Journal of Phytology 2024, 16: 36-40
doi: 10.25081/jp.2024.v16.8007
https://updatepublishing.com/journal/index.php/jp

Research Article

ISSN: 2075-6240

Chemical profiles of essential oils of two cultivars of *Melaleuca cajuputi* leaves and flowers

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Received: September 28, 2022
Revised: February 21, 2024
Accepted: February 24, 2024
Published: March 14, 2024

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ABSTRACT

In the present study, the differences between the chemical compositions of the essential oils obtained from the leaves and flowers of two cultivars of *M. cajuputi* collected from Moc Hoa district, Long An province. By using Gas Chromatography-Mass Spectrometry, a total of 105 components have been identified in the essential oils of four samples of two *M. cajuputi* cultivars such as "Tràm gió" leaves, "Tràm gió" flowers, "Tràm cừ" leaves and "Tràm cừ" flowers. The Agglomerative Hierarchical Cluster (AHC) and Principal Component Analysis (PCA) were performed to show the similarities/dissimilarities in chemical compositions among the four studied samples. As a result, the components of the essential oils of four studied samples were divided into two clusters. Cluster I included two samples such as "Tràm gió" leaf and "Tràm gió" flower with high presence of 1,8-cineole (35.12 and 17.69%), linalool (3.31 and 5.03%), (R)-α-terpinyl acetate (9.17 and 8.1%). Cluster II comprised "Tràm cừ" leaf and "Tràm cừ" flower with the high concentration of α-pinene (9.87 and 12.19%), γ-terpinene (10.48 and 11.3%), p-mentha-2,4(8)-diene (8.8 and 12.7%).

KEYWORDS: *Melaleuca cajuputi*, Essential oils, Tràm gió, Tràm cừ

INTRODUCTION

Melaleuca cajuputi Powell is commonly known as *cajuputi* and belongs to the Myrtaceae family. It is widely distributed in Papua New Guinea, Australia and South-East Asia. This plant is well adapted plants, it is mainly found in periodically flooded soils or marshy, it is also distributed in rocky or dry soil or even in saltwater flooded soils (Van *et al.*, 2019). *M. cajuputi* is a multi-purpose plant and therefore, it is an economically important plant. This plant is used as frame poles and piles in construction, flowers can attract honey bees, leaves are used as essential oil distillation, timber is used as sawn timber, charcoal or paper, etc. (Hai *et al.*, 2019).

M. cajuputi is a highly economic plant in Vietnam. It is widely distributed from North to Southern Vietnam but mainly grows in the southern region (Pham, 2000). There are two cultivars of *M. cajuputi* that grows in Long An province, Southwest Vietnam. Accordingly, the first cultivar is widely found in this region and it is commonly known as "Tràm cừ" in Vietnamese. This cultivar is used as frame poles and piles in construction because of its less branches, tall and straight stem. Meanwhile, the second cultivar is only found in Dong Thap Muoi Herbal Medicine Research, Conservation and Development Center, and it is named "Tràm gió" in Vietnamese. This cultivar possesses a short and curved stem, many branches; leaves are used for essential oil distillation (Van, 2007). Previous studies showed the chemical compositions and biological activities of the essential oils obtained from the leaves of *M. cajuputi* collected from several regions of Vietnam (Motl *et al.*, 1990; Hai *et al.*, 2019; Van *et al.*, 2019; My *et al.*, 2020; Quoc, 2021). In this study, the differences between chemical components of the essential oils isolated from leaves and flowers of two cultivars of *M. cajuputi* grown in the Moc Hoa district, Long An province have been reported for the first time.

MATERIALS AND METHOD

Plant Collection

Specimens of two cultivars of *Melaleuca cajuputi* were collected from Long An province, Vietnam. Accordingly, the samples of "Tràm gió" were collected from Dong Thap Muoi Herbal Medicine Research, Conservation and Development Center, Binh Phong Thanh commune, Moc Hoa district while "Tràm cừ" was collected from Kien Tuong town, Moc Hoa district.

Methods

500 g of specimens were subjected to hydro-distillation for the essential oil extraction for 4 hours in a Clevenger apparatus. The chemical components of the essential oil were determined by Gas Chromatography-Mass Spectrometry (GC-MS) system (Agilent 7890A GC coupled with a 5975C VL MSD Triple-Axis; ZB-5MS capillary non-polar column (30.0 m length x 0.25 mm i.d. x 0.25 µm film thickness)). Helium was used as carrier gas under the constant pressure of 13.209 psi. 0.1 µL essential oil was injected into the system at 250 °C with a split ratio of 10:1. The temperature program in the system was set to increase from 60 °C to 240 °C at 3 °C/min. The chemical compositions of the essential oils were identified based on the comparison between their mass spectra with NIST 2017 library.

Data Analysis

The data were managed in Microsoft Excel 2013 and statistically analyzed using R Studio version 3.5 for Windows. The Principal Component Analysis (PCA) was performed to show the similarities/dissimilarities in chemical compositions among the four collection plants samples. Agglomerative Hierarchical Cluster (AHC) was carried out to group plants with common characteristics into 1 group. Specifically, based on chemical compositions among the nine collection plants samples, to determine the chemical similarities between the various samples.

RESULTS AND DISCUSSION

The chemical constituents of the essential oils obtained from four studied samples were shown in Table 1 in which a total of 105 components have been identified. Accordingly, the major components of the essential oil of "Tràm gió" leaf were mainly composed of 1,8-cineole (35.12%), p-mentha-2,4(8)-diene (14.5%), γ-terpinene (13.71%), (R)-α-terpinyl acetate (9.17%) and eudesm-4(14)-en-11-ol (4.13%). The flower essential oil obtained from "Tràm gió" was characterized by the predominance of eudesm-4(14)-en-11-ol (18.18%), 1,8-cineole (17.69%), epi-γ-eudesmol (8.64%), guaiol (8.23%) and (R)-α-terpinyl acetate (8.1%). In addition, γ-terpinene (10.48%), α-pinene (9.87%), p-Mentha-2,4(8)-diene (8.8%), o-cymene (7.37%), 10-epi-γ-eudesmol (6.43%), caryophyllene oxide (4.77%), citral (4.17%) were the major components in the essential oil obtained from "Tràm cừ" leaf. The essential oil of "Tràm cừ" flower was found to be rich in α-pinene (12.19%), p-mentha-2,4(8)-diene (12.17%), γ-terpinene (11.3%), α-epi-7-epi-5-eudesmol (7.13%) and caryophyllene (4.9%).

Figure 1 showed the PCA biplot (Figure 1a) and the AHC tree diagram (Figure 1b) of the chemical compositions of the essential oils obtained from the two *M. cajuputi* cultivars were high differences. Accordingly, the components of the essential oils of the four studied samples divided into two clusters. Cluster I included two samples as "Tràm gió" leaf and "Tràm gió" flower with high presence of 1,8-cineole (35.12 and 17.69%), linalool (3.31 and 5.03%), (r)-α-terpinyl acetate (9.17 and 8.1%), eudesm-4(14)-en-11-ol (4.13 and 18.18%). Meanwhile, cluster II comprised "Tràm cừ" leaf and "Tràm cừ" flower with the high concentration of α-pinene (9.87 and 12.19%), γ-terpinene (10.48 and 11.3%), p-mentha-2,4(8)-diene (8.8 and 12.7%), caryophyllene (2.47 and 4.9%), humulene (1.96 and 3.37%), caryophyllene oxide (4.77 and 1.44%), ledol (1.14 and 4.26%), hinesol (1.58 and 1.09%).

As mentioned above, the chemical compositions of the essential oils of the flowers of two *M. cajuputi* cultivars grown in Long An province have not been elucidated yet. Meanwhile, the components of the essential oils obtained from the leaves of two *M. cajuputi* cultivars in this study were significant differences from those collected from several regions of Vietnam. Accordingly, the most abundant compounds of the leaf essential oils collected from six regions of Vietnam such as Long An, Tay Ninh, Dong Thap, Kien Giang (Phu Quoc National Park), Kien Giang (U Minh Thuong National Park) and Ba Ria-Vung Tau provinces were α-terpinolene (14.29%), 1,8-cineol (23.02%), eugenol methyl (48.31%), 2,4-nondienal (25.54%), β-selinene (25.63%) and transcaryophyllene (11.08%), respectively (Van *et al.*, 2019). The leaf essential oil of *M. cajuputi* grown in Quang Tri province mainly contained 1,8-cineole (71.83%), *p*-menth-1-en-8-ol (6.01%) and *p*-cymene (2.87%) (Quoc, 2021) while 1,8-cineole (41.1%), α-terpineol (8.7%), *p*-cymene (6.8%) were the major components in the leaf essential oil of this plant collected from Long An province (Motl *et al.*, 1990).

Many chemical components of the essential oil isolated from leaves and flowers of two *M. cajuputi* cultivars in this study have been reported to possess some biological activities. For instance, 1,8-cineole was found to be effective against many bacterial and fungal strains, including *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus faecalis*, *Pseudomonas aeruginosa*, *Mycobacterium smegmatis*, *Cylindrocarpon mali*, *Candida albicans*, *Aspergillus niger*, *SotIyfis cinerea*, *Stereum purpureum* and *Sclerotinia sclerotiorum* (Prudent *et al.*, 1993; Hendry *et al.*, 2009). In addition, many previous studies reported that α-pinene had an inhibitory effect on many tested microorganisms such as *E. coli*, *M. smegmatis*, *C. mali*, *S. aureus*, *S. faecalis*, *P. aeruginosa*, *C. albicans*, *S. sclerotiorum*, *A. niger and S. purpureum* (Prudent *et al.*, 1993; Leite *et al.*, 2007; da Silva *et al.*, 2012). Furthermore, α-pinene has been reported to possess larvicidal and insecticidal effects against fourth stage larvae of *Aedes aegypti* (Santos *et al.*, 2012) and *Lasioderma serricorne*, respectively (Wu *et al.*, 2014).

Linalool exhibited anti-cancer properties against several cancer cells, including DU145 (Sun *et al.*, 2015) and HepG2, A549, SW620, T-47D (Chang & Shen, 2014), RPMI 7932 (Cerchiara *et al.*, 2015), HeLa and U937 (Chang *et al.*, 2015). Moreover, This compound has been reported to have anti-inflammatory activities in LPS-stimulated RAW 264.6 cells (Huo *et al.*, 2013) and BV2 microglia cells (Li *et al.*, 2015). de Oliveira Ramalho *et al.* (2015) provided that γ-terpinene also possessed an anti-inflammatory effect on paw edema in mice (de Oliveira

Table 1: Chemical constituents in the essential oils from four studied samples of *Melaleuca cajuputi*

RT (min)	Components	Code	GL	GH	CL	CH
3.45	2-Thujene	C1	1.91	0.36	0.22	2.75
3.61	α-Pinene	C2	1.85	0.55	9.87	12.19
3.95	Camphene	C3	0.01	-	-	-
4.10	Benzaldehyde	C4	0.04	0.37	-	0.13
4.38	α-Sabinene	C5	0.09	-	0.74	2.25
4.54	β-Pinene	C6	0.59	0.27	0.83	0.72
4.69	Myrcene	C7	0.96	0.42	-	0.17
5.22	3-Thujene	C8	1.14	0.36	-	-
5.29	3-Carene	C9	0.06	-	-	-
5.30	β-Ocimene	C10	-	-	0.26	0.20
5.47	p-Mentha-1,4(8)-diene	C11	2.09	-	-	-
5.49	α-Terpinene	C12	-	0.87	2.45	-
5.50	2-Carene	C13	-	-	-	4.06
5.64	o-Cymene	C14	1.08	1.33	7.37	2.43
5.75	D-Limonene	C15	2.30	1.65	2.44	1.83
5.84	1,8-Cineole	C16	35.12	17.69	2.04	0.67
6.34	γ-Terpinene	C17	13.71	3.65	10.48	11.3
6.53	trans-Linalool oxide	C18	-	-	-	0.03
6.79	p-Mentha-2,4(8)-diene	C19	14.50	3.9	8.8	12.17
6.86	p-Cymenene	C20	-	-	0.16	0.18
6.97	Linalool	C21	3.31	5.03	0.95	0.67
7.21	1,3,8-p-Menthatriene	C22	-	-	-	0.04
7.32	Fenchol	C23	-	-	-	0.03
7.38	4-Isopropyl-1-methylcyclohex-2-enol	C24	-	-	-	0.15
7.54	p-Mentha-1,5,8-triene	C25	-	-	-	0.03
7.59	1,3,8-p-Menthatriene	C26	-	-	-	0.02
7.62	(1R,4R)-4-Isopropyl-1-methylcyclohex-2-enol	C27	0.08	0.05	-	-
7.72	Neoisopulegol	C28	-	0.06	-	-
7.78	3,5-Heptanedione, 2,2,6-trimethyl	C29	-	-	0.17	-
7.82	trans-2-Nonenal	C30	-	0.04	-	0.05
7.83	3,5-Octanedione, 2,7-dimethyl	C31	-	-	0.19	-
7.98	δ-Terpineol	C32	0.14	0.26	-	-
8.03	1,7,7-Trimethylbicyclo[2.2.1]heptan-2-ol	C33	-	-	0.11	0.04
8.04	endo-Borneol	C34	-	0.03	-	-
8.12	Citral	C35	-	0.15	4.17	0.22
8.14	Terpinen-4-ol	C36	2.10	2.03	3.65	2.87
8.17	p-Cymen-8-ol	C37	-	-	0.87	0.18
8.29	(R)-α-Terpinyl acetate	C38	9.17	8.1	2.74	1.87
8.43	trans-Piperitol	C39	-	-	0.1	0.07
8.44	p-Menth-1-en-3-ol, cis-	C40	0.03	0.05	-	-
8.58	cis-p-mentha-1(7),8-dien-2-ol	C41	0.08	0.14	0.32	0.04
8.83	Geraniol	C42	0.23	0.17	-	-
8.90	Citronellic acid	C43	0.03	-	-	-
9.01	Neral	C44	-	0.01	-	-
9.13	Thymol	C45	-	-	0.12	-
9.24	3-Methyl-4-isopropylphenol	C46	-	-	0.09	-
9.30	Carvacrol	C47	-	-	0.13	-
9.31	Thymol	C48	-	0.01	-	-
9.50	Methyl geranate	C49	0.02	-	-	-
9.69	δ-Elemene	C50	0.02	0.03	0.09	0
9.78	Eugenol	C51	-	-	0.29	0.16
9.79	3-Allyl-2-methoxyphenol	C52	0.04	0.27	-	-
9.96	Geranyl acetate	C53	-	0.04	-	-
10.00	Ylangene	C54	-	0.12	-	0.03
10.05	Copaene	C55	-	0.14	-	0.09
10.16	cis-β-Elemene	C56	-	0.08	-	0.12
10.21	trans-α-Bergamotene	C57	-	0.04	-	-
10.42	Caryophyllene	C58	0.14	0.70	2.47	4.9
10.45	γ-Elemene	C59	-	0.10	-	-
10.51	α-Guaiene	C60	-	0.08	-	-
10.54	cis-Isoeugenol	C61	-	0.05	-	-
10.56	Isocaryophyllene	C62	-	0.03	-	-
10.59	isoledene	C63	-	0.07	-	-
10.63	Guaia-6,9-diene	C64	-	0.07	-	-
10.69	Humulene	C65	0.17	0.75	1.96	3.37

(Contd...)

Table 1: *(Continued)*

RT (min)	Components	Code	GL	GH	CL	CH
10.75	2,4,6-Trimethoxytoluene	C66	-	0.07	-	-
10.80	α-Curcumene	C67	-	0.32	-	-
10.79	β-Chamigrene	C68	0.01	-	0.22	-
10.82	α-Amorphene	C69	0.05	0.55	0.15	-
10.86	Calarene epoxide	C70	0.01	0.23	-	-
10.88	β-Maaliene	C71	0.02	0.32	-	-
10.93	Eudesma-4(14),11-diene	C72	0.07	1.00	0.47	0.82
10.98	α-Selinene	C73	0.06	0.86	0.53	0.66
11.00	γ-Muurolene	C74	0.04	0.56	-	-
11.09	Cadina-1(10),4-diene	C75	-	0.50	-	0.34
11.14	Zonarene	C76	-	0.30	-	-
11.20	Guaia-6,9-diene	C77	-	0.24	-	-
11.27	β-Calacorene	C78	-	0.27	-	-
11.29	Hedycaryol	C79	0.15	-	0.13	0.45
11.30	Elemol	C80	-	0.94	-	-
11.34	Calarene epoxide	C81	-	0.25	-	-
11.42	Germacrene B	C82	0.02	0.29	-	-
11.50	Palustrol	C83	-	-	0.12	0.46
11.53	Spathulenol	C84	-	-	0.15	-
11.59	Caryophyllene oxide	C85	-	0.39	4.77	1.44
11.62	Guaiol	C86	1.87	8.23	3.22	3.51
11.65	Viridiflorol	C87	-	-	-	1.37
11.71	Agarospirol	C88	-	0.22	-	-
11.72	Ledol	C89	-	-	1.14	4.26
11.76	Humulene epoxide I	C90	-	-	2.67	0.67
11.78	Rosifoliol	C91	-	0.55	-	-
11.80	Neointermedeol	C92	-	0.50	-	-
11.83	Globulol	C93	0.17	1.84	-	-
11.87	epi-γ-Eudesmol	C94	1.33	8.64	2.39	2.94
11.89	Alloaromadendrene oxide-(2)	C95	-	-	1.99	-
11.91	Cubenol	C96	-	1.51	1.07	1.05
11.92	Hinesol	C97	-	-	1.58	1.09
11.93	Agarospirol	C98	0.23	1.96	-	-
12.03	α-epi-7-epi-5-Eudesmol	C99	-	-	-	7.13
12.03	Eudesm-4(14)-en-11-ol	C100	4.13	18.18	-	-
12.04	10-epi-γ-Eudesmol	C101	-	-	6.43	-
12.06	Guai-1(10)-en-11-ol	C102	0.78	0.44	1.43	2.03
12.30	Eudesm-7(11)-en-4-ol	C103	-	0.32	-	-
13.03	all-trans-Farnesyl acetate	C104	-	-	-	0.04
14.06	n-Hexadecanoic acid	C105	-	-	-	0.18
Total			99.95	99.60	92.54	94.47

GL: "Tràm gió" leaf; GH: "Tràm gió: flower; CL: "Tràm cừ" leaf; CH: "Tràm cừ" flower

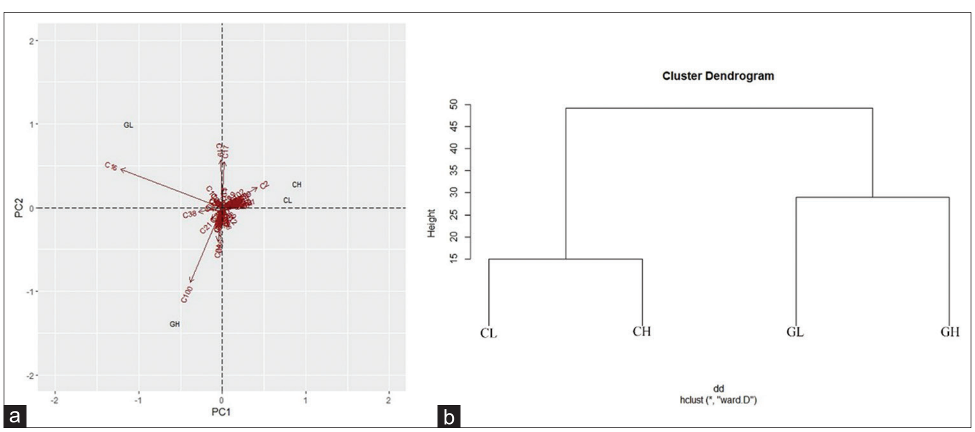

Figure 1: Comparison of chemical compositions of the essential oils obtained from four studied samples. a) PCA biplot presenting the major components of the essential oils, b) The AHC tree diagram presenting the two clusters of the essential oils. Note: GL: "Tràm gió" leaf, GH: "Tràm gió flower, CL: "Tràm cừ" leaf, CH: "Tràm cừ" flower

Ramalho *et al.*, 2015). In addition, d-Limonene was found to be effective against *E. coli*, *S. aureus*, *B. subtilis* and *S. cerevisiae* as well as it also has been proven to have bioactivity against breast cancer (Miller *et al.*, 2013).

CONCLUSION

A total of 105 components were obtained from the essential oils of leaves and flowers of two *M. cajuputi* cultivars collected from Moc Hoa district, Long An province. The most abundant compounds of four studied samples such as "Tràm gió" leaf, "Tràm gió" flower, "Tràm cừ" leaf and "Tràm cừ" flower were 1,8-cineole (35.12%), eudesm-4(14)-en-11-ol (18.18%), γ-terpinene (10.48%) and α-pinene (12.19%), respectively. The chemical components of the essential oils of two cultivars of *M. cajuputi*, including "Tràm gió" and "Tràm cừ" were high differences in which 1,8-cineole linalool, (R)-α-terpinyl acetate and eudesm-4(14)-en-11-ol, etc. presented in "Tràm gió" essential oils whereas "Tràm cừ" essential oils were characterized by the predominance of α-pinene, γ-terpinene, p-mentha-2,4(8)-diene, caryophyllene, humulene, caryophyllene oxide, etc.

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