SHORT REPORT

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Composition and Antimicrobial Activity of Essential Oils from Leaves, Twigs and Ripe Fruits of *Magnolia grandis* (Hu & W.C. Cheng) V.S.Kumar in Ha Giang Province of Vietnam

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Abstract: The leaves, twigs and ripe fruits of *Magnolia grandis* (Hu & W.C.Cheng) V.S.Kumar, growing wild in Ha Giang Province of Vietnam, were hydrodistilled to obtain essential oils which had the respective average yields of 0.09%, 0.25% and 0.62% (v/w), calculated on a dry weight basis. The oils were analyzed using gas chromatography-flame ionization detector (GC-FID) and gas chromatography-mass spectrometry (GC-MS). Major components of these three oil samples were: α-pinene (11.8%), linalool (15.4%) and (*E*)-β-caryophyllene (10.7%) (leaf oil); α-pinene (42.8%) and β-pinene (23.7%) (twig oil); α-pinene (52.2%) (ripe fruit oil). The essential oils from leaves showed stronger inhibitory effects on the seven tested microorganism strains than those from twigs and ripe fruits. To our best knowledge, this is the first time that information on essential oils of *M. grandis* leaves, twigs and ripe fruits is reported.

Keywords: *Magnolia grandis*; antimicrobial activity; essential oil composition; α-pinene.

1. Plant Source

In the framework of intensive studies on plant resources containing essential oils of *Magnolia* species in Vietnam, we investigated composition and antimicrobial activity of essential oils from leaves, twigs and ripe fruits of *M. grandis* in Ta Poc mountains, Tung Vai Commune, Quan Ba District, Ha Giang Province (23°03′21.3″N, 104°50′50.7″E, 1323 m a.s.l), Vietnam in September

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2019. The voucher specimens (HG1924) were deposited at the Herbarium of Institute of Ecology and Biological Resources (HN), Vietnam Academy of Science and Technology. Herein, the chemical composition as well as antimicrobial activity of essential oils from leaves, twigs and ripe fruits of this *Magnolia* species are reported.

2. Previous Studies

Magnolia grandis (syn. *Manglietia grandis* Hu & W.C.Cheng) is a large evergreen tree species that distributes in China and Vietnam [1-3]. The species is used for timber [2] and evaluated as critically endangered species [4]. Some studies involved in this species includes: Sowing seeds and planting the seedlings in Ha Giang province of Vietnam by FFI (Fauna & Flora International) [5], research on seed germination in China [6], and research on genetic diversity and population differentiation in China [7]. Studies on the essential oil of this species are not found until now.

3. Present Study

Hydrodistillation of 1.6-2.5 kg of each of the shredded fresh leaves, twigs and ripe fruits of *M. grandis* produced pale yellow oils with the respective essential oil yields of 0.09 ± 0.01%, 0.25 ± 0.01, and 0.62 ± 0.02 calculated on a dry weight basis.

The average relative densities of the oils were d^{20} = 0.90, 0.87, and 0.87, the refractive indices were n^{20} = 1.48, 1.47, and 1.47; and the optical rotations were $[\alpha]_D^{20}$ = -16.13°, -17.80°, and +20.32°, respectively.

The specific activity of the ripe fruit oil (+20.32°) was remarkably different from those of the leaf and twig oils (-16.13° and -17.80°, respectively), suggesting that there are more of the dextrorotary enantiomers of some of the main constituents (*α*-pinene, *β*-pinene, linalool, and (*E*)-*β*-caryophyllene) in the fruit oil than those in the other oils. It's probably due to *α*-pinene with high concentrations in the oils. Especially, (+)-*α*-pinene with its high value of optical activity (+52.4°) could be the major enantiomer in the fruit oil [8].

The identification of compounds present in the *M. grandis* essential oils collected in Ta Poc Mountains, Tung Vai Commune, Quan Ba District, Ha Giang Province, Vietnam was carried out using mass spectral (MS) and retention index (RI) data. Table 1 presents the identified compounds in order of their elution on the HP-5MS column used for the GC-MS analysis.

A total of 48, 37 and 37 compounds representing 98.0%, 99.7% and 98.3% of the compositions were identified in the leaf, twig and ripe fruit essential oils, respectively, of *M. grandis*. These were comprised of monoterpene hydrocarbons (25.7%, 77.8% and 70.9%), oxygenated monoterpenes (31.8%, 13.3% and 12.5%), sesquiterpene hydrocarbons (23.9%, 5.3% and 9.8%), oxygenated sesquiterpenes (16.2%, 3.2% and 4.5%) of the respective leaf, twig, and ripe fruit oils. Benzenoid aromatics were only 1.0%, 0.6% and 1.2% of respective oil concentrations.

In the leaf oil, the major constituents were *α*-pinene (11.8%), linalool (15.4%), and (*E*)-*β*-caryophyllene (10.7%). Other notable constituents in leaf oil were *β*-pinene (7.4%), 1,8-cineole (4.7%), and *α*-humulene (5.3%). In the twig oil, the major constituents were *α*-pinene (42.8%) and *β*-pinene (23.7%). Following this, limonene (3.7%), 1,8-cineole (5.6%) and (*E*)-*β*-caryophyllene (3.0%) had significant amounts in the twig oil. In contrast to the leaf and twig oils, the ripe fruit oil contained *α*-pinene (52.2%) as its unique major compound. Other constituents that were present in the ripe fruit oil with sizable amounts consisted of: *β*-pinene (7.7%), 1,8-cineole (3.9%), and (*E*)-*β*-caryophyllene (6.0%) (Table 1).

The common feature of these oil samples was that they contain *α*-pinene as a main component of the oils. In addition, all of three analyzed oil samples were richer in monoterpene compounds than in sesquiterpene compounds. The main compounds in the oils of *M. grandis* species in the present study were different to those of other *Magnolias*, for example, (*Z*)-*β*-ocimene (36.5%), (*E*)-*β*-ocimene (30.8%) and germacrene A (9.6%) were the main compounds of *M. acuminata* leaf oil; *β*-pinene (64.4% and 37.4%) of *M. calophylla* and *M. virginiana* leaf oils; (*Z*)-*β*-ocimene (15.2%), germacrene A (12.9%) and *β*-bisabolene (13.3%) of *M. grandiflora* leaf oil [9]; *β*-pinene (23.0%, 32.3%, 12.7%

and 6.9%), 1,8-cineole (4.1%, 4.4%, 4.5% and 12.2%), *β*-elemene (13.6%, 7.7%, 12.9% and 5.7%) were the main compounds of *M. grandiflora* leaf, flower, immature fruit and mature fruit oils [10]; *α*-terpinene (12.73%), bicyclogermacrene (10.25%), and aristolene (28.51%) were the main compounds of *M. figo* leaf oi [11], naphthalene (35.1%), and *α*-bulnesene (10.1%) were the main compounds of *M. ovata* (syn. *Talauma ovata*) fruit oi [12]. The variation of essential oil of one plant species or of different plant species may due to various factors such as seasons in the year [13,14], development stage of plants [15], geographic regions, etc., as well as genetic differences.

Table 1. Essential oil composition (%) of the leaves, twigs and ripe fruits of *M. grandis*

Compounds[a]	RI[b]	RI[c,d,e]	Leaves[f]	Twigs[f]	Ripe fruits[f]
α-Thujene	930	909 – 937[c]	-	0.3	-
α-Pinene	939	912 - 944[c]	11.8	42.8	52.2
Camphene	955	930 - 969[c]	1.2	2.6	1.6
Sabinene	978	945 - 980[c]	0.3	0.3	-
β-Pinene	984	958 - 997[c]	7.4	23.7	7.5
Myrcene	991	955 - 998[c]	0.7	1.7	3.3
α-Phellandrene	1009	966 - 1010[c]	-	-	0.2
α-Terpinene	1021	1007 - 1057[c]	0.2	0.4	-
o-Cymene	1029	1015 - 1039[c]	0.4	0.5	0.5
Limonene	1033	995 - 1037[c]	1.8	3.7	1.9
β-Phellandrene	1035	995 - 1036[c]	0.7	0.7	3.5
1,8-Cineole	1037	1002 - 1046[c]	4.7	5.6	3.9
(*E*)-*β*-Ocimene	1048	1015 - 1056[c]	0.5	0.2	0.2
γ-Terpinene	1062	1023 - 1067[c]	0.3	0.5	-
cis-Sabinene hydrate	1072	1033 - 1099[c]	0.2	-	-
Terpinolene	1094	1052 - 1094[c]	0.2	0.4	-
Linalool	1101	1062 - 1125[c]	15.4	2.2	1.8
trans-Sabinene hydrate	1104	1033 - 1112[c]	0.2	-	0.1
endo-Fenchol	1121	1111 - 1134[c]	-	0.1	-
trans-Sabinol	1148	1136 - 1149[c]	-	-	0.2
cis-Sabinol	1151	1136 - 1149[c]	-	-	0.2
Borneol (=*endo*-Borneol)	1174	1134 - 1205[c]	1.7	0.5	0.6
Terpinen-4-ol	1185	1140 - 1207[c]	1.0	1.3	0.4
α-Terpineol	1197	1153 - 1224[c]	1.9	1.8	1.4
Methyl chavicol (=Estragole)	1203	1190 - 1203[c]	-	-	0.1
Myrtenol	1204	1176 - 1209[c]	-	-	0.1
Citronellol	1228	1212 - 1266[c]	1.1	0.2	1.6
Nerol	1231	1202 - 1237[c]	0.1	-	0.2
Chavicol	1253	1259[c]	0.4	0.1	0.6
Geraniol	1255	1232 - 1301[c]	3.4	0.4	0.5
Geranial	1273	1269 - 1273[c]	0.2	-	-
Bornyl acetate	1293	1236 - 1295[c]	1.0	1.0	1.3
Geranyl acetate	1383	1377 - 1396[c]	1.1	0.2	0.1
α-Copaene	1389	1335 - 1419[c]	0.2	0.2	0.2
Cascarilladiene	1432	1436[d]	1.5	-	-
(*E*)-*β*-Caryophyllene	1437	1395 - 1462[c]	10.7	3.0	6.0
Aromadendrene	1456	1429 - 1472[c]	0.3	-	-
α-Humulene	1471	1418 - 1495[c]	5.3	1.3	2.8
δ-Selinene	1504	1483 - 1500[c]	1.1	0.2	0.2
(*E*,*E*)-*α*-Farnesene	1511	1463 - 1545[c]	2.4	-	-
α-Selinene	1512	1470 - 1503[c]	0.8	0.2	0.2
γ-Cadinene	1530	1470 - 1553[c]	0.2	-	-
δ-Cadinene	1536	1480 - 1562[c]	0.5	0.2	0.4
trans-Calamenene	1537	1495 - 1528[c]	0.2	-	-
Elemol	1562	1545 - 1565[c]	-	0.2	-
(*E*)-Nerolidol	1568	1530 - 1583[c]	4.1	-	-

Table 1 continued..

Eudesma-5,7(11)-diene	1572	1543[e]	0.7	0.2	-
Spathulenol	1596	1543 - 1624[c]	0.3	-	-
Caryophyllene oxide	1603	1548 - 1611[c]	4.2	0.7	2
Humulene epoxide I	1618	1607[c]	0.3	-	-
Humulene epoxide II	1630	1579 - 1652[c]	1.5	0.2	0.8
γ-Eudesmol	1649	1621 - 1647[c]	0.6	0.4	0.2
epi-α-Cadinol (= τ-Cadinol)	1656	1616 - 1665[c]	0.5	-	-
β-Eudesmol	1670	1642 - 1667[c]	1.5	0.8	0.5
α-Eudesmol	1673	1649 - 1670[c]	1.6	0.9	0.7
14-hydroxy-9-*epi*-(*E*)-Caryophyllene	1688	1702[c]	-	-	0.3
(*E*,*E*)-Farnesol	1726	1722 - 1727[c]	1.6	-	-
Total identified			**98.0**	**99.7**	**98.3**
Monoterpene hydrocarbons			25.7	77.8	70.9
Oxygenated monoterpenes			31.8	13.3	12.5
Sesquiterpene hydrocarbons			23.9	5.3	9.8
Oxygenated sesquiterpenes			16.2	3.2	4.5
Others			0.4	0.1	0.6

Note: [a]Elution order on HP-5MS column; [b]Retention indices on HP-5MS column; [c,d,e]Literature retention indices [c][16] on HP-5MS column; [d][17]; [e][18]; [f]Standard deviation were insignificant and excluded from the Table to avoid congestion; (-) Not identified.

Essential oils of *M. grandis* were then determined the minimum inhibitory concentration (MIC) and median inhibitory concentration (IC_{50}) values through microbroth dilution assays [19-20] using 7 strains of microorganisms: *Staphylococcus aureus*, *Bacillus subtilis*, and *Lactobacillus fermentum*, *Salmonella enterica*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Candida albicans.* The results of the assay evaluated after 16-24 hours of incubation are presented in Table 2. Although the antimicrobial activities of *M. grandis* essential oils were marginal at best [20], the leaf essential oil from *M. grandis* expressed stronger inhibitory effects on the seven test microorganisms than the twig and ripe fruit oils. IC_{50} values of the *M. grandis* leaf, twig and ripe fruit oils were from 683 to 7851 µg/mL, from 1499 to 10,012 µg/mL and from 939 to 6599 µg/mL. MIC values of these oils ranged from 2048 to > 16,384 µg/mL, from 8192 to > 16,384 µg/mL and from 4096 to > 16,384 µg/mL, respectively. *S. aureus*, *E. coli*, and *C. albicans* were more sensitive to the essential oils than four other tested microorganisms (Table 2). The higher activity of the *M. grandis* leaf oil could be associated with higher amounts of oxygenated monoterpenes and oxygenated sesquiterpenes than the ones in the twig and ripe fruit oils. Previous study demonstrated that essential oils rich in oxygenated compounds are more active as antimicrobial agents than essential oils with large amounts of hydrocarbons [21].

Table 2. MIC and IC_{50} of essential oils from leaves, twigs and ripe fruits of *M. grandis*

Essential oil samples	**Leaves**		**Twigs**		**Ripe fruits**	
Value (µg/mL)	IC_{50}	MIC	IC_{50}	MIC	IC_{50}	MIC
S. aureus	683	4096	1499	8192	939	4096
B. subtilis	2979	16,384	3959	>16,.384	4578	>16,384
L. fermentum	7851	>16,384	10,012	>16,384	6599	>16,384
S. enterica	4096	16,384	2993	16,384	4876	>16,384
E. coli	1365	4096	1994	8192	1832	8192
P. aeruginosa	3823	>16,384	7877	>16,384	6144	>16,384
C. albicans	728	2048	1950	8192	2010	8192

The antimicrobial activity of essential oils varying on different microorganisms can be derived from their main compounds or the synergism of many of their components. α-pinene, β-pinene, linalool, and (*E*)-β-caryophyllene being main components of essential oil samples in the present study may contribute the great role in antimicrobial activities. In the past, antimicrobial potential of some essential oil components against some tested microbial strains were presented as: Linalyl acetate < limonene < β-pinene < α-pinene < camphor < linalool < 1,8-cineole < menthol < thymol < carvacrol

[22]. Other previous studies indicated the antimicrobial activity of (*E*)-*β*-caryophyllene against different microbial strains including *S. aureus*, *E. coli* [23-25] with its lower antimicrobial activity against *E. coli* than those of *β*-pinene, linalool [24] or higher than those of *α*-pinene, *β*-pinene [25]. It was commented that the magnitude of synergic interaction between different essential oils was low but the synergism between essential oils and food additives was strong [21]. This leads to using smaller amounts of flavored food preservatives that should be helpful for food industry.

In conclusion, the research results provide the first information on the chemical composition and antimicrobial activity of the essential oils from leaves, twigs and ripe fruits of *M. grandis*. Among 48, 37 and 37 compounds identified in of the oils, major components consisted of: *α*-pinene (11.8%), linalool (15.4%), and (*E*)-*β*-caryophyllene (10.7%) (leaf oil); *α*-pinene (42.8%) and *β*-pinene (23.7%) (twig oil); and α-pinene (52.2%) (ripe fruit oil). The leaf essential oil from *M. grandis* had the stronger inhibitory effects on the seven tested microorganisms than the twig and ripe fruit oils. The results of present study can be the basis for future research on the field of food and beverage industry as flavoring and preservative agents.

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Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/journal/records-ofnatural-products

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References

[1] B. N. Tu, Q. H. Nguyen and T. H. Nguyen (2015). Five new records of *Magnoliaceae Juss* for the flora of Vietnam. Proceedings of the 6th National conferences on ecology and biological resources; Oct 21st; Hanoi, Vietnam. Institute of Ecology and Biological Resources, 243-248.

[2] B. L. Chen and H. P. Nooteboom (1993). Notes on Magnoliaceae III: The Magnoliaceae of China, *Ann. Missouri Bot. Gard.* **80**(**4**), 999-1104.

[3] The plant list (2021). http://www.theplantlist.org/tpl1.1/record/kew-344365, (retrieved Dec. 12, 2021).

[4] The IUCN Red List of Threatened Species version 2021-1, 2021: https://www.iucnredlist.org/. Downloaded on 20 May 2021.

[5] D. Gill (2017). Sowing the seeds of hope for critically endangered magnolia tree. Fauna & Flora International. https://www.fauna-flora.org/news/sowing-the-seeds-of-hope-for-critically-endangered-magnolia-tree (retrieved June 2, 2021).

[6] R. Pan and W. B. Sun (2009). Seed dormancy and germination of the critically endangered *Manglietia grandis* Hu et Cheng, *Plant Physiol. Commun.* **45**, 1089–1092.

[7] T. Xu (2020). The genetic diversity and population differentiation of *Magnolia grandis*, an endangered and potential herbal medicine plant in China, *Basic Clin. Pharmacol. Toxicol.* **126**, 38-39.

[8] S. Dev (Ed.) (1982). CRC Handbook of Terpenoids - Monoterpenoids, Volume II, CRC Press, Florida, p. 449.

[9] M. A. Farag, R. S. E. Din and S. Fahmy (2015). Headspace analysis of volatile compounds coupled to chemometrics in leaves from the Magnoliaceae family, *Rec. Nat. Prod*. **9** (**1**), 153-158.

[10] A. Ali, N, Tabanca, B, Demirci, V. Raman, J. M. Budel, K. H. C. Baser and A. A. Khan (2020). Insecticidal and biting deterrent activities of *Magnolia grandiflora* essential oils and selected pure compounds against *Aedes aegypti*, *Molecules* **25**, 1359.

[11] Y. Ma, H. Zhang, R. Song, Z. Wang and B. Yang (2012). Study on antimicrobial activity and chemical components of essential oil from *Michelia figo* Spreng leaves, *Adv. Mat. Res.* **518-523**, 509-515.

[12] M. É. A. Stefanello and R. de Mello-Silva (2005). Volatile constituents of *Talauma ovata* A. St.-Hil. fruits, *J. Essent. Oil Res*. **17**(**4**), 455-456.

[13] J. H. G. Lago, O. A. Fávero and P. Romoff (2009). Chemical composition and seasonal variation of the volatile oils from leaves of *Michelia champaca* L., Magnoliaceae, *Rev. Bras. Farmacogn.* **19**(**4**)**,** 880-882.

[14] M. É. A. Stefanello, M. F. Salvador, I. Y. Ito, A. Wisniewski Jr, E. L. Simionatto and R. de Mello-Silva (2008). Chemical composition, seasonal variation and evaluation of antimicrobial activity of essential oils of *Talauma ovata* A. St. Hil. (Magnoliaceae), *J. Essent. Oil Res.* **20**(**6**), 565-569.

[15] S. Sanimah, R. Suri, R. N. Azizun, A. Hazniza, M. Radzali, I. Rusli and M. D. Hassan (2008). Volatile compounds of essential oil from different stages of *Michelia alba* (cempaka putih) flower development, *J. Trop. Agric. and Fd. Sc.* **36** (**1**), 109–119.

[16] P. J. Linstrom and W. G. Mallard (2021). NIST chemistry webbook, NIST standard reference database number 69, National Institute of Standards and Technology, Gaithersburg M. D., 20899, (retrieved June 2, 2021).

[17] P. Pripdeevech, S. Wongpornchai and A. Promsiri (2006). Highly volatile constituents of *Vetiveria zizanioides* roots grown under different cultivation conditions, *Molecules* **11**(**10**), 817-826.

[18] A. Wajs-Bonikowska, M. Sienkiewicz, A. Stobiecka, A. Maciąg, Ł. Szoka and E. Karna (2015). Chemical composition and biological activity of *Abies alba* and *A. koreana* seed and cone essential oils and characterization of their seed hydrolates, *Chem. Biodivers*. **12** (**3**)**,** 407-418.

[19] F. Hadacek and H. Greger (2000). Testing of antifungal natural products methodologies, comparability of result and assay choice, *Phytochem. Anal*. **11**, 137-147.

[20] P. Cos, A. J. Vlietinck, D. V. Berghe and L. Maes (2006). Anti-infective potential of nature products: How to develop a stronger in vitro 'proof-of-concept', *J. Ethnopharmacol.* **106** (**3**), 290-302.

[21] D. Kalemba and A. Kunicka (2003) Antibacterial and antifungal properties of essential oils. *Current Med. Chem.* **10,** 813–829.

[22] M. Soković, J. Glamočlija, P. D. Marin, D. Brkić and L. J. L. D. van Griensven (2010). Antibacterial effects of the essential oils of commonly consumed medicinal herbs using an *in vitro* model, *Molecules* **15**, 7532-7546.

[23] M. C. S. Neta, C. Vittorazzi, A. C. Guimarães, J. D. L. Martins, M. Fronza, D. C. Endringer and R. Scherer (2017). Effects of β-caryophyllene and *Murraya paniculata* essential oil in the murine hepatoma cells and in the bacteria and fungi 24-h time–kill curve studies. *Pharm. Biol.* **55** (**1**)**,** 190-197.

[24] S. K. Nguikwie, M. A. Nyegue, F. N. F. Belinga, R. A. N. Ngane, B. Romestand, A, Kouzayha, H. Casabianca, P. H. A. Zollo and C. Menut (2013). The chemical composition and antibacterial activities of the essential oils from three *Aframomum* species from Cameroon, and their potential as sources of (*E*)-(*R*)-nerolidol, *Nat. Prod. Commun.* **8** (**6**)**,** 829-834.

[25] C. Yang, D. H. Hu and Y. Feng (2015). Antibacterial activity and mode of action of the *Artemisia capillaris* essential oil and its constituents against respiratory tract infection-causing pathogens, *Mol. Med. Rep.* **11**, 2852-2860.