

## CHEMICAL CONSTITUENTS AND ANTIMICROBIAL ACTIVITY OF *Tsoongiodendron odorum* AND *Manglietia chevalieri* ESSENTIAL OILS FROM VIETNAM

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*Tsoongiodendron odorum* Chun (syn. *Magnolia odora* (Chun) Figlar & Noot.) is one of the valuable trees of Vietnamese flora [1]. Phytochemical studies revealed the isolation of alkaloids, lignans, sesquiterpene lactone and other compounds from *T. odorum* [2–4]. The compounds costunolide, parthenolide, dihydroparthenolide, liriodenine, and 2,3-dihydroxyl-2-methyl butyrolactone isolated from *T. odorum* exhibited cytotoxic activities against a variety of tumor cell strains [5]. *Manglietia chevalieri* Dandy (syn. *Magnolia chevalieri* (Dandy) V.S. Kumar) (Myrtaceae) is a tree that grows up to 10 m tall. The bark is grayish brown, while the leaf blade is reddish brown. Two new neolignan sesquiterpenoids namely, chevalierinol A and chevalierinol B, were characterised recently from the plant [6].

The aim of the present study is to report the chemical constituents and antimicrobial activity of essential oils from the leaves of *T. odorum* and *M. chevalieri* collected in Vietnam for the first time. The leaves of *T. odorum* and *M. chevalieri* were collected at an elevation of 337 m, in April 2020 from Pu Hoat Natural Reserve (GPS 19°42'18"N, 104°49'42"E), Vietnam. Both plants were identified by Assoc. Prof. Le Thi Huong and voucher specimens LTH 883 and 902, respectively, were deposited in the plant specimen room at Vinh University, Vietnam. 2.0 kg of each plant were subjected to separate hydrodistillation using a Clevenger-type apparatus as described previously [7–10].

All experimental procedures used in this study were similar to those described earlier in our previous published studies [7–10]. The identification of constituents from the individual GC/MS spectra of *T. odorum* and *M. chevalieri* was performed, based on retention indices (RI) determined with reference to a homologous series of *n*-alkanes (C<sub>6</sub>–C<sub>40</sub>), under identical experimental conditions [11].

The minimum inhibitory concentration (MIC) and median inhibitory concentration (IC<sub>50</sub>) values were measured by the microdilution broth susceptibility assay [7–10]. DMSO was used as a negative control. Streptomycin was used as the antibacterial standard while nystatin and cycloheximide were used as antifungal standards. All experiments were performed in triplicate.

The essential oils obtained from the leaves of *T. odorum* and *M. chevalieri* were light-yellow coloured. The yields of the essential oils were 0.18% and 0.15%, respectively. The classes of compounds identified in the leaves of *T. odorum* were monoterpene hydrocarbons (25.4%), monoterpene oxygenated (4.3%), sesquiterpene hydrocarbons (45.7%), oxygenated sesquiterpene (10.9%), diterpenes (1.2%), and nonterpenes (3.3%), as shown in Table 1. The major compounds of the essential oil of *T. odorum* were  $\beta$ -pinene (20.4%),  $\beta$ -caryophyllene (6.4%), and  $\alpha$ -humulene (5.6%). Among the terpenes, there are significant quantities of *cis*- $\beta$ -elemene (3.8%),  $\alpha$ -muurolene (3.7%),  $\delta$ -cadinene (3.3%), and  $\alpha$ -pinene (3.2%), while 2-hydroxy-4-methoxyacetophenone (3.3%) was present among the nonterpenes. On the other hand, monoterpene hydrocarbons (5.6%), monoterpene oxygenated (3.9%), sesquiterpene hydrocarbons (61.4%), oxygenated sesquiterpenes (10.4%), and nonterpenes (2.0%) constitute the bulk of the essential oil of *M. chevalieri*. The compounds occurring in higher amounts were *cis*- $\beta$ -elemene (12.7%),  $\beta$ -caryophyllene (8.0%),  $\beta$ -selinene (7.4%),  $\alpha$ -selinene (6.8%), and (*E,E*)- $\alpha$ -farnesene (5.4%).

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TABLE 1. Chemical Constituents of Essential Oils of *Tsoongiodendron odorum* (*T. o.*) and *Manglietia chevalieri* (*M. c.*), %

Compound <sup>a</sup>	RI <sup>b</sup>	<i>T. o.</i>	<i>M. c.</i>	Compound <sup>a</sup>	RI <sup>b</sup>	<i>T. o.</i>	<i>M. c.</i>
$\alpha$ -Pinene	939	3.2	1.6	$\beta$ -Selinene	1503	2.2	7.4
Camphene	955	0.3	0.2	$\gamma$ -Amorphene	1510	0.9	–
$\beta$ -Pinene	984	20.4	1.0	$\alpha$ -Muurolene	1512	3.7	–
6-Methylhept-5-en-2-one	986	–	0.2	( <i>E,E</i> )- $\alpha$ -Farnesene	1513	–	5.4
<i>o</i> -Cymene	1029	0.4	0.3	$\alpha$ -Selinene	1514	–	6.8
Limonene	1034	0.9	0.8	$\delta$ -Amorphene	1522	0.5	0.5
2-Heptylacetate	1039	–	1.4	$\gamma$ -Cadinene	1528	1.8	1.0
( <i>E</i> )- $\beta$ -Ocimene	1049	–	0.6	$\delta$ -Cadinene	1536	3.3	2.1
Terpinolene	1094	0.2	0.4	<i>trans</i> -Calamenene	1539	0.8	0.2
Linalool	1101	–	0.7	<i>trans</i> -Cadin-1,4-diene	1546	0.5	0.9
( <i>E</i> )-4,8-Dimethylnona-1,3,7-triene	1117	–	0.2	$\alpha$ -Cadinene	1552	0.5	–
<i>endo</i> -Fenchol	1122	0.2	0.2	Selina-4(15),7(11)-diene	1554	–	2.1
<i>cis</i> -Sabinol	1149	1.2	–	$\alpha$ -Calacorene	1560	0.8	–
Terpinen-4-ol	1183	–	0.2	Selina-3,7(11)-diene	1561	–	1.9
<i>cis</i> -Pinocamphone	1185	1.2	–	( <i>E</i> )-Nerolidol	1569	0.5	0.6
$\alpha$ -Terpineol	1197	1.0	2.1	( <i>Z</i> )-3-Hexenylbenzoate	1580	–	0.2
Methyl chavicol	1204	–	1.2	Dendrolasin	1583	–	2.2
Geraniol	1255	–	0.2	Palustrol	1589	1.6	–
Bornyl acetate	1294	0.3	–	Caryophyllene oxide	1592	0.7	–
Dihydroedulane	1301	0.2	–	Spathulenol	1595	0.9	0.8
2-Hydroxy-4-methoxyacetophenone	1331	3.3	–	Cubena-11-ol	1614	0.6	–
Myrtenyl acetate	1334	0.2	–	4,5-Dihydro- $\beta$ -caryophyllen-14-al	1621	0.9	–
$\delta$ -Elemene	1348	–	1.3	Globulol	1625	0.6	–
$\alpha$ -Ylangene	1385	0.3	0.8	Humulene epoxide II	1632	0.5	–
$\alpha$ -Copaene	1388	0.5	0.7	1,10-di- <i>epi</i> -Cubenol	1634	0.3	–
<i>cis</i> - $\beta$ -Elemene	1404	3.8	12.7	1- <i>epi</i> -Cubenol	1646	–	2.2
( <i>Z</i> )-Caryophyllene	1423	–	1.7	$\gamma$ -Eudesmol	1650	–	0.4
<i>cis</i> - $\alpha$ -Bergamotene	1426	0.7	–	<i>epi</i> - $\alpha$ -Cadinol	1658	1.1	0.5
$\alpha$ -Santalene	1432	1.9	–	<i>epi</i> - $\alpha$ -Muurolol	1659	0.8	–
$\beta$ -Caryophyllene	1435	6.4	8.0	$\delta$ -Cadinol	1663	0.5	0.4
<i>trans</i> - $\alpha$ -Bergamotene	1446	1.2	0.4	$\beta$ -Eudesmol	1672	–	0.8
$\alpha$ -Guaiene	1455	–	0.9	$\alpha$ -Cadinol	1673	1.3	–
Aromadendrene	1457	0.7	–	<i>neo</i> -Intermedeol	1676	–	2.0
$\beta$ -Santalene	1460	2.4	–	<i>epi</i> - $\alpha$ -Bisabolol	1696	0.6	–
$\alpha$ -Humulene	1470	5.6	1.8	Phytol	2117	1.2	–
9- <i>epi</i> -( <i>E</i> )-Caryophyllene	1478	0.5	0.7	Monoterpene hydrocarbons		25.4	5.6
Drima-7,9(11)-diene	1487	1.3	–	Monoterpene oxygenated		4.3	3.9
$\gamma$ -Muurolene	1489	2.8	–	Sesquiterpene hydrocarbons		45.7	61.4
$\beta$ -Chamigrene	1490	–	3.0	Sesquiterpene oxygenated		10.9	10.4
$\alpha$ -Amorphene	1495	1.1	1.6	Diterpenes		1.2	–
Germacrene D	1497	0.5	–	Non-terpenes		3.3	2.0
Aristolochene	1502	1.0	–	Total		90.8	84.3

<sup>a</sup> Elution order on HP-5MS column; <sup>b</sup> Retention indices on HP-5MS column; –: not identified.

This is the first report on the essential oil constituents of *T. odorum* and indeed the *Tsoongiodendron* species. Also, the composition of the essential oil of *M. chevalieri* is being reported for the first time; however, a noteworthy observation is that terpene compounds predominate in the essential oils, as was previously reported for other oil samples from the Magnoliaceae family grown in Vietnam, including *M. coco* [8] comprised of sabinene (31.9%) and  $\beta$ -pinene (11.8%), *M. fordiana* oils made up of  $\delta$ -cadinene (18.0%), (*E*)-nerolidol (16.7%),  $\alpha$ -copaene (12.8%),  $\alpha$ -selinene (9.1%) and *M. conifera* oils comprised of  $\beta$ -caryophyllene (29.9%), and  $\alpha$ -humulene (7.4%) [12]. The major components of the oils of *M. hookeri* var. *longirostrata* [13] 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). Also, the major components of the oils of *M. insignis* [13] 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).

TABLE 2. Antimicrobial Activity of the Essential Oils of *Tsoongiodendron odorum* and *Manglietia chevalieri*

Microorganism	MIC, $\mu\text{g/mL}^a$		IC <sub>50</sub> , $\mu\text{g/mL}^a$	
	<i>T. odorum</i>	<i>M. chevalieri</i>	<i>T. odorum</i>	<i>M. chevalieri</i>
<i>Enterococcus faecalis</i> ATCC299212	256.0 $\pm$ 0.10	64.0 $\pm$ 0.00	98.98 $\pm$ 0.50	19.56 $\pm$ 0.11
<i>Staphylococcus aureus</i> ATCC25923	–	64.0 $\pm$ 0.00	–	20.43 $\pm$ 0.00
<i>Bacillus cereus</i> ATCC14579	128.0 $\pm$ 0.05	64.0 $\pm$ 0.00	46.21 $\pm$ 0.10	22.45 $\pm$ 0.12
<i>Candida albicans</i> ATCC10231	–	64.0 $\pm$ 0.01	–	20.19 $\pm$ 0.10

–: no activity; <sup>a</sup> mean value of three replicate assays. MIC: streptomycin 1.2–1.5  $\mu\text{g/mL}$ , nystatin 1.8  $\mu\text{g/mL}$  [7, 8].

The essential oils of *M. hypolampra* were dominated by  $\beta$ -pinene (36.5 and 41.3%),  $\alpha$ -pinene (23.7 and 24.4%), and germacrene D (14.6 and 5.8%) [14]. (*E*)- $\beta$ -Ocimene (20.6 and 14.8%), myrcene (7.9 and 11.6%), and (*E*)-nerolidol (4.6 and 7.4%) were the main compounds of *M. kwangsiensis* [15]. It could be observed that the chemical identities of the various terpene compounds present in the *Manglietia* essential oils differed from one species to another, which is an indication of chemical variability in their compositional pattern [8].

The results of the antimicrobial activity of the essential oils are shown in Table 2. The essential oil from the leaves of *M. chevalieri* displayed stronger antimicrobial activity than the leaves of *T. odorum*. The leaf oil of *M. chevalieri* was the most active against *Enterococcus faecalis* ATCC299212, *Staphylococcus aureus* ATCC25923, *Bacillus cereus* ATCC14579, and *Candida albicans* ATCC10231, with MIC value of 64.0  $\mu\text{g/mL}$ . The obtained IC<sub>50</sub> values were 19.56, 20.43, 22.45, and 20.19  $\mu\text{g/mL}$ , respectively. The essential oil of *T. odorum* only exhibited moderate activity towards *E. faecalis* and *B. cereus*, with MIC values of 256.0 and 128.0  $\mu\text{g/mL}$ , respectively; and IC<sub>50</sub> value of 98.98 and 46.21  $\mu\text{g/mL}$ , respectively. The studied essential oils of *T. odorum* and *M. chevalieri* did not exhibit any antimicrobial activity against the Gram-negative microorganisms of *Pseudomonas aeruginosa* ATCC27853, *Escherichia coli* ATCC25922, and *Salmonella enterica* ATCC13076. The observed antimicrobial activity of the essential oils is in agreement with observations that *Manglietia* oil samples selectively inhibited the growth of microorganisms. The essential oil from *M. coco* was active against *S. aureus*, *B. cereus*, and *C. albicans* than *E. faecalis* and *Pseudomonas aeruginosa* [9]. The essential oils from *M. insignis* [13] showed stronger inhibitory effects on the seven test microorganisms, while *M. hypolampra* displayed activity only against *B. subtilis*, *Lactobacillus fermentum*, *Salmonella enterica*, and *P. aeruginosa* [14]. The IC<sub>50</sub> values have also been considered in the range of 10–120  $\mu\text{g/mL}$ , making it eligible for consideration as a good antimicrobial agent [7–9].

The chemical constituents and antimicrobial activity of *T. odorum* and *M. chevalieri* essential oils are being reported for the first time. It is believed that the constituents present in the studied essential oils might have influenced the observed antimicrobial activity of *T. odorum* and *M. chevalieri*. Compounds such as  $\alpha$ -pinene and  $\beta$ -pinene have previously shown antimicrobial activity against broad-spectrum microorganisms [15]. The antibacterial activity of  $\beta$ -caryophyllene against *S. aureus* was recently reported [16].

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