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CHEMICAL COMPOSITION AND POTENTIAL FOR ANTIMICROBIAL PROPERTIES OF *Magnolia citrata* NOOT. & CHALERMGLIN (MAGNOLIACEAE) ESSENTIAL OILS IN CENTRAL HIGHLANDS OF VIETNAM

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ABSTRACT

Magnolia citrata Noot. & Chalermglin was collected in the Central Highlands of Vietnam and examined for its essential oils' chemical compositions and antimicrobial activities. The essential oils were obtained from the leaves, twigs, and stem barks of *M. citrata* using hydrodistillation, then analyzed using Gas Chromatography-Mass Spectrometry (GC-MS) and evaluated for antimicrobial activities. The yields of essential oils were 0.06, 0.51, and 0.16% from twigs, leaves, and stem barks, respectively. A total of forty-three compounds were identified in essential oils, accounting for 98.0, 98.8, and 98.4%, respectively. The major constituents of the oils from leaves and twigs varied slightly. Accordingly, geranial (23.1%), neral (22.5%), linalool (22.5%), and sylvestrene (6.3%) were found in the leaves, while geranial (30.9%), neral (29.6%), sylvestrene (11.8%), and linalool (6.1%) were found in the twigs. In addition, sylvestrene was the major component comprising 51.8% of the stem bark oils. Other compounds present in high quantities in this oil included safrole (11.6%), geranial (6.9%), sabinene (6.9%), and neral (6.6%). The antimicrobial activities of the essential oil samples were tested against six bacterial strains and one yeast strain for the first time. The results indicated that essential oils from *M. citrata* had strong antimicrobial activities. At the concentration of 64 µg/mL, the essential oils from its three parts effectively inhibited the growth of the Gram-positive bacteria *Enterococcus faecalis*, *Staphylococcus aureus*, and *Bacillus cereus*, as well as the yeast *Candida albicans*. Against the Gram-negative bacteria, including *Escherichia coli*, *Pseudomonas aeruginsa*, and *Salmonella enterica*, the effective concentrations of the oils for inhibition varied from 128 µg/mL to 256 µg/mL.

Keywords: Antimicrobial, Central Highlands of Vietnam, essential oils, GC-MS, *Magnolia citrate*.

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INTRODUCTION

Magnoliaceae is one of the earliest groups of flowering plants in Angiosperms, fossil plants identifiably belonging to the Magnoliaceae family dating back 95 million years. Magnoliaceae comprises ca. 220 species of trees or shrubs distributed in temperate and tropical regions from the Himalayas to East Asia and Southeast Asia and the Americas (Nooteboom, 1993, 2000; Figlar & Nooteboom, 2004; Azuma et al., 2011). The genus *Magnolia* belonging to the family Magnoliaceae includes about 61 species native to Vietnam: 25 species from *M.* section *Michelia*, 16 species from *M.* section *Gwillimia*, 15 species from *M.* section *Manglietia*, 2 species from *M.* section *Kmeria*, 2 species from *M.* subgenus *Gynopodium*, and 1 species from *M.* section *Maingola* (Vu, 2020). The species of the genus *Magnolia* are known for producing secondary metabolites such as essential oils, sesquiterpenes, lignans, neolignans, coumarins, and alkaloids (Schühly et al., 2008). The bark or seed cones of the *Magnolia* tree have been used in traditional herbal medicines in eastern Asia and North America for treating conditions such as gastrointestinal-disorders, thrombotic stroke, allergic disease, typhoid fever, anxiety, nervous disturbance, diaphoretic, antimalarial properties (Lee et al., 2011).

Several species of the *Magnolia* genus have been investigated for their ability to repel insects, as well as their antifungal, antimicrobial, and wound healing capability through the use of their essential oils and volatile components such as *Magnolia liliflora* (Fujita, 1989; Bajpai et al., 2009), *Magnolia grandiflora* (Ahmed & Abdelgaleil, 2005; Luo et al., 2005; Farag & Al-Mahdy, 2013; Ali et al., 2020), *Magnolia salicifolia* (Li et al., 2007), *Magnolia virginiana* (Farag & Al-Mahdy, 2013), *Magnolia kobus* (Azuma et al., 2001), *Magnolia sirindhorniae* (Ghosh et al., 2021) and *Magnolia coco* (Nguyen et al., 2020).

The species *Magnolia citrata*, first described in Thailand (Chalermglin & Nooteboom, 2007), was found growing naturally in both the Northern and the Central Highlands of Vietnam and was commonly called "Giổi chanh" in Vietnamese. The epithet *citrata* came from the strong scent of the outer seed coat, which was similar to *Cymbopogon citratus* (DC.) Stapf. The leaves when crushed produced a licorice aroma. This study aims to examine in-depth the components and antimicrobial properties of essential oils from leaves, twigs, and stem barks of the species *Magnolia citrata* in the Central Highlands of Vietnam.

MATERIALS AND METHODS

Plant material

In this research, samples of twigs, leaves, and stem barks of *Magnolia citrata* species were collected from one tree at Bidoup-Nui Ba National Park, Lac Duong district, Lam Dong province in July 2018 which has geographic characteristics of 12º08'23.61"N and 108º41'05.60"E with an altitude of 1,585 m above sea level. A voucher specimen was authenticated by Ms. Tu Bao Ngan (Vietnam National Museum of Nature) and deposited at the Herbarium of the Vietnam National Museum of Nature with the codes TN17/C04-051.

Essential oils extraction

The harvested materials were cleaned, chopped, and subjected to hydrodistillation (ca. 900 g of each plant's material) using a Clevenger apparatus (Thermo Fisher Scientific, Waltham, MA, USA) to extract their essential oil for the duration of three hours as described in Vietnamese Pharmacopoeia V (Ministry of Health, 2017). The slightly yellow oil was dried over anhydrous sodium sulfate to remove any trace of water and stored in sealed glass vials at 4 ºC until further analysis. The yield was then calculated according to the volume of obtained essential oil and was expressed on a fresh weight basis (*v*/*w*).

Gas Chromatography-Mass Spectrometry (GC-MS) analysis

Oils were analyzed by Gas Chromatography-Mass Spectrometer (GC-MS), using a Thermo Scientific Trace 1310 coupled with mass spectrometry Thermo Scientific ITQ

900 equipped with capillary column TG-5MS (30 m × 0.25 mm i.d, 0.25 µm film thickness). The oven temperature was held at 40 ºC, then programmed to 240 ºC (hold 5 min) at a rate of 4 ºC/min. Helium was used as the carrier gas at a flow rate of 1.0 mL/min. The injector temperature was 250 ºC, and the injection volume of 0.1 mL in *n*-hexane, with a split ratio of 1:60. The mass spectra (MS) were operated in electron impact mode (70 eV), and the MS data were acquired in scan mode with a mass range of *m/z* 40–400. The peaks were quantified by calculating the percentage of the peak area of each component by comparison to the sum of the peaks of other compounds. The identification of the components was made on the basis of retention index (RI, determined with reference to a homologous series of *n*-alkanes C_8–C_{30}, under identical experimental conditions), MS library search (NIST 17 version 2.3), and by comparing with MS literature data (Adams, 2017).

Antimicrobial assay

Antimicrobial assays were carried out using *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Salmonella enterica* (ATCC 12228), *Enterococcus faecalis* (ATCC 13124), *Staphylococcus* aureus (ATCC 25923), *Bacillus cereus* (ATCC 13245), and *Candida albicans* (ATCC 1023). Stock solutions of samples were prepared in DMSO, and the assays were carried out in 96-well microtiter plates against the microbial strains (5×10^5 CFU/mL) using a modification of the published method (Andrews, 2001). Essential oil was diluted with DMSO at decreasing concentration ranges of 256, 128, 64, 32, 16, 8, 4, and 2 µg/mL. After incubation for 24 hours at 37 ºC, the absorbance at 650 nm was measured using a microplate reader (BioRad, USA). All the experiments were done in triplicate.

The minimum inhibitory concentrations (**MIC**) were detected as the minimum concentration at which at least 90% of microbial growth was missing. Streptomycin (M = 581) and cyclohexamide (M = 281) were used as positive controls. The microbial inhibition rate expressed as a percentage of inhibition was calculated using the following equation:

$$\%\ \text{inhibition} = \frac{A_o - A_t}{A_o - A_{oc}} \times 100\%$$

$$IC_{50} = H_c - \frac{(H_i - 50\%) \times (H_c - L_c)}{H_i - L_i}$$

Where: A_o: Absorbance of a blank sample; A_{oc}: Absorbance of the culture medium without cells; A_t: Absorbance of the test sample; H_c and L_c: High and low concentration (%) of test agents, respectively; H_i and L_i: Inhibition percentage at high and low concentrations, respectively.

RESULTS AND DISCUSSION

Chemical composition of the essential oils

The yields of hydrodistillation essential oils were 0.06% from twigs, 0.51% from leaves, and 0.16% from stem barks respectively as calculated on a dry weight basis. Three essential oils were yellowish. A total of forty-three compounds were identified from three essential oil samples analyzed, comprising from 98.2% to 98.9% of the total components. The results are shown in detail in Table 1. Monoterpene hydrocarbons and oxygenated monoterpenoids were the main classes of compounds presented in all oils.

The major constituents of the oils from leaves and twigs were similar. Accordingly, geranial (23.1%), neral (22.5%), linalool (22.5%), and sylvestrene (6.3%) were found in the leaves, and geranial (30.9%), neral (29.6%), sylvestrene (11.8%) and linalool (6.1%) were found from the twigs. Meanwhile, the constituents of stem bark oils were different from the leaves and twigs. Sylvestrene was the major component accounting for 51.8%, and the other compounds presented in high quantity included safrole (11.6%), geranial (6.9%), sabinene (6.9%), and neral (6.6%). The components of essential oils from leaves of *M. citrata* collected in central highlands in this study is similar to the components of essential oils from leaves of this species

collected in Ha Giang province, Northern Vietnam in previously published results (Bui et al., 2014; Luu Dam et al., 2021).

Reports on *Magnolia* essential oil compositions in the literature have been documented, and some examples of these are listed in Table 2 for comparison. As was the case with *M. citrata* presented above, most of the *Magnolia* species examined have monoterpenoids dominating their essential oils.

As compared to the data shown in Table 2, the essential oils from *M. citrata* in the present study differed from other species of its genus by the presence of dominant components as geranial (6.9–30.9%), neral (6.6–29.6%), sylvestrene (6.3–51.8%), and safrole (11.6%) with varying quantities in leaves, twigs, and sterm barks. However, another major component, linalool (22.5%), found in *M. citrata* leaves, were also presented with a high amount in the flowers and buds of *M. sirindhorniae*. Minor contents of linalool were found in *M. acuminate*, *M. fraseri*, *M. grandiflora*, *M. tripetala*. The presence of geranial and neral with significant amounts might be a good explanation for the similar smell of *M. citrata* with *Cymbopogon citratus*. Citral (comprising of geranial and neral) was found to be the major content in lemongrass (*C. citratus*) in previous studies (Duong et al., 2020). Interestingly, these variations in *Magnolia* oil compositions (in the same part of trees) could assist in distinguishing *Magnolia* species with similar morphology (Azuma et al., 2001).

Table 1. The chemical composition of *Magnolia citrata* essential oil

No.	RI[a]	RI[b]	Components	Molecular formula	Leaves (%)	Stem barks (%)	Twigs (%)
1	933	939	α-Pinene	$C_{10}H_{16}$	0.2	1.9	0.1
2	948	952	α-Fenchene	$C_{10}H_{16}$	ND	0.1	ND
3	976	975	Sabinene	$C_{10}H_{16}$	0.5	6.9	0.4
4	986	985	6-Methyl-5-hepten-2-one	$C_8H_{14}O$	3.5	0.9	2.3
5	991	990	Myrcene	$C_{10}H_{16}$	ND	0.5	ND
6	1029	1030	Sylvestrene	$C_{10}H_{16}$	6.3	51.8	11.8
7	1038	1037	(*Z*)-β-Ocimene	$C_{10}H_{16}$	3.2	2.7	4.0
8	1048	1050	(*E*)-β-Ocimene	$C_{10}H_{16}$	0.7	0.6	0.9
9	1073	1072	*cis*-Linalool oxide	$C_{10}H_{18}O_2$	0.2	ND	ND
10	1088	1088	Terpinolene	$C_{10}H_{16}$	0.2	0.1	ND
11	1100	1096	Linalool	$C_{10}H_{18}O$	22.5	1.5	6.1
12	1121	1122	*trans*-ρ-Mentha-2,8-dien-1-ol	$C_{10}H_{16}O$	ND	0.1	ND
13	1138	1137	*cis*-ρ-Mentha-2,8-dien-1-ol	$C_{10}H_{16}O$	ND	0.4	ND
14	1145	1144	*trans*-Verbenol	$C_{10}H_{16}O$	0.1	ND	0.2
15	1153	1153	Citronellal	$C_{10}H_{18}O$	0.2	ND	0.1
16	1164	1164	(*Z*)-Isocitral	$C_{10}H_{16}O$	1.0	0.3	1.0
17	1175	1177	Rosefuran epoxide	$C_{10}H_{14}O_2$	0.3	ND	ND
18	1178	1180	Santalone	$C_{11}H_{16}O$	ND	0.1	ND
19	1183	1184	*cis*-Pinocarveol	$C_{10}H_{16}O$	1.5	0.4	1.7
20	1191	1195	Myrtenol	$C_{10}H_{16}O$	ND	0.1	0.2
21	1219	1221	*cis*-Sabinene hydrate acetate	$C_{12}H_{20}O_2$	0.1	0.2	0.1
22	1229	1229	*cis*-Carveol	$C_{10}H_{16}O$	1.3	0.3	1.1
23	1234	1230	*cis*-ρ-Mentha-1(7),8-dien-2-ol	$C_{10}H_{16}O$	ND	ND	0.2
24	1241	1238	Neral	$C_{10}H_{16}O$	22.5	6.6	29.6
25	1255	1257	Linalool acetate	$C_{12}H_{20}O_2$	1.5	0.5	2.8
26	1271	1267	Geranial	$C_{10}H_{16}O$	23.1	6.9	30.9

No.	RI[a]	RI[b]	Components	Molecular formula	Leaves (%)	Stem barks (%)	Twigs (%)
27	1289	1287	Safrole	$C_{10}H_{10}O_2$	3.5	11.6	3.1
28	1324	1324	Methyl geranate	$C_{11}H_{18}O_2$	0.4	0.1	0.3
29	1352	1351	α-Cubebene	$C_{15}H_{24}$	0.6	ND	0.1
30	1379	1376	α-Copaene	$C_{15}H_{24}$	0.3	ND	ND
31	1393	1390	β-Elemene	$C_{15}H_{24}$	0.5	0.1	0.1
32	1423	1419	(*E*)-Caryophyllene	$C_{15}H_{24}$	0.7	0.5	0.3
33	1459	1460	allo-Aromadendrene	$C_{15}H_{24}$	0.2	0.4	0.1
34	1462	1463	*cis*-Cadina-1(6),4-diene	$C_{15}H_{24}$	0.2	ND	ND
35	1500	1500	α-Muurolene	$C_{15}H_{24}$	0.5	0.1	0.2
36	1511	1509	α-Bulnesene	$C_{15}H_{24}$	0.6	0.5	0.2
37	1519	1512	δ-Amorphene	$C_{15}H_{24}$	0.1	ND	ND
38	1527	1529	Zonarene	$C_{15}H_{24}$	0.8	0.5	0.3
39	1557	1557	Elemicin	$C_{12}H_{16}O_3$	0.3	0.9	0.3
40	1582	1578	Spathulenol	$C_{15}H_{24}O$	0.2	ND	0.1
41	1587	1590	β-Copaen-4-α-ol	$C_{15}H_{24}O$	0.2	0.6	0.2
42	1645	1646	Cubenol	$C_{15}H_{26}O$	0.1	0.3	0.1
43	1659	1660	Gymnomitrol	$C_{15}H_{24}O$	0.1	0.1	ND
	Monoterpene hydrocarbons				11.1	64.6	17.2
	Oxygenated monoterpenoids				74.7	17.5	74.3
	Sesquiterpene hydrocarbons				4.5	2.1	1.3
	Oxygenated sesquiterpenoids				0.6	1.0	0.4
	Other compounds				7.3	13.4	5.7
	Total identified				98.2	98.6	98.9

Notes: RI: Retention Index; [a]Experimental value, determined on TG-5MS gas chromatography column; ND: Not detected; [b]Literature value (Adams, 2017).

Table 2. Major components of some species of genus *Magnolia* essential oils

No.	*Magnolia* species	Plant part	Major components	Ref.
1	*Magnolia acuminata*	Follicles (Fruit)	*trans*–nerolidol (20%), bornyl acetate (5.3%), limonene (3.5%), α–myrcene (3.5%), camphor (3.0%), β–caryophyllene (3.0%), linalool (1.0%)	Schühly et al. (2008)
2	*Magnolia biondii*	Flower buds	camphor (10.6%), eucalyptol (25.0%), linalool (5.8%), terpine–4–ol (8.4 %), alphaterpineol (19.8 %), α–cadinol (3.3%), citronellol (2.9%), geraniol (2.3%), and transfarnesol (8.7%)	Guerra-Boone et al. (2013)
3	*Magnolia fraseri*	Follicles (Fruit)	β–pinene (26.3%), β–myrcene (13,1%), limonene (6.3%), bornyl acetate (5.7%), germacrene D (5.7%), linalool (0.6%), β–caryophyllene (1.4%)	Schühly et al. (2008)

No.	*Magnolia* species	Plant part	Major components	Ref.
4	*Magnolia grandiflora*	Flowers	(E, E)–farnesol (18%), 2–phenylethanol (10%)	Farag & Al-Mahdy (2013)
5	*Magnolia grandiflora*	Leaves	bornyl acetate (20.9%), E–caryophyllene (15.1%), germacrene D (15.1%), α–guainene (6.8%), camphor (5.5%), myrcene (4.5%), linalool (2.0%), limonene (1.9%),	Guerra-Boone et al. (2013)
6	*Magnolia grandiflora*	Seed	caryophyllene (19.36%), ketones (21.19%), eucalyptol (10.70%), equilenin (8.02%)	Luo et al. (2012)
7	*Magnolia grandiflora*	Follicles (Fruit)	β–elemene (12.1%), β–caryophyllene (7.4%), caryophyllene oxide (4.3%), bornyl acetate (4.1%), *trans*–pinocarveol (1.8%)	Schühly et al. (2008)
8	*Magnolia kobus*	Fresh and dried fruit	α–pinene (31.6–26.7%), β–pinene (27.9–20.2%), limonen (8.6–10.6%), β–caryophyllene (8.1–3.9%)	Azuma et al. (2001); Sowndhararajan et al. (2016)
9	*Magnolia kwangsiensis*	Peel	*cis*–β–ocimene (30.80 %), p–menth–1–ene (17.76 %), α–terpinene (10.15 %), β–myrcene (7.03 %), α–terpineol (5.18 %)	Zheng et al. (2019)
10	*Magnolia kwangsiensis*	Aril	*cis*–β–ocimene (56.03 %), β–phellandrene (10.96 %), α–terpinene (6.37 %), α–phellandrene (6.16 %), β–myrcene (6.04 %)	Zheng et al. (2019)
11	*Magnolia liliflora*	Leaves	*trans*–α–farnesene (49.0–72.5%), β–caryophyllene (4.9–5.3%), germacrene D (0.4–1.8%), β–bisabolene (0.7–2.5%), δ–cadinene (0.8–1.3%), caryophyllene oxide (0.9–3.2%), nerolidol (3.4–3.9%), α–cadinol (0.3–1.8%)	Fujita (1989); Bajpai et al. (2009); Bajpai, (2012)
12	*Magnolia obovate*	Bark	β–eudesmol (23.61%), cadalene (17.21%), γ–eudesmol (7.32%), bornyl acetate (6.40%), hexanal (4.51%), camphene (3.67%), α–eudesmol (2.95%), caryophyllene oxide (2.94%)	Kameoka et al. (1994)

No.	*Magnolia* species	Plant part	Major components	Ref.
13	*Magnolia officinalis*	Bark	3–eudesmol (17.4%), cadinol (14.6%), guaiol (8.7%)	Pu et al. (1990)
14	*Magnolia sirindhorniae*	Flowers and Buds	linalool (51.0–58.9 %), β–elemene (7.5 %), β–caryophyllene (6.4%), germacrene D (3.0 %)	Ghosh et al. (2021)
15	*Magnolia tripetala*	Follicles (Fruit)	β–caryophyllene (21%), bornyl acetat (17%), α–humulene (11.2%), linalool (4.4%)	Schühly et al. (2008)
16	*Magnolia virginiana*	Flowers	phenylethanol (39,9%), methyl myristate (11.5%), α–humulene (4.6%), β–caryophyllene (3.0)	Farag & al-Mahdy (2013)
17	*Magnolia coco*	Stem	sabinene (31.9 %), β–pinene (11.8%)	Nguyen et al. (2020)

Antimicrobial activities of the essential oils of *Magnolia citrata*

As shown in Table 3, essential oils from leaves, stem barks, and twigs of *M. citrata* exhibited positive activities against six bacteria and one yeast in all tested concentrations. The essential oil showed significant antimicrobial effects with the MIC values ranging from 64 μg/mL to 256 μg/mL. When compared to the reference compound, streptomycin, all essential oils exhibited stronger antimicrobial activity against Gram-positive bacteria including *E. faecalis*, *S. aureus*, and *B. cereus*, similar or weaker activity against Gram-negative bacteria such as *E. coli*, *P. aeruginosa*, and *S. enterica.* The effects of tested essential oils against the yeast *C. albicans* were weaker than the reference compound, cycloheximide.

According to the results obtained, antibacterial and antifungal properties from the essential oils of *M. citrata* is of great interest in both fundamental science and applied science in the development of protective additives as the growing trend of replacing synthesized antimicrobial additives with organic ones.

Table 3. Antibacterial and antifungal activity of the essential oils of *Magnolia citrata*

Essential oils	Gram (+)			Gram (-)			Yeasts
	Enterococcus faecalis ATCC29212	*Staphylococcus aureus* ATCC25923	*Bacillus cereus* ATCC13245	*Escherichia coli* ATCC25922	*Pseudomonas aeruginosa* ATCC27853	*Salmonella enterica* ATCC13076	*Candida albicans* ATCC10231
MIC of the essential oils (μg/mL)							
Twigs	64	128	64	128	256	128	128
Leaves	64	64	64	128	256	64	64
Stem barks	64	64	64	128	256	128	64
Streptomycin	256	256	128	32	256	128	ND
Cycloheximide	ND	ND	ND	ND	ND	ND	32

Note: ND: Not determined.

CONCLUSION

This study is a report on the chemical constituents and potential for antimicrobial properties of the essential oils of *M. citrata* in the Central Highlands region. The compounds geranial (23.1%), neral (22.5%), linalool (22.5%), and sylvestrene (6.3%) were found in the leaves, and geranial (30.9%), neral (29.6%), sylvestrene (11.8%) and linalool (6.1%) were found in the twigs. Meanwhile, sylvestrene was the major component comprising 51.8% of the stem bark oils. Other compounds present in high quantity in this oil included safrole (11.6%), geranial (6.9%), sabinene (6.9%), and neral (6.6%) which corresponds with previous studies in Ha Giang province, northern Vietnam. In addition, the antimicrobial activities of the essential oil samples were tested against six bacterial and one yeast strain for the first time. The results indicated that essential oils from twigs, leaves, stem barks of *M. citrata* had strong antimicrobial activities. At the concentration of 64 μg/mL, essential oils from the three plant parts effectively inhibited the growths of the Gram-positive bacteria *E. faecalis*, *S. aureus*, *B. cereus*, and the yeast *C. albicans*. For the Gram-negative bacteria including *E. coli*, *P. aeruginsa*, and *S. enterica*, the effective concentrations of the oils varied from 128 μg/mL to 256 μg/mL.

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