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 (21.3%), 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. © 2020 ACG Publications. All rights reserved.
1. Plant Source

The leaves and twigs of M. hookeri var. longirostrata were collected in Tung Vai Commune, Quan Ba District, Ha Giang Province (23°07′16.6″N, 104°55′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 (22°52′49.6″N, 105°13′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 shanpaensis, Manglietia insignis, Manglietia insignis var. angustifolia, Manglietia insignis var. latifolia, Manglietia maguanica, Manglietia rafisyncarpa, 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 (6.7%), (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 | RI² | RI² | M. hookeri var. longirostrata | M. insignis |
|-----------|-----|-----|-------------------------------|-------------|
|           | Leaves | Twigs | Leaves | Twigs |
| α-Pinene | 938 | 939 | 0.1 | 0.7 | 0.6 | 1.9 |
| Camphene | 955 | 954 | - | 0.3 | 0.4 | 1.4 |
| β-Pinene | 984 | 982² | - | 0.1 | 0.3 | 0.5 |
| Myrcene | 991 | 991 | - | 0.2 | 0.2 | 0.4 |
| 2,3-Dehydro-1,8-cineol | 995 | 993² | - | 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 | 1038² | 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 (furand) | 1076 | 1073 | 0.2 | 0.1 | - | 0.3 |
| p-Cymenone | 1094 | 1094² | - | 0.1 | - | - |
| Terpinolene | 1094 | 1095² | - | - | - | 0.5 |
| Linalool | 1102 | 1097² | 21.3 | 17.1 | 24.1 | 26.9 |
| Hotrienol | 1106 | 1109² | - | - | - | 0.2 |
| (E)-4,8-Dimethylnona-1,3,7-triene | 1117 | 1116² | - | - | 0.1 | - |
| endo-Fenchol | 1121 | 1119² | - | 0.1 | - | - |
| Camphor | 1155 | 1156² | - | - | - | 0.2 |
| Camphene hydrate | 1158 | 1157² | - | 0.2 | - | 0.4 |
| iso-Isopulegol | 1164 | 1160 | - | - | - | 0.1 |
| δ-Terpinol | 1173 | 1173² | - | 0.1 | - | - |
| Borneol (=endo-Borneol) | 1174 | 1176² | - | - | 0.6 | 1.1 |
| Terpinien-4-ol | 1185 | 1184² | 1.8 | 3.3 | 0.6 | 1.8 |
| α-Terpinol | 1197 | 1196² | 4.6 | 7.9 | 0.3 | 0.8 |
| Methyl salicylate | 1202 | 1203² | - | - | 0.2 | - |
| Citronellol | 1228 | 1226 | 0.3 | - | 1.6 | 1.5 |
| Nerol | 1231 | 1230 | 0.2 | 0.2 | 0.2 | 0.2 |
| Neral | 1245 | 1244² | - | - | 0.2 | 0.3 |
| Geraniol | 1256 | 1253 | 8.4 | 2.3 | 14.9 | 8.5 |
| Piperitone | 1263 | 1263² | - | - | 0.2 | 0.5 |
| Geranial | 1273 | 1273² | - | - | 0.4 | 0.5 |
| Bornyl acetate | 1293 | 1292² | - | 0.2 | 0.5 | 2.8 |
| Geranyl acetate | 1383 | 1383² | 0.3 | 0.2 | 0.1 | 0.7 |
| α-Ylangene | 1384 | 1377² | - | 0.4 | - | - |
| α-Copaene | 1389 | 1387² | - | 0.3 | - | - |
| (E)-Caryophyllene | 1436 | 1433² | - | 0.3 | 0.9 | 0.8 |
| α-trans-Bergamotene | 1445 | 1436² | - | 0.4 | - | - |
| α-Guaicene | 1451 | 1448² | - | 0.2 | - | - |
| Aromadendrene | 1456 | 1449² | - | - | 0.8 | 0.6 |
| (Z)-β-Farnesene | 1459 | 1457² | - | 0.3 | 0.2 | - |
| β-Barbatene | 1464 | 1458² | - | - | - | 0.1 |
### Essential oil of two Magnolia species

| Compound                  | Molecular Formula | Retention Index | Relative Abundance |
|---------------------------|-------------------|-----------------|--------------------|
| α-Humulene                | C19H26O             | 1471            | 1465              |
| β-Chamigrene              | C19H24O             | 1488            | 1490              |
| γ-Murolene                | C19H28O             | 1489            | 1485              |
| ar-Curcumene              | C18H24O             | 1490            | 1488              |
| α-Amorphene               | C18H22O             | 1493            | 1488              |
| α-Zingiberene             | C19H22O             | 1497            | 1497              |
| β-Selinene                | C19H28O             | 1503            | 1498              |
| δ-Selinene                | C18H26O             | 1504            | 1504              |
| trans-Muurola-4(14),5-diene|                   | 1510            | 1494              |
| α-Selinene                | C19H28O             | 1512            | 1504              |
| α-Murolene                | C19H26O             | 1513            | 1514              |
| β-Bisabolene              | C19H24O             | 1517            | 1517              |
| α-Bulnesene (=δ-Guaiene)  | C19H24O             | 1520            | 1526              |
| γ-Cadinene                | C19H24O             | 1529            | 1528              |
| δ-Cadinene                | C19H24O             | 1535            | 1530              |
| trans-Calamenene          | C19H22O             | 1537            | 1532              |
| α-Calacorene              | C20H30O             | 1558            | 1550              |
| Elemicin                  | C18H20O             | 1559            | 1560              |
| (E)-Nerolidol             | C15H24O             | 1569            | 1560              |
| Dendrolasin               | C18H26O             | 1582            | 1581              |
| Caryophyllenyl alcohol    | C15H22O             | 1590            | 1572              |
| Spathulenol               | C18H24O             | 1595            | 1590              |
| Viridiflorol              | C18H24O             | 1603            | 1598              |
| Caryophyllene oxide       | C15H22O             | 1603            | 1601              |
| Guaiol (=Champacol)       | C18H24O             | 1612            | 1603              |
| Cubeab-11-ol              | C18H24O             | 1613            | 1601              |
| Rosifoliol                | C15H22O             | 1620            | 1615              |
| epí-Cedrol                | C16H22O             | 1625            | 1619              |
| Humulene epoxide II       | C19H26O             | 1630            | 1616              |
| 1,10-di-epí-Cubenol       | C17H24O             | 1633            | 1623              |
| Dill apiole               | C15H20O             | 1635            | 1634              |
| 10-epí-γ-Eudesmol         | C15H22O             | 1641            | 1629              |
| 1-epí-Cubenol             | C16H22O             | 1645            | 1629              |
| γ-Eudesmol               | C17H22O             | 1649            | 1647              |
| epí-α-Cadinol (=α-Cadinol)| C17H22O             | 1657            | 1659              |
| epí-α-Murolol (=α-Murolol)| C17H22O             | 1659            | 1660              |
| α-Murolol (=δ-Cadinol)    | C17H22O             | 1662            | 1654              |
| β-Eudesmol               | C17H22O             | 1671            | 1667              |
| α-Cadinol                | C17H22O             | 1672            | 1673              |
| α-Eudesmol               | C17H22O             | 1673            | 1670              |
| neo-Intermedeol          | C18H22O             | 1676            | 1670              |
| Bulnesol                 | C18H24O             | 1684            | 1678              |
| Cadalen                  | C17H22O             | 1692            | 1692              |
| epí-α-Bisabolol           | C17H22O             | 1695            | 1692              |
| α-Bisabolol              | C17H22O             | 1696            | 1696              |
| (E,E)-Farnesol           | C18H24O             | 1727            | 1727              |
| Benzyl benzoate          | C17H18O             | 1779            | 1774              |

| Total identified | 96.5 | 97.4 | 96.6 | 95.2 |

**Note:** *Retention order on HP-5MS column; Retention indices on HP-5MS column; *"*Literature retention indices [15]; [16]; 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 (IC₅₀) 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. IC50 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 IC50 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 IC50 of essential oils from leaves and twigs of *M. hookeri* var. *longirostrata* (HG1919) and *M. insignis* (HG1932)

| Essential oil samples | *M. hookeri* var. *longirostrata* leaves | *M. hookeri* var. *longirostrata* twigs | *M. insignis* leaves | *M. insignis* twigs |
|-----------------------|------------------------------------------|----------------------------------------|---------------------|---------------------|
| Value (µg/mL)         | IC50 | MIC | IC50 | MIC | IC50 | MIC | IC50 | 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 59 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 IC50 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**

Supporting Information accompanies this paper on [http://www.acgpubs.org/journal/records-of-natural-products](http://www.acgpubs.org/journal/records-of-natural-products)

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