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CHEMICAL COMPOSITION AND ANTIMICROBIAL ACTIVITY OF THE ESSENTIAL OIL OF THE LEAVES OF *Machilus cochinchinensis* FROM VIETNAM

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Machilus (family Lauraceae) comprises about 100 species in tropical and subtropical south and Southeast Asia [1]. Twelve species including *Machilus cochinchinensis* Lecomte, syn. *Persea cochinchinensis* (Lecomte) Kosterm. (local name: Khao nam bo) are recorded from Vietnam [2]. The lack of information on the essential oil composition and pharmacological effects of *M. cochinchinensis* prompted this research. Therefore, this paper reports, to our knowledge for the first time, the chemical composition and antimicrobial activity of essential oil hydrodistilled from the leaves of *M. cochinchinensis*.

The chemical constituents of essential oils from *Machilus* species of Vietnamese origin such as *M. velutina* [3], *M. grandifolia* [4], *M. balansae* [4], and *M. japonica* [5] have been reported. In addition, reports on the composition and biological potentials of *M. japonica* [6], *M. zuihoensis* [7], *M. pseudolongifolia* [8], *M. mushaensis* [9], *M. obovatifolia* [10], *M. kusanoi* [11], and *M. philippinensis* shown [12] from Taiwan are available in the literature. Monoterpene hydrocarbons were the main compounds in *M. velutina* [3] and *M. japonica* [5, 6], whereas sesquiterpene hydrocarbons were found in *M. grandifolia* [4]. However, oxygenated sesquiterpenes were the dominant compounds in *M. balansae* [4] and *M. pseudolongifolia* [8]. A mixture of monoterpene and sesquiterpene hydrocarbons occurred in *M. obovatifolia* [10] and *M. philippinensis* [12]. Fatty acids and oxygenated sesquiterpenes were in abundance in *M. zuihoensis* [7], whereas *M. mushaensis* [9] contained aliphatic aldehyde and oxygenated sesquiterpenes. The composition of *M. kusanoi* [11] was a complex mixture of monoterpene, sesquiterpenes, and fatty acids. The leaf oil of *M. grandifolia* exhibited larvicidal action against *Aedes aegypti*, *A. albopictus*, and *Culex quinquefasciatus*, whereas *M. balansae* demonstrated moderate antimicrobial activity [4]. The oil of *M. japonica* [5] from Taiwan showed antioxidant activity toward DPPH with IC_{50} of 51.8 μg/mL, as well as antimicrobial action against *Bacillus cereus* ATCC11778 (MIC 16.12 μg/mL), *Staphylococcus aureus* ATCC6538P, *S. epidermis* ATCC12228, and *Candida albicans* ATCC10231, with MIC values of 31.25 μg/mL [6]. *M. zuihoensis* [7], *M. pseudolongifolia* [8], *M. mushaensis* [9], *M. obovatifolia* [10], and *M. kusanoi* [11] also showed moderate action toward the organisms mentioned. The essential oil of *M. mushaensis* also displayed anticancer activity [9]. The leaf oils of *M. pseudolongifolia* [8], *M. kusanoi* [11], and *M. philippinensis* showed [12] antiwood-decay fungal properties.

The leaves of *M. cochinchinensis* were harvested from Pu Hoat Nature Reserve: Nam Giai Commune (GPS 19°41′38″N, 104°49′31″E), Vietnam, at an elevation of 662 m. Plant samples were harvested on August 2020. The leaf samples were identified and authenticated by Dr. Le Thi Huong. A voucher specimen number LTH 833 was deposited in the plant specimen room, Vinh University, Vietnam. Two kilograms of the leaf sample were subjected to hydrodistillation using a Clevenger-type apparatus as described previously [3, 4, 13–15].

The instrumental analysis of the essential oil was performed by using gas chromatography (GC) on an Agilent Technologies HP 7890 Plus Gas chromatograph equipped with an FID and fitted with HP-5MS column (30 m × 0.25 mm, film thickness 0.25 μm, Agilent Technology). The analytical conditions were as described previously [3, 4, 13–15].

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TABLE 1. Chemical Constituents of Essential Oil from the Leaf of *M. cochinchinensis*

Compound [a]	RI	Concentration, %	Compound [a]	RI	Concentration, %
α-Thujene	930	0.4	β-Chamigrene	1489	3.3
α-Pinene	939	3.3	β-Selinene	1503	7.3
Sabinene	978	11.8	α-Selinene	1511	4.0
β-Pinene	984	4.4	Bicyclogermacrene	1513	10.0
Myrcene	992	1.0	γ-Cadinene	1528	0.2
α-Terpinene	1021	0.6	δ-Cadinene	1536	0.4
o-Cymene	1029	0.1	Caryophyllene alcohol	1589	0.2
Limonene	1034	1.2	Caryophyllene oxide	1602	0.2
β-Phellandrene	1035	4.8	Guaiol	1612	1.3
(Z)-β-Ocimene	1037	1.6	γ-Eudesmol	1649	1.0
(*E*)-β-Ocimene	1049	22.6	*epi*-α-Cadinol	1656	0.3
γ-Terpinene	1063	1.0	β-Eudesmol	1656	1.0
Terpinolene	1093	0.3	α-Eudesmol	1672	0.9
Linalool	1101	0.5	*neo*-Intermedeol	1674	1.0
Terpinen-4-ol	1185	0.8	Bulnesol	1684	0.2
α-Terpineol	1197	0.3	Total		95.2
Decanal	1206	0.5	Monoterpene hydrocarbons		53.1
δ-Elemene	1347	0.2	Monoterpene oxygenated		1.6
β-Caryophyllene	1437	6.6	Sesquiterpene hydrocarbons		34.4
Aromadendrene	1445	0.5	Sesquiterpene oxygenated		6.1
α-Humulene	1470	1.8	Non-terpenes		0.5
α-Amorphene	1470	0.1	Yield (v/w)		0.32

[a] Elution order on HP-5MS column.

An Agilent Technologies HP 7890N Plus Chromatograph fitted with capillary HP-5 MS column (30 m × 0.25 mm, film thickness 0.25 μm) and interfaced with a mass spectrometer HP 5973 MSD was used for this gas chromatography-mass spectrometry (GC-MS) experiment, under the same conditions as those used for gas chromatography analysis as described above. The MS conditions were as follows: ionization voltage 70 eV; emission current 40 mA; acquisitions scan mass range of 35–350 amu at a sampling rate of 1.0 scan/s. The identification of constituents from the GC-MS spectra of *M. cochinchinensis* was performed based on retention indices (RIs) determined with reference to a homologous series of *n*-alkanes (C_4–C_{40}), under identical experimental conditions. The mass spectral fragmentation patterns were checked with those of other essential oils of known composition [16].

The minimum inhibitory concentration (MIC) and median inhibitory concentration (IC_{50}) values were measured by the microdilution broth susceptibility assay [13–15]. Stock solutions of the oil were prepared in DMSO. Dilution series (2^{14}, 2^{13}, 2^{12}, 2^{11}, 2^{10}, 2^9, 2^7, 2^5, 2^3, and 2^1 μg/mL) were prepared in sterile distilled water inside the micro-test tubes, from where they were transferred separately to 96-well microtiter plates. Bacteria grown in double-strength Mueller–Hinton broth or double-strength tryptic soy broth, and fungi sustained in double-strength Sabouraud dextrose broth, were standardized to 5×10^5 and 1×10^3 CFU/mL, respectively. DMSO was used as a negative control. Streptomycin was used as the antibacterial standard while nystatin and cycloheximide were used as antifungal standards. All experiments were performed in triplicate. After incubation at 37°C for 24 h, the MIC values were determined as the lowest concentration of essential oils of *M. cochinchinensis*, which completely inhibited the growth of the microorganisms. The IC_{50} values were determined by the percentage of microorganism-inhibited growth based on the turbidity measurement data of the EPOCH2C spectrophotometer (BioTeK Instruments, Inc. Highland Park Winooski, VT, USA) and Rawdata computer software (Belgium).

A light-yellow essential oil (yield, 0.32%) was obtained from the hydrodistillation of the *M. cochinchinensis* leaf. The main classes of compounds identified in the essential oil were monoterpene hydrocarbons (53.1%) and sesquiterpene hydrocarbons (34.4%). The oxygenated monoterpenes and sesquiterpenes were present in lower quantities (1.6% and 6.1%, respectively) as seen in Table 1. The main constituents of *M. cochinchinensis* leaf essential oil were (*E*)-β-ocimene (22.6%), sabinene (11.8%), bicyclogermacrene (10.0%), β-selinene (7.3%), β-caryophyllene (96.6%), β-phellandrene (4.8%), and β-pinene (4.4%). To our knowledge, this is the first report on the essential oil constituents of *M. cochinchinensis*.

TABLE 2. Antimicrobial Activity of the Leaf Oil of *M. cochinchinensis*

Microorganism	MIC, μg/mL	IC_{50}, μg/mL
Enterococcus faecalis ATCC299212	15.98 ± 1.10	32.0 ± 0.11
Staphylococcus aureus ATCC25923	100.22 ± 1.50	256.0 ± 1.21
Bacillus cereus ATCC14579	88.78 ± 0.50	128.0 ± 0.50
Pseudomonas aeruginosa ATCC27853	56.77 ± 0.55	128.0 ± 0.55
Candida albicans ATCC10231	128.35 ± 1.50	256.0 ± 1.00

TABLE 3. Comparison of the MIC Values (μg/mL) of the Leaf Oil of *M. cochinchinensis* and Some *Machilus* Oil Samples against the Microbial

Essential oils	*Enterococcus faecalis*	*Bacillus cereus*	*Staphylococcus aureus*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Candida albicans*	References
M. cochinchinensis	15.98	88.78	100.22	–	56.77	128.35	This study
M. japonica	N.a.	16.12	31.25	125	125	31.25	6
M. zuihoensis	N.a.	125	125	500	500	125	7
M. pseudolongifolia	N.a.	250	125	750	750	250	8
M. mushaensis	N.a.	500	375	1000	1000	500	9
M. kusanoi	N.a.	250	125	500	500	250	11

–: No activity; N.a.: data not available.

As expected, terpene compounds constitute the bulk of the contents of the essential oil, in accordance with data reported for previously analyzed *Machilus* oil samples from Vietnam [3–5] and Taiwan [6–12]. The chemical identities of the terpene compounds present in the *Machilus* essential oils differed from one species to another. This is an indication of chemical variability in their compositional pattern. The compositional pattern of *M. cochinchinensis* was similar to that of *M. obovatifolia* [10] and *M. philippinensis* [12] owing to high contents of monoterpene and sesquiterpene hydrocarbons.

The essential oil of the leaves of *M. cochinchinensis* displayed varying antibacterial activity against gram-positive and gram-negative microorganisms; and anticandidal activity against the yeast (Table 2). The leaf oil exhibited the highest antimicrobial activity against *Enterococcus faecalis* ATCC299212, with a MIC value of 15.98 μg/mL. The IC_{50} value obtained was 32.0 μg/mL. The essential oil also displayed good activity toward *Pseudomonas aeruginosa* ATCC27853 (MIC, 56.77 μg/mL) and *Bacillus cereus* ATCC14579 (MIC, 88.78 μg/mL). The corresponding IC_{50} value was 128.0 μg/mL. Potential antibacterial and anticandidal activities were also shown by the essential oil against *Staphylococcus aureus* ATCC25923 (MIC, 100.22 μg/mL) and *Candida albicans* ATCC10231 (MIC, 128.35 μg/mL), with IC_{50} values of 256.0 μg/mL. The reference compounds namely streptomycin for Gram-positive bacteria exhibited activity with MIC values within the range 0.5–1.0 μg/mL, whereas cycloheximide used as an antifungal had MIC values within the range 1.2–3.7 μg/mL. Also, nystatin, an anticandidal compound, displayed activity, with MIC values within the range 0.8–2.3 μg/mL. The essential oil of *M. cochinchinensis* can be adjudged to have good antimicrobial activity, with MIC values within the range 500–100 μg/mL, according to the rules guiding the efficacy of antimicrobial substances [14, 15, 17]. Thus, essential oil of *M. cochinchinensis* exhibited good activity against *E. faecalis*, *S. aureus*, *B. cereus*, *P. aeruginosa*, and *C. albicans*.

Comparing the antimicrobial activities of the leaf essential oils of *Machilus* plants (Table 3), the leaf essential oil of *M. cochinchinensis* was superior to that of *M. zuihoensis* [7], *M. pseudolongifolia* [8], *M. mushaensis* [9], and *M. kusanoi* [11], but not that of *M. japonica* [6]. The chemical constituents and antimicrobial activity of the essential oils of *M. cochinchinensis* are being reported to our knowledge for the first time. It is believed that the constituents present in the essential oils studied might have influenced the observed antimicrobial activity of *M. cochinchinensis*. A section of the chemical compounds identified in the essential oils had been previously reported to possess antimicrobial activity [13–15].

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