Determination of major chemical constituents and antimicrobial activities of essential oils extracted from Nonsaleable Grade (NSG) spices of *Cymbopogon nardus*, *Rosmarinus officinalis*, *Thymus vulgaris* and *Coriandrum sativum* seeds

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Abstract

Four non-saleable grade (NSG) plant spices, including *Cymbopogon nardus* (citronella), *Rosmarinus officinalis* (rosemary), *Thymus vulgaris* (thyme), and *Coriandrum sativum* (coriander) seeds were extracted using hydro-distillation. The chemical compositions of essential oils were analyzed using gas chromatography coupled with mass spectrometry (GC-MS) and the antimicrobial activities were tested to against the microbes of *Escherichia coli* (E. coli)*, Staphylococcus aureus* (S. aureus) and *Candida albicans* (C. albicans). Four essential oils are corresponding to the main functional substances of citronellal (29.562%), 1, 8- Cineole (62.267%), thymol (42.579%), and linalool (76.512%). Both the chemical constituents and antimicrobial activity of NSG essential oils were similar to both commercial products and those reported in previous studies, some of NSG essential oils even present better antimicrobial activity than commercial ones. This new approach of using NSG spices can help to reduce agricultural waste and increase the revenue of spice farmers.

Keywords: Agricultural chemistry, antimicrobial activity, chemical constituents, essential oil, spices

1. Introduction

Plant materials including seeds, fruits, roots, stems and rhizomes can be used as spices. Many spices are primarily used for flavoring, coloring or preserving food (1). Although spice is one of the most significant agricultural products in China, not all the spices are of saleable grade. For those spices with wormholes, stale or dysplasia, they are generally treated as fodder or organic fertilizer. Their value is not high. In order to increase their value, these non-saleable grade (NSG) spices may be used for extracting essential oils, which is a common mean of processing a spice and convert it into commodity. Some essential oils were reported to present antimicrobial activity, which is mainly contributed by the major groups of components including predominantly terpenes, as well as phenylpropanoids and other components, which is also known to be influenced by configuration, quantity and the possible interaction (2-3). Thus, essential oils are commonly used for food preserving and cosmetic production .

Cymbopogon nardus (citronella), belonging to the family Poaceae, is originated from Sri Lanka and South Africa (4). It is one of the most widely used essential oils in aromatherapy and medical treatment, which can also be used as an aromatic agent for refreshing indoor air. Citronellal was reported to be the most abundant component in the citronella essential oil, and presented antimicrobial, antioxidant, and depressant properties (5). Excellent antimicrobial activities of citronella essential oil were found towards E. coli and S. aureus (6).

Rosmarinus officinalis (rosemary), belonging to the family Labiatae, is an evergreen shrub which mainly distributed in Tunisia, Morocco, and Italy (7). It can be used broadly in the food, pharmaceutical, and cosmetic industries. 1, 8-Cineole was found to be one the most effective components in rosemary essential oil, which contributes to its antimicrobial activity (8). According to the previous studies, it was found to present antimicrobial (towards E. coli and S. aureus) and antioxidant properties (9).

Thymus vulgaris (thyme), a kind of plant belonging to the family Lamiaceae, is native to southern Africa (3). The extract from stem and leaves of thyme have been used in food industry for a long time as flavoring and preservative agents because of its antioxidant and antimicrobial properties. It can also be used as stimulants and bactericidal substances. It has been reported that more than 30 kinds of chemical components were found in thyme essential oil (10). Thymol, the major component of thyme essential oil, showed good antimicrobial and inhibitory activities towards E. coli, S. aureus and C. albicans (11, 12).

Coriandrum sativum (Coriander), an annually herbaceous plant, belongs to the Apiaceae family (13). It is a native aromatic, medicinal and condimental plant species of southern Europe and western Mediterranean region but cultivated all over the world (14). The extracts of coriander seeds are usually used for flavoring candies, and commonly used for cookery, perfumery, beverage, tobacco , pharmacy and medical industries (1). Its seed oil was found to be high in α-Pinene, which is a kind of Pinene-type monoterpene hydrocarbons and has been reported for its antimicrobial activities to against some gram-positive and gram-negative bacteria (3, 14, 15).

In this study, the major chemical constituents and antimicrobial activity of the essential oils extracted from NSG spices of citronella, rosemary, thyme and coriander seeds will be determined. To evaluate the quality of these essential oils, the determined properties will be compared with those obtained from some commercially available essential oils. If this new approach for recycling and treating these non-saleable grade spices turns out to be valuable, this new approach not only stimulates the use of essential oils extracted from NSG spices for making non-edible products, but also provide another source of income for spice farmers or dealers. In addition, it can reduce the impacts of these agricultural waste on the environment.

2. Materials and methods

2.1.Sample collection and treatment

The NSG spices materials (stems and leaves of citronella, whole herbs of rosemary and thyme, and coriander seeds) were collected from local markets. For comparison, four commercial essential oils were purchased. Citronella and rosemary essential oils were obtained from Flavor Life Co., Ltd (Japan), whereas thyme and coriander seeds essential oils were obtained from Oshadhi Ltd. (Germany) and Base Formula Ltd. (United Kindom) , respectively.

2.2.Extraction of essential oil and yield calculation

Hydro-distillation was used for the extraction of essential oil from the spices. The spices were grinded into small pieces of maximum 5 mm in length. Fifty grams of each grinded spice and 100 ml of deionized water were placed together into a Clevenger apparatus and heated in an Electro-thermal furnace for at least 3 hours until the volume of the distillate did not increase further. After distillation, the spices residues were removed using anhydrous sodium sulfate whereas the essential oil was separated and transferred into an air-tight bottle and stored at 4 °C in a refrigerator.

The calculation of essential oil yield is referred to equation (1) :

$$\text{Oil yield \% } (\text{w}/w) = \frac{M1}{M2} \times 100\% \quad (1)$$

Where M1 represents the weight of essential oils after dehydration and M2 represents the weight of dried spice. The unit for M1 and M2 are both in grams (g).

2.3. GC-MS analysis

The chemical composition of the essential oils was analyzed by gas chromatographic coupled with mass spectrometry (GC-MS, Agilent 5970 MSD, USA). Helium was employed as the carrier gas (0.8 ml/min). Essential oil samples were diluted with n-hexane. The injection volume was 1.0 μl. The injector temperature was set at 260 ℃, and the split ratio was 10:1. Different chemical constituents were separated by a 30 m × 25 μm × 0.25 μm HP-5MS capillary column. The temperature of the column was increased from 50 ℃ (holding for 2 min) to 160 ℃ (holding for 2 min) with a rate of 5 ℃/min, and then further increased with a rate of 30 ℃/min to 280 ℃ (holding for 5 min). The ionization energy was 70 eV. Transfer line to MSD was 280 ℃, and MSD (EI) was 350 ℃. The mass detector was set at mass scan mode with a mass range of 45-500 m/z, with a scan rate of 0.52 scans/s.

2.4. Antimicrobial assay

The antimicrobial activity of essential oils was determined using the microbial inhibiting zone method (QB/T 2738-2012). Escherichia coli (ATCC25922), Staphylococcus aureus (ATCC6538) and Candida albicans (ATCC10231) were obtained from Microorganism

Germplasm Resources, Guangdong Biological Germplasm Resource Bank. Bacterial suspensions were adjusted to 10^4 CFU ml-1 and smeared on nutrient agar and Sabouraud's agar by using sterilized cotton swabs. Five sterilized pieces of filter paper discs (5 mm, Xinhua 1#) were impregnated with 20 µl of essential oils and air dried at room temperature, then placed on the surface of agar in Petri dishes. The Petri dishes were incubated at 37 ℃ ± 0.5 ℃ for 18 hours. The determination was conducted in triplicate. Antimicrobial activity was evaluated by measuring the diameters of microbial inhibiting zones.

3. Results and discussion

3.1. Yield of essential oil

The yields of essential oils are presented in Table 1. Citronella has the highest yield of essential oil (1.81%) whereas coriander seed has the lowest (0.31%) among the four spices in this study. Compared with previous studies, the oil yields in three of the four spices obtained in this study are slightly lower. However, the oil yield of rosemary is higher than that from previous study. The differences may be due to different extraction methods, cultivation environment and spice species. Oil yield is considered to be the most important value which influences the economic benefit of the spices. The results in this study show that these NSG spices have competitive oil yield. Therefore, they still have significant economic value.

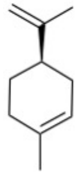
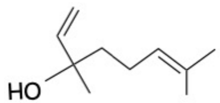
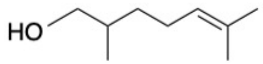
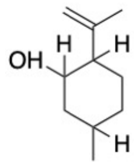
Table 1 Yields of essential oils

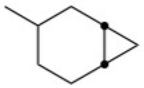
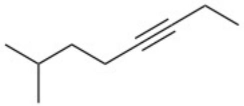
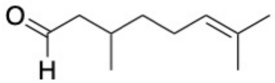
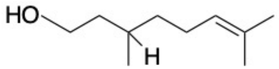
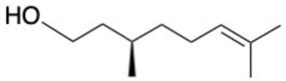
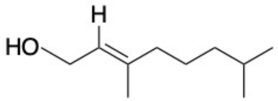
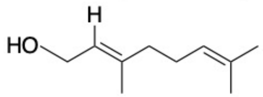
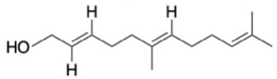
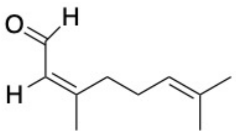
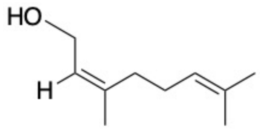
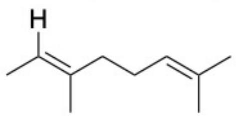
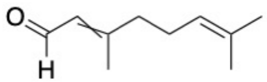
Oil yield (w/w %)	Previous study	This study
Citronella	3.0[14]	1.81
Rosemary	0.44[29]	1.18
Thyme	0.8[10]	0.54
Coriander seeds	0.39[30]	0.31

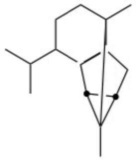
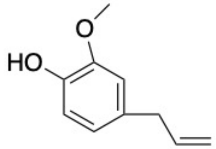
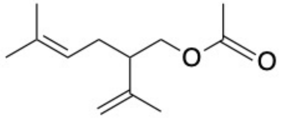
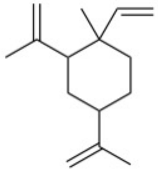
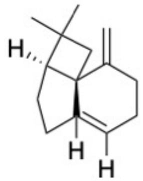
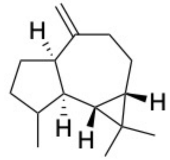
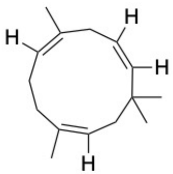
3.2.*Essential oils extracted from NSG spices versus commercial products*

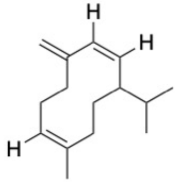
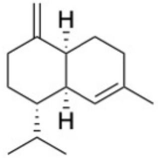
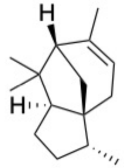
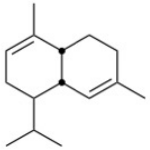
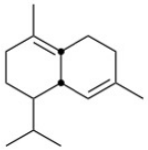
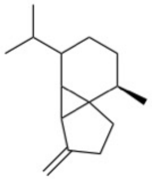
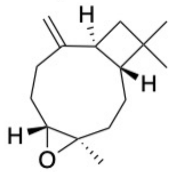
Table 2 shows the chemical constituents of the NSG spices and commercial products. The number of chemical constituents present in the essential oils extracted from rosemary and thyme is less than those in the commercial ones, but in the essential oils extracted from citronella and coriander seed, it is more (Fig. 1-4). The major chemical constituents in the NSG citronella essential oil are citronellal and geraniol, while citral and neral are the two major components in the commercial products. Linalool is found to have the highest percentage yield in commercial thyme essential oil while thymol is the most abundant one in the essential oil extracted from NSG thyme. In rosemary and coriander seeds, the major constituents are the same in both commercial and NSG essential oils. They are 1, 8-cineole (35.081%commercial and 62.267%$_{NSG}$) and linalool (67.317%commercial and 76.512%$_{NSG}$).

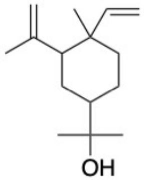
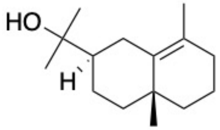
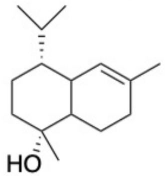
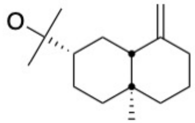
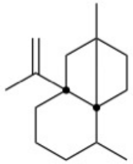
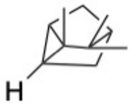
Table 2 Chemical compositions of essential oils from NSG (extracted samples of this work) and commercial products

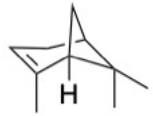
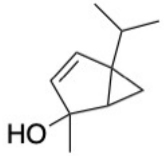
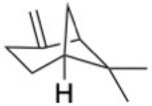
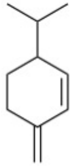
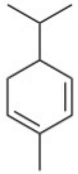
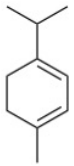
No.	Chemical name	Structure	Qualifying and quantitation ions (m/z)	Percentage		Previous study#
				Commercial*	NSG**	
Citronella essential oil						
1	D-Limonene		68, 93, 121, 136		3.639%	14
2	Camphene		67, 79, 93, 121	1.336%		
3	Borneol		41, 95, 110, 139	0.585%		26
4	1, 6-Octadien-3-ol, 3, 7-dimethyl- **(Linalool)**		71, 93, 121, 136		2.211%	26
5	5-Hepten-1-ol, 2,6-dimethyl- (Melonol)		41, 69, 82, 109		0.428%	
6	Cyclohexanol, 5-methyl-2-(1-methylethenyl)-, [1R-(1α, 2β, 5α)]- (Isopulegol)		67, 81, 93, 121		1.667%	26

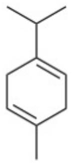
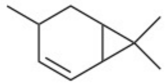
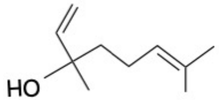
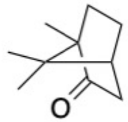
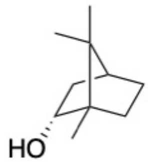
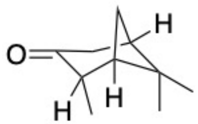
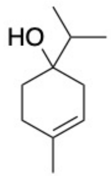
7	Bicyclo[4.1.0]heptane, 3-methyl- (3-Methylnorcarane)		81, 109, 123, 151	0.586%		
8	3-Octyne, 7-methyl-		41, 67, 95, 109	1.112%		
9	6-Octenal, 3,7-dimethyl- (Citronellal)		41, 69, 95, 121		29.562%	14
10	6-Octen-1-ol, 3,7-dimethyl- (Citronellol)		41, 69, 82, 95	0.190%		14
11	6-Octen-1-ol, 3,7-dimethyl-, (R)- **(6-Octen-1-ol, 3,7-dimethyl-, (3R)-)** (D-Citronellol)		69, 81, 95, 123		15.751%	
12	2-Octen-1-ol, 3,7-dimethyl-		69, 81, 95, 123		0.425%	
13	2, 6-Octadien-1-ol, 3,7-dimethyl- (Geraniol)		69, 93, 123, 154		24.601%	26
14	6,11-Dimethyl-2,6,10-dodecatrien-1-ol		41, 69, 81, 123		0.854%	
15	2,6-Octadienal, 3,7-dimethyl-,(z)- (Neral)		41, 69, 94, 109	36.373%		26
16	2,6-Octadien-1-ol, 3,7-dimethyl-, (Z)- (Nerol)		69, 93, 121, 136	4.745%	0.277%	15
17	2, 6-Octadiene, 2,6-dimethyl-		81, 95, 123, 138		1.368%	14
18	2,6-Octadienal,3,7-dimethyl- (Citral)		41, 69, 84, 94	41.189%		4

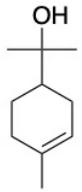
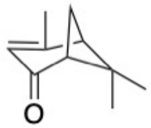
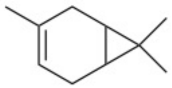
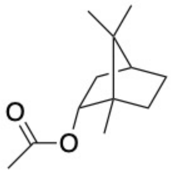
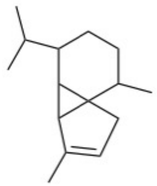
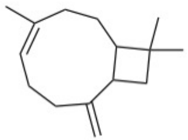
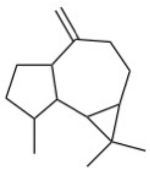
19	1,2,4-Metheno-1H-indene, octahydro-1,7a-dimethyl-5-(1-methylethyl)-, [1S-(1α, 2α, 3aβ, 4α, 5α, 7aβ, 8S*)]-(Cyclosativene)		105, 119, 161, 204	0.262%		
20	Eugenol		77, 103, 149, 164		0.400%	
21	4-Hexen-1-ol, 5-methyl-2-(1-methylethenyl),acetate (Lavandulyl acetate)		43, 69, 93, 121	2.854%	1.435%	
22	Cyclohexane, 1-ethenyl-1-methyl-2, 4-bis(1-methylethenyl)-, [1S-(1α,2β,4β)]-(β-Elemene, (-)-)		81, 93, 107, 121		2.158%	14
23	Caryophyllene		79, 93, 105, 133	2.991%		14
24	1H-Cycloprop[e]azulene, decahydro-1,1,7-trimethyl-4-methylene-, [1aR-(1aα,4aβ,7.α.,7aβ, 7bα)]-(Alloaromadendrene)		91, 105, 161, 204		0.140%	
25	α-Caryophyllene		41, 80, 93, 121	0.323%	0.104%	

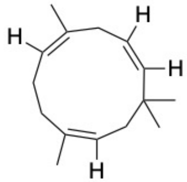
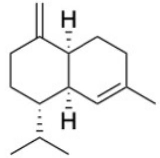
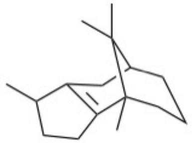
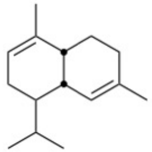
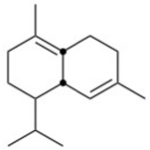
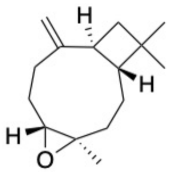
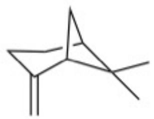
26	1,6-Cyclodecadiene, 1-methyl-5-methylene-8-(1-methylethyl)-, [s-(E, E)]- (Germacrene D)		91, 105, 119, 161	0.391%	1.488%	26
27	Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1-(1-methylethyl)-, (1α, 4aα, 8aα)- (γ-Muurolene)		41, 93, 105, 204		0.108%	14
28	α-Cedrene		41, 119, 161, 204		0.136%	25
29	Naphthalene,1,2,4a,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-(1α, 4aα, 8aα)- (α-Muurolene)		41, 105, 161, 204	2.024%	3.660%	14
30	Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, (1S-cis)- (D-Amorphene)		119, 134, 161, 204	0.525%	0.677%	14
31	1H-Cyclopenta[1, 3]cyclopropa[1, 2]benzene, octahydro-7-methyl-3-methylene-4-(1-methylethyl)-, [3aS-(3aα, 3bβ,4β,7α,7aS*)]- (β-Cubebene)		91, 105, 161, 204		0.324%	
32	Caryophyllene oxide		41, 79, 93, 107	0.584%		16

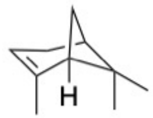
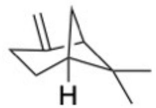
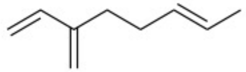
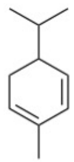
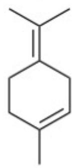
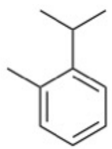
33	Cyclohexanemethanol, 4-ethenyl-α,α,4-trimethyl-3-(1-methylethenyl)-, [1R-(1α, 3α,4β)]-		59, 93, 161, 189		6.903%	
34	2-Naphthalenemethanol, 1,2,3,4,4a,5,6,7-octahydro-α,α, 4a, 8-tetramethyl-, (2R-cis)- (γ-Eudesmol)		59, 161, 189, 204		0.191%	
35	.tau.-Cadinol		105, 161, 189, 204		0.384%	
36	2-Naphthalenemethanol, decahydro- α,α, 4a-trimethyl-8-methylene-, [2R-(2α,4aα, 8aβ)]- (β-Eudesmol)		59, 108, 149, 164		0.367%	
37	1, 4-Methano-1H-indene, octahydro-1,7a-dimethyl-4-(1-methylethenyl)-, [1S-(1α,3aβ,4α,7aβ)]-		95, 161, 189, 204		0.741%	
Rosemary essential oil						
1	Tricyclo[2.2.1.0 (2,6)]heptane, 1,7,7-trimethyl- (Tricyclene)		41, 79, 93, 121	0.210%		17
2	Bicyclo[3.1.0]hex-2-ene, 2-methyl-5-(1-methylethyl)- (α-Thujene)		41, 77, 93, 136	0.236%		17

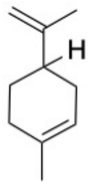
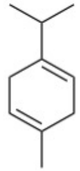
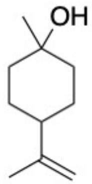
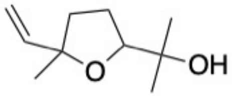
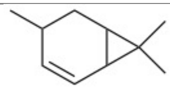
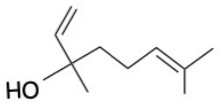
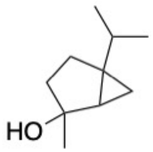
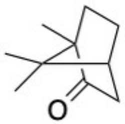
3	α-Pinene		41, 77, 93, 121	9.296%	12.267%	17
4	Camphene		39, 79, 93, 121	4.132%	3.910%	17
5	Bicyclo[3.1.0]hex-3-en-2-ol, 2-methyl-5-(1-methylethyl)-, (1α,2α, 5α)- (cis-2-Thujen-4-ol)		43, 91, 109, 119	0.147%		
6	Bicyclo [3.1.1]heptane, 6,6-dimethyl-2-methylene-,(1S)- (L-β-Pinene)		41, 69, 79, 93	6.321%	2.651%	
7	β-Phellandrene		41, 77, 93, 136	1.356%		29
8	α -Phellandrene		41, 77, 93, 136	0.676%		17
9	1, 3-Cyclohexadiene, 1-methyl-4-(1-methylethyl)- (α-Terpinene)		77, 93, 121, 136	1.094%		17
10	Eucalyptol (1,8-Cineole)		43, 81, 108, 139	35.081%	62.267%	17

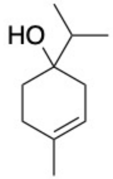
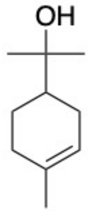
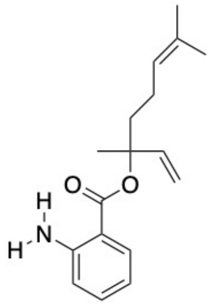
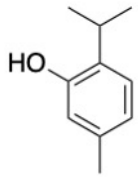
11	1,4-Cyclohexadiene, 1-methyl-4-(1-methylethyl)- (γ-Terpinene)		77, 93, 121, 136	1.934%		17
12	4-Carene		79, 93, 121, 136	0.570%		
13	1,6-Octadien-3-ol, 3,7-dimethyl- (Linalool)		41, 71, 93, 121	2.081%		17
14	Bicyclo[2.2.1] heptan-2-one, 1,7,7-trimethyl-, (1R)- (Camphor)		81, 95, 108, 152	14.874%	14.268%	17
15	Bicyclo[3.1.1]heptan-3-one, 2,6,6-trimethyl- (3-Pinanone)		55, 69, 83, 95	0.186%		
16	Bicyclo [2.2.1] heptan-2-ol, 1,7,7-trimethyl-, (1S-endo)- (Linderol)		41, 95, 110, 139	4.056%	4.073%	
17	Bicyclo[3.1.1]heptan-3-one, 2,6,6-trimethyl-, (1α, 2β, 5α)- (Isopinocamphono)		55, 69, 83, 152	0.339%		
18	3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)- (Terpinen-4-ol)		43, 71, 93, 111	1.293%		

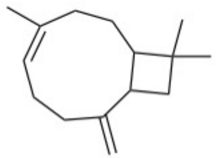
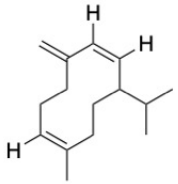
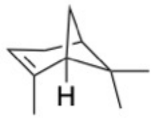
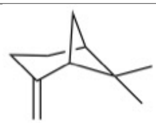
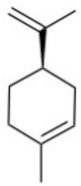
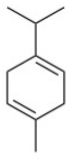
19	3-Cyclohexene-1-methanol,α,α,4-trimethyl- (α-Terpineol)		59, 93, 121, 136	3.305%		17
20	Bicyclo[3.1.1]hept-3-en-2-one, 4,6,6-trimethyl-, (1S)- (Verbenone)		91, 107, 135, 150	0.469%		17
21	Bicyclo[4.1.0]hept-3-ene, 3,7,7-trimethyl-, (1S)- (3-Carene)		77, 93, 121, 136	0.302%		17
22	Bicyclo[2.2.1] heptan-2-ol, 1,7,7-trimethyl-, acetate, (1S-endo)- (Bornyl acetate)		43, 95, 121, 136	1.054%		17
23	α-Cubebene		105, 119, 161, 204	1.006%		
24	β-Caryophyllene		93, 133, 161, 189	6.594%	0.565%	17
25	1H-Cycloprop[e]azulene, decahydro-1,1,7-trimethyl-4-methylene (Aromadendrene)		69, 93, 161, 204	0.198%		

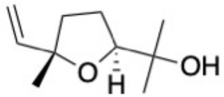
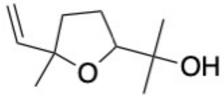
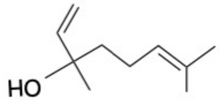
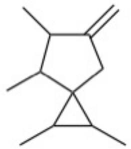
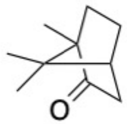
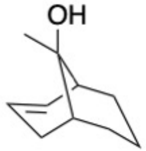
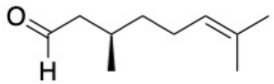
26	α-Caryophyllene		80, 93, 121, 147	0.862%	7
27	Naphthalene,1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1-(1-methylethyl)-,-(1α,4aα,8aα)- (γ-Muurolene)		105, 119, 161, 204	0.636%	
28	4,7-Methanoazulene, 1,2,3,4,5,6,7,8-octahydro-1,4,9,9-tetramethyl-, [1S-(1α, 4α,7α)]- (β-Patchoulene)		105, 119, 161, 189	0.372%	
29	Naphthalene, 1,2,4a, 5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)- (α- Muurolene)		91, 105, 161, 204	0.277%	17
30	Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, (1S-cis)- (delta-Amorphene) (D-Amorphene)		81, 134, 161, 204	0.858%	
31	Caryophyllene oxide		41, 79, 93, 107	0.185%	29
Thyme essential oil					
1	β-Pinene		41, 69, 77, 93	0.345%	19

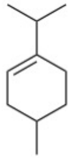
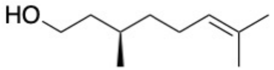
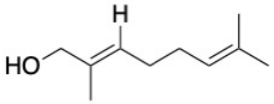
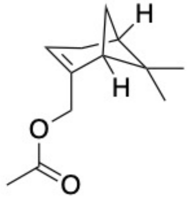
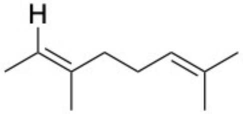
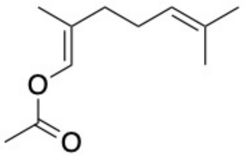
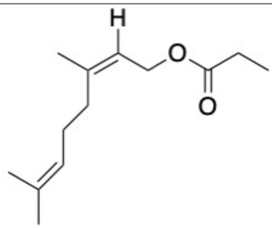
2	Bicyclo[3.1.0]hex-2-ene, 2-methyl-5-(1-methylethyl)- (α-Thujene)		41, 77, 93, 136	0.345%	2.615%	19
3	α-Pinene		41, 77, 93, 121	4.626%	1.646%	13
4	Camphene		39, 79, 93, 121	1.308%	0.883%	19
5	Bicyclo[3.1.1]heptane, 6,6-dimethyl-2-methylene-, (1S)- (L-β-Pinene)		41, 69, 79, 93	1.900%	0.447%	
6	β-Myrcene		41, 69, 79, 93	5.264%	0.944%	21
7	α-Phellandrene		41, 77, 93, 136	0.532%		19
8	Cyclohexene, 1-methyl-4-(1-methylethylidene)- (Terpinolene)		39, 93, 121, 136	4.942%	2.905%	19
9	Benzene, 1-methyl-2-(1-methylethyl)- (O-Cymene)		41, 91, 119, 134	2.344%	15.997%	21

10	Limonene		39, 68, 93, 136	5.907%		22
11	1, 4-Cyclohexadiene, 1-methyl-4-(1-methylethyl)- (γ-Terpinene)		77, 93, 121, 136	7.915%	28.005%	19
12	Terpineol, cis-β- (β-Terpineol)		43, 71, 93, 111	3.862%		
13	2-Furanmethanol, 5-ethenyltetrahydro-α,α,5-trimethyl- (Linalyl oxide)		43, 59, 94, 111	0.945%		
14	4-Carene		79, 93, 121, 136	2.294%		
15	1,6-Octadien-3-ol, 3,7-dimethyl- (Linalool)		41, 55, 71, 93	37.722%		19
16	Bicyclo[3.1.0]hexan-2-ol, 2-methyl-5-(1-methylethyl)-, (1α,2β,5α)- (4-Thujanol)		43, 71, 93, 121	0.600%		
17	Bicyclo[2.2.1]heptan-2-one, 1,7,7-trimethyl-, (1R)- (Camphor)		81, 95, 108, 152	0.972%		19

18	Borneol		41, 95, 110, 139	2.528%	0.968%	19
19	3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)- (Terpinen-4-ol)		71, 93, 111, 154	11.458%		13
20	p-menth-1-en-8-ol (α-Terpineol)		59, 93, 121, 136	1.712%		20
21	1,6-Octadien-3-ol, 3,7-dimethyl-, 2-aminobenzoate (Linalyl anthranilate)		43, 80, 93, 121	0.645%		
22	Thymol		91, 117, 136, 150		42.579%	19

23	β-Caryophyllene		93, 133, 161, 189	1.832%	2.521%	13
24	1,6-Cyclodecadiene, 1-methyl-5-methylene-8-(1-methylethyl)-, [s-(E, E)]- (Germacrene D)		41, 105, 161, 204		0.492%	19
Coriander seed essential oil						
1	1S-α -Pinene		41, 77, 93, 121	12.423%	5.228%	
2	Camphene		39, 79, 93, 121	0.268%	0.124%	11
3	β-Pinene		41, 69, 77, 93	0.382%	0.760%	11
4	D-Limonene		68, 93, 121, 136	10.738%	0.541%	1
5	1,4-Cyclohexadiene, 1-methyl-4-(1-methylethyl)- (γ-Terpinene)		77, 93, 121, 136		0.553%	11

6	Linalool oxide trans		43, 59, 94, 111	0.611%	0.316%	
7	2-Furanmethanol, 5-ethenyltetrahydro-α,α, 5-trimethyl-, cis- (Linalyl oxide)		43, 59, 68, 94	0.922%		
8	1,6-Octadien-3-ol, 3,7-dimethyl- (Linalool)		55, 71, 93, 121	67.317%	76.512%	11
9	Spiro[2,4]heptane, 1,2,4,5-tetramethyl-6- methylene-		41, 79, 93, 108		0.523%	
10	trans-Pinocarveol		70, 92, 119, 134		0.370%	
11	Bicyclo[2.2.1]heptan-2-one, 1,7,7-trimethyl-, (1R)- (Camphor)		81, 95, 108, 152	7.338%		11
12	Bicyclo[3.3.1]non-2-en-9-ol, 9-methyl-		43, 67, 108, 134		0.705%	
13	6-Octenal, 3,7-dimethyl-, (R)- ((+)-Citronellal)		41, 69, 95, 121		0.485%	
14	Borneol		41, 95, 110, 139		0.741%	11

15	Cyclohexene, 4-methyl-1-(1-methylethyl)- (p-menth-3-ene) (3-Menthene)		79, 93, 107, 136	0.357%	
16	6-Octen-1-ol, 3,7-dimethyl-, (R)- (D-Citronellol)		41, 69, 81, 95	0.618%	
17	2,6-Octadien-1-ol, 2,7-dimethyl-		41, 55, 69, 93	0.584%	
18	Myrtenyl acetate		43, 91, 119, 134	0.266%	11
19	2,6-Octadiene, 2,6-dimethyl-		69, 81, 95, 123	0.434%	
20	2,6-Octadien-1-ol, 3,7-dimethyl-, acetate, (E)- (Geranyl acetate)		41, 69, 93, 121	10.496%	11
21	2,6-Octadien-1-ol, 3,7-dimethyl-, propanoate, (Z)- (Neryl propionate)		57, 69, 93, 121	0.388%	

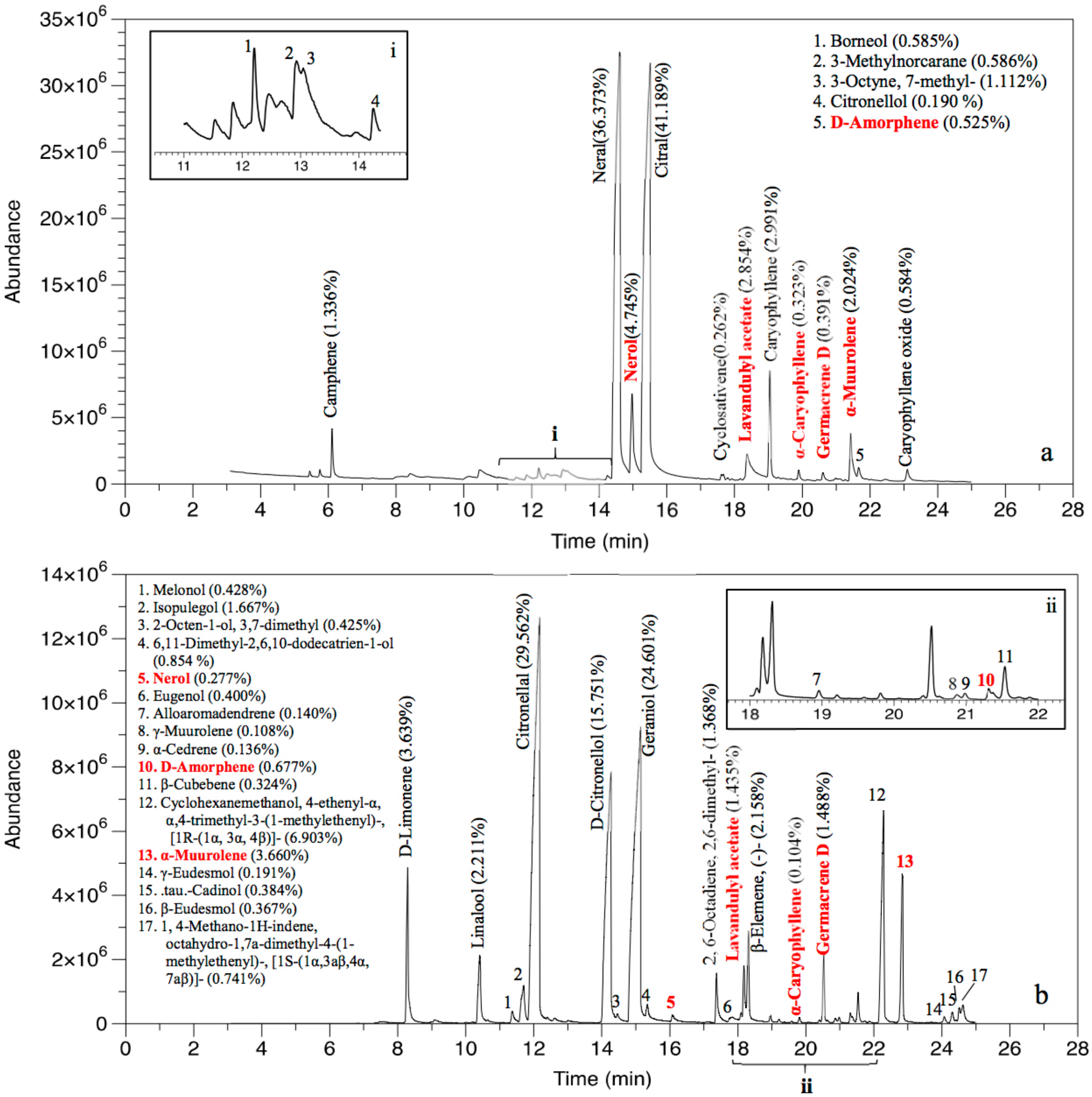


Figure 1 Total ion chromatogram of citronella essential oils, (a) commercial citronella essential oil (i. chromatogram for 11-14.5 min) and (b) NSG citronella essential oil (ii. chromatogram for 18-22 min) (The names in red color are the common chemical components in both commercial and NSG essential oils)

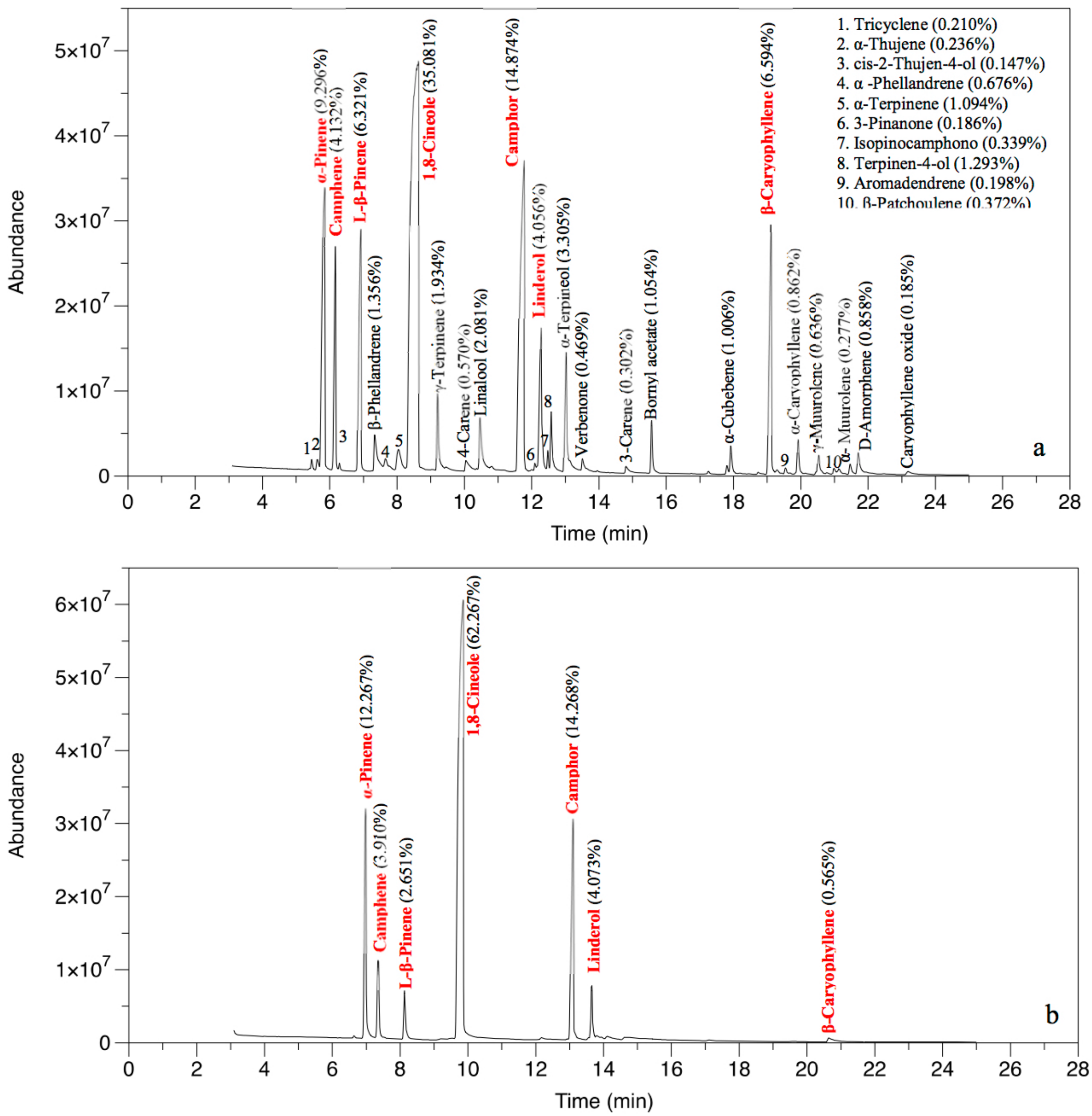


Figure 2 Total ion chromatogram of rosemary essential oils, (a) commercial rosemary essential oil, and (b) NSG rosemary essential oil (The names in red color are the common chemical components in both commercial and NSG essential oils)

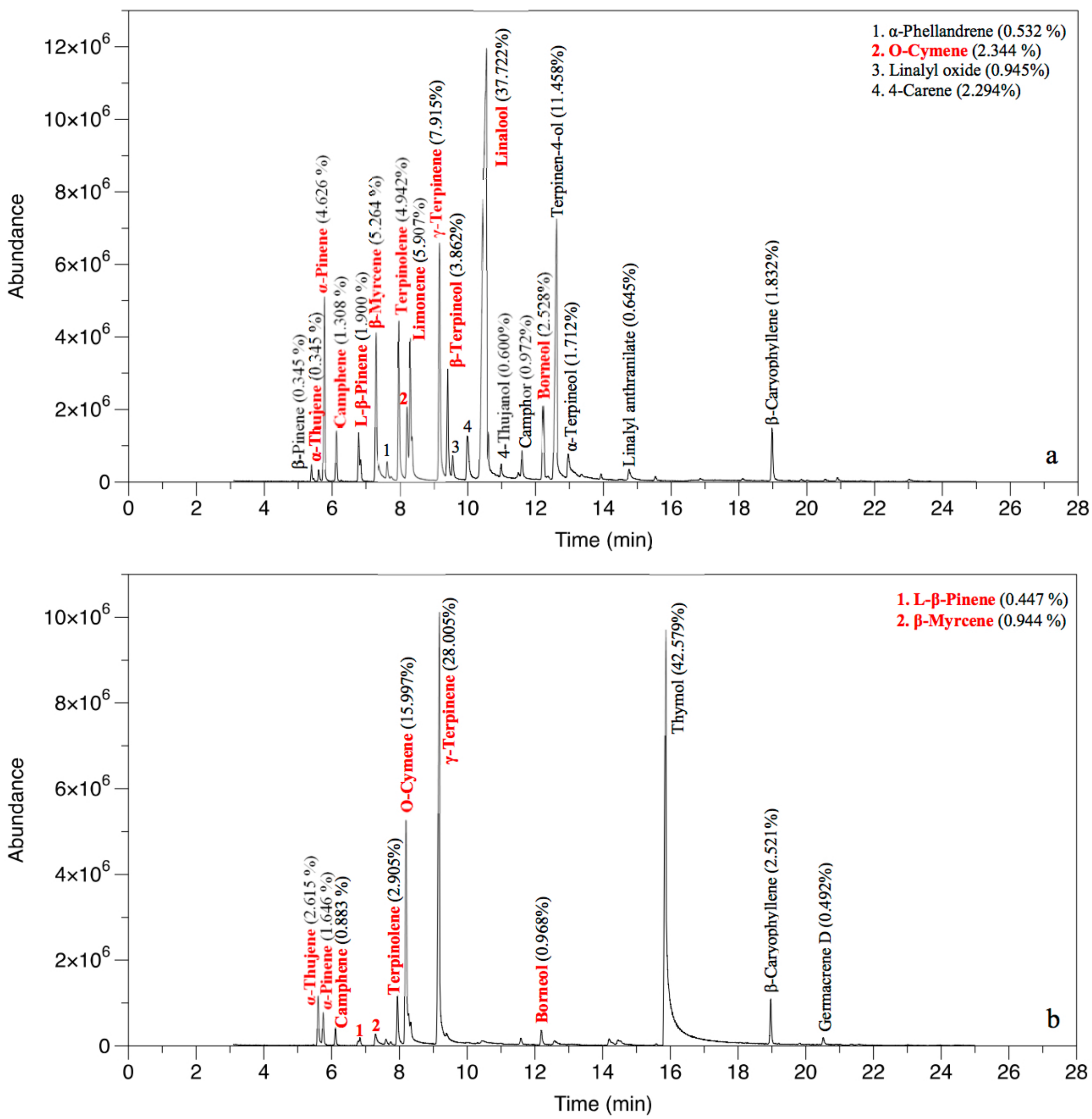


Figure 3 Total ion chromatogram of thyme essential oils, (a) commercial thyme essential oil and (b) NSG thyme essential oil (The names in red color are the common chemical components in both commercial and NSG essential oils)

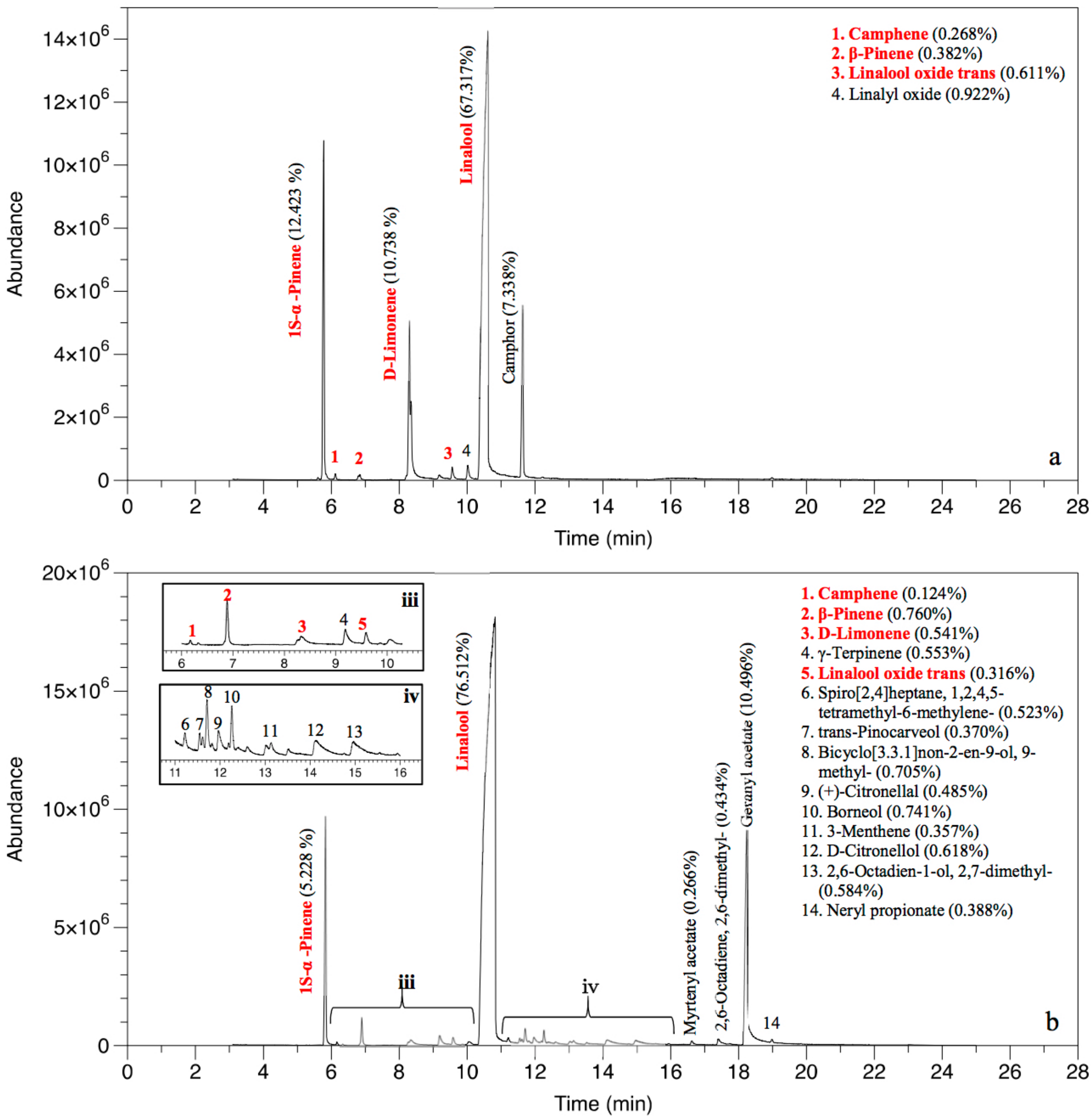


Figure 4 Total ion chromatogram of coriander seed essential oils, (a) commercial coriander seed essential oil and (b) NSG coriander seed essential oil (iii. chromatogram for 6-10 mins. iv. chromatogram for 11-16 mins) (The names in red color are the common chemical components in both commercial and NSG essential oils)

Overall, the essential oils extracted from NSG spices are similar to those commercial products. The loss of some chemical constituents in the essential oil of the NSG spices may attribute to the factors such as long-term storage and dysplasia. In addition, according to previous studies, parts of plant, cultivation environment and species make significant effects on the chemical compositions of essential oils, which can further impact on their antimicrobial activities (10, 14). In this study, the NSG and commercial essential oils were extracted from whole herb of citronella, thyme and the seeds of coriander. However, for NSG rosemary, the essential oil was extracted from the leaves while the commercial one was extracted from both flowers and leaves. Not only the parts of plant are different, we also believe that cultivation environment (the quality of soil, sunshine duration, precipitation, water for irrigation, fertilizing techniques), freshness and species of spices could be different because the sample spices were grown in different areas in the world. Furthermore, it is not certain that the heating temperature used for hydro-distillation extraction in this study is the same as that used for extracting the commercial ones. As a result, the chemical constituents in essential oils are different. Nevertheless, the results in this study show that the essential oils extracted from NSG spices contain significant amounts of major chemical constituents.

3.3.Essential oils extracted in this study versus essential oils extracted in other studies

Some of the chemical constituents found in the essential oils extracted in the NSG spices in this study were reported by previous studies (Table 2), and the percentage yields of some components are varied.

Citronellal is the most abundant chemical constituent in the citronella essential oil (Fig. 1), which mainly contributes to the special lemongrass aroma (16). Compared with the percentage of citronellal reported by Wei and Wee 29.6% (16), similar result was obtained in the present study (29.562%). However, the percentage of that mentioned by Nakahara et al. was much lower (5.8%) (17). Discrepancies in the chemical constituents were also noticed for geraniol in NSG extract in this study (24.601%) compared to those described by Nakahara et al. (35.7%) and Budzyńska et al. (22.4%), respectively (17-18). Besides, 3.639% of D-limonene was determined in the NSG extract in this study. None was reported by Nakahara et al., but only 2.96% was found in the study of Chen et al. (4, 17).

1, 8-Cineole was agreed to be the most abundant substance in rosemary essential oil according to Jiang et al. (26.54%), Vasile et al., (43.1%) and Ladu et al. (13.26%) (7, 8, 19). In this study the percentage yield (62.267%) is even higher than those in their studies (Fig. 2). Camphor in the NSG essential oils (14.268%) extracted in this study was also higher than those obtained by Jiang et al. (12.88%), Vasile et al. (11.3%) and Ladu et al. (7.19%) (7, 8, 19), but lower than that reported by Okoh et al. (16.89%) (20). Differences can also be noticed in the percentage of β-caryophyllene in the NSG extract from rosemary (0.565%) compared to those reported by Vasile et al. (3.2%), Ladu et al. (1.52%), and Okoh et al. (1.11%), (8, 19-20). This substance was not even found in the study by Jiang et al. (7).

Similarly, thymol was found to be the most abundant substance in the thyme essential oil by Mancini et al. (46.2-63.0%) and Sharafzadeh et al. (53.70-63.63%) (11, 21), of which the percentages are higher than that of this study (Fig. 3). In contrast, only 0.24% of thymol was found in another study (15). Differences can also be noticed in the percentage of γ-terpinene in the NSG extract from thyme (28.005%) compared to those reported by Al-Asmari et al. (1.18%) and Gedikoğlu et al. (13.25%) (22, 23). O-Cymene determined in this study (15.997%) was much higher than the result (0.39%) obtained by Al-Asmari et al. (22). The percentage of borneol determined in this study (0.968%) was lower than the result of Sharafzadeh et al.(4.91%) (21) but higher than that obtained by Mancini et al. (0.5%) (11).

Linalool was found to be the most abundant in coriander seed essential oils according to Hassanen et al. (54.08%), Teneva et al. (58.141%), and Sourmaghi et al. (66.29%) (1, 13, 24), but these percentages are lower than that obtained in this study (Fig. 4). However, Linalool acetate was suggested to be the major component in the study of Bogavac et al. (43.1%) (25), which was not found in the NSG coriander seed essential oils in this study. D-Limonene was analyzed to be 4.94% by Hassanen et al. (1), which is higher than the percentage in NSG citronella seed essential oil (0.541%) in this study. Geranyl acetate reported by Hassanen et al. (2.04%), Teneva et al. (3.906%), and Sourmaghi et al. (0.06%) (1, 13, 24) were much lower than that of the NSG essential oil in the present study (10.496%). But the result of Laribi et al. indicated that the percentage of geranyl acetate from essential oil extracted from coriander seed originated from Bangladesh was as much as 17.57% (26).

In conclusion, the percentage of only some chemical constituents in the NSG essential oils are lower than those presented in previous studies, while the percentage of other chemical constituents in the NSG essential oils are either similar or even higher than those mentioned in previous studies. The loss of certain kinds of chemical constituents might due to poor storage environment, long-term storage duration, dysplasia, etc. as mentioned before, but these factors do not make great effects on the overall chemical composition. The cultivation environment, species, and distillation methods are more important factors contributed to the variation in chemical constituents of essential oils.

3.4.Antimicrobial activity of the extracted essential oils

To further confirm the quality of the NSG essential oils, the antimicrobial activity was determined and compared to that of the commercial ones. The average diameters of inhibitory zones of essential oil samples are compared in Fig. 5. All kinds of essential oils performed antibacterial activities towards S. aureus, and the NSG thyme essential oil revealed the best. Inhibitory effects to E. coli can be observed from both commercial and NSG thyme and commercial coriander seed essential oils, with the inhibitory effect of NSG thyme essential oil being the highest. It ought to be pointed out that for rosemary, thyme and coriander seed essential oils, the commercial products all had no antimicrobial activity to C. albicans, but all the NSG essential oils did. Commercial and NSG citronella essential oils, and NSG coriander seed essential oils presented very strong inhibitory effects, with the inhibition zone diameter of 45.0 mm towards C. albicans. According to Lara et al., the differences in antimicrobial activity of the same essential oil can be explained by the influences from different extraction methods, the changes in season and climate, and the geographic distribution on the growth of spices (9). These factors may lead to the changes in chemical compositions of essential oils and consequently alter their antimicrobial activities.

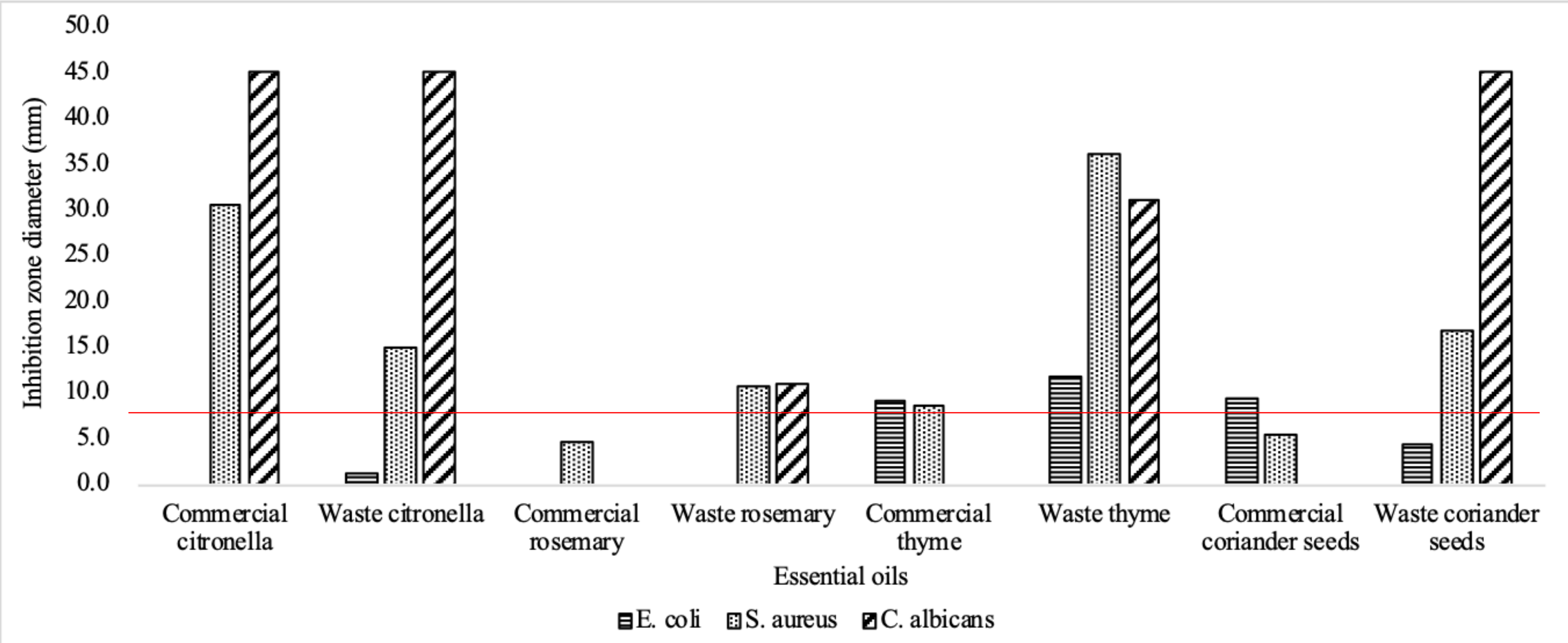


Figure 5 Antimicrobial activity of essential oils. Zone of growth inhibition values is presented as mean ± standard deviation ($p < 0.05$). The error bars represent the standard errors (SD). The red line represents the inhibition zone diameter (d) of 7.0 mm, which is a standard to evaluate whether the essential oil has inhibitory effect (d > 7.0 mm: the essential oil has inhibitory effect; d ≤ 7.0 mm: the essential oil has no inhibitory effect)

It was suggested that citronella essential oil can inhibit the growths of E. coli and S. aureus effectively (6). Similar results were also obtained by Wei & Wee (16). According to Budzyńska et al. and Taweechaisupapong et al. (18, 27), citronella essential oil had good inhibitory effect to C. albicans. Compared to their findings, both commercial and NSG citronella essential oils of this study were found to have no inhibitory effect towards E. coli (Fig. 5). According to Taweechaisupapong et al. and Ganjewala, citronellal, citronellol and geraniol contribute to the antimicrobial activity of the citronella essential oil (27), which are analyzed in the NSG citronella essential oil in this study (Fig. 1).

For rosemary essential oils, the NSG extracts performed antimicrobial activities only towards S. aureus and C. albicans but not towards E. coli, whereas commercial products had no inhibitory effects towards all these three microbes (Fig. 5). Rosemary essential oil had inhibitory effect to E. coli, S. aureus and C. albicans (7), and similar results were also obtained by Nieto, Vasile et al. and Tahri et al., who found that rosemary essential oil presented good inhibitory effect to E. coli (8). The inhibitory effect towards E. coli in this study was found to be in accordance with that of Lara et al. (9), who indicated that rosemary essential oil has no antibacterial effect against E. coli. It was reported that the antimicrobial activities of rosemary essential oil were contributed by the major components 1, 8-Cineole and α-Pinene (20). Since all chemicals analyzed in NSG rosemary essential oil were found in the commercial product (Fig. 2), there might be some substances existing in the commercial product that can inhibit the antimicrobial activities, especially 1, 8-Cineole and α-Pinene.

NSG thyme essential oil presented strong inhibitory effects to S. aureus and C. albicans, which obviously stronger compared to those of the commercial products. The antimicrobial activities of NSG extracts towards E. coli was relatively weaker, but still stronger than those of the commercial products (Fig. 5). According to the literature review, about sixteen studies suggested that thyme extracts have strong inhibitory effect to E. coli, seventeen studies suggested that they have strong antimicrobial activity to S. aureus, and ten studies indicated that they have strong inhibitory effect to C. albicans (12). Similar results were also obtained by Cutillas et al. and El Bouzidi et al. (10, 28). These results are all corresponding similarly to the results obtained, while deviation occurred only for commercial thyme essential oil in this study. It was reported that the inhibitory activity towards S. aureus was found to be the strongest, and the inhibitory activity towards the other two microorganisms were found to be similar (10). By contract, El Bouzidi et al. indicated that thyme essential oils presented the strongest inhibitory effect towards C. albicans, followed by S. aureus, and the inhibitory activity to E. coli was the weakest (28). However, the results in this study were different from those studies, which indicated that the inhibitory effect of thyme essential oil towards S. aureus is the strongest, followed by C. albicans (slightly weaker), and the antimicrobial activities to E. coli was the weakest. Besides, Marchese suggested that thymol is the major effective substance that contributes to the antimicrobial activity of thyme to E. coli, S. aureus and C. albicans (12), which was found in the NSG essential oil but not in commercial essential oil (Fig. 3). Hence, though the spices have poor appearance and poor quality, the active components to against microbes are still remained, and the NSG spices can be used for producing antimicrobial essential oils.

The commercial coriander seed essential oils showed inhibitory effect only to E. coli, whereas NSG coriander seed essential oils exhibited good inhibitory effects to S. aureus and C. albicans, but not to E. coli (Fig. 5). The results of NSG coriander seed essential oils obtained in this study are in consistence with the findings of Teneva et al. , who suggested that the inhibitory effect of coriander seed essential oil is more effective to inhibit the growth of Gram-positive bacteria compared with Gram-negative bacteria (13). According to Hassanen et al., the diameters of inhibition zone of 100% of coriander seed essential oil to E. coli and S. aureus are 47 mm and 30 mm, respectively (1), which are larger than the results obtained in this study. Sourmaghi et al. suggested that the antimicrobial activities of coriander seed essential oil were much stronger against S. aureus than E. coli and C. albicans, as the later ones are similar to each other (24). Linalool, analyzed in NSG coriander seed essential oil (Fig. 4), has strong antimicrobial activity (24-25).

According to Hashemi et al., the outer membrane of Gram-negative bacteria represents an effective hurdle, thus gram-positive bacteria are more sensitive to essential oils (2). This finding can explain the result in this study that each kind of essential oil sample (except commercial thyme and commercial coriander seed essential oils) showed stronger inhibitory activities towards S. aureus compared to E. coli. According to this finding, a possibility that the sensitivity towards microorganism by essential oils is arranged as: fungi > gram positive-bacteria > gram-negative bacteria (2). Commercial citronella essential oil, NSG citronella essential oil, and NSG coriander seed essential oil were found to obey this rule since their antimicrobial activities against C. albicans were found to be the strongest among the three microorganisms tested. Since the major effective chemicals of the four essential oils reported by previous studies were all analyzed in the NSG essential oils, the poor appearance and poor quality of the NSG spices does not significantly affect the antimicrobial activity. Therefore, the NSG spices are valuable to be recovered and the essential oils extracted from them are still good antimicrobial products.

In this study, the chemical constituents and antimicrobial activities of essential oils extracted from NSG spices were determined. These properties were compared with those from commercial products. The chemical constituents of NSG essential oils were similar to both commercial products and those reported by previous studies. It is worth in noting that the antimicrobial activity of the essential oils extracted from NSG spices was similar or even better than those extracted from the commercial ones used in this study. NSG thyme essential oil has good inhibitory effect on the three microbes tested (E. coli, S. aureus and C. albicans), and NSG citronella and coriander seed essential oils have very strong antimicrobial activity towards C. albicans. The essential oils extracted from NSG spices showed good quality for non-edible purposes.

The results of this study indicated that NSG spices can certainly be used to extract essential oils. Compared to the commercial ones, the properties in flavoring, coloring, preserving, and particularly antimicrobial activity as the focus of this study are similar or even better. Essential oils are trendy nowadays, which are commonly viewed as predominant quality products, produced with fine crude materials, and consequently cost. This approach can help to increase the awareness for environmental protection and enrich local education in sustainable development. Its possibility is sensible, in any case it ought to be joined by specialized support. This work can be a turning point for NSG spices treatment, which can highly contribute to the environmental health, public health and economic development.

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