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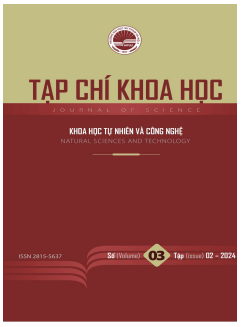

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Essential oil of the leaves of *Cinnamomum subpenninervium* Kosterm. ex H. H. Pham and its antimicrobial activity

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Abstract

The current study first describes the chemical composition of the leaf essential oil of *Cinnamomum subpenninervium* Kosterm. ex H.H. Pham collected from Nghe An province, Central Vietnam. By the GC-FID/MS (gas chromatography-flame ionization detection/mass spectrophotometry) analysis, more than thirty compounds were identified, representing 99.92%. The obtained essential oil from the fresh leaves was dominated by non-terpenic compounds (57.07%), monoterpene hydrocarbons (23.03%) and oxygenated monoterpenes (13.34%), as well as *E*-cinnamaldehyde (38.56%), linalool (12.20%), benzyl benzoate (11.33%), and α-pinene (8.12%). *C. subpenninervium* showed strong antimicrobial activity against the fungus *Fusarium oxysporum* ATCC 46591 with the MIC (minimum inhibitory concentration) value of 64 µg/mL.

Keywords: *Cinnamomum subpenninervium*, Lauraceae, *E*-cinnamaldehyde, essential oil, antimicrobial

1. Introduction

Cinnamomum is a genus of scented, evergreen trees and shrubs of the Lauraceae family. More than 300 species in the genus can be found in tropical and subtropical areas of Australia, Asia, Oceania, North America, Central America, and South America [1]–[3]. Numerous bioactive phytochemicals have been found in plants of this genus. The volatile contents are usually terpenic and non-terpenic compounds. *E*-cinnamaldehyde is the main compound of some species, consisting of *C.*

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paciflorum, *C. pubescens*, *C. aromaticum,* and *C. zeylanicum* [1]. On the other hand, compounds such as linalool in *C. camphora* var. *linaloolifera*, safrole and benzyl benzoate in *C. parthenoxylon*, 2-methylene-3-buten-1-yl-benzoate in *Cinnamomum* sp., and eugenol/1,8-cineole in *Cinnamomum albiflorum* have been identified in higher proportions [4]–[7].

With the presence of cinnamyl derivatives, *Cinnamomum* essential oils have given rise to a wide range of biological activities such as cytotoxicity, antioxidant, anti-biofilm, and anti-inflammation, especially antimicrobial activity [8].

Cinnamomum subpenninervium Kosterm. ex. H.H.Pham, locally named Re long chim, is widely distributed in the tropical rainforests of Southeast Asia [9], [10]. Its flowers are small and white, whereas seeds are small, dark brown, and have a hard outer shell. The seedlings are tiny and green with thin stems and small leaves. It is used as an ornamental plant in gardens and as a shade tree. It is also used in traditional herbal remedies [9]. Our current study aims to report chemical compositions in the leaf essential oil of this species, which was gathered from Nghe An - Viet Nam. The obtained essential oil was further subjected to an antimicrobial assay.

2. Experimental

2.1. Materials

C. subpenninervium fresh leaves were collected from Pu Huong - Nghe An, Vietnam, in 2023. The Latin name was identified by co-author Dr. Do Ngoc Dai. The voucher specimen CS-L has been deposited in the Faculty of Chemistry, Hanoi Pedagogical University 2 (HPU2).

2.2. Distillation

The fresh leaves (1.0 kg) were cut into small pieces and then submitted to water distillation using a Clevenger-type apparatus for 2.3 h to afford a yellowish oil (0.45% w/w). The obtained oil was dried over Na_2SO_4, and maintained in a sealed vial at -5°C for further analysis.

2.3. The GC-FID/MS analytical procedures

Chemical compounds in essential oil were identified by the GC-FID/MS analysis as previously reported by the Department of Agricultural Biochemistry and Essential Oils, Institute of Natural Products Chemistry - Vietnam Academy of Science and Technology (VAST) [11]. The GC analysis was performed using an HP 7890A Plus Agilent Technologies gas aided by an FID, and coupled with an HP-5MS column (60 m × 0.25 mm i.d., s 0.25 µm film thickness). The temperature of the oven was kept at 55°C for 3 min and then operated at 240°C at a rate of 3°C/min. The corresponding temperatures of the injector and detector were 260 and 270°C. Helium (1.0 mL/min) was used as a carrier gas. The injection volume of the oil sample was 1.0 µL.

The GC-MS analysis has been carried out using the same HP-5MS column, and coupled with a mass HP 5973 MSD spectrometer. The temperature of the oven was 55-240°C at a rate of 3°C/min. He (1 mL/min), split ratio 9:1, ionization energy of 70 eV, emission current of 40 mA, sampling rate of 1.0 scan/s, and mass scanning of 50–450 amu was established. An EI source at 240 ºC was used, while the interface temperature was 270°C. The retention index (RI) of each compound was calculated by a comparison between the experimental result and a homologous series of *n*-alkanes C7–C40. Each chemical was identified by comparing its RI value to that in literature [11]. Based on the NIST 20 and WILEY 12 Libraries, the MS fragmentations were compared to those of other essential oils with

known compositions. In contracst, the peak area in the GC-FID was used to calculate the relative percentage (%) without any correction factor.

2.4. Antimicrobial assay

The antimicrobial activity of essential oil was evaluated against eight strains [12], including Gram-positive [*Staphylococcus aureus* ATCC 25923, and *Bacillus cereus* ATCC 14579], Gram-negative [*Escherichia coli* ATCC 25922, and *Pseudomonas aeruginosa* ATCC 27853], filamentous fungi [*Aspergillus niger* ATCC 1015 and *Fusarium oxysporum* ATCC 46591], and yeasts [*Candida albicans* ATCC 10231 and *Saccharomyces cerevisiae* ATCC 4098] at the Bioactives department, Institute of Natural Products Chemistry - VAST. All strains were acquired from American Type Culture Collection (ATCC). Each strain was sub-cultured for one day on either Tryptic soil agar at 37°C (bacteria) or potato dextrose agar at 35°C (yeasts). The assays were performed in Mueller-Hinton broth (bacteria) and RPMI 1640 culture medium (yeasts). The inoculum was adjusted to 5×10^5 CFU/mL for bacteria and 2.5×10^3 CFU/mL for yeasts.

The tested oil sample was dissolved in dimethyl sulfoxide (DMSO) (5%) and diluted in a culture medium to achieve concentrations from 400 µg/mL to 4 µg/mL. Inoculated wells with and without antimicrobial agents were assayed to control the adequacy of the broth for microorganism growth and medium sterility, respectively. The final concentration of DMSO (5%) was also evaluated. The microplates were incubated at either 37°C (bacteria) or 35°C (yeasts) for 24 h. After that, resazurin (aqueous solution 0.02%) was added to the microplates to indicate the microorganism viability. Before that, aliquots were aseptically removed from each well, plated onto an adequate culture medium, and incubated as previously described. The lowest concentration that allowed no discernible growth of the tested microorganism was identified as the minimum inhibitory concentration (MIC). Streptomycin and tetracycline served as the standards for Gram-positive and -negative bacteria, respectively, while nystatin was used as the standard for fungi and yeasts. DMSO at 5% was used as a negative control. Each experiment was performed in triplicates.

3. Results and discussion

Hydro-distillation of the fresh leaves of Vietnamese *C. subpenninervium* resulted in a yellowish oil (0.45% yield, w/w). A total of 32 compounds were identified, which accounted for 99.92% (Table 1 and Figure 1). Non-terpenic compounds (57.07%), monoterpene hydrocarbons (23.03%), and oxygenated monoterpenes (13.34%) were the main chemical classes, followed by sesquiterpene hydrocarbons (3.12%), diterpene hydrocarbons (1.89%), and oxygenated sesquiterpenes (1.47%).

E-Cinnamaldehyde (38.56%), linalool (12.20%), benzyl benzoate (11.33%), and α-pinene (8.12%) were the principal compounds in this essential oil. Some compounds were found to reach greater than 1%, including limonene (4.83%), β-pinene (4.19%), 1,8-cineole (4.14%), *E*-cinnamyl acetate (4.13%), *E*-caryophyllene (2.64%), kaurene (1.89%), benzaldehyde (1.44%), and benzenepropanal (1.04%). The remaining compounds are represented as trace amounts (less than 1%).

The studies on chemical compositions of *Cinnamomum* essential oils have always been considered to be a topic of interest for a long time. There have been plenty of previous reports on essential oils of other *Cinnamomum* species. For instance, the major compounds in the leaf essential oil of Chinese *C. osmophloeum* were 1,8-cineole (17.0%), spathulenol (15.7%), santolina triene (14.2%), and caryophyllene oxide (11.2%) [13].

Table 1. Chemical constituents in essential oil of *C. subpenninervium* leaves.

No.	R_t	RI_E	RI_L	Constituents	Leaves (%)	Classification
1	9.87	940	932	***α*-Pinene**	**8.12**	MH
2	10.35	956	946	Camphene	0.37	MH
3	10.69	967	952	Benzaldehyde	1.44	NT
4	11.06	979	969	Sabinene	0.57	MH
5	11.23	985	974	*β*-pinene	4.19	MH
6	11.48	993	988	Myrcene	0.43	MH
7	12.73	1030	1022	*P*-cymene	0.23	MH
8	12.88	1035	1024	Limonene	4.83	MH
9	13.00	1038	1026	1,8-cineole	4.14	MH
10	13.87	1064	1054	*γ*-terpinene	0.14	MH
11	15.28	1105	1095	**Linalool**	**12.20**	OM
12	15.43	1109	1109	Hotrienol	0.10	OM
13	17.07	1156	1141	Camphor	0.15	OM
14	17.59	1170	1162	Benzenepropanal	1.04	NT
15	17.69	1173	1168	Pinocarvone	0.15	OM
16	18.18	1187	1174	Terpinen-4-ol	0.38	OM
17	18.63	1200	1186	*α*-terpineol	0.20	OM
18	18.91	1208	1201	Decanal	0.11	NT
19	19.64	1230	1217	*Z*-cinnamaldehyde	0.29	NT
20	20.64	1259	1249	Geraniol	0.16	OM
21	21.57	1285	1267	***E*-cinnamaldehyde**	**38.56**	NT
22	22.64	1317	1317	*E*-cinamyl alcohol	0.17	NT
23	25.03	1389	1374	*α*-copaene	0.16	SH
24	26.54	1437	1417	*E*-caryophyllene	2.64	SH
25	27.07	1453	1443	*E*-cinnamyl acetate	4.13	NT
26	27.63	1471	1467	*α*-humulene	0.21	SH
27	29.63	1537	1522	*δ*-cadinene	0.11	SH
28	30.66	1571	1561	*E*-nerolidol	0.69	OS
29	31.66	1605	1582	Caryophyllene oxide	0.59	OS
30	32.29	1627	1600	Cedrol	0.19	OS
31	36.63	1784	1759	**Benzyl benzoate**	**11.33**	NT
32	43.90	2078	2042	Kaurene	1.89	DH
Total					**99.92**	
Monoterpene hydrocarbons (MH)					23.03	
Oxygenated monoterpenes (OM)					13.34	
Sesquiterpene hydrocarbons (SH)					3.12	
Oxygenated sesquiterpenes (OS)					1.47	
Diterpene hydrocarbons (DH)					1.89	
Non-terpenic compounds (NT)					57.07	

R_t: *Retention time,* **RI_E**: *Retention indices relative to n-alkanes (C_7-C_{30}) on HP-5 MS column,* **RI_L**: *Retention indices from Adam's book* [14] *and the NIST standard database* [15].

By the GC-MS analysis, essential oil from *C. zeylanicum* barks, collected from Turkey, was reported to include the major agents *E*-cinnamaldehyde (68.95%), benzaldehyde (9.94%) and *E*-cinnamyl acetate (7.44%) [16]. *E*-cinnamaldehyde, *trans*-cinnamic acid, cinnamyl acetate, and benzaldehyde were also among the primary agents of essential oil from Vietnamese *C. verum* aerial parts [8]. Antibacterial activity has been studied from *Cinnamomum balansae* [17] and *Cinnamomum cassia* [18] plants grown in Vietnam.

The obtained essential oil has further been subjected to antimicrobial experiments and the result is outlined in Table 2. *C. subpenninervium* leaf essential oil exhibited inhibitions against the growth of the Gram-positive bacteria *B. subtilis* and *S. aureus* with the MIC values of 128-256 µg/mL, as compared with those of the positive control streptomycin (MIC 6.25-12.5 µg/mL). However, this

essential oil did not show activity to the Gram-negative bacteria *E. coli* and *P. aeruginosa* (inactive, MIC > 400 µg/mL). In contrast to the result regarding the fungus *A. niger* (inactive), *C. subpenninervium* leaf essential oil show strong activity against the fungus *F. oxysporum* with the MIC value of 64 µg/mL. In the final case, the studied essential oil showed antimicrobial activity against the yeasts *C. albicans* and *S. cerevisiae* with the same MIC value of 256 µg/mL.

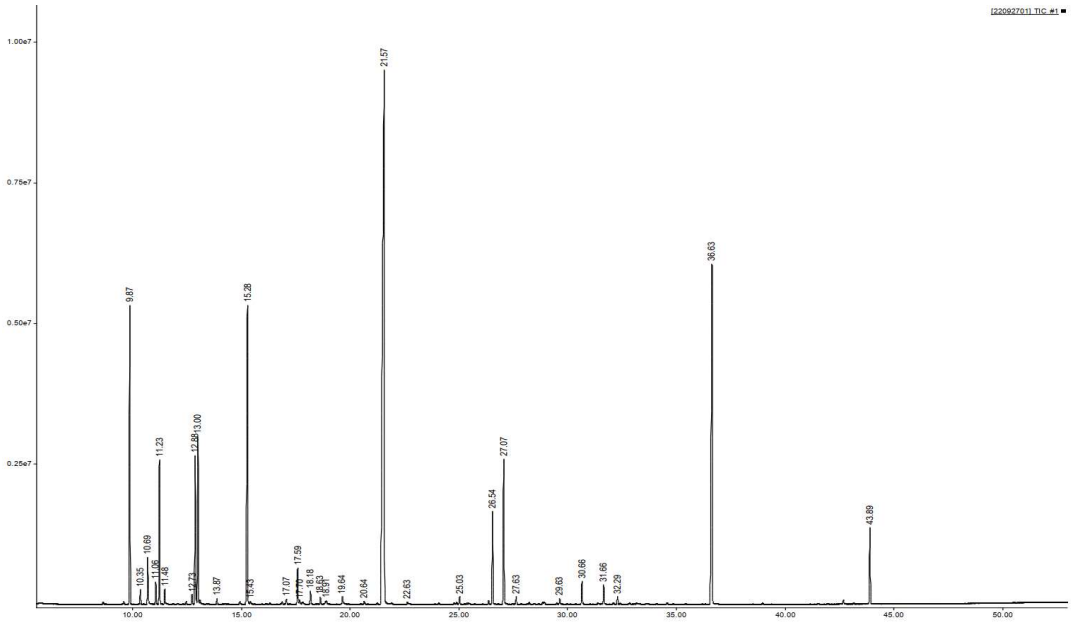

Figure 1. GC-FID/MS spectrum of *C. subpenninervium* leaf essential oil.

Table 2. Antimicrobial activity of the studied essential oil.

Microbial strains		**Minimum Inhibitory concentration (MIC: µg/mL)**			
		Essential oil	**Streptomycin**	**Tetracycline**	**Nystatin**
Gram (+)	*B. subtilis*	256	6.25		
	S. aureus	128	12.5		
Gram (-)	*E. coli*	> 400		6.25	
	P. aeruginosa	> 400		12.5	
Fungi	*A. niger*	> 400			25.0
	F. oxysporum	64			12.5
Yeasts	*C. albicans*	256			12.5
	S. cerevisiae	256			6.25

Previously, much attention has been paid to antimicrobial activities of *Cinnamomum* essential oils. *C. Zeylani cum* bark essential oil containing *E*-cinnamaldehyde (71.50%) exerted the MIC value of less than 6.25 mg/mL against *B. cereus*, *S. aureus*, *E. coli*, and *P. aeruginosa* [19]. The leaf essential oil of *C. osmophloeum* possessed the same MIC value of 250 µg/mL against *S. aureus*, *E. coli*, and *P. aeruginosa* [20]. Essential oil of Vietnamese *C. tonkinense* leaves rich in *β*-phellandrene (23.1%) and linalool (32.2%) exhibited good antimicrobial activity with the MIC value of 32 µg/mL against *Enterococcus faecalis* and *Candida albicans* [21]. Our current result, once again, confirms the potential in the use of *Cinnamomum* essential oils for infectious diseases.

4. Conclusion

For the first time, the current research reports the chemical compositions of essential oil from Vietnamese *C. subpenninervium* leaves. By the GC-MS/FID analysis, non-terpenic compounds (57.07%), monoterpene hydrocarbons (23.03%), and oxygenated monoterpenes (13.34%) were the main chemical classes. *E*-cinnamaldehyde (38.56%), linalool (12.20%), benzyl benzoate (11.33%), and α-pinene (8.12%) were the principal compounds in this essential oil. The obtained results showed antimicrobial activity against *B. subtilis, S. aureus*, *F. oxysporum*, *C. albicans*, and *S. cerevisiae* with the MIC value of 64-256 µg/mL.

References

[1] L. C. Son, D. N. Dai, T. H. Thai, D. D. Huyen, T. D. Thang, and I. A. Ogunwande, "The leaf essential oils of four Vietnamese species of *Cinnamomum* (Lauraceae)," *J. Essent. Oil Res.*, vol. 25, no. 4, pp. 267–271, Aug. 2013, doi: 10.1080/10412905.2013.775673.

[2] K. D. Nguyen, Ed. *Flora of Vietnam vol 20: Lauraceae Juss*. Hanoi: Publishing House for Science and Technology, 2017.

[3] Ministry of Health, *Pharmacopoeia Vietnamica*. Hanoi: Medicinal Publishing House, 2009, p. 962.

[4] N. X. Dung, P. Van Khien, H. T. Chien, and P. A. Leclercq, "The essential oil of *Cinnamomum camphora* (L.) Sieb. var. linaloolifera from Vietnam," *J. Essent. Oil Res*., vol. 5, no. 4, pp. 451–453, Jul. 1993, doi: 10.1080/10412905.1993.9698258.

[5] N. X. Dung, L. D. Moi, N. D. Hung, and P. A. Leclercq, "Constituents of the essential oils of *Cinnamomum parthenoxylon* (Jack) Nees from Vietnam," *J. Essent. Oil Res*., vol. 7, no. 1, pp. 53–56, Jan. 1995, doi: 10.1080/10412905.1995.9698462.

[6] N. X. Dung, P. Van Khien, N. T. Q. Vinh, H. T. Le, L. J. M. Van De Ven, and P. A. Leclercq, "2-Methylene-3-buten-1-yl benzoate: The major constituent of the leaf, stem and wood oils from "Re Gúng" a Vietnamese *Cinnamomum* sp. Tree," *J. Essent. Oil Res*., vol. 9, no. 1, pp. 57–61, Jan. 1997, doi: 10.1080/10412905.1997.9700715.

[7] N. X. Dung, N. Sothy, V. N. Lo, and L. Pa, "Chemical composition of the wood oil of *Cinnamomum albiflorum* nees from Kampuchea," *J. Essent. Oil Res.*, vol. 6, no. 2, pp. 201–202, Mar. 1994, doi: 10.1080/10412905.1994.9698357.

[8] H. H. Phu, K. P. Van, T. H. Tran, and D. T. N. Pham, "Extraction, chemical compositions and biological activities of essential oils of *Cinnamomum verum* cultivated in Vietnam," *Processes*, vol. 10, no. 9, p. 1713, Aug. 2022, doi: 10.3390/pr10091713.

[9] Selina Wamucii, "Plants » Selina Wamucii," *Selina Wamucii*, Jul. 26, 2023. https://www.selinawamucii.com/plants/lauraceae/cinnamomum-subpenninervium/ (accessed Mar. 15, 2024).

[10] "Cinnamomum subpenninervium Kosterm. ex H. H. Pham | Plants of the World Online | Kew Science," *Plants of the World Online*. https://powo.science.kew.org/taxon/urn:lsid:ipni.org:names:3272262-4 (accessed Apr. 16, 2024).

[11] D. Q. Huan *et al.,* "The leaf oils of *Beilschmiedia tonkinensis* (Lecomte) Ridl. and *Lindera gracilipes* H. W. Li: chemical composition, cytotoxicity, antimicrobial activity, and docking study," *Nat. Prod. Commun.*, vol. 19, no. 1, p. 1934578X231224995, Jan. 2024, doi: 10.1177/1934578X231224995.

[12] L. T. Huong *et al.,* **"**Essential oils of the ginger plants *Meistera caudata* and *Conamomum Vietnamense*: chemical compositions, antimicrobial, and mosquito larvicidal activities," *Z. Naturforsch C. J. Biosci*, vol. 78, no. 9-10, pp. 337–344, May 2023, doi: 10.1515/znc-2022-0244.

[13] L. K. Chao, K.-F. Hua, H.-Y. Hsu, S.-S. Cheng, J.-Y. Liu, and S.-T. Chang, "Study on the antiinflammatory activity of essential oil from leaves of *Cinnamomum osmophloeum*," *J. Agric. Food Chem.,* vol. 53, no. 18, pp. 7274–72788, Aug. 2005, doi: 10.1021/jf051151u.

[14] R. P. Adams, *Identification of essential oil components by gas chromatography/mass spectrometry*. 4th ed. Allured Publishing Corporation, 2007.

[15] "Chemical name search." https://webbook.nist.gov/chemistry/name-ser (accessed Mar. 15, 2024).

[16] M. Unlu, E. Ergene, G. V. Unlu, H. S. Zeytinoglu, and N. Vural, "Composition, antimicrobial activity and

in vitro cytotoxicity of essential oil from *Cinnamomum zeylanicum* Blume (Lauraceae)," *Food Chem. Toxicol.*, vol. 48, no. 11, pp. 3274–3280, Nov. 2010, doi: 10.1016/j.fct.2010.09.001.

[17] L. V. Quang, H. V. Thang, and N. Vien, "Chemical composition and antimicrobial activity of *Cinnamomum balansae* growing in Vietnam," *Chem. Nat. Compd.,* vol. 58, no. 6, pp. 1161–1163, Nov. 2022, doi: 10.1007/s10600-022-03894-5.

[18] V.-D. Le, V.-T. Tran, V.-S. Dang, D.-T. Nguyen, C.-H. Dang, and T.-D. Nguyen, "Physicochemical characterizations, antimicrobial activity and non-isothermal decomposition kinetics of *Cinnamomum cassia* essential oils," *J. Essent. Oil Res.*, vol. 32, no. 2, pp. 158–168, Dec. 2020, doi: 10.1080/10412905.2019.1700834.

[19] B. A. Behbahani, F. Falah, F. L. Arab, M. Vasiee, and F. T. Yazdi, "Chemical composition and antioxidant, antimicrobial, and antiproliferative activities of *Cinnamomum zeylanicum* bark essential oil," *Evid. -based Complement. Altern. Med.*, vol. 2020, pp. 1–8, Apr. 2020, doi: 10.1155/2020/5190603.

[20] S. T. Chang, P. F. Chen, and S. C. Chang, "Antibacterial activity of leaf essential oils and their constituents from *Cinnamomum osmophloeum*," *J. Ethnopharmacol.*, vol. 77, no. 1, pp. 123–127, Sep. 2001, doi: 10.1016/S0378-8741(01)00273-2.

[21] D. N. Dai *et al.,* "Chemical compositions, mosquito larvicidal and antimicrobial activities of essential oils from five species of *Cinnamomum* growing wild in North Central Vietnam," *Molecules*, vol. 25, no. 6, p. 1303, Mar. 2020, doi: 10.3390/molecules25061303.