SHORT REPORT

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Composition and Antimicrobial Activity of Essential Oils from Leaves and Twigs of *Magnolia hookeri* var. *longirostrata* D.X.Li & R. Z. Zhou and *Magnolia insignis* Wall. in Ha Giang Province of Vietnam

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Abstract: The essential oils from leaves and twigs of *Magnolia hookeri* var. *longirostrata* D.X.Li & R.Z.Zhou and *Magnolia insignis* Wall., growing wild in Ha Giang Province of Vietnam, were obtained by hydrodistillation and analyzed by gas chromatography-flame ionization detector (GC-FID) and gas chromatography-mass spectrometry (GC-MS). The respective yields of the *M. hookeri* var. *longirostrata* leaf and twig oils were 0.14% and 0.05% (v/w), and of the *M. insignis* leaf and twig oils were 0.16% and 0.05% (v/w), calculated on a dry weight basis. Major components of the oils of *M. hookeri* var. *longirostrata* were: Linalool (21.3%), (*E*)-nerolidol (12.2%) and *neo*-intermedeol (13.5%) (leaf oil); 1,8-cineole (13.3%) and linalool (17.1%) (twig oil). Major components of the oils of *M. insignis* were: Linalool (24.1%), geraniol (14.9%) and (*E*)-nerolidol (22.5%) (leaf oil); 1,8-cineole (9.5%) and linalool (26.9%) (twig oil). The essential oils from *M. insignis* showed stronger inhibitory effects on the seven test microorganisms than those from *M. hookeri* var. *longirostrata*. *Candida albicans* and *Lactobacillus fermentum* were more sensitive to the essential oils than the other tested microorganisms. This is the first time information on essential oils of *M. hookeri* var. *longirostrata* leaves and twigs and of *M. insignis* twigs are reported.

Keywords: *Magnolia hookeri* var. *longirostrata*; *Magnolia insignis*; antimicrobial activity; essential oil.

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1. Plant Source

The leaves and twigs of M. hookeri var. longirostrata were collected in Tung Vai Commune, Quan Ba District, Ha Giang Province (23º07˙16.6"N, 104º55'26.5"E, 1088m a.s.l), Vietnam in September 2019. The leaves and twigs of M. insignis were collected in Du Gia Nature Reserve, Du Gia Commune, Yen Minh District, Ha Giang Province (22º52'49.6"N, 105º13'38.1"E, 1631m a.s.l), Vietnam in November 2019. Botanical identification were performed by Assoc. Prof. Dr. Vu Quang Nam (at the Vietnam National University of Forestry, Ha Noi) and the voucher specimens (HG1919 and HG1932) were deposited at the Herbarium of Institute of Ecology and Biological Resources (HN), Vietnam Academy of Science and Technology.

2. Previous Studies

The two species are large evergreen trees belonging to *Magnolia* genus. *M. hookeri* var. *longirostrata* is a new variety that was found in China [1] and recorded later in Vietnam [2]. *M. insignis* distributes in Nepal, India, China, Burma, Myanmar, Thailand, and Vietnam [3-5] with different synonyms (syn. *Magnolia insignis* var. *angustifolia*, *Magnolia insignis* var. *latifolia*, *Magnolia shangpaensis*, *Manglietia insignis*, *Manglietia insignis* var. *angustifolia*, *Manglietia insignis* var. *latifolia*, *Manglietia maguanica*, *Manglietia rufisyncarpa*, *Manglietia yunnanensis*) [6]. In traditional medicine, *M. insignis* is used for treating chest and abdominal pain, indigestion, asthma, and dysentery [7]. Some phytochemical studies on chemical composition, structure, and bioactivity of compounds isolated from leaves and/or twigs of *M. insignis* have been presented in the literature [8,9]. Studies on the essential oil of these two *Magnolia* species are limited except the composition and antibacterial and antitumor activities of essential oil distilled from leaves of *M. insignis* [10,11].

3. Present Study

Hydrodistillation of fresh leaves and twigs of two *Magnolias* produced light yellow oils. Essential oil yields of 0.14 ± 0.01% and 0.05 ± 0.01% (v/w, leaves and twigs of *M. hookeri* var. *longirostrata*), and 0.16 ± 0.01% and 0.05 ± 0.01% (v/w, leaves and twigs of *M. insignis*) calculated on a dry weight basis were obtained, respectively. 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 35 and 59 compounds representing 96.5% and 97.4% of the compositions were identified in the leaf and twig essential oils, respectively, of *M. hookeri* var. *longirostrata*. These were comprised of monoterpene hydrocarbons (1.9% and 7.1%), monoterpenoids (41.5% and 45.1%), sesquiterpene hydrocarbons (13.5% and 11.1%), sesquiterpenoids (39.6% and 33.9%) of the respective leaf and twig oils. In the leaf oil, the major constituents were linalool (21.3%), (*E*)-nerolidol (12.2%) and *neo*-intermedeol (13.5%). Additionally, the most abundant minor components of the leaf oil were geraniol (8.4%) and α-selinene (5.5%). In the twig oil, the major constituents were 1,8-cineole (13.3%) and linalool (17.1%). In addition, significant quantities of β-eudesmol (5.7%), α-eudesmol (5.7%), and bulnesol (6.8%) were also present in the twig oil (Table 1).

On the other hand, 54 and 56 compounds representing 96.6% and 95.2% of the compositions were identified in the leaf and twig essential oils of *M. insignis*, respectively. These consisted of monoterpene hydrocarbons (3.8% and 9.3%), monoterpenoids (46.5% and 56.3%), sesquiterpene hydrocarbons (7.6% and 5.2%), sesquiterpenoids (38.1% and 24.4%) of the leaf and twig oils, respectively. The major components of the leaf oil were linalool (24.1%), geraniol (14.9%) and (*E*)-nerolidol (22.5%). In the twig oil, 1,8-cineole (9.5%) and linalool (26.9%) were the major components. In addition, geraniol (8.5%) had significant amount in the twig oil (Table 1).

The common feature of these oil samples was that linalool was the predominant component of the oils. In addition, all of four analyzed oil samples contained higher amount of terpenoids than those of hydrocarbons. The high contents of compounds containing oxygen in the essential oils of these two species are in agreement with the oil of some Magnoliaceae samples [12] but are different from oil constituents of some others [13]. The main compounds in the oils of two *Magnolia* 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 [13].

The present results of leaf oil of *M. insignis* are different from data in previous reports [10,11]. In this study, 54 compounds were identified in the oil with linalool (24.1%), geraniol (14.9%) and (*E*)-nerolidol (22.5%) as main components. While, in the previous study, among 16 constituents, (*E*)-nerolidol (38.8%), 2,2-dicyclohexylpropanedinitrile (the identification of this compound is doubtful; the compound is not found in the *Dictionary of Natural Products* (2019)) [14] (13.2%), δ-cadinene (7.8%), geraniol (6.4%) were the main compounds of the oil [10]. In another report, 53 constituents were identified in the oil with germacrene B (7.7%), α-cadinol (6.70%), (*E*)-nerolidol (6.1%) and globulol (5.6%) were its main components [11].

Table 1. Essential oil composition (%) of the leaves and twigs of *M. hookeri* var. *longirostrata* (HG1919) and *M. insignis* (HG1932)

Compounds[a]	RI[b]	RI[c]	*M. hookeri* var. *longirostrata*		*M. insignis*	
			Leaves[e]	Twigs[e]	Leaves[e]	Twigs[e]
α-Pinene	938	939	0.1	0.7	0.6	1.9
Camphene	955	954	-	0.3	0.4	1.4
β-Pinene	984	982[d]	-	0.1	0.3	0.5
Myrcene	991	991	-	0.2	0.2	0.4
2,3-Dehydro-1,8-cineol	995	993[d]	-	0.1	-	-
α-Terpinene	1021	1017	-	-	-	0.4
p-Cymene	1029	1026	1.1	3.4	0.4	1.5
Limonene	1033	1029	0.7	2.3	0.5	1.9
β-Phellandrene	1035	1030	-	-	-	0.2
1,8-Cineole	1037	1038[d]	4.4	13.3	2.8	9.5
(*E*)-β-Ocimene	1048	1050	-	-	1.3	0.2
γ-Terpinene	1063	1060	-	-	0.1	0.4
trans-Linalool oxide (furanoid)	1076	1073	0.2	0.1	-	0.3
p-Cymenene	1094	1094[d]	-	0.1	-	-
Terpinolene	1094	1095[d]	-	-	-	0.5
Linalool	1102	1097[d]	21.3	17.1	24.1	26.9
Hotrienol	1106	1109 [d]	-	-	-	0.2
(*E*)-4,8-Dimethylnona-1,3,7-triene	1117	1116 [d]	-	-	0.1	-
endo-Fenchol	1121	1119[d]	-	0.1	-	-
Camphor	1155	1156[d]	-	-	-	0.2
Camphene hydrate	1158	1157[d]	-	0.2	-	0.4
iso-Isopulegol	1164	1160	-	-	-	0.1
δ-Terpineol	1173	1173[d]	-	0.1	-	-
Borneol (=*endo*-Borneol)	1174	1176[d]	-	-	0.6	1.1
Terpinen-4-ol	1185	1184[d]	1.8	3.3	0.6	1.8
α-Terpineol	1197	1196[d]	4.6	7.9	0.3	0.8
Methyl salicylate	1202	1203[d]	-	-	0.2	-
Citronellol	1228	1226	0.3	-	1.6	1.5
Nerol	1231	1230	0.2	0.2	0.2	0.2
Neral	1245	1244[d]	-	-	0.2	0.3
Geraniol	1256	1253	8.4	2.3	14.9	8.5
Piperitone	1263	1263[d]	-	-	0.2	0.5
Geranial	1273	1273[d]	-	-	0.4	0.5
Bornyl acetate	1293	1292[d]	-	0.2	0.5	2.8
Geranyl acetate	1383	1383 [d]	0.3	0.2	0.1	0.7
α-Ylangene	1384	1377[d]	-	0.4	-	-
α-Copaene	1389	1387[d]	-	0.3	-	-
(*E*)-Caryophyllene	1436	1433[d]	-	0.3	0.9	0.8
α-*trans*-Bergamotene	1445	1436[d]	-	0.4	-	-
α-Guaiene	1451	1448[d]	-	0.2	-	-
Aromadendrene	1456	1449[d]	-	-	0.8	0.6
(*Z*)-β-Farnesene	1459	1457[d]	-	0.3	0.2	
β-Barbatene	1464	1458 [d]	-	-	-	0.1

α-Humulene	1471	1465[d]	-	0.1	0.6	0.5
β-Chamigrene	1488	1490[d]	1.4	0.3	0.2	
γ-Muurolene	1489	1485[d]	-	0.5	0.2	0.3
ar-Curcumene	1490	1488[d]	-	0.3	-	-
α-Amorphene	1493	1488[d]	-	0.3	-	-
α-Zingiberene	1497	1497[d]	-	-	0.7	-
β-Selinene	1503	1498[d]	4.4	1.5	0.4	0.6
δ-Selinene	1504	1504[d]	-	-	0.3	-
trans-Muurola-4(14),5-diene	1510	1494	-	-	-	0.3
α-Selinene	1512	1504[d]	5.5	2.2	-	0.6
α-Muurolene	1513	1514[d]	-	-	1.7	0.3
β-Bisabolene	1517	1517[d]	-	0.4	-	-
α-Bulnesene (=δ-Guaiene)	1520	1526[d]	-	0.2	-	-
γ-Cadinene	1529	1528[d]	0.3	0.6	0.4	0.3
δ-Cadinene	1535	1530[d]	0.5	1.5	0.9	0.6
trans-Calamenene	1537	1532[d]	0.5	0.6	0.3	0.2
α-Calacorene	1558	1550[d]	0.4	0.4	-	-
Elemicine	1559	1560 [d]	-	-	0.2	-
(*E*)-Nerolidol	1569	1560[d]	12.2	2.7	22.5	5.2
Dendrolasin	1582	1581[d]	0.6	-	0.5	-
Caryophyllenyl alcohol	1590	1572	0.2	0.4	0.2	0.5
Spathulenol	1595	1590[d]	-	0.1	-	-
Viridiflorol	1603	1598[d]	-	0.3	2.4	1.9
Caryophyllene oxide	1603	1601[d]	0.2	-	-	-
Guaiol (=Champacol)	1612	1603[d]	0.6	2.3	2.7	2.4
Cubeban-11-ol	1613	1601[d]	-	-	-	-
Rosifoliol	1620	1615[d]	-	-	0.5	-
epi-Cedrol	1625	1619	-	0.5	-	-
Humulene epoxide II	1630	1616[d]	-	-	-	0.5
1,10-di-*epi*-Cubenol	1633	1623[d]	-	-	0.5	0.2
Dill apiole	1635	1634[d]	-	-	0.1	-
10-*epi*-γ-Eudesmol	1641	1629[d]	-	-	0.7	0.5
1-*epi*-Cubenol	1645	1629	0.4	0.3	0.2	0.2
γ-Eudesmol	1649	1647[d]	0.7	3.9	0.7	2.1
epi-α-Cadinol (=τ-Cadinol)	1657	1659[d]	1.7	0.9	1.3	1.7
epi-α-Muurolol (=τ-Muurolol)	1659	1660[d]	0.6	0.8	-	-
α-Muurolol (=δ-Cadinol)	1662	1654[d]	-	0.5	0.7	0.8
β-Eudesmol	1671	1667[d]	-	5.7	-	3.3
α-Cadinol	1672	1673[d]	2.1		1.4	-
α-Eudesmol	1673	1670[d]	-	5.7	2.6	4.2
neo-Intermedeol	1676	1670[d]	13.5	0.3	-	-
Bulnesol	1684	1678[d]	1.5	6.8	0.8	0.8
Cadalene	1692	1684[d]	0.5	0.3	-	-
epi-α-Bisabolol	1695	1692[d]	0.2	0.2	-	-
α-Bisabolol	1696	1696[d]	0.3	0.2	-	-
(*E*,*E*)-Farnesol	1727	1727[d]	4.8	2.3	0.4	0.1
Benzyl benzoate	1779	1774[d]	-	0.2	-	-
Total identified			96.5	97.4	96.6	95.2
Monoterpene hydrocarbons			1.9	7.1	3.8	9.3
Monoterpenoids			41.5	45.1	46.5	56.3
Sesquiterpene hydrocarbons			13.5	11.1	7.6	5.2
Sesquiterpenoids			39.6	33.9	38.1	24.4
Benzenoid aromatics			0	0.2	0.5	0
Others			0	0	0.1	0

Note: [a]Elution order on HP-5MS column; [b]Retention indices on HP-5MS column; [c,d]Literature retention indices [c][15]; [d][16]; [f]Standard deviation were insignificant and excluded from the Table to avoid congestion; (-) Not identified.

The essential oil samples were then subjected to microbroth dilution assays [17-18] to determine the minimum inhibitory concentration (MIC) and median inhibitory concentration (IC_{50}) values 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 obtained after 16-24 hours of incubation are presented in Table 2. The leaf and twig essential oils from *M. insignis* showed stronger inhibitory effects on the seven test microorganisms than those from *M. hookeri* var. *longirostrata*. MIC values of the *M. insignis* leaf and twig oils were from 512 to 4096 µg/mL. IC_{50} values of the *M. insignis* leaf and twig oils ranged from 9.2 to 825 µg/mL and from 25 to 951 µg/mL, respectively. The oil from *M. hookeri* var. *longirostrata* twigs had the lowest inhibitory effects on test microorganisms with MIC and IC_{50} values were from 2048 to more than 8192 µg/mL and from 491 to 3662 µg/mL, respectively. *C. albicans* and *L. fermentum* were more sensitive to the essential oils than the other tested microorganisms (Table 2).

Table 2. MIC and IC_{50} of essential oils from leaves and twigs of *M. hookeri* var. *longirostrata* (HG1919) and *M. insignis* (HG1932)

Esential oil samples	***M. hookeri* var. *longirostrata* leaves**		***M. hookeri* var. *longirostrata* twigs**		***M. insignis* leaves**		***M. insignis* twigs**	
Value (µg/mL)	IC_{50}	MIC	IC_{50}	MIC	IC_{50}	MIC	IC_{50}	MIC
S. aureus	994	4096	1896	8192	582	1024	750	2048
B. subtilis	452	2048	924	4096	161	1024	304	1024
L. fermentum	278	1024	491	2048	37	512	62	512
S. enterica	1536	8192	3288	> 8192	819	4096	941	4096
E. coli	1399	4096	2003	8192	647	2048	805	4096
P. aeruginosa	1831	8192	3662	> 8192	825	4096	951	4096
C. albicans	896	4096	1920	8192	9.2	512	25	512

In the previous study, leaf oil of *M. insignis* had some antibacterial activities to *Rhodotorula glutinis*, but had no inhibition against *E. coli* and *S. aureus* [10]. 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. Linalool, 1,8-cineole, geraniol, (*E*)-nerolidol, and *neo*-intermedeol being main components of essential oil samples in the present study may contribute the great role in antimicrobial activities because they belong to group of oxygenated terpenes as previously attributed [19]. In the past, antimicrobial activities of linalool and 1,8-cineole against some tested microbial strains were shown with their MIC values from 4 to 7 µg/mL [19] and from lower than 90 to 380 µg/mL [20]. Other researches indicated that the respective MIC values of geraniol and (*E*)-nerolidol against some tested microbial strains were from 30 to 70 µg/mL [21] and from 125 to 500 µg/mL [22]. The research results of antimicrobial activity of essential oils, especially leaf oil of *M. insignis* can be the basis for future applied research on adding to food as flavoring and preservative agents.

As a conclusion, The present study is the first of its kind that provided information on the chemical composition and antimicrobial activity of the essential oils from leaves and twigs of *M. hookeri* var. *longirostrata* and from twigs of *M. insignis*. Among 35 and 59 compounds identified in of the oils of *M. hookeri* var. *longirostrata*, major components consisted of: Linalool (21.3%), (*E*)-nerolidol (12.2%) and *neo*-intermedeol (13.5%) (leaf oil); 1,8-cineole (13.3%) and linalool (17.1%) (twig oil). Major components of the oils of *M. insignis* were: Linalool (24.1%), geraniol (14.9%) and (*E*)-nerolidol (22.5%) among 54 compounds of the leaf oil; 1,8-cineole (9.5%) and linalool (26.9%) among 56 compounds of the twig oil. The leaf essential oil from *M. insignis* had the strongest inhibitory effects on the seven test microorganisms with respective IC_{50} and MIC values from 9.2 to 825 µg/mL and from 512 to 4096 µg/mL. The results of present study can be the basis for future research on the field of food industry as flavoring and preservative agents.

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Supporting Information

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