ACADEMIA JOURNAL OF BIOLOGY 2020, 42(3): 135–144
DOI: 10.15625/2615-9023/v42n3.14739

CHEMICAL COMPOSITION AND ANTIMICROBIAL ACTIVITY OF THE ESSENTIAL OIL FROM LEAVES OF *Magnolia coriacea* (Hung T. Chang & B. L. Chen) Figlar GROWING IN VIETNAM

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Received 27 December 2019, accepted 6 August 2020

ABSTRACT

Leaf essential oil of *Magnolia coriacea* (Hung T. Chang & B. L. Chen) Figlar growing wild in the Bat Dai Son Nature Reserve, Ha Giang Province, Viet Nam was obtained by hydrodistillation and its chemical composition was analyzed using GC/MS. In total, 45 compounds were detected in the essential oil, accounting for 87.1% of the oil, in which 37 compounds were identified accounting for 66.9%. Bicyclogermacrene (12.6%) and spathulenol (17.0%) were the main components of the leaf essential oil of *M. coriacea*. Antimicrobial activity of the essential oil sample was tested against three microorganism strains using an agar disk diffusion method. The results show that the inhibitory zone diameters ranged from 8.5 to 20.5 mm. Median inhibitory concentration (IC_{50}) and minimum inhibitory concentration (MIC) of the essential oil was determined using microdilution broth susceptibility assay against seven test microorganism strains. *Bacillus subtilis* had the highest sensitivity with IC_{50} and MIC values of 185.9 and 512 µg/mL, respectively.

Keywords: Magnoliaceae, *Magnolia coriacea*, antimicrobial activity, essential oil composition, Nature Reserve.

Citation: Chu Thi Thu Ha, Bui Van Thanh, Dinh Thi Thu Thuy, 2020. Chemical composition and antimicrobial activity of the essential oil from leaves of *Magnolia coriacea* (Hung T. Chang & B. L. Chen) Figlar growing in Vietnam. *Academia Journal of Biology*, 42(3): 135–144. https://doi.org/10.15625/2615-9023/v42n3.14739.

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INTRODUCTION

Magnolia coriacea (Hung T. Chang & B.L.Chen) Figlar is known as Giổi lá dai, Giổi đá in Vietnam. *Michelia coriacea* Hung T.Chang & B.L.Chen, *M. nitida* B. L. Chen and *M. polyneura* C. Y. Wu ex Y. W. Law & Y. F. Wu are the synonyms of *M. coriacea*. This tree species belonging to the genus of *Magnolia* L., family of Magnoliaceae Juss. can grow up to 15–20 m high, leaves alternate, coriaceous, green, glossy above, slightly wavy leaf margins. Young twigs and stipules have pale white to light yellowish gray pubescences. Cylindrical shoots are covered with thick fuzz, silvery-white to light yellow-gray, before young leaves are present, the buds are crooked like tea hooks; young leaves do not curl up in the bud. Petioles are without stipule scar. Flower buds appear in January to April, flowers bloom in May and June. Fruit ripen and contain mature seeds in September to October of the year (Chen, 1988; Tu et al., 2014). *Magnolia coriacea* grow in evergreen forest, limestone mountain areas at 1,000–1,700 m a.s.l. In the past, *M. coriacea* was considered as endermic species of China (Chen, 1988), then its distribution in Vietnam was recorded in 2014 (Tu Bao Ngan et al., 2014). In addition to Ha Giang Province (Quan Ba District), distribution of *M. coriacea* was also recorded in Cao Bang and Son La provinces of Vietnam. *Magnolia coriacea* was ranked at level of critically endangered-CR B2ab(i,ii,iii,v) (Cicuzza et al., 2007) and at level of endangerd-EN B1ab (iii,v) (IUCN, 2014).

The previous topics of studies on *M. coriacea* focused on karyomorphology (Zhang & Xia, 2007), sexual development (Zhao et al., 2009) and genetic diversity (Zhao et al., 2012). Study on the volatile compositions of leaf and twig essential oil of *M. coriacea* sampled in China indicated that essential oil consists of 7 main constituents: α-farnesene, β-maaliene, aromadendrene, germacrene B, germacrene D, valencene, and β-elemene (Ma et al., 2011) or it consists of four main constituents: α-farnesene, β-maaliene, germacrene B, and valencene (Ma et al., 2012). In the present study, the authors would like to report on chemical composition and antimicrobial activity of leaf essential oil of *M. coriacea* growing in Ha Giang Province, Vietnam.

MATERIALS AND METHODS

Plant material

Fresh leaves of *M. coriacea* were collected in April 2018 at near the top of a lime stone mountain in Bat Dai Son commune belonging to the Bat Dai Son Nature Reserve, Quan Ba District, Ha Giang Province, Vietnam (N23º08.050’; E104º59.761’; 1.161 m a.s.l.). Botanical identification was performed indivisually by Dr. Nguyen Tien Hiep, Centre for Plant Conservation of Vietnam, Ha Noi and MSc. Trinh Ngoc Bon, Vietnamese Academy of Forest Sciences, Ha Noi. A voucher specimen (HG1801) was deposited to the Herbarium of the Institute of Ecology and Biological Resources (HN), Vietnam Academy of Science and Technology, Ha Noi.

Hydrodistilation of essential oil

An amount of 1.3 kg sample of fresh leaves were shredded and hydrodistilled for 4 hours using a Clevenger type apparatus. The principle of hydrodistilation was based on Ministry of Health (2009). Then, essential oil was separated and stored at (-)5 ºC until analysis.

Microbial strains

The antimicrobial activity of the essential oils was evaluated using 1 strain each of Gram-positive test bacteria *Staphylococcus aureus* (ATCC 13709), Gram-negative test bacteria *Escherichia coli* (ATCC 25922) and yeast *Candida albicans* (ATCC 10231). The minimum inhibitory concentration (MIC) and median inhibitory concentration (IC_{50}) values of the essential oil sample was determined using three above mentioned strains of microorganisms and two other strains of Gram-positive test bacteria, *Bacillus subtilis* (ATCC 6633) and *Lactobacillus fermentum* (VTCC N4), and two other strains of Gram-negative test bacteria, *Salmonella enterica* (VTCC) and *Pseudomonas aeruginosa* (ATCC 15442). The ATCC strains were obtained from American Type Culture Collection; the VTCC strains were obtained from the Vietnam Type Culture Collection, Institute of Microbiology and Biotechnology, Vietnam National University, Ha Noi.

Gas chromatography - mass spectrometry

Composition analysis of the essential oil was carried out 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 with an interphase temperature of 270 °C. The split ratio was 1:100, the detector temperature was 270 °C, and the injection volume was 1 μL. The MS interface temperature was 270 °C, MS mode, E.I. detector voltage 1200 V, 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 contents were calculated based on total ion current without standardization. Data processing software was MassFinder4.0 (Adams, 2002; König et al., 2019).

Screening of antimicrobial activity

The agar disk diffusion method was used to test the antimicrobial activity of essential oil (Bauer et al., 1966; Jorgensen & Ferraro, 2009; Balouiri et al., 2016). 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 hours. The presence or absence of growth around each hole containing antimicrobial agent on each plate culture was observed. The values of inhibition growth zone diameters 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 (Mothana & Lindequist, 2005; Philip et al., 2009).

Minimum inhibitory concentration (MIC) and median inhibitory concentration (IC_{50}) values were measured by the microdilution broth susceptibility assay (Hadacek & Greger, 2000; Cos et al., 2006). Stock solutions of the oil were prepared in dimethylsulfoxide (DMSO). Dilution series were prepared from 512 μg/mL to 2 μg/mL (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 hours, 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:

$$\%\text{inhibition} = \frac{\text{OD}_{\text{control}(+)} - \text{OD}_{\text{test agent}}}{\text{OD}_{\text{control}(+)} - \text{OD}_{\text{control}(-)}} \times 100\%$$

$$\text{IC}_{50} = \text{High}_{\text{Conc}} - \frac{\left(\text{High}_{\text{Inh\%}} - 50\%\right) \times \left(\text{High}_{\text{Conc}} - \text{Low}_{\text{Conc}}\right)}{\left(\text{High}_{\text{Inh\%}} - \text{Low}_{\text{Inh\%}}\right)}$$

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. $\text{High}_{\text{Conc}}$/$\text{Low}_{\text{Conc}}$: Concentration of test agent at high concentration/low concentration; $\text{High}_{\text{Inh\%}}$/$\text{Low}_{\text{Inh\%}}$: % 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 µg/mL to 1.2 µg/mL, Cefotaxime for Gram-negative bacterial strains with MIC values in the range of 0.07–19.23 µg/mL, Nystatine for fungal strain with MIC value of 2.8 µg/mL.

Statistical Analysis

Average and standard seviation values of diameters of microorganism inhibition zone in the test were calculated using software Excel.

RESULTS AND DISCUSSION

Chemical composition of *Magnolia coriacea* essential oil

By hydrodistillation, esential oil from leaves of *M. coriacea* obtained was pale yellow liquid having lower density than water. The chemical composition of the leaf essential oil of *M. coriacea* from Bat Dai Son Nature Reserve is summarized in table 1.

Essential oil yield of 0.074% (v/w), calculated on a dry weight basis, was obtained from the leaves of *M. coriacea*. A total of 45 compounds were found in the essential oil, representing 87.1%, in which 37 compounds were identified representing 66.9% of the oil compositions.

Sesquiterpenoids were predominant in the leaf essential oil of *M. coriacea* representing 65.0% of the 66.9% of identified components. Among them, sesquiterpene hydrocarbons consisted of 19 compounds representing 33.7%, and oxygenated sesquiterpenoids consisted of 11 compounds representing 31.4%. In contrast, the amount of monoterpenoids was very small (2.0%) in the leaf essential oil of *M. coriacea*, in which monoterpene hydrocarbons comprised 3 compounds accounting for 0.5%, and oxygenated monoterpenoids comprised 1 compound accounting for 0.7%. Total amount of benzenoids was 0.4% (2 compounds). Other compounds consisted of 3 constituents representing 0.7% of essential oil concentration.

Bicyclogermacrene and spathulenol were the main constituents of the leaf essential oil of *M. coriacea* accounting for respective 12.6% and 17.0% of oil concentration. The most abundant minor components were; *cis*-β-elemene (5.11%) and humulene epoxide II (5.4%). The rest of the identified components of the leaf essential oil of *M. coriacea* were present at the amount ranging from 0.1–3.7% (Table 1).

Previous study indicated that bicyclogermacrene had an anti-mosquito effect. Specifically, the 50% lethal concentration (LC_{50}) of this substance for the exposed *Anopheles subpictus* (a vector of malaria), *Aedes albopictus* (a vector of virus), and *Culex tritaeniorhynchus* (a vector of Japanese encephalitis) were 10.3 µg/mL, 11.1 µg/mL and 12.5 µg/mL, respectively (Govindarajan & Benelli, 2016). Spathulenol has *in vitro* growth inhibition and bactericidal activity against *Mycobacterium tuberculosis* (Dzul-Beh et al., 2019). β-elemene has anti-inflammatory and anti-cancer effects; β-elemene improves motor disability and reduces optic neuritis in rats with encephalitis and spondylitis-a type of autoimmune disease tested (Zhang et al., 2011).

Table 1. Compositions of the leaf essential oil of *Magnolia coriacea*

Nº	RI	Components	%
1	855	(*Z*)-Hex-3-en-1-ol	0.26
2	984	β-Pinene	0.20
3	993	2-Pentylfuran	0.27
4	1034	Limonene	0.16
5	1049	(*E*)-β-Ocimene	0.10
6	1103	Linalool	0.71
7	1161	unknown (124, 124, RI 1161)	1.06
8	1348	δ-Elemene	0.94
9	1385	α-Ylangene	0.54
10	1390	α-Copaene	0.14
11	1400	β-Bourbonene	0.27
12	1404	*cis*-β-Elemene	5.11
13	1409	Methyl eugenol	0.20
14	1437	(*E*)-β-Caryophylene	0.73
15	1446	β-Gurjunene (=Calarene)	0.47
16	1457	α-Guaiene	0.17
17	1469	unknown (204, 204, RI 1469)	1.00
18	1472	α-Humulene	2.42
19	1479	9-*epi*-(*E*)-Caryophyllene	0.84
20	1499	Germacrene D	3.51
21	1505	β-Selinene	1.84
22	1513	(*E,E*)-α-Farnesene	1.95
23	1516	Bicyclogermacrene	12.64
24	1531	γ-Cadinene	0.45
25	1538	δ-Cadinene	0.88
26	1549	Zonarene	0.17
27	1562	α-Calacorene	0.16
28	1566	Elemol	0.41
29	1572	(*E*)-Nerolidol	0.63
30	1579	Germacrene B	0.43
31	1584	Dendrolasin	0.20
32	1602	Spathulenol	17.04
33	1607	Caryophyllene oxide	3.73
34	1622	Humulene epoxide I	0.44
35	1630	*epi*-Cedrol	0.33
36	1634	Humulene epoxide II	5.35
37	1650	unknown (119, 220, RI 1650)	2.51
38	1655	unknown (81, 220, RI 1655)	1.00
39	1660	unknown (119, 220, RI 1660)	3.83
40	1676	α-Cadinol	2.08
41	1685	*neo*-Intermedeol	0.75
42	1693	unknown (205, 220, RI 1693)	1.07
43	1696	Cyperol	0.41

44	1877	unknown (43, 250, RI 1877)	4.58
45	1884	unknown (79, 248, RI 1884)	5.15
Monoterpene hydrocarbons			0.46
Oxygenated monoterpenoids			0.71
Sesquiterpene hydrocarbons			33.66
Oxygenated sesquiterpenoids			31.37
Other compounds			0.73
Benzenoids			0.36
Unknown compounds			66.93
Total			87.13

Note: RI: Retention indices.

Comparison of the results of the chemical composition analysis of the leaf essential oil of *M. coriacea* in this study with the previously published data showed the remarkable difference. Ma et al. (2011, 2012) reported that the composition of volatile compounds in *M. coriacea* leaves in China under the synonyms of *Michelia polyneura* C. Y. Wu ex Law et Y. F. Wu (Ma et al., 2011) and of *Michelia coriacea* H. T. Chang et B. L. Chen (Ma et al., 2012) consisted of 26 and 20 compounds, respectively. In their reports, the main compounds of two samples of *M. coriacea* leaf essential oils have different points, including 7 constituents: α-farnesene, β-maaliene, aromadendrene, germacrene B, germacrene D, valencene, and β-elemene (Ma et al., 2011) or including only 4 constituents: α-farnesene, β-maaliene, germacrene B, and valencene (Ma et al., 2012). In the present study, leaf essential oil of *M. coriacea* in Vietnam contained bicyclogermacrene (12.6%) and spathulenol (17.0%) as the main constituents, and *cis*-β-elemene (5.1%) and humulene epoxide II (5.4%) as the most abundant minor components. (*E*,*E*)-α-farnesene and germacrene B presented at very low concentration (2.0% and 0.4%, respectively). β-maaliene, aromadendrene, germacrene D or valencene were not detected in the *M. coriacea* leaf essential oil in the present study. These results showed the variety of chemical compositions of the essential oils of different *M. coriacea* leaf samples, despite the common biosynthetic precursors, possibly due to different habitat and sample collection times.

The chemical composition of the *M. coriacea* leaf oil in this study is different from the chemical composition of other essential oils in the genus of *Magnolia* L. Only in a few species, for example, *M. gloriensis* (syn. *Talauma gloriensis*), its essential oil composition is rich in sesquiterpenoids (Haber et al., 2008) like in the case of *M. coriacea* species in the current study. Many studied species in the genus *Magnolia* L. have monoterpenoid content that accounts for the majority of essential oils including: *M. acuminata*, *M. calophylla*, *M. virginiana* (Farag et al., 2015), *M. hypolampra* (Liu et al., 2007; Chu et al., 2019), *M. kwangsiensis* (Huang et al., 2010; Zheng et al., 2015; Zheng et al., 2019), and *M. sieboldii* (Sun et al., 2014). However, *M. grandiflora* and *M. ovata* are different from the above mentioned species because their essential oil constituents may be monoterpenoids (Apel et al., 2009; Farag et al., 2015) or sesquiterpenoids (Wang et al., 2009; Scharf et al., 2016).

Antimicrobial activity of *Magnolia coriacea* leaf essential oil

The antimicrobial activity of the *M. coriacea* leaf essential oil was assessed using the standard agar disk diffusion method against three test microorganisms. The results obtained after 18–24 hours of incubation are presented in table 2.

M. coriacea leaf essential oil exhibited moderate inhibitory activity against *Escherichia coli*, and strong activity against *Staphylococcus aureus* and *Candida albicans*

(Mothana & Lindequist, 2005; Philip et al., 2009) with the inhibitory zone diameters ranging from 8.5 to 20.5 mm. Of the three strains tested, *E. coli* was more tolerant to the *M. coriacea* leaf essential oil than the other two strains. The value of the diameter of the microbiological inhibitory zone was 8.5 ± 0.70 mm for *E. coli*, whereas that was 16 ± 1.41 mm for *S. aureus* and 20.5 ± 0.70 mm for *C. albicans*.

Table 2. Anti-yeast and antibacterial activity of leaf essential oil of *Magnolia coriacea* (average ± standard deviation, n = 2)

Inhibition zones (mm)		
Staphylococcus aureus	*Escherichia coli*	*Candida albicans*
16 ± 1.41	8.5 ± 0.70	20.5 ± 0.7

Then, the minimum inhibitory concentration (MIC) and median inhibitory concentration (IC_{50}) values of the *M. coriacea* leaf essential oil were determined using seven strains of microorganisms. The results obtained after 16–24 hours are presented in table 3. The IC_{50} values of *M. coriacea* leaf essential oil for *B. subtilis* and *S. aureus* are 186 and 451 µg/mL, respectively. Other five strains of tested microorganisms were more resistant to *M. coriacea* leaf essential oil, with IC_{50} values higher than 512 µg/mL. The MIC value of the leaf essential oil for *B. subtilis* was 512 µg/mL and those for six other microorganisms tested were higher than 512 µg/mL (Table 3). Thus, out of seven strains of microorganisms studied, *B. subtilis* is the most sensitive bacteria for *M. coriacea* leaf essential oil.

Table 3. Microbial minimum inhibitory (MIC) concentrations and median inhibitory (IC_{50}) concentrations of leaf essential oil of *Magnolia coriacea*

Mico-organisms	IC_{50} (µg/mL)	MIC (µg/mL)
Staphylococcus aureus	450.7	> 512
Bacillus subtilis	185.9	512
Lactobacillus fermentum	> 512	> 512
Salmonella enterica	> 512	> 512
Escherichia coli	> 512	> 512
Pseudomonas aeruginosa	> 512	> 512
Candida albicans	> 512	> 512

The antimicrobial activity of essential oils extracted from different species of the genus *Magnolia* L. exhibited varying intensities and properties. *Magnolia liliflora* essential oil inhibited the growth of tested strains of fungi with MIC and minimum fungicide concentration (MFC) from 125 µg/mL to 500 µg/mL and from 125 µg/mL to 1,000 µg/mL, respectively (Bajpai and Kang, 2012). *Magnolia grandiflora* leaf oil had MIC for *Staphylococcus aureus* and *Streptococcus pyogenes* bacteria of 500 µg/mL and 125 µg/mL, respectively (Guerra-Boone et al., 2013). In addition, the antimicrobial activity of essential oils of the same plant may vary seasonally throughout the year, as was the case for *M. ovata* (syn. *Talauma ovata*). Specifically, essential oil from its leaves collected in October was the most active, inhibiting 19 of the 22 tested strains of microorganisms, while essential oil from its bark collected in January had the growth inhibiting activity against 15 out of 22 strains of tested microorganisms (Stefanello et al., 2008).

CONCLUSIONS

The content of essential oil obtained from *M. coriacea* leaves was 0.074% (v/w) calculated on a dry weight basis. In the chemical composition of *M. coriacea* leaf essential oil, 37 compounds were identified in total of 45 constituents discovered. Among them, two compounds, bicyclogermacrene (12.6%) and spathulenol (17.0%), were the main components of *M. coriacea* leaf essential oil.

M. coriacea leaf essential oil had the strongest growth inhibitory activity against *C. albicans* among three microorganisms tested using the standard agar disk diffusion method, with inhibitory zone diameter of 20.5 mm. The microdilution broth susceptibility assay for seven strains of microorganisms tested showed that *B. subtilis* is the most sensitive bacteria for *M. coriacea* leaf essential oil.

Acknowledgements:

This work was supported by the Vietnam National Foundation for Science and Technology Development (NAFOSTED) under Grant 106.03-2019.16.

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