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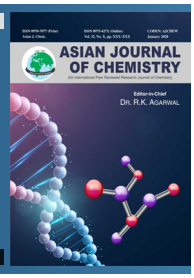

Essential Oil Profile of Vietnamese *Callicarpa candicans* (Burm. f.) Hochr

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In this study, the essential oil profile of *Callicarpa candicans* (Burm. f.) Hochr was presented. Samples were collected from Vietnam and the oils were extracted from fresh leaves and dry leaves by hydrodistillation method. GC-MS data and retention indices were used to identify the chemical composition. The essential oil performance achieved 0.263% for fresh leaves and 1.503% for dry leaves. A total of 47 components were identified from fresh leaves oil and 39 constituents from dry leaves oil. Chemical compositions of the two essential oils were similar, with the major components being α-gurjunene, δ-cadinene, ε-caryophyllene (β-caryophyllene) and α-selinene.

Keywords: Vietnamese *Callicarpa candicans* Hochr, Essential oil.

INTRODUCTION

The Callicarpa genus, whose family has been reclassified from Verbenaceae to Lamiaceae *via* recent molecular and micro-morphological observations, comprises about 190 species [1,2]. The genus was geographically distributed mainly in Oceania, America, Australia, the Pacific Islands, tropical and subtropical Asia including India, Burma, Thailand, Indochina and Vietnam [3-5]. Among species in the genus, several plants have been used as traditional and ethnomedicines. *C. arborea* Roxb. Bank, for example, has been widely utilized in India in medicinal formulae for treatment of skin diseases. Another species, *C. formasana* Rolfe, is an important ingredient in Taiwanese folk medicine to cure hepatitia, oral infections, intestinal and stomach disorders [1]. Other recognized functionalities of plants in the *Callicarpa* genus consist of treatment of inflammation, rheumatism, heumaturia, hematemesis, fractures, women amenorrhea, gastrointestinal and scrofula [6]. Such benefits are mostly due to abundance of chemical compounds such as terpenoids (especially diterpenoids), flavonoids, volatile oils, lignans and phenylethanoids, several of which have been shown to exhibit cytotoxic, mosquito repellent, antibacterial, antiviral and anthelmintic activities [3,7]. In a previous compound identification attempt involving the *C. longissimi* species, four new compounds have been discovered in the ethanolic extract of leaves and twigs of the species. In addition, four compounds among identified compounds were found to exhibit potent anti-inflammatory activities [7]. Similarly, other six new clerodane diterpenes were also isolated in the methanolic extract from various parts of another *Callicarpa* species, *C. americana*. Three of which have been determined to feature potent cytotoxicity against six human cancer cell lines [2]. In the EtOAc extract of leaves of *C. nudiflora*, a traditional plant used in China for treatment of antibiosis, antiphlogosis and hemostasis, four flavonoids (ayanin, apigenin, luteolin and quercetin) were found in significant quantities [8].

Plant essential oil are mixtures of plant secondary compounds, consisting mainly of terpenoids (especially monoterpenes and sesquiterpenes), aromatic phenol, oxides, ethers, alcohols, esters, aldehydes and ketone. Depending on the composition, aroma and biological activities of essential oils could vary [9, 10], defining their uses in industry and in medicine [9,11]. In

the *Callicarpa* genus, several species have been demonstrated to offer valuable essential oils. For example, in essential oil derived from leaves at different maturity stages and fruits of *Callicarpa macrophylla*, a total of 45 compounds were identified, in which β-selinene, α-selinene, *cis*-calamenene, dendrolasin, cedr-8(15)-en-9α-ol, β-copaen-4α-ol, α-muurolol and selin-11-en-4α-ol and phyllocladene were the major constituents [12]. The essential oil of *C. americana*, an ornamental shrub species, has been found to contain α-humulene, humulene epoxide II, intermedeol and callicarpenal, a new terpenoid. This results was further extended in another study where 67 components, accounting for > 78% of the essential oil, was detected in the steam-distilled oil of *C. americana* with humulene epoxide II (13.9%) as the most abundant compounds. Other major compounds were α-humulene (10.0%), 7-epi-α-eudesmol (9.4%), β-pinene (8.8%) and 1-octen-3-ol (8.5%) [13]. Similar to *C. americana* in terms of composition, essential oil of *C. japonica* was shown to contain callicarpenal, intermedeol and spathulenol, which are all effective in deterring *A. stephensi* and *A. aegypti* [14]. However, these compositional results of *C. japonica* seem to contradict another study where 84 compounds were found in *C. japonica* essential oil and abundant constituents were spathulenol (18.1%), followed by germacrene B (13.0%), bicyclogermacrene (11.0%), globulol (3.3%), viridiflorol (2.6%), α-guaiene (2.3%) and γ-elemene (2.0%) [13]. Another species in the *Callicarpa* genus, *C. nudiflora*, has been also been investigated for essential oil composition [15].

Callicarpa candicans (Burm. f.) Hochr is a species of the genus Callicarpa, distributed in various South East Asian countries such as Vietnam, Thailand, Cambodia, Philippines and Laos [16]. Due to the widespread availability of the species in Vietnam, the species has been used in Vietnamese traditional medicine for treatment of trunks, pimples and ulceration and in formulae for maternal care [17,18]. In Philippines, the leaf of *Callicarpa candicans* was used to cure stupefy due to the strong toxicity of callicarpone, one main component in its leaves [19]. So far, compositional determination attempts involving *Callicarpa candicans* have focused on the ethyl actate extract and *n*-hexane extract of the plant. To be specific, it was found that ethyl actate extract comprised ursolic acid (**1**), 2α-hydroxy-ursolic (**2**), 2α,3β,23-trihydroxyurs-12-en-28-oic acid (**3**), genkwanin (**4**) and luteolin-7-O-β- D-glucopyranoside (**5**) and *n*-hexane extract contained 5-hydroxy-7,4′-dimethoxyflavone (**1**), 5-hydroxy-3′,4′,7-trimethoxyflavone (**2**) and ursolic acid (**3**) [18-20]. Hence, in the current study, we report the chemical composition of essential oil from *Callicarpa candicans* (Burm. f.) Hochr growing in the wild in Vietnam through gas chromatography-mass spectrometry (GC-MS) analysis.

EXPERIMENTAL

Fresh *Callicarpa candicans* leaves were divided into two parts which were chopped fresh leaves and chopped dry leaves. Dry leaves were formed by drying fresh leaves under the shade.

Isolation of essential oils: Each part of *Callicarpa candicans* leaves was weighed to 200 g and put in a pressure cooker, followed by addition of clean water with the water:material ratio of 3:1. Following that, hydrodistillation took place in a Clevenger type distillation apparatus for 6-7 h. Obtained oil was centrifuged to remove water and then pure oil was transferred into vial, which was then cooled in a fridge for further analysis.

GC-MS analysis of essential oils: GC-MS analysis of the essential oils was carried out on an Agilent Technologies HP7890A GC equipped with a mass spectrum detector (MSD) Agilent Technologies HP5975C and a HP5-MS column (60 m × 0.25 mm, film thickness 0.25 μm, Agilent Technologies). The injector and detector temperature was set at 250 and 280 ºC, respectively. The column temperature progress initiated at 60 ºC, followed by an increase to 240 ºC at 4 ºC/min. The carrier gas was helium at a flow rate of 1 mL/min. Samples were injected by splitting. The split ratio was 100:1. The volume injected was 1 μL of essential oils. The MSD conditions were as follows: ionization voltage 70 eV, emission current 40 mA, acquisitions scan mass range 35-450 amu under full scan. A homologous *n*-alkane series was used as the standard to calculate retention time indices (RI) of each component. The relative amounts of individual components were calculated based on the GC peak area (MSD response) without correction.

Identification of constituents: MassFinder 4.0 software connected to the HPCH1607, W09N08 libraries and the NIST Chemistry WebBook was used to match mass spectra and retention indices. To confirm these results, further comparison was made with data of authentic compounds reported in the original literature.

RESULTS AND DISCUSSION

By hydrodistillation, the yield of *Callicarpa candicans* essential oils for fresh leaves was 0.263% and dry leaves was 1.503%. The yield discrepancy between two types of leaves was possibly due to the difference in moisture. In addition, the essential oil productivity of *Callicarpa candicans* fresh leaves was higher than that of *C. americana* (0.11%) and *C. japonica* (0.1%) [14,21]. This might be due to species difference, distillation method and miscellaneous factors such as growing habitat or experimental conditions.

Identification of constituents in the obtained oils were made by GC/MS analysis. The chromatogram profiles are shown in Fig. 1, while the identity, retention index and percent composition of the oil of two leaves are presented in Table-1. Visually, primary compounds detected in the samples are sesquiterpene hydrocarbons and oxygenated sesquiterpenes, which are largely responsible for the pleasant and desirable odor of the materials.

Analysis of the essential oil derived from dry leaves showed a total of 39 compounds, accounting for 92.57% of the total content. Among the identified components, there were 25 sesquiterpene hydrocarbons (62.98%) and 11 oxygenated sesquiterpenes (22.46%). The three unidentified compounds were detected at 1626, 1653, 1672 (RI), representing 2.44%, 1.98% and 2.71% the total content, respectively. The main compound identified in the oil was α-gurjunene (21.97%), followed by δ-cadinene (7.78%), α-selinene (5.13%), α-cadinol (5.13%), ε-caryophyllene (= β-caryophyllene) (5.09%), α-copaene (4.43%), β-selinene (3.90%), γ-muurolene (3.84%), 1-epi-cubenol (3.60%),

TABLE-1
CHEMICAL COMPOSITION OF *C. candicans* ESSENTIAL OIL FROM DIFFERENT TYPES OF LEAVES

RI	Compound	Fresh leaves (%)	Dry leaves (%)
984	β-Pinene	0.24	–
1103	Linalool	0.31	–
1204	Methyl salicylate	0.13	–
1348	δ-Elemene	0.27	–
1360	α-Cubebene	0.40	0.31
1385	α-Ylangene	0.70	0.59
1389, 1390	α-Copaene	5.39	4.43
1400	β-Bourbonene	0.17	–
1426, 1427	α-Gurjunene	21.31	21.97
1437, 1438	*E*-Caryophyllene (β-caryophyllene)	6.32	5.09
1445	β-Gurjunene (=Calarene)	0.94	0.76
1446	α-*trans*-Bergamotene	0.94	0.76
1457	Aromadendrene	0.61	0.53
1471, 1472	α-Humulene	0.47	0.36
1479	9-epi-(*E*)-Caryophyllene	0.39	0.30
1488	*trans*-Cadina-1(6),4-diene	0.62	0.55
1491	γ-Muurolene	5.66	3.84
1494	α-Amorphene	0.53	0.34
1498	Germacrene D	0.25	–
1505	β-Selinene	4.23	3.90
1509, 1510	γ-Amorphene	1.25	0.76
1513, 1514	α-Selinene	5.71	5.13
1518	β-Bisabolene	0.56	0.30
1530, 1531	γ-Cadinene	2.17	2.36
1537, 1538	δ-Cadinene	7.70	7.78
1539	*cis*-Calamenene	0.82	0.46
1541	Zonarene	0.21	–
1548, 1549	*trans*-Cadina-1,4-diene	0.46	0.49
1553	α-Cadinene	0.46	0.42
1560, 1561	α-Calacorene	0.80	0.70
1580	β-Calacorene	–	0.22
1589, 1590	Ledol	1.30	2.54
1598	Spathulenol	0.43	0.77
1601	Axenol (=Gleenol)	0.21	0.47
1605	Caryophyllene oxide	1.25	2.00
1626	Unknown (109, 222, RI 1626)	1.55	2.44
1635	1,10-di-epi-Cubenol	0.65	1.08
1647	1-epi-Cubenol	2.51	3.60
1653	Unknown (161, 222, RI 1653)	1.52	1.98
1660	epi-α-Cadinol (T-cadinol)	1.40	1.66
1661, 1662	epi-α-Muurolol (T-muurolol)	1.42	1.77
1664, 1665	α-Muurolol (δ-cadinol)	0.75	0.91
1672	Unknown (162, 220, RI 1672)	2.53	2.71
1674, 1675	α-Cadinol	4.17	5.13
1678	neo-Intermedeol	1.98	2.53
1691	14-Hydroxy-9-epi-(*E*)-caryophyllene	0.43	–
1695	Cadalene	0.50	0.63
2120	Phytol	0.55	–
	Monoterpene hydrocarbons	0.24	–
	Oxygenated monoterpenes	0.31	–
	Sesquiterpene hydrocarbons	69.84	62.98
	Oxygenated sesquiterpenes	16.50	22.46
	Derivatives of benzen (benzenoid)	0.13	–
	Derivatives of diterpene	0.55	–
	Unknown	5.60	7.13
	Total	93.17	92.57

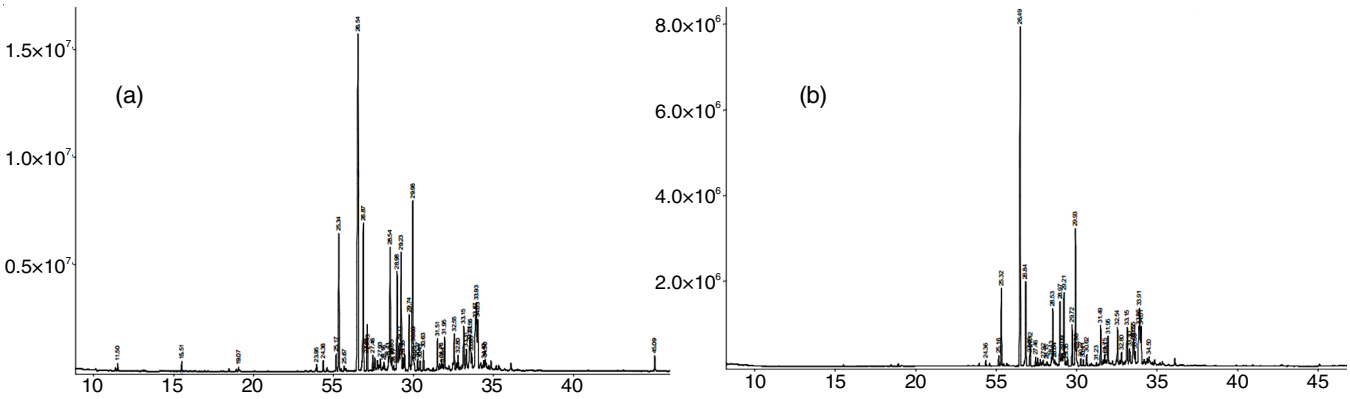

Fig. 1. GC-MS analysis results of chemical compounds present in the *C. candicans* essential oils in (a) fresh leaves, (b) dry leaves

ledol (2.54%), neo-intermedeol (2.53%), γ-cadinene (2.36%) and caryophyllene oxide (2.00%).

In the essential oil of fresh leaves, 47 components representing 93.17% of the total oil content were found. Among them, there were 28 sesquiterpene hydrocarbons (69.84%), 12 oxygenated sesquiterpenes (16.50%), 1 monosesquiterpene hydrocarbon (0.24%), 1 oxygenated monosesquiterpene (0.31%), 1 derivative of diterpene (0.55%) and 1 benzenoid (0.13%). In addition, the fresh leaf oil also contained 3 unidentified compounds detected at 1626, 1653, 1672 (RI) accounting for 1.55%, 1.52% and 2.53%, respectively. The identified compound occupying the largest content was α-gurjunene (21.31%), followed by δ-cadinene (7.70%), ε-caryophyllene (= β-caryophyllene) (6.32%), α-selinene (5.71%), γ-muurolene (5.66%), α-copaene (5.39%), β-selinene (4.23%), α-cadinol (4.17%), 1-epi-cubenol (2.51%), γ-cadinene (2.17%), neo-intermedeol (1.98%).

From the result, it was clear that essential oil compositions of two types of leaves shared considerable similarities. To be specific, both samples had 38 similar components and were constituted by a large amount of α-gurjunene, δ-cadinene, ε-caryophyllene (= β-caryophyllene), α-selinene, γ-muurolene, α-copaene, β-selinene, α-cadinol, 1-epi-cubenol, γ-cadinene, neo-intermedeol. In addition, most components in dry leaves essential oil were present in fresh leaves essential oil, except for β-calacorene. A total of 9 compounds including diterpene derivative, monoterpens hydrocarbon and oxygenated monoterpens were found in fresh leaves exclusively. This may be due to evaporation of small compounds (lower than 1%) during drying.

Fig. 1 also showed that the peak having the greatest intensity was found at the retention time of 26.54 and 26.49 min, corresponding to essential oil sample derived from fresh leaves and dry leaves, respectively. This indicates that the component corresponding to these peak is of great importance in the oils. By comparing the retention time with the mass spectrometry database, it was determined that this component was α-gurjunrene. The identified composition of *Callicarpa candicans* essential oil was starkly different from those of other species of the *Callicarpa* genus. In comparision with other studies, it is found that the essential oils composition of *C. candicans* shares around 20 components with that of *C. americana*.

To be specific, Tellez *et al*. [21] found that humulene epoxide II, α-humulene, β-pinene were the major components of the essential oil of *Callicarpa americana* (L.) leaves. The main compounds of the essential oil from the leaves of *Callicarpa japonica* were spathulenol, germacrene B [14]. Among them, α-humulene, β-pinene, germacrene B and spathulenol were present in the essential oil of *C. candicans* leaves, but with the content of less than 1%. Both *C. candicans* and *C. macrophylla* essential oil had α-selinene and β-selinene as the major compounds. There are several factors that may result in difference in compounds of essential oil including species discrepancy, extraction method, growing habitat and used part of the plant.

Conclusion

The essential oils of *Callicarpa candicans* (Burm. f.) Hochr leaves were obtained from hydrodistillation method with yield of 0.263% (fresh leaves) and 1.503% (dry leaves). By GC-MS method, the chemical composition of the oils were determined, it was found that the essential oil of *Callicarpa candicans* fresh leaves are made up of a complex mixture of sesquiterpenes, oxygenated sesquiterpenes, monosesquiterpene, oxygenated monosesquiterpene, diterpene derivative and benzenoid. In addition, the compositions of the two oils are similar. Besides, by comparing with essential oils of other species in the genus, it is found that common components among them were spathulenol, α-selinene and β-selinene.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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