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Original Article

Essential oils from Taiwan: Chemical composition and antibacterial activity against *Escherichia coli*

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ARTICLE INFO

Article history:
Received 15 May 2015
Received in revised form
4 September 2015
Accepted 25 December 2015
Available online 11 April 2016

Keywords:
antibacterial activity
chemical composition
Escherichia coli
essential oil
gas chromatography-mass spectrometry

ABSTRACT

The chemical compositions of seven essential oils from Taiwan were analyzed by gas chromatography-mass spectrometry. The eluates were identified by matching the mass fragment patents to the National Institute of Standards and Technology (NIST) 08 database. The quantitative analysis showed that the major components of lemon verbena are geranial (26.9%) and neral (23.1%); those of sweet marjoram are γ-terpinene (18.5%), thymol methyl ether (15.5%), and terpinen-4-ol (12.0%); those of clove basil are eugenol (73.6%), and β-(Z)-ocimene (15.4%); those of patchouli are carvacrol (47.5%) and *p*-cymene (15.2%); those of rosemary are α-pinene (54.8%) and 1,8-cineole (22.2%); those of tea tree are terpinen-4-ol (33.0%) and 1,8-cineole (27.7%); and those of rose geranium are citronellol (28.9%) and 6,9-guaiadiene (20.1%). These components are somewhat different from the same essential oils that were obtained from other origins. Lemon verbena has the same major components everywhere. Tea tree, rose geranium, and clove basil have at least one major component throughout different origins. The major components and their amounts in sweet marjoram, patchouli, and rosemary vary widely from one place to another. These results demonstrate that essential oils have a large diversity in their composition in line with their different origins. The antibacterial activity of essential oils against *Escherichia coli* was evaluated using the optical density method (turbidimetry). Patchouli is a very effective inhibitor, in that it completely inhibits the growth of *E. coli* at 0.05%. Clove basil and sweet marjoram are good inhibitors, and the upper limit of their minimum inhibitory concentration is 0.1%.

1. Introduction

Essential oils have a long history of being used by people in their daily lives for both spiritual and practical reasons. Plant products were the principal sources of pharmaceutical agents used in traditional medicine. Some medicinal plants are rich in antimicrobial reagents [1]. Several essential oils derived from varieties of medicinal plants are known to possess

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http://dx.doi.org/10.1016/j.jfda.2015.12.006

insecticidal, antifungal, anti-inflammatory, and antioxidant activities [2–5]. Using essential oils from flowering plants (angiosperms) as food preservatives can be traced back to the ancient Egyptians [6]. In modern society, pure chemicals are developed for use as insecticides, food preservatives, and antibacterial reagents. The practical functions of essential oils have been completely replaced by industrial products. However, in recent years, many concerns have been raised regarding the overuse of chemicals as preservatives and additives in food products. The urge to search for healthy chemical substitutes has drawn massive attention to essential oils [7]. Addition of essential oils into food, drugs, and cosmetics is now desired in various products. Many remarkable results have been made possible by the advent of essential oil products. It is therefore important to scientifically evaluate the possible applications of essential oils.

One of the intriguing properties of essential oils is their antibacterial property. Depending on the plants' growth condition, such as location and climate, the chemical composition of the same essential oils may vary. Hence, it is important to determine the chemical composition of each essential oil. Documentation of the contents of essential oils not only makes the investigation of the influence between essential oils and claims possible, but it also builds a common ground for discussion.

In this paper, we report the chemical compositions of seven essential oils that are extracted from the plants that were grown and harvested in Ji-an Town, Hualien, Taiwan, and the potential of these essential oils for inhibition against *Escherichia coli*.

2. Materials and methods

2.1. Chemicals

Ethyl acetate was ACS grade and was purchased from Mallinckrodt (Dublin, Ireland). Normal paraffin C7, C8, C9, C10 mix and C10, C12, C14, C16 mix were purchased from Aldrich (St. Louis, USA). Sodium chloride was purchased from Merck (Darmstadt, Germany). Tryptone (casein hydrolysate) enzymatic digest, yeast extract, and agar were purchased from USB Corporation (Cleveland, USA). All reagents were used as received without further purification.

2.2. Plant material

Fresh leaves of *Lippia citrodora* (Paláu) Kunth (lemon verbena), *Origanum majorana* L. (sweet marjoram), *Ocimum gratissimum* L. (clove basil), *Pogostemon cablin* Benth. (patchouli), *Rosmarinus officinalis* L. (rosemary), *Melaleuca alternifolia* Cheel (tea tree), and *Pelargonium graveolens* L'Hér. (rose geranium) were collected from Ji-an Town, Hualien, Taiwan. The plant samples were identified, and the voucher specimens were deposited at the Herbarium of the Department of Life Science, National Taiwan Normal University. The voucher numbers are as follows: *P. cablin* Benth (TNU055241); *P. graveolens* L'Hér (TNU055242); *O. majorana* L. (TNU055243); *O. gratissimum* L. (TNU055246); *M. alternifolia* Cheel (TNU055247); *L. citrodora* (Paláu) Kunth (TNU055248); *R. officinalis* L. (TNU055249). All seven essential oils were extracted from air-dried leaves of plants by hydrodistillation for 40 minutes using a Clevenger-type apparatus. The oil samples obtained were dried over anhydrous sodium sulfate and stored in sealed vials in a cool and dark place prior to analyses. Essential oils were volumetrically diluted to a thousand times in ethyl acetate prior to gas chromatography (GC) injection.

2.3. GC and GC-mass spectrometry analysis

GC was performed on Agilent Technologies 6850 Series II equipped with a flame ionization detector. The capillary column was HP-5MS cross bond (5% diphenyl-polysiloxane and 95% dimethyl-polysiloxane; 30 m × 250 μm × 0.25 μm). The injector and detector temperatures were both set at 250°C. Nitrogen was used as the carrier gas, and the flow rate was set to constant mode (1 mL/min). Injection volume was 1 μL and the injection mode was splitless. The temperature was raised by a step gradient that starts at 60°C for 15 minutes then quickly rises up to 80°C in 4 minutes, followed by a slow rise to 135°C in 55 minutes, and finally a rapid rise to 260°C in 8 minutes and hold for 2 minutes. GC-mass spectrometry (GC-MS) analysis was performed using a Hewlett-Packard 6890 Gas Chromatograph and Hewlett-Packard 5973 Mass Selective Detector. The capillary column was also HP-5MS cross bond. The injector and detector temperatures were set at 250°C and 230°C, respectively. Helium was used as the carrier gas, and the flow rate was set to constant mode (1 mL/min). The injection volume was 1 μL, and the injection mode was split with a 50:1 ratio. The temperature gradient was the same as in the GC condition. Ionization voltage was 70 eV by electron impact. The acquisition mass range was set to 30–650 amu. The mass spectra were searched and compared through the database of National Institute of Standards and Technology (NIST) 08 libraries for characterization (the matching quality between the experimental data and the database is more than 90%). For further confirmation, Kovats retention index (RI) was calculated relative to standard *n*-alkanes of *n*-paraffin mix C7, C8, C9, C10 and C10, C12, C14, C16. The calculated RI was compared with those reported by Pino et al [8] and Pitarokili et al [9]. The relative percentage amounts were calculated on the basis of peak areas for both GC and GC-MS analysis, and the results were very similar. To confirm the high percentage components, standards were used to spike into the sample.

2.4. Antibacterial test

Sterilized lysogeny broth (LB) was prepared for the growth of *E. coli*. Frozen stock of strain ATCC 13676 was grown at 37°C on a 1.5% agar plate overnight. An *E. coli* stock solution was prepared by suspending a selected colony to 1 mL sterilized LB [10]. For each test, tubes with 5 mL sterilized LB were inoculated with 40 μL of *E. coli* stock solution. Essential oils were added to the tubes directly to give the appropriate concentrations: 0.01%, 0.05%, and 0.1% (v/v). The antibacterial activity of the essential oils against *E. coli* was evaluated using the optical density method (turbidimetry) [11]. During the incubation (37°C and 150 rev/min), the optical density of the inoculated broths at 600 nm was measured using Amersham Pharmacia Biotech Novaspec II Visible Spectrophotometer

Table 1 – Volatile components of the seven essential oils and their relative abundance.

RI[a]	RI[b]	RI[c]	Compounds[d]	Lemon verbena	Sweet marjoram	Clove basil	Patchouli	Rosemary	Tea tree	Rose geranium
				% of total[e]						
916	923	905	Tricyclene					t		
919	931	909	α-Thujene	t	1.7		0.7	t	t	
925	939	918	α-Pinene	0.9	1.5		t	54.8	2.6	
939	953	934	Camphene					4.7		
963	976	955	Sabinene	2.1	4.8				t	
968	978	—[f]	1-Octen-3-ol		t					
969	980	962	β-Pinene	t			t	2.6	t	
977	985	—	6-Methyl-5-hepten-2-one	t						
985	991	976	β-Myrcene	t	2.4		1.1	t	t	
1005	1005	990	α-Phellandrene				t	t	t	
1016	1018	1005	α-Terpinene		8.1		1.9	t	5.8	
1026	1026	1010	*p*-Cymene		9.2		15.2	t	6.0	
1031	1031	—	Limonene	16.1	2.6		t	3.7	2.9	
1033	1032	1024	1,8-Cineole	4.6			2.4	22.2	27.7	
1041	1040	—	β-(*Z*)-Ocimene	t	3.5	15.4				
1053	1050	1032	β-(*E*)-Ocimene	2.4						
1061	1062	1043	γ-Terpinene	t	18.5		10.7	t	14.0	
1075	—	1051	*cis*-Sabinene hydrate	t	1.9			t		
1087	1087	1072	Terpinolene		3.2			t	2.2	
1097	—	1086	*trans*-Sabinene hydrate		9.9					
1102	1098	1088	Linalool	t				t		t
1110	—	—	*cis*-Rose oxide							t
1117	—	—	Chrysanthenone					t		
1123	—	—	*trans*-Rose oxide							t
1139	1143	1136	Camphor					t		
1149	—	—	Isomenthone							t
1158	1154	—	Menthone							2.4
1158	—	—	*cis*-Chrysanthenol	0.7						
1164	1164	1152	Borneol	t				3.7		
1167	—	1162	*cis*-Pinocamphone					t		
1174	1177	1162	Terpinen-4-ol	t	12.0		t	t	33.0	
1177	—	—	(*E*)-Isocitral	1.1						
1182	1173	—	Menthol							t
1189	1189	1174	α-Terpineol	1.0	t			t	5.9	
1205	1204	—	Verbenone	t				4.5		
1218	—	1204	Nerol	0.4						
1224	—	—	Citronellol	t						28.9
1227	—	—	Thymol methyl ether		15.5					
1230	1240	—	Neral	23.1						
1234	1242	1229	Carvone				4.9			
1244	1255	—	Geraniol	0.7				t		2.4
1259	1270	—	Geranial	26.9						
1267	1275	—	Citronellyl formate							11.7
1273	1285	1269	Bornyl acetate	t				3.9		
1291	—	—	Geranyl formate							t
1292	1298	1287	Carvacrol		2.2		47.5			
1334	—	1341	α-Cubebene							t
1336	1356	1342	Eugenol			73.6				
1345	1354	—	Citronellyl acetate							t
1356	1376	1357	α-Copaene	t		t				0.9
1368	1383	1365	Geranyl acetate	0.9						
1368	—	1365	β-Bourbonene	t						1.5
1378	1391	—	β-Elemene							t
1399	1409	—	α-Cedrene	t						
1404	1418	1406	β-Caryophyllene	3.9	3.1	t	10.9	t		9.3
1406	—	—	β-Cedrene	t						
1424	1439	1423	α-Guaiene							1.3
1429	—	—	6,9-Guaiadiene							20.1
1435	—	—	*cis*-Muurola-3,5-diene							1.1
1439	1454	1435	α-Humulene	t			2.7			1.8
1443	—	—	*allo*-Aromadendrene	t						t

Table 1 – (*continued*)

RI[a]	RI[b]	RI[c]	Compounds[d]	Lemon verbena	Sweet marjoram	Clove basil	Patchouli	Rosemary	Tea tree	Rose geranium
				% of total[e]						
1462	1477	1457	γ-Muurolene							t
1465	1480	1461	Germacrene D	t		8.3				4.5
1472	1487	1461	ar-Curcumene	6.2						
1472	1484	1466	β-Selinene							t
1474	—	1476	Bicyclogermacrene	3.9	t					1.0
1486	—	1480	α-Muurolene							t
1490	—	—	Isodaucene							t
1499	—	1494	γ-Cadinene							0.9
1501	—	1492	β-Curcumene	1.3						
1506	1523	1503	δ-Cadinene							2.1
1556	1568	1544	(*E*)-Nerolidol	0.6						1.0
1557	—	1557	Spathulenol	1.8						1.8
1561	1581	1565	Caryophyllene oxide	1.5			2.1			1.7
1566	—	—	Furopelargone A							1.7
1597	1642	1592	1,10-di-*epi*-Cubenol							t

t = Trace, <0.5%.
[a] Calculated Kovats retention index on the HP-5MS column.
[b] Kovats retention index (RI) from Pino et al [8].
[c] Kovats RI from Pitarokili et al [9].
[d] The chemical compositions were identified by Database NIST 08.
[e] Integral of peak area in the GC chromatograms is used.
[f] Not found in the literature.

every hour. The process was carried out for 10 hours. After 10 hours, the spectra were taken at 24 and 28 hours. All spectra were taken against a blank solution of LB medium only.

3. Results and discussion

3.1. *Composition analysis of essential oils*

GC-MS chromatography with database NIST 08 was used to identify the chemical compositions of the essential oils. The same GC analyses with flame ionization detector were used to integrate the peak area. The chemical components of seven essential oils, ranked according to their Kovats retention indices, and their relative abundance (above 0.5%) are listed in Table 1. Although most of the essential oils have many components, clove basil and patchouli have only one dominate species each (eugenol and carvacrol, respectively). Lemon verbena has geometric isomers of citral (the common name is geranial and neral for the *E* and *Z* form, respectively) in high percentage, which together comprise the major species. Sweet marjoram contains about 18.5% of γ-terpinene, 15.5% of thymol methyl ether, and 12.0% of terpinen-4-ol. Rosemary contains about 54.8% of α-pinene and 22.2% of 1,8-cineole. Tea tree contains about 33.0% of terpinen-4-ol and 27.7% of 1,8-cineole. Rose geranium contains about 28.9% of citronellol and 20.1% of 6,9-guaiadiene. The main components mentioned above constitute at least half of the essential oils. These high percentage contents indicate potential for special applications.

The growing condition of plants has a definite role in the amount of each component in a particular essential oil [12,13]. It is of interest to compare the composition of species

Table 2 – Comparison of major components of essential oils from tea trees of various origins.

Compounds	Australia[a]	USA[b]	India[c]	Brazil[d]	Taiwan
	% of total[e]				
Terpinen-4-ol	36.7	42.9	36.4	53.7	33.0
γ-Terpinene	22.2	19.4	23.0	18.9	14.0
α-Terpinene	10.1	10.4	11.9	7.6	5.8
1,8-Cineole	2.5	0.5	4.5	—[f]	27.7

[a] Reported by Shelli et al [25].
[b] Reported by Kawakami et al [26].
[c] Reported by Verghese et al [27].
[d] Reported by Silva et al [28].
[e] Integral of peak area in the GC chromatograms is used.
[f] Not found.

grown in Taiwan and other species grown elsewhere. By comparing with the literature data, we found that the major components for lemon verbena are the same everywhere—that two structural isomers of citral (geranial and neral) consist of at least half essential oil [14]. Clove basil and rose geranium have at least one major component conserved around the world. Clove basil grown in Taiwan contains primarily eugenol along with other minor components, whereas that grown in Brazil contains a relative abundance of (Z)-α-bisabolene or 1,8-cineole in addition to eugenol [15,16]. Rose geranium in Taiwan is abundant in citronellol and 6,9-guaiadiene, but the one grown in United States contains citronellol and geraniol [17]. Components from Taiwan's sweet marjoram, patchouli, and rosemary essential oils are very different from those of their counterparts in other parts of the world. The essential oil of sweet marjoram from Taiwan contains thymol methyl ether and γ-terpinene, whereas that from Greece or Germany primarily contains either carvacrol or limonene, depending on the species [18,19]. It is interesting to note that thymol and carvacrol are structural isomers that have the hydroxyl group at different positions of the aromatic ring. Patchouli essential oils from Brazil and the United States have been reported to contain α-guaiene, δ-guaiene, and patchoulol [20,21], whereas those from Taiwan contain mainly carvacrol. Although both are alcohols, the structures of carvacrol and patchoulol are very different and indicates little to no relationship between them. Rosemary essential oils have been reported to have a high percentage of 1,8-cineole (Greece) [22], three major components of camphor, limonene, and α-pinene (Serbia) [23], and α-pinene and 1,8-cineole (Taiwan). These results further demonstrate the complexity of essential oils.

M. alternifolia essential oil, commonly known as narrow-leaved tea-tree essential oil, is the primary species for commercial production of tea tree essential oil. It is native to Australia and has been cultivated around the world. It has been reported to have many desirable applications, such as smoothing, antifungal, anti-inflammatory, antimicrobial, and antioxidant [24]. This wide range of functions could be attributed to the fact that its chemical composition is dramatically different from that of other essential oils produced in other areas of the world. Table 2 summarizes the major compositions of tea trees from Australia, United States, India, Brazil, and Taiwan. Although terpinen-4-ol, γ-terpinene, and α-terpinene are three major components for all originally, except for that in Taiwan, the percentage of each major component varies widely. The most abundant component, terpinen-4-ol, is found to comprise 53.7% of the essential oil from the Brazilian species to about 35% in Australian, Indian, and Taiwanese species. It is rather remarkable to find that 1,8-cineole is abundant in the species from Taiwan in spite of its scarcity in others.

3.2. *Antibacterial activity against* E. coli

Because of the large amount of phenol-type compounds found in essential oils from Taiwan, we decided to assess their antibacterial activity. *E. coli* is the most widely studied prokaryotic model organism in biochemistry and is used as an indicator for bacteriological water analysis. All seven essential oils have been tested for their inhibition of *E. coli* growth. The growth curves of *E. coli* with 0.1% of seven essential oils are shown in Figure 1. The inhibitory effect is defined as:

$$\left(1 - \frac{OD_{EO}}{OD_{control}}\right) \times 100, \quad (1)$$

where OD_{EO} is the optical density at 600 nm in the presence of essential oil and $OD_{control}$ is the optical density of the *E. coli* sample without any essential oil after 24 hours of incubation. Figure 1 shows a very high inhibitory effect for patchouli, clove basil, and sweet marjoram. To fully assay the inhibitory effect, concentrations of 0.01%, 0.05%, and 0.1% essential oils were added to the *E. coli* solution. The minimum inhibitory concentration (MIC) is the lowest concentration of an antibiotic that will inhibit the visible growth of a microorganism after overnight incubation [29]. Although the exact value of MIC cannot be obtained directly, it is evaluated to be between 0.01% and 0.05% for patchouli, 0.05–0.1% for clove basil and sweet marjoram, and >0.1% for lemon verbena, tea tree, rosemary, and rose geranium. Rosemary and rose geranium show a poor inhibitory effect such that even at 0.1%, *E. coli* growth reduces by only about 30% after an overnight incubation. Tea tree is well known for its broad-spectrum antimicrobial activity [24]. It is expected to restrain the growth of *E. coli*. The MIC of the Australian species was reported to be 0.25% for *E. coli* strain AG100 [30]. The species from Taiwan completely inhibits the *E. coli* growth in the first 10 hours of incubation at 0.1%. However, after 10 hours, *E. coli* regains its growth to about 50% of no essential oil control. This phenomenon could result from the consumption of essential oils by enzymes secreted from *E. coli* over time. Terpinen-4-ol is the major component in both Australian and Taiwanese species and may be the key component for inhibitory effect.

4. Conclusion

We have demonstrated that GC-MS with NIST 08 database is an ideal tool for the routine analysis of essential oils. Although

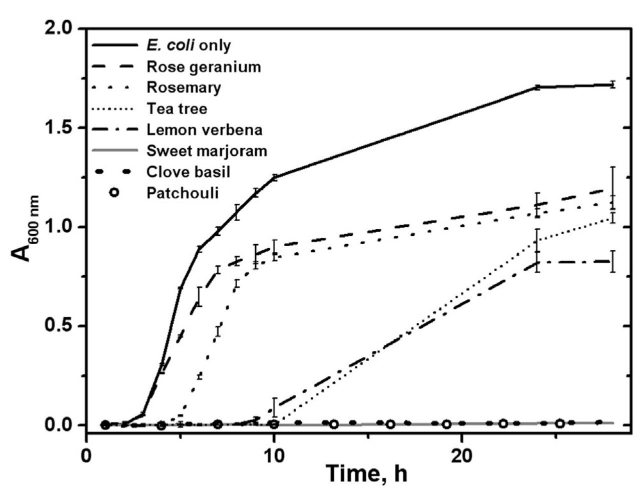

Figure 1 – Growth curves of *Escherichia coli* in lysogeny broth (LB) medium in the absence and presence of 0.1% of seven essential oils.

seven essential oils from Taiwan show complex compositions, some of them contain a high percentage of a few compounds. These essential oils are subject to specific applications. For example, patchouli and clove basil have a high content of carvacrol and eugenol, respectively. Both compounds are very effective antibacterial reagents, and it is not surprising that patchouli and clove basil exhibit excellent inhibition to *E. coli*. The compositions of essential oils are highly dependent on the growing conditions of the plants. We found that tea tree essential oils from Taiwan have a high percentage of 1,8-cineole, and account for its unique flavor.

Conflicts of interest

All authors declare no competing financial interest.

Acknowledgments

The financial support from the Ministry of Science and Technology of the Republic of China (NSC 98-2622-M-003-002-CC3) is gratefully acknowledged. Professor Y.C. Wang's (Department of Life Science) generous offer of making laboratory facilities available for our use is very much appreciated. Professor J.C. Wang's (Department of Life Science) help on the preparation of plant specimens is also highly appreciated.

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