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THE LEAF ESSENTIAL OILS OF *Neolitsea vuquangensis*: A RICH RESOURCE OF β-(*E*)-OCIMENE

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The genus *Neolitsea* contains about 100 species of evergreen trees or small shrubs in the family Lauraceae, which is mainly distributed in tropical Asia [1, 2]. It is recognized that plants of this genus are a rich resource of essential oils, in which terpene derivatives are the main chemical class. The steam volatile constituents of *N. pallens* leaf included furanogermenone (30.6%), β-caryophyllene (19.3%), and germacrene D (12.7%) [3]. Su et al. indicated that the anticancer activity of *N. variabillima* leaf oil is due to the role of the main components *trans*-β-ocimene (13.4%), α-cadinol (10.5%), and terpinen-4-ol (9.3%) [4]. *N. sericea* leaf oil with a high amount of sericenine (32.3%) showed an anti-inflammatory effect in a model of lipopolysaccharide-stimulated Raw 264.7 macrophage cells by inhibiting the NF-κB/MAPK signaling pathway [5].

Neolitsea vuquangensis Mitsuyuki & Yahara was identified as a new species in 2018 [6]. This species is a medium tree with scaly terminal buds, scales with dense golden yellow hairs, light golden yellow young branchlets, and alternate leaves [6]. *N. vuquangensis* is now available in Vietnam [6]. For the first time to our knowledge, the current paper reports chemical compositions in its leaf oils, and the application of these oils in the antimicrobial assay.

N. vuquangensis leaves were collected from both Pu Huong Nature Reserve and Pu Hoat Nature Reserve in Nghean, Vietnam, in 03/2022. Plant materials were namely identified by our co-author Dr. Do Ngoc Dai. Two voucher specimens 8941-L (Pu Huong) and 8951-L (Pu Hoat) were deposited in the Faculty of Chemistry, HPU2. The fresh leaves of each sample (1.5 kg) were hydro-distilled for 2.5 h using a Clevenger apparatus. The distilled oils were collected with yellow colors, and were then dried over anhydrous Na_2SO_4 before being kept at 5°C until analysis. The extraction yields were 0.3% for the 8941-L sample and 0.45% for the 8951-L sample.

The essential oils were analyzed using the GC/MS-FID apparatus, an Agilent 7890A gas chromatograph with an Agilent 5975C mass detector fitted with an HP-5MS fused silica column (60 m × 0.25 mm with a 0.25-m fixed phase). The establishments for the analysis were performed as previously described [7–10]. The chemical compounds were identified by comparing the retention index (RI) relative to *n*-alkanes (C_7–C_{30}) with those in the literature, and by matching the mass spectra with the NIST 21, MassFinder 4.0, and Wiley GC-MS library [7–10]. The relative percentage of each component was calculated as the GC peak area relative to the total peak area.

The extraction of Pu Huong *N. vuquangensis* leaves 8941-L gave a yellow oil with 13 identified compounds, which accounted for 99.9% (Table 1).

This sample was dominated by monoterpene hydrocarbons (98.7%), whereas the remaining chemical classes included oxygenated monoterpenes (0.4%), sesquiterpene hydrocarbons (0.6%), and oxygenated sesquiterpenes (0.2%). Remarkably, β-(*E*)-ocimene (92.5%) appeared as the representative for this oil sample. Some compounds were obtained with percentages of greater than 1.0%, including limonene (1.7%) and β-(*Z*)-ocimene (3.5%). In the same manner, all 14 identified compounds in the 8951-L oil sample belong to terpene derivatives, which represented 99.7%.

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TABLE 1. Chemical Composition of Essential Oils from *N. vuquangensis* Leaves, %

Compound	RI	8941-L	8951-L	Compound	RI	8941-L	8951-L
α-Pinene	939	0.2	0.2	Germacrene D	1498	–	0.2
Myrcene	991	0.5	0.4	β-Selinene	1505	0.2	0.5
3-δ-Carene	1016	0.3	–	α-(*E*,*E*)-Farnesene	1512	–	0.1
Limonene	1034	1.7	–	α-Selinene	1513	0.2	0.4
β-(*Z*)-Ocimene	1038	3.5	2.7	δ-Cadinene	1537	–	0.1
β-(*E*)-Ocimene	1052	92.5	94.2	Spathulenol	1597	0.2	0.1
Rosefuran	1098	0.2	0.1	Total		99.9	99.7
Linalool	1101	0.2	0.2	Monoterpene hydrocarbons		98.7	97.5
cis-β-Elemene	1403	–	0.4	Oxygenated monoterpenes		0.4	0.3
(*E*)-Caryophyllene	1437	0.1	0.1	Sesquiterpene hydrocarbons		0.6	1.8
Aromadendrene	1457	0.1	–	Oxygenated hydrocarbons		0.2	0.1

RI: Retention index relative to *n*-alkanes (C_7–C_{30}) on an HP-5MS column.

TABLE 2. Antimicrobial Activity of Essential Oils from *N. vuquangensis* Leaves, µg/mL

Microbial strains	IC_{50}/MIC	8941-L	8951-L	Streptomycin
Enterococcus faecalis ATCC299212	IC_{50}	20.42	19.45	50.34
	MIC	64	64	256
Staphylococcus aureus ATCC25923	IC_{50}	25.12	20.45	45.24
	MIC	64	64	256
Bacillus cereus ATCC14579	IC_{50}	18.54	45.34	20.45
	MIC	64	128	128
Escherichia coli ATCC25922	IC_{50}	-	-	9.45
	MIC	-	-	32
Pseudomonas aeruginosa ATCC27853	IC_{50}	-	-	41.46
	MIC	-	-	256
Salmonella enterica ATCC13076	IC_{50}	-	18.78	45.67
	MIC	-	64	128
Candida albicans ATCC10231	IC_{50}	10.34	23.12	
	MIC	32	64	

Monoterpene hydrocarbons reached up to 97.5%, whereas the remaining classes ranged from 0.1 to 1.8% (Table 1). In this oil, β-(*E*)-ocimene was still the principal compound with 94.2%, followed by its isomer β-(*Z*)-ocimene (2.7%). The remaining compounds were obtained with minor amounts of less than 0.5%.

3-δ-Carene, limonene, and aromadendrene were only detected in the 8941-L sample, whereas *cis*-β-elemene, germacrene D, α-(*E*,*E*)-farnesene, and δ-cadinene were only observed in the 8951-L sample. In another approach, the current research matches well with previous reports as β-(*E*)-ocimene can be seen as the principal compound of various *Neolitsea* plants. For instance, this compound achieved from 49.3 to 84.7% in essential oils of *N. sericea* var. *aurata* leaf, twig, and fruit [11]. β-(*E*)-Ocimene (85.6%) was also a characteristic compound of *N. polycarpa* leaf oil [12]. Collectively, from a chemotaxonomic view, monoterpene hydrocarbon β-(*E*)-ocimene could be seen to be a good agent to confirm a close relationship among *Neolitsea* plants.

Antimicrobial activity of *N. vuquangensis* essential oils was performed against Gram-negative and -positive bacteria, fungi, and yeasts using the micro-dilution method (Table 2) [7, 8, 13]. As shown in Table 2, both oil samples were generally comparable with the positive control streptomycin against three Gram-positive bacteria, *Enterococcus faecalis*, *Staphylococcus aureus*, and *Bacillus cereus*, with IC_{50} = 18.54–45.34 µg/mL/MIC = 64–128 µg/mL. Regarding Gram-negative bacteria, only the 8951-L sample showed activity against *Salmonella enterica*, with IC_{50} = 18.78 µg/mL/MIC = 64 µg/mL. The different results between Gram-positive and Gram-negative bacteria can be explained by the fact that the cell wall of Gram-negative bacteria is a complex envelope that is structurally formulated by the cytoplasmic membrane, periplasm, and

outer membrane [14]. The 8941-L oil sample was also associated with IC_{50} = 10.34 µg/mL/MIC = 32 µg/mL against fungus *Candida albican*s, compared with that of the 8951-L sample (IC_{50} = 23.12 µg/mL/MIC = 64 µg/mL), and the positive control cycloheximide (IC_{50} = 10.46 µg/mL/MIC = 32 µg/mL). Our current result is in agreement with similar reports, as Vietnamese *Neolitsea* essential oils could be potential agents for antimicrobial treatments. For instance, *N. ellipsoidea* leaf essential oil, collected from Vu Quang National Park-Vietnam, also demonstrated notable antibacterial activity against *E. faecalis* and *B. cereus* with the same MIC value of 16 µg/mL [15].

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