Chemical diversity of the *Melaleuca cajuputi* leaf oils from six locations in southern Vietnam

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Summary

Melaleuca cajuputi Powell, a member of Myrtaceae family, is a popular and valuable plant in Vietnam, especially in the southern region. In this study, gas chromatography-mass spectrometry was used to determine the diversity in chemical composition of *M. cajuputi* leaf oil collected from six sites in the South of Vietnam. A total of sixty compounds has been identified from the essential oils of *M. cajuputi* leaf, such as α-thujene, α-pinene, benzaldehyde, β-pinene, β-myrcene, α-phellandrene, α-terpinene, benzene, 1-methyl-2-(1-methylethyl), 1,8-cineole, γ-terpinene, etc. Among those, five compounds were found in all six samples including α-thujene, α-pinene, α-phellandrene, γ-terpinene, and α-terpinolene, whereas other compounds were only present in some samples with varying amounts. Principal Component Analysis (PCA) has been performed to examine the similarities in the composition of leaf oils among the collection sites using XLSTAT software. The results from PCA suggested that six samples of leaf oil could be classified into 2 chemotypes with different chemical constituents. Note that 1,8-cineole only occurred in chemotype group 1 whereas it was omitted in chemotype group 2. This finding explains the diversity of bioactivity of *M. cajuputi* collected from different regions and provide more information on *M. cajuputi* for further application in medicine and food industry.

Key words

Melaleuca cajuputi, chemical diversity, essential oils, Southern Vietnam

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Received: April 12, 2019 | Accepted: July 23, 2019

Introduction

Melaleuca cajuputi Powell, which is commonly known as white *samet* or *cajuputi*, is a member of Melaleucae tribe, Myrtoideae family, which comprises of nearly 230 species all over the world (Craven and Lepschi, 1999; Wilson et al., 2005). *M. cajuputi* is divided into 3 sub-species: subsp. *cajuputi*, subsp. *cumingiana* (Turcz) Barlow, and subsp. *platyphylla* Barlow. These species are usually medium to large trees, typically grow to 25 m and sometimes reach up to 40 m. They are well adapted plants; they are mainly distributed in marshy or periodically flooded soils, but sometimes they are found in dry and rocky soils or even in salt-water flooded soils (Turnbull, 1986). In Vietnam, only one species of the *Melalauca* genus, *M. cajuputi* Powell, is known and recorded (Pham-hoang, 2000). It is one of the most widespread species, extending from North to South Vietnam, but mainly growing in the southern region (Pham-hoang, 2000). They have been utilized to make charcoal, supporting and construction materials, and produce cajuput oil, which is the remedy used in both traditional and modern medicine to relieve headache, convulsion, toothache, rheumatism or to repel the insect (Ogata, 1969).

Previous studies have determined chemical constituents in *M. cajuputi* leaf oil to suggest the potential application of this plant (Barbosa et al., 2013), whereas other studies used chemical constituents as a chemotaxonomic tool which supports the morphological taxonomy to classify sub-species of this species. However, the diversity of chemical composition of *M. cajuputi* leaf oil in Vietnam has not been studied yet. This study investigated the chemical composition of *M. cajuputi* leaf oil collected from six sites in the southern region of Vietnam using as an instrument Gas Chromtaograph-Mass Spectrometer (GC-MS) as well as the diversity of *M. cajuputi* in Vietnam.

Materials and Methods

Materials

Fresh leaves of *M. cajuputi* were collected from six collection sites in the southern region of Vietnam (Table 1 and Figure 1), including Mephydica Center, Moc Hoa District, Long An Province; Lo Go-Xa Mat National Park, Tan Bien District, Tay Ninh Province; Tram Chim National Park, Tam Nong District, Dong Thap Province; Phu Quoc National Park, Phu Quoc Island, Kien Giang Phu Quoc National Park; U Minh Thuong National Park, U Minh Thuong District, Kien Giang Phu Quoc; and Binh Chau-Phuoc Buu Nature Reserve, Bung Rieng District, Ba Ria-Vung Tau Province.

Methods

Distillation of the Essential Oils

The leaf oil of *M. cajuputi* was prepared by the method adapted from Tran et al. (2018) with some modifications. In brief, fresh leaves of *M. cajuputi* (500 g) were ground into fine powder by a blender and then distributed into a five-liter flask; the samples were continuously soaked in 1 liter of distilled water for 1 hour. The essential oils were obtained by hydrodistillation at normal pressure for 4 hours. The volatile oils distilled in water and were collected in the receiver arm of the apparatus into clean dark glass bottles. The oils were stored at 4°C in dark chamber until further analyses.

Table 1. Collection sites for samples of *M. cajuputi*

Collection site	Code of Samples	Latitude	Longitude
Mephydica Center	MC	10°42'55.7"N	106°05'04.0"E
Binh Chau-Phuoc Buu Nature Reserve	BC	10°31'04.6"N	107°28'03.7"E
Tram Chim National Park	TC	10°42'35.3"N	105°30'48.7"E
Phu Quoc National Park	PQ	10°19'52.9"N	104°01'49.1"E
U Minh Thuong National Park	UM	9°35'55.8"N	105°05'27.0"E
Lo Go-Xa Mat National Park	LG	11°36'01.8"N	105°53'58.1"E

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

Chemical composition of the essential oil from *M. cajuputi* leaf was analyzed by GC-MS mass spectrometry. In brief, gas chromatography was carried out using an Agilent Technologies HP 6890 Plus Gas chromatograph fitted with a flame ionization detector (FID and HP-5MS (30 m 0.25 mm) column. Hydrogen (1 mL/min) was used as the carrier gas. The injector and detector temperatures were maintained at 250 and 260°C, respectively. Oil samples were injected into the GC by splitting mode with the split ratio of 100:1, while 1.0 mL of each individual oils was injected into the GC with inlet pressure at 6.1 kPa. Each analysis was performed in triplicates. The relative amounts of individual components were calculated based on the GC peak area (FID response) without using correction factors.

An HP 6890 Plus Chromatograph (Agilent Technologies) equipped with a HP-5MS column (30 m x 0.25 mm, film thickness 0.25 μm) and a mass spectrometer HP 5973 MSD were used to perform the GC-MS analysis. The analytical conditions were similar to those of GC analysis, except that the carrier gas was helium (1 mL/min). The ionization voltage was 70 eV with the emission current of 40 mA. The acquisitions scan mass range of MS was 35-350 amu with the sampling rate of 1.0 scan/s.

Data Analysis

Principal Component Analysis (PCA) was performed to investigate the similarities/dissimilarities among the six collection sites in term of chemical compositions of *M. cajuputi* leaf oil. The XLSTAT software (XLSTAT-Ecology, Addinsoft, USA) was used to run PCA.

Results and Discussions

The chemical compositions of essential oil from *M. cajuputi* leaf collected from six collection sites in Vietnam using GC-MS are presented in Table 2. A total of sixty compounds have been identified. These results explained the divergence in bioactive compounds content and bioactivity of essential oils of *M. cajuputi* leaf collected from different regions. Among six regions, TC samples had high abundance of eugenol methyl (48.31%)

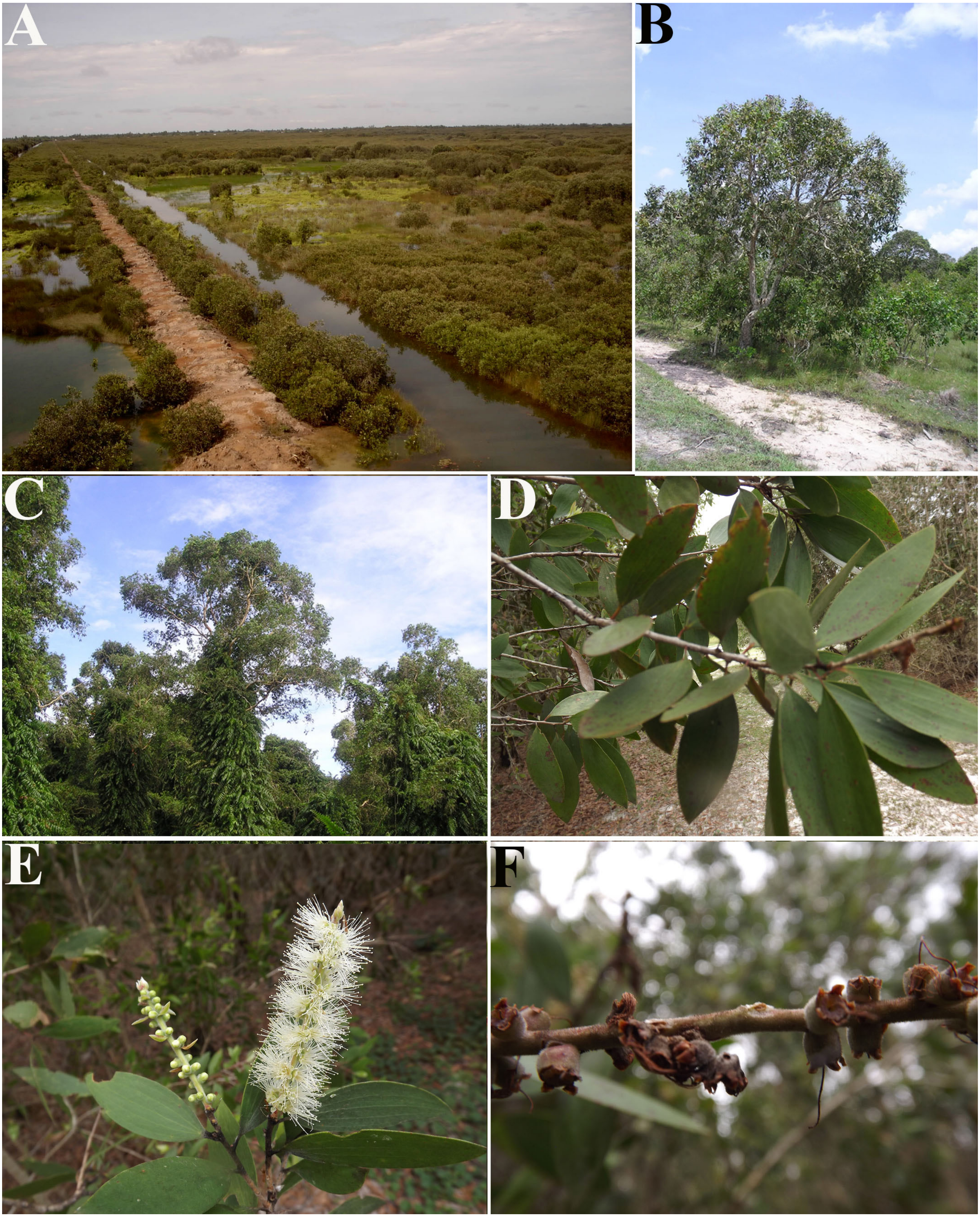

Figure 1. *Melaleuca cajuputi*. A – The habitat of the studied species in Mephydica Center, B – the habitat in Binh Chau-Phuoc Buu Nature Reserve and C – the habitat in U Minh Thuong National Park. D – Leaves. E – Flowers. F – Fruits

Table 2. Chemical compositions in the essential oils from *M. cajuputi* leaf collected from six collection sites in the Southern region of Vietnam

Compounds	RT	The amount percentage in samples (%)					
		MC	LG	TC	PQ	UM	BC
α-thujene	7.77	4.15	3.06	0.25	0.19	0.24	0.97
α-pinene	8.07	2.95	2.78	3.58	0.75	0.60	3.58
Benzaldehyde	8.86	0.66	-	-	-	-	-
β-pinene	9.28	0.59	1.45	0.30	-	-	0.21
β-myrcene	9.77	0.98	1.8	0.17	-	-	-
α-phellandrene	10.30	4.26	2.52	0.20	0.35	0.18	0.69
α-terpinene	10.56	5.38	-	0.42	-	0.46	1.29
Benzene, 1-methyl-2-(1-methylethyl)	10.89	-	3.86	-	1.16	2.42	5.16
1,8-cineole	11.48	7.30	23.02	-	-	-	-
γ-terpinene	12.35	12.97	7.49	2.89	1.97	3.72	6.60
α-terpinolene	13.15	14.29	8.91	2.44	2.47	3.82	6.68
1,3,8-p-menthatriene	13.69	0.19	-	-	-	-	-
δ-3-carene	13.90	0.12	2.58	0.6	0.13	0.22	
4-carene	15.58	-	-	-	-	2.12	1.32
3-carene	15.6	-	3.33	-	-	1.13	0.21
β-phellandrene	15.92	-	-	-	0.83	-	-
2-cyclohexen-1-ol, 2-methyl-5-(1-methylethyl)-	14.40	0.24	-	-	-	-	-
3-cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-	15.72	3.65	-	-	-	-	-
2-carene	16.02	-	-	2.39	-	-	-
Camphene	16.30	3.29	12.20	-	-	-	0.68
1,3,6-heptatriene, 2,5,5-trimethyl camphene	19.50	-	-	-	0.22	-	-
Phenol, 2-methyoxy-4-(2-propenyl)-	19.95	0.31	-	-	-	-	-
α-ylangene	20.18	0.36					
α-copaenen	20.27	0.22	-	-	-	0.19	0.49
β-elemene	20.55	-	0.57	-	-	-	-
trans-caryophyllene	21.14	2.44	-	-	10.04	10.43	11.08
caryophyllene	21.46	-	-	-	-	-	1.02
Eugenol methyl	21.59	-	-	48.31	-	-	0.70
α-humulene	21.69	2.40	-	-	6.91	9.42	8.61
α-murolene	22.02	2.15	-	-	-	-	-
β-selinene	22.25	2.35	-	-	-	-	-

Compounds	RT	The amount percentage in samples (%)					
		MC	LG	TC	PQ	UM	BC
β-guaiene	22.24	-	-	-	6.21	4.31	-
Eremophilene	22.30	-	-	-	-	-	5.57
δ-cadinene	22.67	0.62	-	-	2.01	-	1.57
Selina-3, 7 (11) -deene	22.98	0.84	-	-	-	-	-
Isohomogenol	23.29	-	-	34.46	-	-	-
α-guaiene	23.35	-	-	-	-	-	1.09
γ-gurjunene	23.68	-	-	-	-	-	6.01
aromadendrene	23.80	-	3.54	-	-	-	-
agarospirol	23.89	5.93	-	-	-	-	-
β-selinene	24.22	-	-	-	-	25.63	-
calarene	24.26	-	4.35	-	-	-	9.13
δ-selinene	24.29	-	-	-	-	-	2.66
β-gualene	24.32	4.88	-	-	-	-	-
α-farnesene	24.38	-	-	-	-	-	2.32
2,4-nondienal	24.51	-	-	-	25.54	-	-
Eudesmol	24.61	-	9.34	-	-	-	-
Guaiol	24.78	10.32	-	-	-	-	8.16
Ylangene	24.80	-	-	0.52	-	-	-
EPI-bicyclosesquiphellandrene	24.95	-	-	0.39	-	-	-
α-eudesmol	24.99	-	-	-	-	22.22	-
β-gurjunene	25.08	-	-	-	9,57	-	-
Eremophilene	25.10	0.25	-	-	-	-	-
1, 6, 10-dodecatrien-3-ol, 3, 7, 11-trimethyl	25.32	-	-	-	-	2.46	-
1-Acetyl-4-methyldibenzofuran	25.67	-	-	-	12.92	-	-
Isoxazolo[4,3-a]phenazin-1[3H]-one 9,10-Anthracenedione, 1-amino-2-methyl-	26.01	-	-	-	-	-	4.73
9, 10-anthracenedione, 1-amino-2-methyl	26.11	-	-	-	-	6.65	2.52
1, 1-bis (p-tolyl) ethane	26.20	-	-	-	2.46	-	-
Tetradecanoic acid	27.71	-	-	-	0.22	-	-
10-methylanthracene-9-carboxaldehyde	28.32	-	-	-	4.19	-	-
Total		89.94	87.74	96.67	78.57	96.22	93.05

Note: Mephydica Center (MC), Binh Chau-Phuoc Buu Nature Reserve (BC), Tram Chim National Park (TC), Phu Quoc National Park (PQ), U Minh Thuong National Park (UM), Lo Go-Xa Mat National Park (LG) and isohomogenol (34.46%) whereas β-selinene (25.63%) and α-eudesmol (22.22%) were the most abundant compounds in UM samples. Moreover, α-eudesmol (23.02%) and camphene (12.20%) accounted for over 35% of samples from LG whereas both compounds were not present in samples of TC, PQ, UM (Table 2). On the other hand, the most abundant compounds of samples also varied among six different regions, such as α-terpinolene in MC samples (14.29%), 1,8-cineol in LG samples (23.02%), eugenol methyl in TC samples (48.31%), 2,4-nondienal in PQ samples (25.54%), β-selinene in UM samples (25.63%), trans-caryophyllene in BC samples (11.08%). Furthermore, there were some compounds only existing in samples in particular regions; this finding suggests a possibility to apply these compounds as chemotaxonomic tools to classify subspecies of *M. cajuputi* based on their chemical composition.

Figure 2 presents the PCA biplot that showed the association between the chemical constituents and the collection sites. The first dimension of the PCA biplot separated the samples into two groups. Group 1, which consists of samples collected from MC and LG, was characterized by compounds such as α-thujene; α-pinene,; β-myrcene; α-phellandrene; and 1,8-cineol, γ-terpinene, etc. whereas group 2 including samples of BC, UM, PQ, and TC was characterized by compounds such as trans-caryophyllene, α-humulene, β-guaiene, etc. The amounts of some compounds in the samples of group 2 such as trans-caryophyllene, and α-humulene, β-guaiene are higher than those of group 1 (Table 2). Among the compounds which are used to distinguish group 1 from group 2; 1,8-Cineole showed a remarkable difference between the two groups. It accounted for 7.30% in MC sample and 23.02% in LG sample, but was not found in other samples (BC, UM, PQ, and TC). 1,8-cineole is a natural organic compound, and a member of cyclic ethers and monoterpenoid. It has a mint-like fragrance; therefore, it is widely used to produce perfume and cosmetics. 1,8-Cineole is the main component of essential oil of *Eucalyptus* species and *M. cajuputi* in some regions over the world (Boland et al.,1991; Southwell et al., 2003; Barbosa et al., 2013). The similarity between the two samples MC and LG may be accounted for by the similar ecological characteristics of the two regions Mephydica Center (MC) and Lo Go-Xa Mat National Park (LG). On these two sites, *M. cajuputi* has grown in acidic soil with low pH (2.9-3.2). MC and LG are also quite close in term of geographic location (Pham-hoang, 2000; Le, 2003). On the other hand, Binh Chau-Phuoc Buu Nature Reserve (BC) has the similar ecological conditions with Phu Quoc National Park (PQ). Both cajuput forests of BC and PQ are evergreen forests on low hill areas mainly containing sandy soil mixed with marine sediment. Moreover, cajuput forests of Tram Chim National Park (TC) and

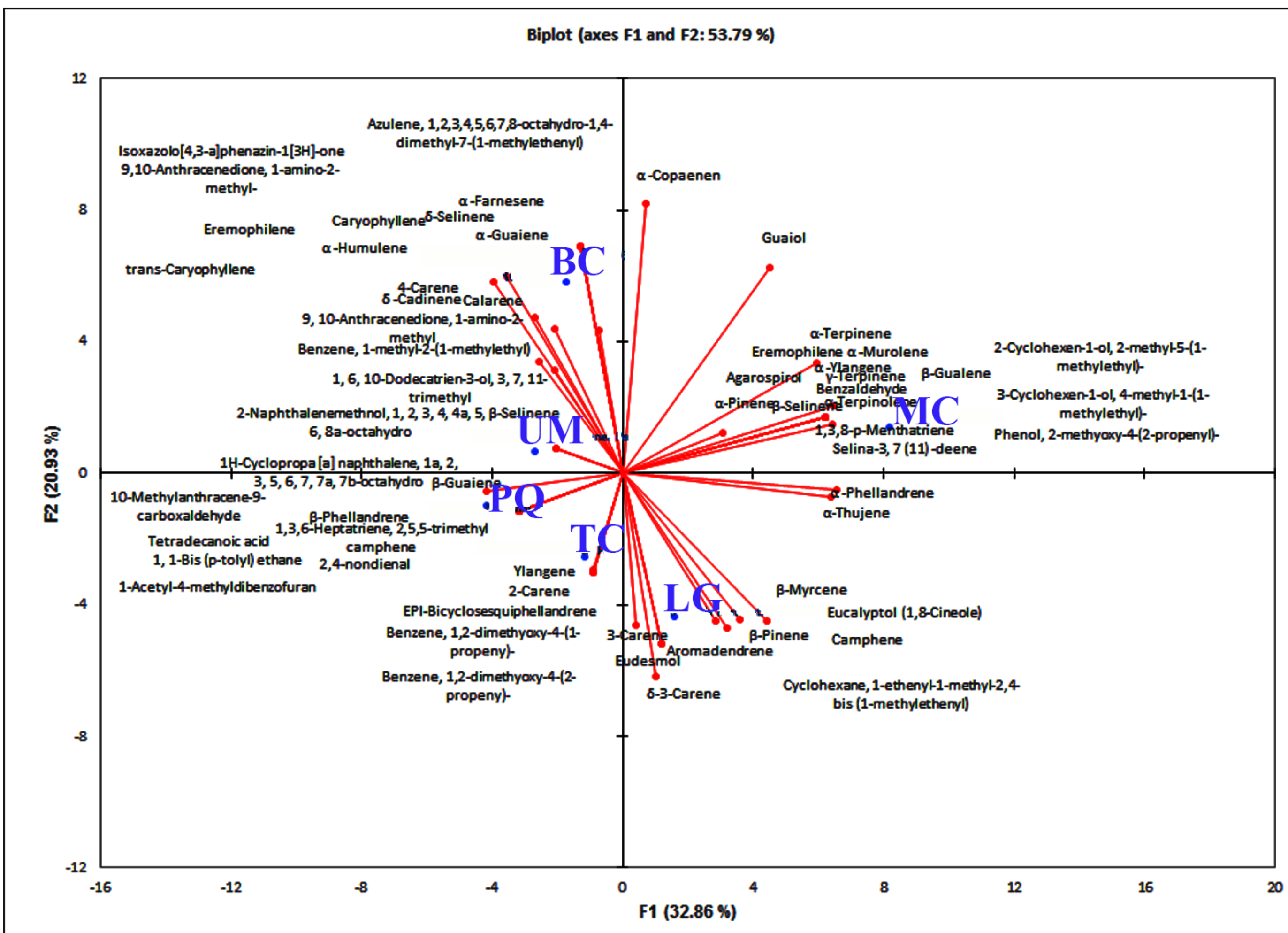

Figure 2. Comparison of chemical composition of essential oils of *M. cajuputi* from different regions using PCA in XLSAT software

U Minh Thuong National Park (UM) have grown in flooded soil. However, U Minh Thuong National Park (UM) is located in fresh water flooded region mainly containing peat soil whereas Tram Chim National Park is located in flooded region of the Mekong Delta which consists of acidic soil, alluvial soil, and acrisols. Therefore, there are some differences in ecological conditions of these areas, which may account for the dissimilarity of chemical compositions of the essential oils collected from TC versus UM, as well as MC and LG versus BC, PQ, TC, and UM.

The results from this study are similar with those from Kim et al. (2005) in which the authors determined the chemical compositions of the essential oil of *M. cajuputi* leaves from six regions of Thailand. They proved that both γ-terpinene and α-terpinolene were found in all six samples and 1,8-cineole was present in three samples from Ban Lubosama, Ban Mai, and Ban Tha Se with 0.71, 1.19, and 2.88%, respectively. In contrast, the essential oils from other three samples did not have 1,8-cineole in their chemical compositions. The content of 1,8-cineole in the samples of MC and GC in this study was higher than those in Kim et al.'s study (2005).

Sakasegawa et al. (2003) have classified the essential oils of *M. cajuputi* into three chemotypes based on 1,8-cineole abundance: chemotype 1 containing high concentration of 1,8-cineole (50-70%), chemotype 2 containing medium concentration of 1,8-cineole (about 31%), and chemotype 3 omitting 1,8-cineole in its composition. In previous study, Silva (2007) determined that essential oil of *M. cajuputi* leaf collected in Brazil comprised of 44% 1,8-cineole, whereas its content in samples collected from Indonesia was over 55% (Sakasegawa et al., 2003). Based on the method of Sakasegawa et al. (2003), *M. cajuputi* in the southern regions of Vietnam could be classified into two chemotypes: chemotype 1 consisting of 1,8-cineole (MC and LG samples) and chemotype 2 omitting that compound (TC, PQ, UM, and BC samples). These results support the utilization of 1,8-cineole as a chemotaxonomic indicator to classify *M. cajuputi* sub-species.

Conclusion

A total of 60 compounds has been identified from the essential oils of *M. cajuputi* collected in six different sites in the south of Vietnam, α-thujene; α-pinene; benzaldehyde; β-pinene; β-myrcene; α-phellandrene; α-terpinene; benzene, 1-methyl-2-(1-methylethyl); 1,8-cineole; γ-terpinene; α-terpinolene; 1,3,8-p-menthatriene; δ-3-carene; 4-carene; 3-carene; β-phellandrene, to name a few. The most abundant compounds of samples also varied among six different regions, such as α-terpinolene (MC samples), 1,8-cineol (LG samples), eugenol methyl in (TC samples), 2,4-nondienal (PQ samples), β-selinene (UM samples), and trans-caryophyllene (BC samples). Moreover, the chemical constituents could be used as chemotaxonomic indicators to classify *M. cajuputi* into two chemotypes: chemotype 1 (MC and GC), which was characterized by compounds such as α-thujene, α-pinene, β-myrcene, α-phellandrene, 1,8-cineol, γ-terpinene, etc., and chemotype 2 (samples collected from BC, UM, PQ, and TC), which was characterized by other compounds such as trans-caryophyllene, α-humulene, β-guaiene, etc. This finding somehow demonstrated the diversity of bioactivity of *M. cajuputi* collected from different regions, proved the possibility of application of chemical constituent as chemotaxonomic indicator to support morphological analysis, and provided more information on *M. cajuputi* for further application in medicine and food industry.

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aCS84_47