genus *Citrus*, family Rutaceae.<sup>1</sup> The scientific name is *Citrus hystrix* DC. Common names of *Citrus*

**Abbreviations:** KLO, Kaffir lime oil; KLO, Kaffir lime leaf oil; MIC, Minimal inhibitory concentration; MBC, Minimal bactericidal concentration; °C, Degree Celsius; mg, milligram; g, gram; ml, milliliter;  $\mu$ l, microliter; %v/v, percent in volume by volume;  $\mu$ m, micrometer; mm, millimeter; ppm, part per million; min, minute.

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*hystrix* DC are kaffir lime, leech lime and makrut (Thai).<sup>2</sup> Kaffir lime leaves have been used in Southeast Asian recipes since they provide unique and strong aroma.<sup>2</sup>

Analyses by using gas chromatography-mass spectrometry (GC-MS), with headspace SPME-GC-MS techniques showed that the volatile compounds in Kaffir lime belonged to the terpenoids group. Related biological and pharmacological effects included cardioprotective, hepatoprotective,<sup>3</sup> anticholinesterase activities,<sup>4</sup> antibacterial effect,<sup>5</sup> etc.

In a previous study, Kaffir lime fruit peel oil, a volatile oil from fruit peel of *C. hystrix* DC., was analyzed for its constituents, using GC-MS. The major constituents were  $\iota$ -limonene,  $\alpha$ -terpineol, 2- $\beta$ -pinene, terpinene-4-ol,  $\gamma$ -terpinene,  $\alpha$ -terpinene, and  $\alpha$ -terpinolene. The minimal inhibitory concentration (MIC) against

*Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, and *Salmonella typhimurium* ATCC 13311 were 0.1, 0.3, 0.4, and 0.6% v/v, respectively.<sup>6</sup> In another study, two essential oils from *C. hystrix* DC., Kaffir lime fruit peel oil and Kaffir lime leaf oil were reported to exhibit the antibacterial activities against 411 isolates of groups A, B, C, F, G streptococci, *S. pneumoniae*, *H. influenzae*, *S. aureus* (methicillin-resistant and methicillin-sensitive *S. aureus*) and *Acinetobacter baumannii*, obtained from patients with respiratory tract infections, with MIC ranges of 0.03–17.40 and 0.06–68 mg/ml, respectively. The major compounds in Kaffir lime fruit peel oil were  $\alpha$ -terpineol, terpinene-4-ol, and *l*-limonene whereas Kaffir lime leaf oil contained citronellal as the major compound.<sup>7</sup>

Sore throat (pharyngitis) has an infectious or non-infectious etiology.<sup>8</sup> Most cases of sore throat are infectious.<sup>9</sup> In a mild to moderate stages which do not require antibiotics prescription, the patients are often prescribed a mild antiseptic/anti-inflammatory spray to reduce the pain and the mild infection. This study aimed to develop antibacterial oral sprays from Kaffir lime fruit peel oil and Kaffir lime leaf oil and to test the activity of these sprays against the bacteria that caused sore throat.

## 2. Materials and methods

### 2.1. Chemical analysis of the constituents of Kaffir lime fruit peel oil (KLO) and Kaffir lime leaf oil (KLLO)

Kaffir lime fruit peel oil (KLO) (Batch no. 5209234/2009; density 0.87 g/ml) and Kaffir lime leaf oil (KLLO) (Batch no. 5209234–1/2009) were purchased from Thai China Flavours and Fragrances Co. Ltd. and stored at 4 °C. The products were prepared by steam distillation. The diluted oils (100 ppm) were analyzed by gas chromatography-mass spectrometry (GC-MS),<sup>10</sup> using a Hewlett-Packard HP 6890 Series GC System and Hewlett-Packard HP 5973 Mass selective detector, as stated in details in our previous study.<sup>7</sup> The standard compounds in analytical grade, citronellal (density = 0.86 g/ml), limonene (density = 0.84 g/ml), terpinene-4-ol (density = 0.93 g/ml),  $\alpha$ -terpineol (density = 0.94 g/ml) were purchased from Sigma Chemical Co, USA.

### 2.2. Preparation of antibacterial clear oral sprays

KLO was formulated into 6, 10, and 13% v/v clear oral sprays, using a solubilizing agent and a co-solvent; with the addition of flavoring agents and coloring agents. KLLO was also formulated into 4, 8, 12% v/v clear sprays, in the same manner.

### 2.3. Chemical analysis of KLO oral sprays and KLLO oral sprays

Three KLO oral sprays and three KLLO oral sprays were extracted, using hexane, at the proportion of 1:4 (oral spray: hexane) in separatory funnels for 5 times. The hexane extracts were combined and analyzed with GC-MS as in 2.1. The analysis was carried out in triplicate, comparing with the corresponding standard curves,  $\alpha$ -terpineol (0.5, 1, 3, 5, 8, and 10 ppm) and citronellal (5, 10, 20, 40, 60, and 80 ppm), for KLO and KLLO, respectively.

### 2.4. Antibacterial activity of KLO oral sprays and KLLO oral sprays

All six oral sprays were diluted and tested for their antibacterial activity, using broth microdilution method according to the standard microbial techniques.<sup>11,12</sup> One Group A streptococcal clinical isolate was obtained from respiratory tract specimens of the patients with respiratory symptoms at Siriraj Hospital, a tertiary care center in Bangkok, as previously described.<sup>7</sup> The specimens were

discarded samples from the hospital's routine laboratory identification use with no links to the source/names and thus exempted from the ethical committee approval. Three standard strains,<sup>7</sup> *Streptococcus pneumoniae* ATCC 49619, *Staphylococcus aureus* ATCC 29213, and *Haemophilus influenzae* ATCC 49247 were also used. MIC and MBC of all samples were recorded.

## 2.5. Stability test

### 2.5.1. Antibacterial activity of KLO oral sprays and KLLO oral sprays during 4-month storage

All six oral sprays were transferred into 10-ml injection bottles, crimped, and stored at 4, 30 and 45 °C, under 75% relative humidity in closed containers. Samples were collected at 1, 2, 3, and 4 months for the antibacterial testing, using broth microdilution method according to standard microbiological techniques.<sup>11,12</sup> The bacteria used were the same as in 2.4. MIC and MBC of the oral sprays were recorded.

### 2.5.2. Correlation between active compounds and their antibacterial activity of selected KLO oral sprays and KLLO oral sprays during a 4-month accelerated stability test

Selected formulae (10%v/v KLO and 12%v/v KLLO) were collected at 0, 1, 2, 3, and 4 months, extracted and determined by comparing with the standard curves of the corresponding markers ( $\alpha$ -terpineol and citronellal, respectively), using GC-MS, under the condition as previously described. Contents of the markers were compared with the antibacterial activity results of both oral sprays at 0, 1, 2, 3, and 4 months.

## 3. Result and discussion

### 3.1. Chemical constituents of KLO and KLLO

From GC-MS analysis of KLO, the essential oil consisted of 3 major constituents, *l*-limonene (40.65%), terpinene-4-ol (13.71%),  $\alpha$ -terpineol (13.20%), and other constituents (Table 1). In our previous study,<sup>7</sup>  $\alpha$ -terpineol showed the strongest antibacterial activity (MIC range = 0.07–2.40 mg/ml) and was chosen as a marker for the subsequent GC-MS analysis of KLO oral sprays.

For KLLO, citronellal (80.04%) was the major constituent (Table 1). In our previous study,<sup>7</sup> citronellal exhibited a strong antibacterial activity against pathogens, with the MIC range of 0.3–1.1 mg/ml, and was chosen to be the marker for subsequent GC-MS analysis of KLLO oral sprays.

### 3.2. Preparations and chemical analysis of KLO oral sprays and KLLO oral sprays

All oral sprays were clear. It was shown that 6, 10, 13%v/v KLO contained 3.06, 4.28, and 5.11 ppm of  $\alpha$ -terpineol, respectively, whereas 4, 8, 12%v/v KLLO contained 53.90, 88.77, and 148.32 ppm of citronellal, respectively. The attempt was to try to obtain the highest concentrations of both essential oils with acceptable aroma and also the lowest concentration that would effectively work against the pathogens, thus 3 varying formulae for each essential oil were developed. Strong undesirable flavor and odor of both oils were completely masked. Future sensory evaluation is needed to confirm the desirable attributes of the oral sprays.

### 3.3. Antibacterial activity of KLO oral sprays and KLLO oral sprays

The antibacterial activity of all oral sprays was shown in Table 2. All oral sprays showed the strongest antibacterial activity against *H. influenzae*. Upon converting the unit of MIC and MBC of

**Table 1**  
Constituents of Kaffir lime fruit peel oil and Kaffir lime leaf oil as analyzed by GC-MS.

Kaffir lime fruit peel oil				Kaffir lime leaf oil			
Peak number	Retention time(min)	Constituents	Area (%)	Peak number	Retention time(min)	Constituents	Area (%)
1	7.69	unidentified	3.47	1	8.05	l-limonene	0.52
2	8.06	l-limonene	40.65	2	8.50	trans-beta-ocimene	0.38
3	8.93	gamma-terpinene	7.24	3	8.92	gamma-terpinene	0.20
4	9.31	linalool oxide	1.73	4	9.30	linalool oxide	0.54
5	9.80	alpha-terpinolene	5.80	5	9.80	cis-carane-cis-4-ol	0.31
6	10.19	linalool	0.60	6	10.18	linalool	3.04
7	10.92	D-fenchyl alcohol	0.48	7	11.97	citronellal	80.04
8	11.43	3-terpinen-1-ol	0.20	8	12.25	isopulegol	1.29
9	11.90	isopulegol	4.38	9	12.97	terpinene-4-ol	0.94
10	12.23	isopulegol	1.95	10	13.44	alpha-terpineol	0.29
11	12.69	borneol	0.45	11	14.43	beta-citronellol	4.13
12	12.97	terpinene-4-ol	13.71	12	18.55	citronellol acetate	5.46
13	13.44	alpha-terpineol	13.20	13	18.84	unidentified	0.08
14	14.42	beta-citronellol	0.45	14	19.48	neryl acetate	0.70
15	18.55	citronellyl propionate	0.55	15	20.95	caryophyllene	0.89
16	19.51	alpha-copaene	1.56	16	22.08	alpha-humulene	0.14
17	19.90	unidentified	0.61	17	23.31	bicyclogermacrene	0.33
18	20.94	caryophyllene	0.69	18	23.96	cadinene	0.23
19	22.07	alpha-humulene	0.22	19	25.21	nerolidol	0.49
20	22.86	germacrene D	0.36				
21	23.96	delta-cadinene	1.69				

**Table 2**  
Antibacterial activity of three Kaffir lime fruit peel oil oral sprays and three Kaffir lime leaf oil oral sprays against one Group A streptococcal clinical isolate and 3 standard strains of respiratory tract pathogenic bacteria, using broth microdilution method.

Oral sprays	Group A streptococcal clinical isolate		<i>S. aureus</i> ATCC 29213		<i>S. pneumoniae</i> ATCC 49619		<i>H. influenzae</i> ATCC 49247	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
6%v/v KLO	4	4	16	16	2	2	0.5	1
10%v/v KLO	4	4	16	16	1	1	0.5	0.5
13%v/v KLO	4	4	8	16	1	1	0.25	0.25
4%v/v KLLO	8	8	8	8	4	4	0.5	0.5
8%v/v KLLO	4	4	8	8	0.5	0.5	0.25	0.5
12%v/v KLLO	4	4	8	8	0.25	0.25	1	0.5

Concentration in  $\mu\text{l}$  of oral spray/100  $\mu\text{l}$  of broth.

the three KLO oral sprays into “mg of KLO/ml”, the MIC and MBC ranges against all tested bacteria were 0.26–13.92 and 0.28–18.10 mg KLO/ml, respectively. They were compared to those of the essential oil KLO (MIC(MBC) = 0.03–17.40 mg of KLO/ml) in the previous study.<sup>7</sup> As for the three KLLO oral sprays, the converted MIC(MBC) range was 0.26–8.26 mg KLLO/ml. The range was compared to those of the essential oil KLLO in the previous study<sup>7</sup>(MIC(MBC) = 0.03–68 mg KLLO/ml). The sprays showed slightly weaker antibacterial effect than their corresponding essential oils.

### 3.4. Stability test

#### 3.4.1. Antibacterial activity of KLO oral sprays and KLLO oral sprays during a 4-month accelerated stability test

All oral sprays were stored at 4, 30 and 45 °C, under 75% relative humidity. The ranges of MIC and MBC of the six oral sprays, covering all 3 temperatures, were 0.06–16 and 0.125–32  $\mu\text{l}$  of oral spray/100  $\mu\text{l}$  of broth, respectively. The MIC and MBC ranges (combining the three temperatures) of the six oral sprays at 1, 2, 3, and 4 months were compared with those at initial. Except for those against *S. aureus* which were slightly weaker than those at initial, the results implied that the antibacterial activities of all oral sprays were mostly retained at 4, 30 and 45 °C, under 75% relative humidity, for at least 4 months.

#### 3.4.2. Correlation between active compounds and their antibacterial activity of selected KLO oral sprays and KLLO oral sprays during a 4-month accelerated stability test

The results were as shown in Table 3.

Ten-percent v/v KLO oral sprays. There was no significant changes ( $p > 0.05$ ) in the active compound,  $\alpha$ -terpineol, during the first two months at 4 and 30 °C. By the end of 4 months, the active compound decreased ( $p < 0.05$ ) to 82.01% and 74.77%, at 4 and 30 °C, respectively. Contrastingly, at 45 °C, the active compound concentration decreased drastically. The MIC and MBC of the samples during the 4-month storage were mostly retained, with some exceptions. Other compounds (Table 1) that might be contributing to the antibacterial effect included l-limonene,<sup>7</sup> terpinene-4-ol,<sup>7,13</sup>  $\gamma$ -terpinene,<sup>14</sup> isopulegol,<sup>14</sup> delta-cadinene,<sup>15</sup> etc.

Twelve-percent v/v KLLO oral spray. The active compound, citronellal, was more stable at 4 °C; there were only slight decreases ( $p < 0.05$ ) of citronellal during the first 3 months. At 30 and 45 °C, the concentrations at one month decreased more than at 4 °C. By the end of 4 months, the active compound decreased to 80.06, 60.82, and 33.54% ( $p < 0.05$ ), at 4, 30 and 45 °C, respectively. Similarly, the oral spray still maintained its antibacterial activity throughout the storage time of 4 months. Other compounds (Table 1) that might be contributing to the antibacterial effect included beta-citronellol,<sup>13</sup> linalool,<sup>13,14</sup> isopulegol,<sup>14</sup> etc.

**Table 3**  
Correlation between the active compounds (alpha-terpineol/citronellal) and the antibacterial activity of Kaffir lime fruit peel oil oral spray (10%v/v KLO) and Kaffir lime leaf oil (12%v/v KLO) against Gr. A streptococcal clinical isolate, *S. aureus* ATCC 29213, *S. pneumoniae* ATCC 49619, and *H. influenzae* ATCC 49247, during 4-month storage, at 4, 30, and 45 °C, under 75% relative humidity.

Test formulae	Storage temp. (°C)	Storage time (months)	Active compounds <sup>a</sup>	Bacterial strains								
				Gr.A streptococcal clinical isolate		<i>S. aureus</i> ATCC 29213		<i>S. pneumoniae</i> ATCC 49619		<i>H. influenzae</i> ATCC 49247		
				MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	
10% v/v KLO	4	0	4.28 ± 0.02 (100%) A	4	4	16	16	1	1	0.5	0.5	
		1	4.22 ± 0.07 (98.60%) A	8	8	8	16	2	2	0.25	0.25	
		2	3.88 ± 0.67 (90.65%) ABC	2	2	8	16	0.5	0.5	1	1	
		3	3.58 ± 0.54 (83.64%) BCD	2	2	4	8	0.25	0.5	0.25	0.25	
		4	3.51 ± 0.04 (82.01%) CD	4	4	4	16	0.25	0.5	0.25	0.5	
	30	1	4.13 ± 0.07 (96.50%) AB	8	8	8	8	1	1	0.125	0.125	
		2	3.75 ± 0.214 (87.62%) ABCD	2	2	4	16	1	2	0.5	0.5	
		3	3.56 ± 0.23 (83.18%) BCD	2	2	4	16	0.5	2	0.25	0.25	
		4	3.20 ± 0.31 (74.77%) D	2	4	4	8	0.5	0.5	0.5	0.5	
	45	1	2.53 ± 0.41 (59.11%) E	8	8	8	8	2	2	0.25	0.25	
		2	2.25 ± 0.21 (52.57%) EF	2	2	16	16	0.5	1	0.5	0.5	
		3	2.04 ± 0.21 (47.66%) EF	8	4	16	16	0.5	0.5	0.5	1	
		4	1.75 ± 0.32 (40.89%) F	4	4	8	32	0.5	0.5	0.5	1	
	12% v/v KLO	4	0	148.32 ± 1.57 (100%) a	4	4	8	8	0.25	0.25	1	0.5
			1	144.68 ± 2.69 (97.55%) b	8	8	8	8	1	1	0.25	0.25
			2	144.65 ± 1.07 (97.53%) b	2	2	4	8	0.5	0.5	0.06	0.25
3			144.65 ± 1.07 (97.53%) b	2	2	4	16	0.5	0.5	0.125	0.125	
4			118.74 ± 1.07 (80.06%) f	2	2	4	4	0.25	0.25	0.25	0.25	
30		1	142.03 ± 0.98 (95.76%) c	8	8	8	8	1	1	0.125	0.25	
		2	98.33 ± 0.99 (66.30%) g	2	2	4	16	1	1	0.25	0.5	
		3	98.77 ± 0.54 (66.59%) g	2	2	4	16	0.5	1	0.125	0.125	
		4	90.21 ± 1.03 (60.82%) h	2	2	4	4	0.5	0.5	0.125	0.25	
45		1	135.62 ± 1.00 (91.44%) d	ND	ND	ND	ND	ND	ND	ND	ND	ND
		2	87.28 ± 0.89 (58.85%) i	2	2	8	8	0.5	0.5	0.25	0.5	
		3	53.70 ± 0.64 (36.21%) j	2	2	8	8	0.5	0.5	0.25	0.5	
		4	49.74 ± 0.14 (33.54%) k	2	2	4	8	0.25	0.25	0.25	0.25	

<sup>a</sup> α-Terpineol in ppm (in bracket, as %) in 10% v/v KLO oral spray, citronellal in ppm (%) in 12% v/v KLO oral spray, triplicate analysis, means not sharing the same alphabets differ significantly (p < 0.05); MIC = Minimal inhibitory concentration, MBC = Minimal bactericidal concentration, in µl of oral spray/100 µl of broth, ND = not determined.

#### 4. Conclusions

Six clear oral sprays, three from Kaffir lime fruit peel oil (6, 10, 13%v/v KLO) and three from Kaffir lime leaf oil (4, 8, 12%v/v KLO), were developed. The active compounds in KLO and KLO oral sprays were  $\alpha$ -terpineol and citronellal, respectively. All oral sprays exhibited antibacterial activity against a Group A streptococcal clinical isolate and three respiratory pathogens (*S. aureus* ATCC 29213, *S. pneumoniae* ATCC 49619, and *H. influenzae* ATCC 49247), with the strongest activity against *H. influenzae* ATCC 49247. The antibacterial activity of all oral sprays was retained in an accelerated stability test throughout the 4-month storage.

#### Conflicts of interest

Authors declare that there is no conflict of interest regarding the publication of this paper.

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#### References

- Bown D. *The Royal Horticultural Society New Encyclopedia of Herbs & Their Uses*. Great Britain. London: Dorling Kindersley; 2002.
- Wongpornchai S. Chapter 16. Kaffir lime leaf. In: Peter KV, ed. *Handbook of Herbs and Spices*. second ed. vol. 2. Oxford: Woodhead Publishing; 2012: 319–328.
- Putri H, Nagadi S, Larasati YA, Wulandari N, Hermawan A. Cardioprotective and hepatoprotective effects of *Citrus hystrix* peels extract on rats model. *Asian Pac J Trop Biomed*. 2013;3:371–375.
- Seeka C, Sutthivaiyakit P, Youkwan J, et al. Prenylfuranocoumarin-HMGA-flavonol glycoside conjugates and other constituents of the fruit peels of *Citrus hystrix* and their anticholinesterase activity. *Phytochemistry*. 2016;127:38–49.
- Nanasombat S, Lohasupthawee P. Antibacterial activity of crude ethanolic extracts and essential oils of spices against Salmonellae and other enterobacteria. *KMITL Sci Tech J*. 2005;5(3):527–538.
- Srisukh V, Bunyapraphatsara N, Pongpan A, et al. Fresh produce antibacterial rinse from Kaffir lime oil. *Mahidol Univ J Pharm Sci*. 2012;39(2):24–36.
- Srisukh V, Tribuddharat C, Nukoolkarn V, et al. Antibacterial activity of essential oils from *Citrus hystrix* (makrut lime) against respiratory tract pathogens. *Sci Asia*. 2012;38:212–217.
- Renner B, Mueller CA, Shephard A. Environmental and non-infectious factors in the aetiology of pharyngitis (sore throat). *Inflamm Res*. 2012;61:1041–1052.
- Middleton DB. *Pharyngitis*. *Prim Care*. 1996;23(4):719–739.
- Chanthaphon S, Chanthachum S, Hongpattarakere T. Antimicrobial activities of essential oils and crude extracts from tropical *Citrus* spp. against food-related microorganisms. *Songklanakaraj J Sci Technol*. 2008;30(Suppl 1):125–131.
- Clinical and Laboratory Standards Institute. *Performance Standards for Antimicrobial Susceptibility Testing: Twenty-Fifth Informational Supplement, CLSI Document M100 S25*. Wayne, PA: CLSI; 2015.
- Jorgensen JH, Turnidge JD. Chapter 71. Susceptibility test methods: dilution and disk diffusion methods. In: Jorgensen JH, Pfaller MA, Carroll KC, et al., eds. *Manual of Clinical Microbiology*. eleventh ed. Washington DC: ASM Press; 2015: 1253–1273.
- Kotan R, Kordali S, Cakir. Screening of antibacterial activities of twenty-one oxygenated monoterpenes. *Z Naturforschung*. 2007;62c:507–513.
- Erdogan Orhan I, Ozcelik B, Kartal M, Kan Y. Antimicrobial and antiviral effects of essential oils from selected Umbelliferae and Labiatae plants and individual essential oil components. *Turk J Biol*. 2012;36:239–246.
- Pérez-López A, Cirio AT, Rivas-Galindo VM, Aranda RS, de Torres NW. Activity against *Streptococcus pneumoniae* of the essential oil and delta-cadinene isolated from *Schinus molle* fruit. *J Essent Oil Res*. 2011;23:25–28.