MELALEUCA CAJUPUTI AND *CYMPOBOGON CITRATUS* ESSENTIAL OILS FROM THE CENTRAL VIETNAM: NEW INSIGHTS INTO PHYTOCHEMICALS AND ANTIMICROBIAL ACTIVITY

TINH DẦU TRÀM GIÓ (*MELALEUCA CAJUPUTI)* VÀ SẢ CHANH (*CYMPOBOGON CITRATUS)* Ở MIỀN TRUNG VIỆT NAM: NHỮNG KẾT QUẢ MỚI VỀ THÀNH PHẦN HÓA HỌC VÀ HOẠT TÍNH KHÁNG VI SINH VẬT

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ABSTRACT

The extractions of *Melaleuca cajuputi* and *Cympobogon. citratus* essential oils (EOs) cultivated in the Central Vietnam were accomplished by hydrodistillation method with yields 0.78% and 0.51%, respectively. The obtained EOs were then screened for chemical compositions using gas chromatography-mass spectrometry (GC-MS). The results indicated 33 compounds which constitute 98.94% of the total content of the *M. cajuputi* EO in which 1,8-cineole (46.95%) and *α*-terpineol (12.54%) were major ingredients. For the *C. citratus* EO, 19 compounds accounting for 96.65% of the EO content were analyzed with major components include citral (a mixture of two isomers neral and geranial, 73.90%) and myrcene (6.96%). These EOs were also tested for *in vitro* antimicrobial activity. Both of EOs displayed antimicrobial effect against four microbial strains *E. coli, S. aureus, A. niger and S. cerevisiae*. While *M. cajuputi* EO gave the same MIC values (50µg/ml) against these four strains, the identical MIC values (100µg/ml) were obtained in case of *C. citratus* EO.

Keywords*: Essential oils, Melaleuca cajuputi, Cymbopogon citratus, phytochemical, antimicrobial.*

TÓM TẮT

Các tinh dầu tràm gió (*Melaleuca cajuputi)* và sả chanh (*Cympobogon. Citratus)* thu hái ở miền Trung Việt Nam thu được bằng phương pháp chưng cất lôi cuốn hơi nước với hiệu suất tương ứng là 0,78% and 0,51%. Các tinh dầu này sau đó được khảo sát thành phần hóa học sử dụng phương pháp sắc kí khí kết hợp khối phổ (GC-MS). Các kết quả đã chỉ ra 33 hợp chất được tìm thấy chiếm 98,94% hàm lượng của tinh dầu tràm gió (*M. cajuputi),* trong đó hai thành phần chính là 1,8-cineole (46,95%) và *α*-terpineol (12,54%). Còn đối với tinh dầu sả chanh (*C. citratus),* 19 hợp chất chiếm 96,65% hàm lượng đã được phát hiện với các thành phần chính bao gồm citral (một hỗn hợp của hai đồng phân neral và geranial, chiếm 73,90%) và myrcene (6,96%). Những tinh dầu này sau đó cũng được thử nghiệm hoạt tính kháng vi sinh vật *in vitro*. Theo đó, cả hai tinh dầu đều thể hiện hoạt tính chống lại bốn dòng vi sinh vật gồm *E. coli, S. aureus, A. niger* và *S. cerevisiae*. Trong khi tinh dầu tràm gió (*M. cajuputi)* cho giá trị MIC giống nhau 50µg/ml chống lại bốn dòng vi sinh vật ở trên, các giá trị MIC như nhau 100µg/ml đã đạt được trong trường hợp của tinh dầu sả chanh (*C. citratus)*.

Từ khóa*: Tinh dầu, tràm gió (Melaleuca cajuputi), Sả chanh (Cymbopogon citratus), thành phần hóa học, kháng vi sinh vật.*

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1. INTRODUCTION

Melaleuca cajuputi, belonged to the Myrtaceae family, was widely used in traditional medicine [1]. According to modern medicine, *M. cajuputi* essential oil possesses important biological activities such as antioxidant, anticancer, antiviral, and antimicrobial activities [2-4]. The chemical compositions of *M. cajuputi* essential oil have been extensively investigated where 1,8-cineole, γ-terpinene, terpinolene, and caryophyllene were principal components [4, 5].

Cymbopogon (or lemongrass), a member of the Poaceae family, was originated from Southwest Asia, then dispersed all over the world including Vietnam [6]. *Cymbopogon citratus* is one of the most important species displayed interesting pharmacological activities - antibacterial, antifungal, anti-inflammatory, and cytotoxic activities [7-9] - which might be granted by the presence of phytochemicals in *C. citratus*, especially essential oil with citral as the most notable main component [7, 10].

In this study, we aimed to examine the phytochemicals using gas chromatography-mass spectrometry (GC-MS) and evaluate *in vitro* antimicrobial activity of *M. cajuputi* and *C. citratus,* two of the most abundant and valuable EOs grown in the Central Vietnam. The latest

finding can be an important scientific evidence for further applications of these EOs in industry as well as medicine.

2. EXPERIMENTAL

2.1. Materials

M. cajuputi and *C. citratus* were harvested in Quang Tri province in September 2021. In this study, *M. cajuputi* EO was obtained from twigs and leaves whereas the aerial parts were utilized to produce *C. citratus* EO. 300 gram of the fresh selected parts of each plant were subjected to a preliminary treatment including removing damaged parts, rinsing, draining and chopping into 1 - 2mm pieces in prior to the extraction.

Dimethylsulfoxide (DMSO), anhydrous sodium sulfate and *n*-hexane (analytical grade) were purchased from Sigma-Aldrich, USA. Tryptic soy broth (TSB) and saboraud-2% dextrose broth (SDB) were supplied from Merck, Germany.

The pathogenic microbial strains utilized in the study were purchased from American Type Culture Collection (ATCC, VA, USA).

2.2. Essential oils isolation

EO extractions from above prepared *M. cajuputi* and *C. citratus* materials were performed by the hydrodistillation method for 8 hours and 4 hours at 100^{0}C, respectively, using a Clevenger-type apparatus. The EOs were then collected and dried with anhydrous sodium sulfate and stored in amber vials at below 10°C until GC-MS analyses. The EO extractions were repeated three times and the calculated yields are the average values of three extractions.

2.3. Phytochemical analyses of Eos

Phytochemical analyses of the essential oils were conducted by the Gas Chromatography-Mass Spectrometry/Flame Ionization Detection method (GC-MS/FID) on an Agilent Technologies HP7890A GC coupled with a flame ionization detector, Agilent Technologies HP5975C and a HP5-MS column (60m × 0.25mm, film thickness 0.25µm, Agilent Technologies) using MassFinder 4.0 software.

2.4. Antimicrobial assay

Antimicrobial activity of the EOs was evaluated by the method described by Vanden Berghe and Vlietinck [11]. DMSO (5%) was used as negative control. Streptomycin and tetracycline were used as positive control for Gram (+) and Gram (-) bacteria, respectively, whereas nystatin was positive control for fungi and yeasts.

3. RESULTS AND DISCUSSION

3.1. Extraction and phytochemical analysis of *M. cajuputi* EO

The EO *of M. cajuputi* leaves was obtained as pale yellow liquid by the hydrodistillation method with an extraction yield 0.78%. This yield was good in comparison to literatures [4, 12].

Chemical constituents from *M. cajuputi* EO leaves, determined by GC-MS/FID, were represented in the Table 1. 39 compounds were found and contributed 98.94% total content of EO. Major components were also identified, in order of the decrease of contents, as 1,8-cineole (or eucalyptol, 46.95%), α-terpineol (12.54%), limonene (5.96%), linalool (5.86%), and guaiol (4.00%) while others were only detected in low percentages. Table 1 also showed monoterpenoids (60.29%) were dominated in *M. cajaputi* EO. Notably, content of 1,8-cineole in the studied EO was relatively high in correlation to the previously reported values for example 23.59% [2], 27.51% [4], and 43.00% [12]. These differences might be contributed by factors such as plant species, cultivation conditions as well as geographical region.

Table 1. Phytochemicals in *M. cajuputi* EO

No	RT (min)	RI	Compounds	Classification	Content (%)
1	10.51	938	α-Pinene	Monoterpene	2.22
2	11.30	964	Benzaldehyde	Aldehyde	0.68
3	11.90	984	β-Pinene	Monoterpene	1.02
4	12.12	991	Myrcene	Monoterpene	0.69
5	13.12	1021	α-Terpinene	Monoterpene	0.21
6	13.38	1029	*o*-Cymene	Monoterpene	0.27
7	13.55	1034	Limonene	Monoterpene	5.96
8	13.71	1038	1,8-Cineole	Monoterpenoid	46.95
9	14.54	1063	γ-Terpinene	Monoterpene	0.65
10	15.61	1094	Terpinolene	Monoterpene	0.30
11	15.85	1101	Linalool	Monoterpenoid	5.86
12	18.42	1174	Terpinen-4-ol	Monoterpenoid	0.22
13	18.82	1185	Santalone	Monoterpenoid	0.72
14	19.27	1198	α-Terpineol	Monoterpenoid	12.54
15	21.24	1255	Linalool acetate	Ester	1.78
16	25.59	1385	α-Ylangene	Sesquiterpene	0.12
17	27.25	1437	β-Caryophyllene	Sesquiterpene	0.67
18	28.33	1472	α-Humulene	Sesquiterpene	0.68
19	28.91	1490	β-Chamigrene	Sesquiterpene	0.13
20	29.04	1494	α-Amorphene	Sesquiterpene	0.36
21	29.35	1504	β-Selinene	Sesquiterpene	0.56
22	29.38	1505	γ-Selinene	Sesquiterpene	0.56
23	29.61	1513	α-Selinene	Sesquiterpene	0.76
24	29.87	1522	γ-Amorphene	Sesquiterpene	0.13
25	31.00	1560	α-Calacorene	Sesquiterpene	0.11
26	31.03	1561	Selina-3,7(11)-diene	Sesquiterpene	0.11
27	32.33	1604	Caryophyllene oxide	Sesquiterpenoid	0.24
28	32.60	1614	Guaiol	Sesquiterpenoid	4.00
29	33.11	1632	Humulene epoxide II	Sesquiterpenoid	0.35
30	33.64	1651	γ-Eudesmol	Sesquiterpenoid	3.09
31	34.26	1673	β-Eudesmol	Sesquiterpenoid	3.03

32	34.33	1675	α-Eudesmol	Sesquiterpenoid	3.11
33	34.63	1686	Bulnesol	Sesquiterpenoid	0.86
			Total		**98.94**

3.2. Extraction and phytochemical analysis of C. citratus EO

The extraction and phytochemical analysis of the studied *C. citratus* EO (whole plant) were carried out similarly to those for the above *M. cajuputi* EO. The isolation yield, in this case, was 0.51%. The analyzed chemical compositions were summarized in the Table 2. 19 individual components, accounting for 96.65% total content of *C. citratus* EO, were discovered where one compound was unknown. Unsurprisingly, citral was present in the studied *C. citratus* EO with the highest content 73.90% (31.70% of neral and 42.20% of geranial) followed by myrcene (6.96%) and geraniol (6.09%). Table 2 revealed that monoterpenoids (77.28%) were dominated in *C. citratus* EO whereas sesquiterpenoids only constituted a tiny percentage of 0,16%, even group of sesquiterpenes was not found. Phytochemical profile from *C. citratus* EO was well documented in which percentages of citral varied from 47.4 – 79.5% for aerial parts [13-15]. Clearly, the studied *C. citratus* EO showed an excellent content of citral compared to the previous studies. Again, factors including plant species, geographical region and cultivation conditions might be considered as reasons for the observed significant differences.

Table 2. Phytochemical in C. citratus EO

No	RT (min)	RI	Compounds	Classification	Content (%)
1	11.95	985	6-Methylhept-5-en-2-one	Ketone	0.94
2	12.13	991	Myrcene	Monoterpene	6.96
3	12.27	996	2,3-Dehydro-1,8-cineol	Pyran	0.12
4	13.67	1037	β-(Z)-Ocimene	Monoterpene	0.42
5	14.05	1048	α-(E)-Ocimene	Monoterpene	0.21
6	15.84	1101	Linalool	Monoterpenoid	0.71
7	17.46	1147	Lavandulol	Monoterpenoid	0.30
8	17.75	1155	Citronellal	Monoterpenoid	0.18
9	17.79	1156	Z-Chrysanthemol	Monoterpenoid	0.13
10	18.14	1166	Isoneral	Monoterpenoid	0.93
11	18.77	1184	Isogeranial	Monoterpenoid	1.32
12	20.32	1229	Citronellol	Monoterpenoid	0.68
13	20.96	1247	Neral	Monoterpenoid	31.70
14	21.27	1256	Geraniol	Monoterpenoid	6.09
15	21.96	1276	Geranial	Monoterpenoid	42.20
16	24.51	1352	Ethyl nerolate	Ester	0.71
17	25.55	1384	Geranyl acetate	Ester	1.83
18	32.35	1605	Caryophyllene oxide	Sesquiterpenoid	0.16
19	33.31	1639	Unknown	Unknown	1.06
			Total		**96.65**

3.3. Antimicrobial activity evaluation

Table 3. Antimicrobial activity of M. cajuputi and C. citratus EOs

Samples	Minimum Inhibitory Concentration (MIC, μg/ml) for microbial strains							
	Gram (-) bacteria		Gram (+) bacteria		Fungi		Yeasts	
	E. coli	*P. aeruginosa*	*B. subtilis*	*S. aureus*	*A. niger*	*F. oxysporm*	*C. albicas*	*S. cerevisie*
M. cajaputi	50	(-)	(-)	50	50	(-)	(-)	50
C. citratus	100	(-)	(-)	100	100	(-)	(-)	100
Positive control	5.0	11.00	7.19	14.38	23.12	11.56	5.78	11.56

The result for antimicrobial activity evaluation of two tested EOs was represented in the Table 3. The growth of four microbial strains including *E. coli, S. aureus, A. niger* and *S. cerevisiae* were inhibited by both EOs while none of EOs indicated inhibition against four remain strains. As compared to the positive control, antimicrobial effect of the *M. cajuputi* EO was promising with the same MIC values 50μg/ml. Many findings attested that antimicrobial effect of *M. cajuputi* EO was related to active components such 1,8-cineole, terpine-4-ol, linalool [16]. Comparative antimicrobial activity of the studied *M. cajuputi* EO was not out of our expectation and demonstrated an excellent connection from the view of the above phytochemical profile.

The *M. cajuputi* EO expressed more effective activity than the *C. citratus* EO which gave the identical MIC values of 100μg/ml. S. Burt reported that EOs expressed the best antibacterial effects against pathogen microorganisms contained a high content of phenolic ingredients, for example 1,8-cineole, terpine-4-ol, linalool as in *M. cajuputi* while citral was lied on the bottom half in the rank of antimicrobial activity [17]. The weaker activity of *C. citratus* compared to *M. cajuputi* was, therefore, logical. However, it was fair to state that antimicrobial effect of the studied *C. citratus* EO was potential in comparison to the previous reports [18-19].

4. CONCLUSION

Analyses of phytochemical profiles determined 1,8-cineole (a phenolic monoterpenoid) and citral (an aldehyde monoterpenoid) as major components in *M. cajuputi* and *C. citratus* EOs, respectively. The evaluation of antimicrobial activity of these EOs showed the promising effects with MIC values from 50 - 100μg/ml against four among eight tested pathogenic microbial strains. The relationship between chemical compositions and antimicrobial property of EOs was well evidenced. The obtained results suggested potential applications of two of the most valuable EOs in Vietnam as antimicrobial agents in food protection and medicine.

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