



Essential Oil of *Glycosmis stenocarpa* (Drake) Leaves Grown in Hai Duong Province, Vietnam

XUAN DUY LE¹, PHUONG THAO LAI PHUONG¹, QUOC TOAN TRAN^{1,2,*}, THI NGOC ANH HOANG¹,
MANH CUONG NGUYEN^{1,2,†}, THI KIM NGAN TRAN^{3,†} and THIEN HIEN TRAN^{3,*†}

¹Institute of Natural Products Chemistry, Vietnam Academy of Science and Technology (VAST), Hanoi City, Vietnam

²Graduate University of Science and Technology, Vietnam Academy of Science and Technology (VAST), Hanoi City, Vietnam

³Institute of Applied Technology and Sustainable Development, Nguyen Tat Thanh University, Ho Chi Minh City, Vietnam

*Corresponding authors: Tel: +84 37 5643345; E-mail: tranquoctoan2010@gmail.com; hientt@ntt.edu.vn

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In this study, the extraction of essential oil of Vietnamese *Glycosmis stenocarpa* leaves was conducted using two different methods such as conventional microwave-assisted hydrodistillation (MAHD) and hydrodistillation (HD). The obtained products were analyzed by gas chromatography-mass spectrometry (GC-MS). The essential oil quality was attained in a comparable manner in both samples respectively. In addition, the GC-MS analysis showed that the hydrodistillation essential oil contains a total of 29 chemical compounds, whereas the MAHD essential oil contained 28 constituents. Moreover, the chemical constituents of the two essential oils were similar, with the major components being citronellal, neryl acetate, citronellyl acetate and geranyl acetate.

Keywords: *Glycosmis stenocarpa* (Drake), Essential oil, GC-MS.

INTRODUCTION

The genus *Glycosmis*, which belongs to the Rutaceae family, comprises about 125 species. The genus is geographically distributed primarily in the south and southeastern Asia, Taiwan and South China as well as northern Australia and New Guinea, the tropical rainforest of Asia, Africa, Americas [1-3]. The shrubs or small trees have pinnate or simple leaves with translucent punctate glands emitting an aromatic odour when crushed [4-9]. Among species in the genus, some plants have been used as traditional medicine to against many diseases [10-13]. For example, stem and fruits of *Glycosmis pentaphylla* have been used in Bangladesh for the treatment of rheumatoid arthritis or its roots have been used in India against facial inflammation, rheumatism, jaundice and anaemia [14].

Another species, *G. citrifolia* (Willd.) Lindley, is an key factor in folk medicine to treat skin itch, scabies, boils and skin ulcers [15]. Others pharmacological functions of plant in this genus are treatment of cough, stomach pain, fever, liver complaints, jaundice, eczema and diarrhea [16]. These benefits are due to the abundance of phytochemical compounds such as terpenoids, alkaloids, flavonoids, sterols, amides, coumarins,

imides, carbazoles, acridone, quinoline and quinazolines, some of them have shown bioactivities such as antiviral, antitumour, anti-inflammatory, hepatoprotective, antioxidant, antibacterial, anticancer, mosquito repellent, larvicidal activity [7,17-20], etc. Rahman *et al.* [21] reported about *G. pentaphylla* (Retz.) correa leaves extract has shown neuroprotective and antioxidant effects and it can be a therapeutic potential of neurological diseases. Similarly, the methanol, acetone, ethyl acetate and chloroform solvent leaf extracts of this species have been effective against the larvae of three important vector mosquitoes *viz.* *An. stephensi*, *Cx. quinquefasciatus* and *Ae. aegypti* [22]. Six novel amides have been isolated from the lipophilic leaf extracts of *Glycosmis cf. cyanocarpa*, *Glycosmis cf. mauritiana* and *Glycosmis crassifolia* and the known imide ritigalin have displayed pronounced antifungal and/or insecticidal activity against *Spodoptera littoralis* and *Cladosporium herbarum* [23].

Not only have some species in *Glycosmis* genus had much value from solvent extracts, but also been illustrated to offer valuable plant essential oil. For instance, essential oil of bark, leaves and seeds of *G. pentaphylla* were isolated from India and around 60 phytochemical compounds were identified [24]. Among them, benzaldehyde oxime (15.66%), bicyclo[6.1.0]-

non-1-ene (18.93%), caryophyllene oxide (7.47%), caryophyllene oxide (7.47%) and aromandendrane (0.30%) were the major constituents. All of them were the excellent antioxidant and radical scavenging properties and they help oil become a potential and valuable larvicidal compound [22,23]. Predominantly the compositions in these oils were aliphatic ketones, aliphatic and monocyclic compounds [25]. In essential oil derived from *G. lucida* Wall.ex Huang, 27 constituents were isolated, accounting for about 92.2% of the total. It was found that the main components contains β -caryophyllene (6.87%), verbenone (10.32%), spathulenol (10.68%), anethole (12.05%) followed by elixene (19.81 %). Especially, anethole and verbenone were strongly repellent against *T. castaneum* and *L. bostrychophila* after a 2 h treatment [26]. Similarly, essential oil of *G. parviflora* was shown to contain (*Z*)-caryophyllene, (*Z*)- β -ocimene methyl isoeugenol and nerolidol, which are all effective in exhibiting insecticidal, strong nematicidal activity against *M. incognita* and contacting toxicity against *T. castaneum* and *S. zeamais* adults [27].

Glycosmis stenocarpa, a species of *Glycosmis* genus, popularly known as “Com ruou trai hep” in Vietnam, is a small shrub, 0.75-1.00 m, intense aroma and grows in limestone areas in some northern provinces of Vietnam [28]. So far, the compositional and bioactive determination attempts involving *G. stenocarpa* have focused on carbazole. To be specific, methanol extract from the leaves and stem of *G. stenocarpa* exhibit the antifungal, anticancer and antibacterial activities. Murrayafoline A and its derivative have been extracted and purified from the roots of the Vietnamese plant *G. stenocarpa* and the content of murrayafoline A in the root was 0.38% (w/w). Hence, in this study, we attempted to recover the essential oil of *G. stenocarpa* leaves by conventional hydrodistillation (HD) and microwave assisted hydrodistillation (MAHD) methods. These new extraction method is advantageous in terms of cost and extraction time, eco-friendly and energy efficiency [29,30]. And then, the chemical compositions of isolated essential oil *via* two methods were identified through gas chromatography-mass spectrometry (GC-MS).

EXPERIMENTAL

Plant samples of *G. stenocarpa* were collected at Hoang Hoa Tham commune, Hai Duong province (20°55'59.99" N, 106°19'0.01" E), Vietnam in May 2023. The leaves were collected and chopped into suitable size for the next distillation experiment.

Isolation of essential oil

Conventional hydrodistillation: The samples were weighed accurately and then added to a 2 L round-bottomed flask containing a suitable volume of water and then connected to the Clevenger apparatus. Essential oils were extracted by hydrodistillation for 4 h. The resulting essential oil was centrifuged to remove water and dried in anhydrous sodium sulfate, the resulting pure essential oil was transferred to a dark vial and then cooled in a refrigerator for further analysis.

Microwave-assisted hydrodistillation: The microwave assisted hydrodistillation was conducted using the microwave

oven below 100 °C and atmospheric pressure. The microwave operates on many modes and has a frequency of 2450 MHz with a maximum power of 900 W. A quantity of weighed sample was subjected to hydrodistillation for a duration of 30 min using 2 L of distilled water. The duration was adequate to extract all the crucial oils from the sample.

GC-MS analysis of essential oils: the 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 \times 0.25 mm, film thickness 0.25 μ m, Agilent Technologies). The injector and detector temperature was set at 250 and 280 °C, respectively. The column temperature progress initiated at 60 °C, followed by an increase to 240 °C at 4 °C/min. The carrier gas was helium at a flow rate of 1 mL/min. Samples were injected by splitting and the split ratio was kept at 100:1. The volume injected was 1 μ L 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 the constituents: The Mass Downloader 4.0 software was connected to the HPCH1607, W09N08 and NIST electronic chemistry e-books that have been used to match the spectral index and maintenance volume. The result of the determination is made based on comparison with the data of the certified compounds reported in the original document.

RESULTS AND DISCUSSION

Both conventional hydrodistillation (HD) and microwave-assisted hydrodistillation (MAHD) isolated the essential oils from *G. stenocarpa* leaves with yields of 0.19 and 0.21%, respectively possesses a viscosity, pale yellow in colour and has characteristic fragrance. But these values were much lower than that of *G. parviflora* (0.64% v/w dry weight) and *G. lucida* (0.35%) [31,32]. This difference might due to the species discrepancy, extraction method, growing habitat and used part of the plant.

G. stenocarpa essential oil obtained by two methods were analyzed by GC-MS. Fig. 1 displays the chromatogram profiles, while Table-1 summarizes the identity, retention index and percent component in the oils. The samples mainly contain monoterpene hydrocarbons and oxygenated monoterpenes, which are responsible for characteristic odour and bioactivity of the material [33].

The essential oil isolated from the conventional hydrodistillation method contains 29 compounds, which account for 98.93% of the total content, were detected. Among them, 28 constituents were identified and 1 unknown compound was found at 1421 (RI), accounted 1.97% of the total essential oil. The identified compounds belong to six categories including 15 oxygenated monoterpenes (82.04%), 8 monoterpene hydrocarbons (13.83%), 1 sesquiterpene hydrocarbon (0.14%), 2 oxygenated sesquiterpenes (0.26%), 1 aliphatic (0.39%), 1

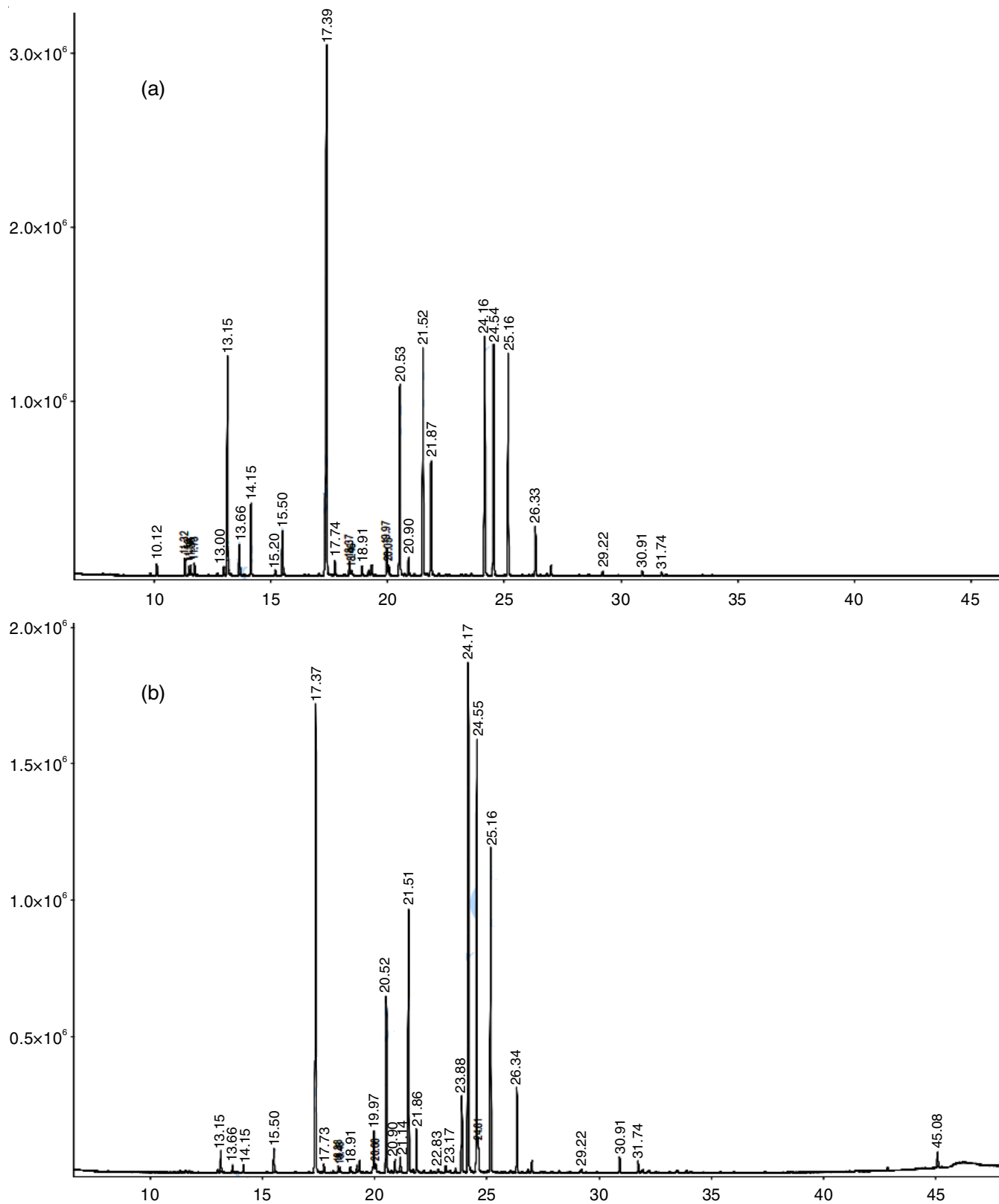


Fig. 1. GC-MS analysis results of chemical compounds present in the *G. stenocarpa* essential oils in (a) conventional hydro-distillation method, (b) microwave-assisted hydro-distillation method

benzenoid (0.3%). The principle compositions of *G. stenocarpa* extracted by hydrodistillation were citronellal (25.9%), followed by geranial (9.89%), citronellyl acetate (9.76%), neryl acetate (9.31%), geranyl acetate (8.81%), limonene (8.23%), neral

(7.94%), perilla aldehyde (4.9%), γ -terpinene (2.63%), linalool (1.6%), (*E*)- β -ocimene (1.1%) and citronellol (1.05%).

The essential oil obtained through MAHD exhibit a total of 28 compositions, constituting 98.45% of the oil. Among

TABLE-1
CHEMICAL INGREDIENTS OF *G. stenocarpa* LEAVES
ESSENTIAL OIL OBTAINED BY DIFFERENT METHOD

RI	Compound	HD (%)	MAHD (%)
938	α -Pinene	0.37	–
978	Sabinene	0.58	–
984	β -Pinene	0.31	–
987	6-Methylhept-5-en-2-one	0.39	–
992	Myrcene	0.42	–
1030	<i>o</i> -Cymene	0.30	–
1034	Limonene	8.23	0.80
1049	(E)- β -Ocimene	1.10	0.24
1063, 1064	γ -Terpinene	2.63	0.27
1094	Terpinolene	0.19	–
1103	Linalool	1.60	0.82
1156, 1157	Citronellal	25.9	18.24
1166	iso-Isopulegol	–	0.32
1167	Isoneral	0.63	–
1184, 1185	Isogeranial	0.50	0.22
1187	Terpinen-4-ol	0.16	0.17
1200	α -Terpineol	0.40	0.20
1230, 1231	Citronellol	1.05	1.56
1233	Nerol	0.46	0.36
1246, 1247	Neral	7.94	6.40
1257, 1258	Geraniol	0.73	0.51
1265	Piperitone	–	0.59
1275	Geranial	9.89	9.93
1285	Perilla aldehyde	4.90	1.75
1314	Citronellic acid	–	0.17
1324	vinyl-Guaiacol	–	0.29
1346	Unknown (81, 154, RI 1346)	–	3.04
1354, 1355	Citronellyl acetate	9.76	19.09
1366	Neryl acetate	9.31	15.48
1368	Unknown (81, 172, RI 1368)	–	1.27
1385	Geranyl acetate	8.81	11.78
1421	Unknown (93, 196, RI 1421)	1.97	3.12
1513, 1514	Bicyclogermacrene	0.14	0.15
1570	(E)-Nerolidol	0.15	0.55
1598	Spathulenol	0.11	0.42
2118	Phytol	–	0.71
	Monoterpene hydrocarbons	13.83	1.31
	Oxygenated monoterpenes	82.04	87.59
	Sesquiterpene hydrocarbons	0.14	0.15
	Oxygenated sesquiterpenes	0.26	0.97
	Benzenoids	0.30	0.29
	Diterpenoids	–	0.71
	Aliphatics	0.39	–
	Unknown	1.97	7.43
	Total	98.93	98.45

the identified components, there were 17 oxygenated monoterpenes (85.59%), 3 monoterpene hydrocarbons (1.31%), 1 sesquiterpene hydrocarbon (0.15%), 2 oxygenated sesquiterpenes (0.97%), 1 diterpenoid (0.71%) and 1 benzenoid (0.29%). In addition, the MAHD essential oil also contains three unknown compounds spotted at 1346, 1368, 1421 (RI), representing 3.04%, 1.27%, 3.12%, respectively. In the essential oil, the highest proportions consists of citronellyl acetate (19.09%), citronellal (18.24%), neryl acetate (15.48%), geranyl acetate (11.78%), geranial (9.93%), neral (6.4%), perilla aldehyde (1.75%) and citronellol (1.56%).

It is clearly found that the essential oil isolated from the MAHD method have considerably similar constituents. To be specific, both isolated samples had 21 similar compounds and consist of mostly monoterpenoids. However, some quantitative differences were also observed. For example, the citronellal percentage in conventional hydrodistillation was 25.9%, whereas in MAHD it was 18.24%. Similarly, citronellyl acetate content obtained in MAHD was 19.09%, which is much higher than that obtained in conventional hydrodistillation (9.76%). Furthermore, the MAHD essential oil contained more polar compounds that contained oxygen since the microwave influences polarized molecules, which assist these components to elevate temperatures very quickly, leaving cells and coming into contact with water vapour more easily [34].

In *Glycosmis* genus, some species were extracted essential oil and analyzed their chemical compounds. In fact, compared to other species, the identified constituent of essential oil from *G. stenocarpa* was totally different. According to previous research, around six compounds identical between the essential oil content of *G. pentaphylla* and that of *G. stenocarpa* leaves were isolated. However, Prakasia & Nair [16] found that phytol, 1,19-eicosadiene, caryophyllene oxide, (–)-spathulenol and bicyclogermacrene were the major constituents of *Glycosmis pentaphylla* leaves essential oil. In case of *G. crassifolia*, the main constituents were δ -cadinene, geyren, spathulenol, neryl salicylate, (E)- β -ocimene and benzyl benzoate. Among them, phytol, spathulenol, bicyclogermacrene, (E)- β -ocimene were also isolated in *G. stenocarpa* but their content less was than 1%. It is apparent that the essential oil of *G. stenocarpa* also possesses excellent biological activity due to the presence of spathulenol, phytol and nerolidol, as reported earlier [3,4] to have immunomodulatory effects, strong antioxidant properties, and insecticidal and acaricidal activities, respectively.

Conclusion

The essential oils of *Glycosmis stenocarpa* leaves were obtained from conventional hydrodistillation and microwave-assisted hydrodistillation method with yield of 0.19% and 0.21% respectively. By GC-MS method, the chemical constituents of the oils were determined, it was found that the essential oil of *G. stenocarpa* leaves contained large amounts of monoterpene hydrocarbons and oxygenated monoterpenes. Since, the compositions of two methods were similar and by comparing with other species, it was found that the common constituents of them were limonene, linalool, α -pinene, β -pinene, nerolidol, spathulenol and phytol.

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CONFLICT OF INTEREST

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

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