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Chemical composition and antimicrobial activity of the leaf essential oils of *Magnolia kwangsiensis* Figlar & Noot growing in Vietnam

Thi Thu Ha Chu, Huy Thai Tran, Thi Hien Nguyen, Thi Thu Thuy Dinh, Van Thanh Bui, Van Du Nguyen, Quang Nam Vu and William N Setzer

Abstract
The leaf essential oils of two individual trees of *Magnolia kwangsiensis*, growing wild in Bat Dai Son Nature Reserve, Ha Giang province of Vietnam, were obtained by hydrodisitillation and analyzed by gas chromatography-mass spectrometry (GC-MS). Monoterpenoids (56.2% and 58.9%) and sesquiterpenoids (31.3% and 32.7%) were the main groups in both samples, the main compounds being (*E*)-β-ocimene (20.6% and 14.8%), myrcene (7.9% and 11.6%), and (*E*)-nerolidol (4.6% and 7.4%). Antibiotic activity of the essential oil samples was tested against three test microorganism strains using an agar disk diffusion method that showed the inhibition zones from 19.5–46.5 mm and 20–36.5 mm. Minimum inhibitory concentration (MIC) of the essential oils was determined using micro dilution broth susceptibility assay against seven test microorganism strains. MIC values of the essential oil from the first tree were from 4.1 to 16.4 mg/mL, while those from the second tree were from 8.2 to 16.4 mg/mL.

Keywords Magnoliaceae, *Magnolia kwangsiensis*, essential oil composition, antimicrobial activity, Bat Dai Son Nature Reserve

1. Introduction
Magnolia kwangsiensis Figlar & Noot. (syn *Kmeria septentrionalis* Dandy, syn. *Woonyoungia septentrionalis* (Dandy) Y.W Law) (Vietnamese name is Giổi quảng tây) is a timber tree belonging to *Magnolia* L. of family Magnoliaceae. Over the last century due to the potential use and significant value of *Magnolia* species in traditional health-care systems as well as in fragrance industry, they have been the subject of numerous phytochemical, pharmacological and essential oil investigations [1-3]. *M. kwangsiensis* was listed in vulnerable category (VU) in IUCN red list 2015 [4] and was considered as endemic genus located only in Guangxi, Guizhou and Yunnan provinces of China [5]. In fact, this plant species has been recently found growing in Vietnam [6]. The height (H) and diameter ($D_{1.3}$) of mature plant are up to 18 m and 40 cm [7], even up to 40 m and 60-80 cm, respectively [6]. The species has grey bark. The twig is green, at first appressed pubescent. Stipular scar nearly reaches the apex of the petiole. Petiole's length is from 2.0–3.5 cm, at first greyish pubescent, later glabrescent; leaf blade is elliptic-oblong to obovate-oblong, 8–22 × 3.5–11 cm, leathery, both surfaces are glabrous or when young abaxially sparsely pilose at base, adaxially green and glossy, number of secondary veins is from 12-17 on each side of midvein, reticulate veins are prominent on both surfaces, base is broadly cuneate, apex obtuse and slightly emarginate. In Vietnam, the plant flowers from May to June.

The subjects of previous studies on *M. kwangsiensis* included the genetic structure among isolated relic populations [8], the chloroplast genome sequence [9], the extraction of polysaccharides and its antitumor activity [10], the volatile constituents from testa collected in China [11], the essential oil composition from flower and leaf collected in Guangxi, China [5], and the essential oil composition from peel and aril collected in China [12].

To the best of our knowledge, limited investigation has been done on the essential oil from leaf of *M. kwangsiensis*and its antimicrobial activity has not yet been reported in the literature to date. The purpose of this work is to characterize the volatile components of *M. kwangsiensis* trees from Vietnam and their antimicrobial activity.

2. Materials and Methods

2.1 Plant material

The leaves of *M. kwangsiensis* growing wild in 2 mountains of Bat Dai Son Nature Reserve in Dau Cau 1 village, Can Ty commune, Quan Ba district, Ha Giang province, North of Vietnam were collected in April 2018 with the details as follow:

(a) Timber tree, height (H) = 5 m, diameter at breast height ($D_{1.3}$) < 8 cm (HG1803), at Xi Sinh Ho mountain, 23º06.677′ N, 105º00.995′ E, 1029 m above sea level.

(b) Timber tree, height (H) = 10 m, diameter at breast height ($D_{1.3}$) = 15 cm (HG1813), at Hang Tang Chong mountain, 23º05.116′ N, 105º02.161′ E, 1208 m above sea level.

The plants were identified by Dr. Tien Hiep Nguyen (Center for plant conservation, Nghia Do, Cau Giay, Ha Noi, Vietnam) and Assoc. Prof. Dr. Quang Nam Vu (Vietnam National University of Forestry, Xuan Mai, Chuong My, Ha Noi, Vietnam) individually; voucher specimens (HG1803 and HG1813) have been deposited at the Herbarium of Institute of Ecology and Biological Resources (HN), Vietnam Academy of Science and Technology 1.1-kg and 1.4-kg samples of the fresh leaf materials, respectively, were shredded and hydrodistilled for 3 h using a Clevenger type apparatus. After that the essential oils were separated and dried with anhydrous $MgSO_4$. The obtained oils were stored at -5 ºC until analysis.

2.2 Gas chromatography–mass spectrometry

Analysis of the essential oils was performed by GC/MS using an Agilent GC7890A system with Mass Selective Detector (Agilent 5975C). A HP-5MS fused silica capillary column (60 m × 0.25 mm i.d. × 0.25 µm film thickness) was used. Helium was the carrier gas with a flow rate of 1.0 mL/min. The inlet temperature was 250 °C and the oven temperature program was as follows: 60 °C to 240 °C at 4 °C/min. The split ratio was 1:100, the detector temperature was 270 °C, and the injection volume was 1µL. The MS interface temperature was 270 °C, MS mode, E.I. detector voltage 1200V, and mass range 35–450 Da at 1.0 scan/s. Identification of components was achieved based on their retention indices and by comparison of their mass spectral fragmentation patterns with those stored on the MS library (HPCH1607, NIST08, Wiley09). Component relative concentrations were calculated based on total ion current without standardization. Data processing software was Mass Finder 4.0.

2.3 Microbial strains

The antimicrobial activities of the essential oils were evaluated using *Staphylococcus aureus* ATCC 13709 (a strain of Gram-positive test bacteria), *Escherichia coli* ATCC 25922 (a strain of Gram-negative test bacteria) and *Candida albicans* ATCC 10231 (a strain of yeast). Minimum inhibitory concentration (MIC) and median inhibitory concentration (IC_{50}) values were determined using 3 strains of Gram-positive test bacteria including *Staphylococcus aureus* ATCC 13709, *Bacillus subtilis* ATCC 6633, and *Lactobacillus fermentum* VTCC N4, 3 strains of Gram-negative test bacteria including *Salmonella enterica* VTCC, *Escherichia coli* ATCC 25922, and *Pseudomonas aeruginosa* ATCC 15442, and 1 strain of yeast *Candida albicans* ATCC 10231.The ATCC strains were obtained from American Type Culture Collection, The VTCC strains were obtained from Vietnam Type Culture Collection–Vietnam National University, Hanoi.

2.4 Screening of antimicrobial activity

The agar disk diffusion method was used to test the antimicrobial activity of essential oil [13-15]. Testing media included Mueller-Hinton Agar (MHA) used for bacteria, and Sabouraud Agar (SA) used for fungi. Microorganisms were stored at -80 °C and activated by culture medium prior to testing to reach a concentration of 1.0×10^6 CFU/mL. A 100-µL inoculum solution was taken and spread evenly over the surface of the agar. Two holes were made on agar plates (about 6 mm in diameter each hole) using an aseptic technique. 50 µL essential oil was put into each hole using a pipette. The petri dishes were kept at room temperature for 2-4 hours and then incubated at 37 °C for 18-24 h. The presence or absence of growth around each antimicrobial disk on each plate culture was observed. The diameters of inhibition growth zones values were measured using a ruler with millimetre markings. The zone of inhibition is the point at which no growth is visible to the unaided eye. An inhibition zone of 14 mm or greater (including diameter of the hole) was considered as high antibacterial activity [16-17]. Minimum inhibitory concentration (MIC) and median inhibitory concentration (IC_{50}) values were measured by the micro dilution broth susceptibility assay [18, 19]. Stock solutions of the oil were prepared in dimethylsulfoxide (DMSO). Dilution series were prepared from 16384 µg/mL to 2 µg/mL (2^{14}, 2^{13}, 2^{12}, 2^{11}, 2^{10}, 2^9, 2^7, 2^5, 2^3, 2^1 µg/mL) in sterile distilled water in micro-test tubes, from where they were transferred to 96-well microtiter plates. Bacteria grown in double-strength Mueller-Hinton broth or double-strength tryptic soy broth, and fungi grown in double-strength Sabouraud dextrose broth were standardized to 5×10^5 and 1×10^3 CFU/mL, respectively. The last row, containing only the serial dilutions of sample without microorganisms, was used as a negative control. Sterile distilled water and medium served as a positive control. After incubation at 37 °C for 24 h, the MIC values were determined at well with the lowest concentration of agents completely inhibit the growth of microorganisms. The IC_{50} values were determined by the percentage of microorganisms inhibited growth based on the turbidity measurement data of EPOCH2C spectrophotometer (BioTeK Instruments, Inc Highland Park Winooski, USA) and Rawdata computer software (Belgium) according to the following equations:

$$\%\ \text{inhibition} = \frac{OD_{control(+)} - OD_{test\ agent}}{OD_{control(+)} - OD_{control(-)}} \times 100\% \quad (1)$$

$$IC_{50} = High_{Conc} - \frac{(High_{Inh\%} - 50\%) \times (High_{Conc} - Low_{Conc})}{(High_{Inh\%} - Low_{Inh\%})} \quad (2)$$

Where:

OD: Optical density; control (+): only cells in medium without Antimicrobial agent, test agent coresponds to a known concentration of Antimicrobial agent; control (-) culture medium without cells. High $_{Conc}$ Low $_{Conc}$: Concentration of test agent at high concentration/low concentration; High $_{Inh\%}$ Low $_{Inh\%}$ % inhibition at high concentration % inhibition at low concentration).

Reference materials: Ampicillin for Gram-positive bacterial strains with MIC values in the range of 0.004 to 1.2 µg/mL,

Cefotaxime for Gram-negative bacterial strains with MIC values in the range of 0.07-19.23µg/mL, Nystatine for fungal strain with MIC values of about 2.8-5.0µg/mL.

3. Results and discussion

3.1. Chemical composition of essential oil

By hydrodisitillation, essential oil yields of 0.101% (v/w) and 0.096% (v/w), calculated on a dry weight basis, were obtained from the leaves of the first tree (HG1803– smaller one) and the second tree (HG1813–larger one) of *M. kwangsiensis*, respectively. Both essential oils were pale yellow liquids having lower densities than water.

The chemical compositions of the leaf essential oils from two individuals (HG1803 and HG1813) of *M. kwangsiensis* from Bat Dai Son Nature Reserve in Vietnam are summarized in Table 1 and their chromatograms are presented in Figures 1 and 2. A total of 75 and 62 compounds were identified in the essential oils, representing 92.3%, and 95.4% of the compositions, respectively. Monoterpenoids (56.1% and 58.9%) and sesquiterpenoids (31.3% and 32.8%) made up the bulk of the essential oil compositions, with (*E*)-β-ocimene (20.6% and 14.8%), myrcene (7.9% and 11.6%), linalool (6.5% and 7.4%), (*Z*)-β-ocimene (5.9% and 6.1%), and (*E*)-nerolidol (4.6% and 7.4%) as major components of the leaf oils of the two samples (HG1803 and HG1813), respectively. Chemical compositions of essential oils from leaves of both *M. kwangsiensis* trees had a similar pattern in that (*E*)-β-ocimene was the most abundant major constituent. In addition, another 52 compounds were also present as the constituents of both oils with varying amounts except 22 constituents were present only in the 1st leaf oil(HG1803); and 9 constituents (and 1 unknown compound) were found only in the 2nd leaf oil (HG1813).

Table 1: Essential oils compositions of the leaf of *Magnolia kwangsiensis* Figlar & Noot (HG1803 and HG1813)

RI[a]	Components	HG1803 (%)	HG1813 (%)
852	(*E*)-2-Hexenal	0.2	-
855	(*Z*)-Hex-3-en-1-ol	0.4	-
866	*n*-Hexanol	0.4	-
955	Camphene	0.6	0.7
985	*p*-Menth-3-ene (= Menthomenthene)	0.1	0.1
987	6-Methylhept-5-en-2-one	0.2	0.2
992	Myrcene	7.9	11.6
1006	δ-2-Carene	0.5	0.7
1011	α-Phellandrene	-	0.8
1022	α-Terpinene	1.3	2.3
1027	*p*-Menth-1-ene	0.6	0.7
1030	*o*-Cymene	2.5	3.0
1034	Limonene	4.0	4.0
1036	β-Phellandrene	0.3	0.3
1038	(*Z*)- β-Ocimene	5.9	6.1
1050	(*E*)- β-Ocimene	20.6	14.8
1063	γ-Terpinene	0.3	0.4
1078	*trans*-Linalool oxide (furanoid)	0.1	-
1094	Terpinolene	0.8	0.9
1104	Linalool	6.5	7.4
1124	*endo*-Fenchol	0.2	0.3
1130	*cis*-*p*-Menth-2-en-1-ol	0.3	0.2
1145	*allo*-Ocimene	0.2	-
1148	*trans*-*p*-Menth-2-en-1-ol	0.2	0.2
1162	Camphene hydrate	-	0.2
1170	Isoborneol (=*exo*-Borneol)	-	0.2
1178	Borneol (=*endo*-Borneol)	0.2	0.5
1187	Terpinen-4-ol	0.5	0.7
1201	α-Terpineol	2.5	2.9
1217	*trans*-Piperitol	0.1	-
1234	Thymol methyl ether	0.2	-
1239	Carvacrol methyl ether	0.1	0.2
1258	Geraniol	0.3	0.2
1290	*trans*-Linalool oxide acetate	0.3	0.3
1294	Bornyl acetate	2.0	2.7
1342	Dimethoxy-(*E*)-citral	0.1	-
1365	Neryl acetate	0.3	0.3
1384	Geranyl acetate	0.5	0.2
1390	α-Copaene	0.1	0.1
1429	4,8-β-Epoxy-caryophyllane	0.2	-
1437	(*E*)-β-Caryophyllene	1.2	0.3
1451	α-Guaiene	0.3	0.2
1457	Aromadendrene	-	0.4
1461	(*Z*)-β-Farnesene	1.0	1.4
1465	α-Acoradiene	0.3	0.2
1472	α-Humulene	0.2	-
1489	γ-Curcumene	0.1	0.9
1491	γ-Muurolene	0.9	1.2
1494	α-Amorphene	0.8	0.3
1505	β-Selinene	1.0	1.6
1512	*trans*-Muurola-4(14), 5-diene	-	1.0
1513	γ-Amorphene	0.9	-
1514	α-Muurolene	1.8	2.3
1518	β-Bisabolene	1.5	2.1
1522	δ-Amorphene	0.3	-
1531	γ-Cadinene	0.5	0.5
1535	Myristicin	0.1	-
1538	δ-Cadinene	2.6	2.9
1540	*cis*-Calamenene	0.3	0.2
1542	Zonarene	0.7	-
1543	(*E*)-γ-Bisabolene	-	0.8
1549	*trans*-Cadina-1,4-diene	0.3	0.3
1551	(*E*)-α-Bisabolene	0.3	2.5
1554	α-Cadinene	0.4	-
1561	α-Calacorene	0.5	0.2
1572	(*E*)-Nerolidol	4.6	7.4
1594	Caryophyllenyl alcohol	1.2	-
1600	Spathulenol	0.2	0.1
1602	Axenol (Gleenol)	-	0.2
1606	Caryophyllene oxide	1.3	-
1607	Viridiflorol	-	0.5
1616	Cubeban-11-ol	0.3	-
1630	Cedrol	0.3	-
1637	1,10-di-*epi*-Cubenol	0.2	-
1647	unknown (161, 220, RI 1647)	-	1.0
1649	1-*epi*-Cubenol	1.1	0.9
1653	γ-Eudesmol	0.3	-
1663	*EPI*-α-Muurolol (=τ-Muurolol)	1.8	1.1
1665	α-Muurolol (=δ-Cadinol)	-	0.6
1666	*EPI*-α-Cadinol (=τ-Cadinol)	1.8	-
1676	α-Cadinol	1.1	0.8
1679	*neo*-Intermedeol	0.4	0.6
1699	*EPI*-α-Bisabolol	0.4	0.5
1701	α-Bisabolol	0.3	0.5
1730	(*E,E*)-Farnesol	0.1	0.2
	Monoterpene hydrocarbons	45.7	46.4
	Oxygenated monoterpenoids	10.5	12.5
	Sesquiterpene hydrocarbons	15.9	19.2
	Oxygenated sesquiterpenoids	15.3	13.5
	Benzenoids	3.7	3.4
	Unknown compounds	-	1.0
	Total identified	92.3	95.4

[a] RI: Retention Index

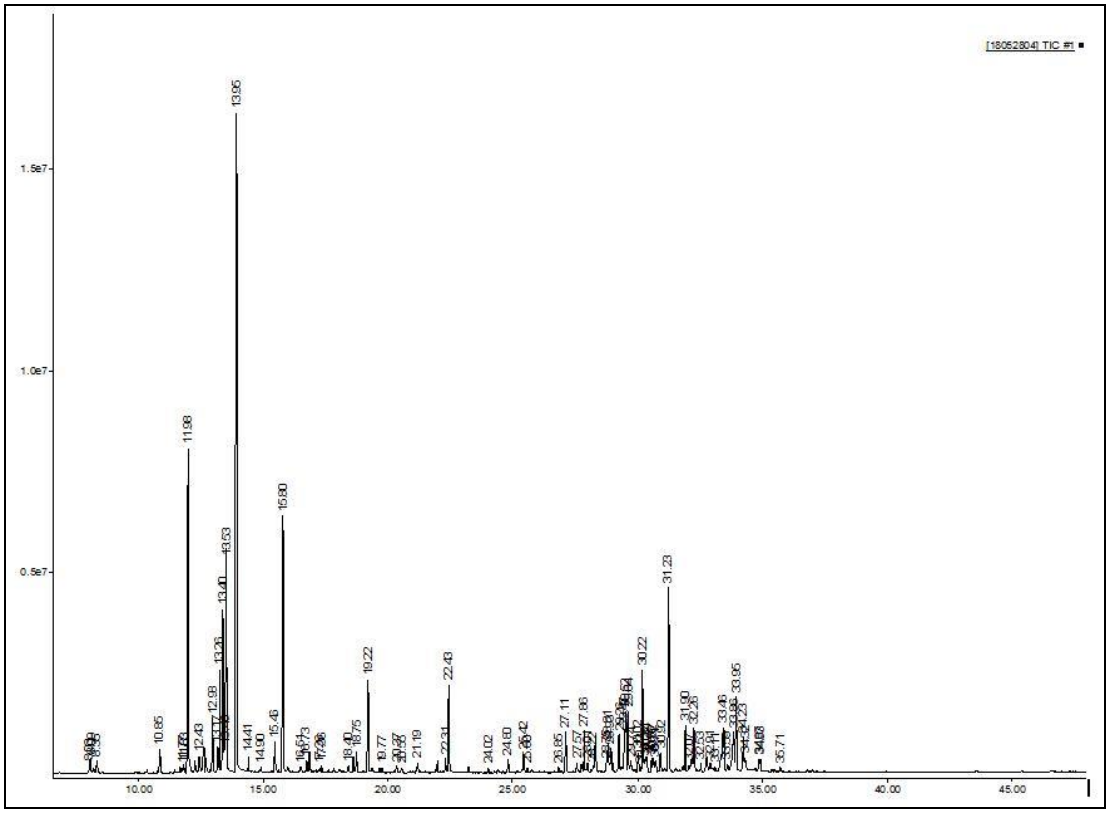

Fig 1: Gas chromatogram of the leaf essential oil of *Magnolia kwangsiensis* Figlar & Noot (HG1803)

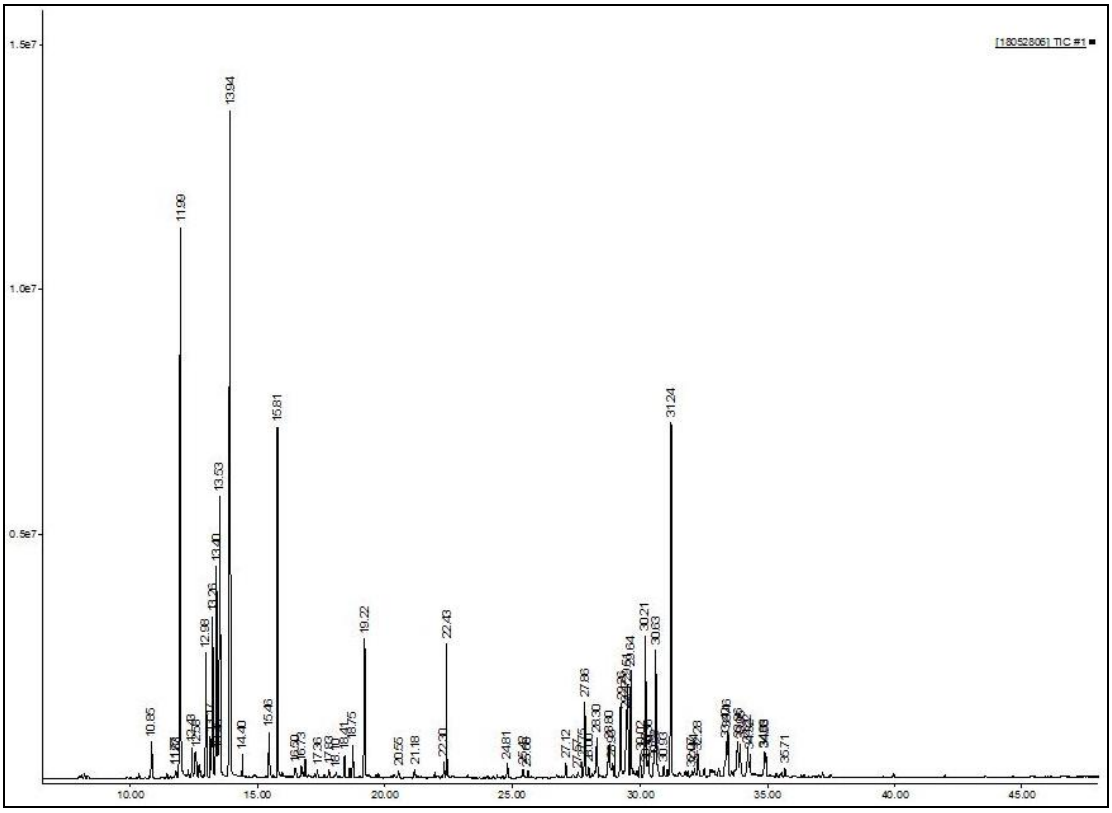

Fig 2: Gas chromatogram of the leaf essential oil of *Magnolia kwangsiensis* Figlar & Noot (HG1813)

The chemical compositions of the essential oils obtained from the leaf, male flower, and female flower of *M. kwangsiensis* growing in China were previously reported [5]. These authors reported 26 constituents in the leaf oil of *M. kwangsiensis* identified with the major components to be β-terpineol (28.9%), γ-terpinene (18.1%), myrcene (15.9%), α-terpineol (5.4%), limonene (4.7%), α-eudesmol (4.4%) and *p*-menth-1-ene (2.8%). They also identified 31 and 27 constituents in the male flower and female flower oils with limonene (18.5%, 20.8%), α-terpinene (13.0%, 7.1%), α-cadinol (12.2%, 11.5%), τ-muurolol (9.9%, 8.4%), δ-cadinene (7.2%, 8.5%), (*Z*)-β-ocimene (8.1%, 9.5%), and myrcene (6.4%, 6.6%) as the major components. In this present work, we find no evidence for either β-terpineol or α-eudesmol in the leaf essential oils. While the amounts of γ-terpinene, α-terpineol, and *p*-menth-1-ene in the leaf oil of the first and the second

trees were, in comparison to the previous data, at lower levels, which were 0.3% and 0.4%, 2.5% and 2.9%, 0.6% and 0.7%, respectively. The findings for the leaf oil of *M. kwangsiensis* in this work were obviously different from the leaf oil in the previous report. The difference in growing location and the sampling time or age of the tree may play an important role in the difference in chemical composition of the essential oil of *M. kwangsiensis*. The *M. kwangsiensis* leaf samples in the previous research [5] were collected in Guangxi, China in April, 2014 while the samples in the present study were collected in Ha Giang, Vietnam in April 2018. It has been shown thatvariation in essential oil compositions can be due to developmental stage of growth [20], irrigation interval and harvest time [21], or geographical region [22].

The essential oils from other parts of *M. kwangsiensis* including test, peel and aril were used to analyze their chemical components. Huang *et al.* (2010) reported α-ocimene (37.3%), limonene (9.0%), *p*-cymene (8.1%), myrcene (7.8%), and (*E*)-β-ocimene (4.1%), *p*-menth-1-ene (4.0%) as major constituents of its testa essential oil. Zheng *et al.* (2019) [12] identified (*Z*)-β-ocimene (30.8%), *p*-menth-1-ene (17.8%), α-terpinene (10.2%), myrcene (7.0%), and α-terpineol (5.2 %) as major compounds of essential oil of *M. kwangsiensis* peel, and (*Z*)-β-ocimene (56.0%), β-phellandrene (11.0%), α-terpinene (6.4%), α-phellandrene (6.2%), and myrcene (6.0 %) as major compounds of aril essential oil from *M. kwangsiensis*.

The other analyzed parts of *M. kwangsiensis* have monoterpenoids dominating their essential oils [5, 11-12] as same with leaf of *M. kwangsiensis* in the present work. There have been some investigations on essential oil compositions of other *Magnolia* species reported in the literature. As was the case with *M. kwangsiensis* in this present work, some of the *Magnolia* species examined have monoterpenoids dominating their essential oils, including *M. acuminata*, *M. calophylla*, *M. virginiana* [23]. *M. grand flora* and *M. ovata* are differently characterized with their essential oils dominated by either monoterpenoids [23-24] or sesquiterpenoids [25-26], while a sample of *M. ovata* fruit had hexadecanoic acid as its major component [26]. The major components of leaf essential oil from *M. acuminata* were (*Z*)-β-ocimene (36.5%), (*E*)-β-ocimene (30.8%) and germacrene A (9.6%); while those from *M. calophylla* were β-pinene (64.4%), α-phellandrene (7.0%), and limonene (7.0%); those from *M. Grandi flora* were unknown monoterpene (19.5%), (*Z*)-β-ocimene (15.2%), β-bisabolene (13.3%), germacrene A (12.9%); and those from *M. virginiana* were β-pinene (37.4%), *p*-cymene (7.6%), (*Z*)-β-ocimene (7.6%), α-terpinolene (6.3%), 2-phenylethyl alcohol (6.3%) [23]. For the case of *M. virginiana* leaf essential oil, Wang *et al.* (2009) identified γ-elemene (15.7%), α-pinene (11.6%), β-caryophyllene (9.0%), and spathulenol (6.5%) as its major components that were different in comparison to those reported by Farag *et al.* (2015) [23].

It was reported that the leaf essential oil of *M. ovata* (syn. *Talauma ovata*) contained major compounds such as limonene (34.8%), α-pinene (11.3%), β-bisabolene (10.7%), germacrene D (10.0%), δ-cadinene (4.8%), and β-caryophyllene (4.5%) (Apel *et al.* 2009). The fruit samples of *M. ovata* had either of spathulenol (19.3%), β-eudesmol (8.0%), hexadecanoic acid (7.6%), and germacrene D (6.4%) or hexadecanoic acid (52.0%), β-eudesmol (7.6%), 1-hexadecanol (4.3%), and 1-pentadecanol (4.1%) dominating in their essential oil composition [26].

3.2. Antimicrobial activity

M. kwangsiensis essential oil extracts were used to screen the antimicrobial activity using the standard agar disk diffusion method against three test microorganisms. Table 2 presents the results of the test that were obtained after 18-24 hours. Both of investigated essential oils from the leaves of *M. kwangsiensis* showed strong inhibition [16, 17] against all three microorganism strains tested in this study with inhibition zones of more than 14.0 mm in diameter. The leaf essential oils from the 1st tree (HG1803) and the 2nd tree (HG1813) were found to be strongly active against *S. aureus*, *E. coli*, and *C. albicans* with the inhibitory zone diameters of 23.0, 19.5 and 46.5 mm (HG1803), and 22.5, 20.0 and 36.5 mm (HG1813), respectively (Table 2). Anti-yeast activity of the leaf essential oil from the first tree is much strongerthan that of the second tree *M. kwangsiensis*.

Table 2: Anti-yeast and antibacterial activity of leaf essential oils of *Magnolia kwangsiensis* Figlar & Noot (HG1803 and HG1813)

Sample	Inhibition zones (mm)		
	Staphylococcus aureus	***Escherichia coli***	***Candida albicans***
HG1803	23.0±1.41	19.5±0.70	46.5±0.70
HG1813	22.5±2.12	20.0±1.41	36.5±0.70

The results of antimicrobial activity test through the standard agar disk diffusion method showed strong activity of both essential oil samples against all of the 3 strains of microorganisms tested. Therefore, these essential oils were then subjected to microbroth dilution assays to determine the minimum inhibitory concentration (MIC) and median inhibitory concentration (IC_{50}) values using 7 strains of microorganisms. Table 3 presents the results of the assay that were obtained after 16-24 hours 4.

Table 3: Microbial minimum inhibitory concentrations (MIC) and median inhibitory concentrations (IC_{50}) of leaf essential oils of *Magnolia kwangsiensis* Figlar & Noot (HG1803 and HG1813)

Mico-organisms	HG1803		HG1813	
	IC_{50} (mg/mL)	**MIC (mg/mL)**	**IC_{50} (mg/mL)**	**MIC (mg/mL)**
Staphylococcus aureus	2.7	8.2	2.9	8.2
Bacillus subtilis	0.3	4.1	0.3	8.2
Lactobacillus fermentum	0.7	4.1	0.8	8.2
Salmonella enterica	1.9	16.4	1.8	8.2
Escherichia coli	2.3	8.2	2.1	8.2
Pseudomonas aeruginosa	1.6	8.2	1.6	8.2
Candida albicans	2.6	16.4	3.0	16.4

The essential oil from leaves of the first *M. hypolampra* (HG1803) showed stronger inhibitory effects on the three Gram-positive test microorganisms and one test fungus than that from the second one (HG1813). This is in contrast to the case of the three Gram-negative test microorganisms. MIC values of the first oil were from 4.1 to 16.4 mg/mL, while those of the second oil were from 8.2 to 16.4 mg/mL. IC_{50} values of the first and second oils ranged from 0.3 to 2.7 mg/mL and from 0.3 to 3.0 mg/mL, respectively. *B. subtilis* and *L. fermentum* were more sensitive to the essential oils than the other tested microorganisms (Table 3). *S. aureus* is a bacterium that can cause pains, burns, sore throats, pus infections on the skin and internal organs; *B. subtilis* can contaminate food; *L. fermentum* is used for a wide variety of

applications that include food and feed fermentation because it is a "friendly" bacterium in animals, *S. enterica*can cause some diseases such as: gastroenteritis, bacteremia, enteric fever, and an asymptomatic carrier state, *E. coli* can cause some gastrointestinal diseases including gastritis, colitis, enterocolitis, bacillary dysentery, *P. aeruginosa* can cause infections in some organs such as urinary tract, respiratory system, soft tissue, bone and joint, gastrointestinal tract and some other diseases like dermatitis and bacteremia, while *C. albicans*can cause thrush in children and gynecological diseases.

The first major component in *M. kwangsiensis* leaf essential oils, β-ocimene, is one of the most ubiquitous volatiles in floral scents [27] that has a pleasant or and is used in perfumery. Screening of antimicrobial activity of β-ocimene showed its MIC values of 1.3, 1.3, and 2.5 mg/mL against *Bacillus cereus*, *Staphylococcus aureus*, and *Escherichia coli* [28] using micro broth dilution technique. While the second major component, myrcene, was identified as analgesic component in the essential oil [29] and presented sedative as well as motor relaxant effects [30]. Research on antimicrobial activity of mycrene against *Escherichia coli*, and *Escherichia coli* showed MIC values > 20 µg/mL [31]. Other study reported that myrcene had MIC values from 0.5 - > 1.0% against *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Escherichia coli*, and *Haemophilus influenza* [32]. Myrcene was evaluated not antibacterial against *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli*, and *Klebsiella pneumonia* [33] or antifungal against *Candida albicans*, that had MIC values > 8% and > 2% and minimum fungicidal (MFC) values also > 8% and > 2% by the broth micro dilution and macro dilution methods, respectively [34]. The analgesic and fragrant properties of β-ocimene and myrcene, two major components of *M. kwangsiensis* leaf essential oil should be the potential for the use in health-care and fragrance fields.

Antimicrobial activities of essential oil from other *Magnolia* species were reported in literature. It was indicated that magnolol, honokiol, and 3,5′-diallyl-2′-hydroxy-4-methoxy biphenyl of *M. grand flora* exhibited significant activity against Gram-positive and acid-fast bacteria and fungi [35]. Other research reported that essential oil from *M. grand flora* leaves had antimicrobial activity against *Staphylococcus aureus* and *Streptococcus pyogenes* with MIC of 500 µg/mL and 125 µg/mL, respectively [36]. Volatile oil of aerial parts of *M. foveolata* (syn. *Michelia foveolata*) exhibited a significant antibacterial activity against *Salmonella enterica*, *Staphylococcus epidermidis*, *Staphylococcus aureus* and *Bacillus cereus* [37]. Antimicrobial activity of essential oil of *M. ovata* (syn. *Talauma ovata*) changed during year: The oil from leaves collected in October was the most active and the oil from trunk bark collected in January had the highest activity [38]. The leaf essential oil of *M. gloriensis* (syn. *Talauma gloriensis*), with myrcene (31.7%) and germacrene D (43.5%) as the major components, was devoid of antibacterial activity against *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* [39]. In another study, the essential oil of *M. lili flora* inhibited growth of test fungal strains with the MIC concentration from 125 to 500 µg/mL and MFC concentration from 125 to 1,000 µg/mL of the essential oil determined [40].

4. Conclusions

The present work reports, for the first time, chemical composition and antimicrobial activity of two essential oil samples from leaves of *Magnolia kwangsiensis* Figlar & Noot collected in Vietnam. In total, 75 and 62 compounds were identified in two oil samples. The oils exhibited antimicrobial activity against 7 tested microbial strains with MIC values ranged from 4.1 to 16.4 mg/mL and from 8.2 to 16.4 mg/mL, and IC_{50} values ranged from 0.3 to 2.7 mg/mL and from 0.3 to 3.0 mg/mL.

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