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CHEMICAL COMPOSITIONS AND ANTIMICROBIAL ACTIVITY OF ESSENTIAL OILS OF *Schisandra henryi* subsp. *hoatii* FROM VIETNAM

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Schisandra henryi subsp. *hoatii* N. S. Ly & X. T. Nguyen, is a new subspecies recently described from Tay Nguyen (Central Highlands), Vietnam [1]. It differs from the subsp. *marginalis* and the typical subspecies by its flowers with 9–10 tepals, suborbicular largest tepals, essentially free stamens 19–22 and carpels 21–25; and differs from *S. henryi* subsp. *yunnanensis* in having leaf blades abaxially glausecent, yellow flowers, and 19–22 essentially free stamens [1]. The lack of literature information on the chemical compositions and biological activities of both volatile and non-volatile constituents of *S. henryi* subsp. *hoatii* has led to this study. However, the compositions of essential oils from other *Schisandra* plants have been described – essential oil from the berries of *S. chinensis* are comprised of α-*cis*-bergamotene (10.7%), selina-4,11-diene (5.2%), and α-cadinol (5.1%), while the ylangene (10.1%), β-himachalene (9.4%) and *di-epi*-α-cedrene (8.9%) were found in the seed oil. The major compounds of the fruit oils [3] were ylangene (50.1%), β-himachalene (10.7%), α-bergamotene (9.5%), and β-chamigrene (5.4%). All essential oils from various parts of *S. chinensis* showed differing antioxidant activity [2, 3]. The fruit essential oil displayed antibacterial activity against both Gram-positive (*Staphylococcus epidermidis*, *Staphylococcus aureus*, *Bacillus subtilis*) and Gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus vulgaris*) bacteria [3]. Also the sesquiterpene δ-cadinene (25.6%) was the most abundant constituent of the fruit of *S. sphenanthera*, exhibiting concentration-dependent cytotoxicities towards HepG2 cells with IC_{50} of 189 μg/mL, and antimicrobial activity against Gram-positive bacteria [4]. A previous report [5] indicated the abundance of β-caryophyllene (*S. grandiflora*), α-bulnesene (*S. rubriflora*), and α-chamigrene (*S. propinqua*). It seems that sesquiterpene hydrocarbon dominated the compositional pattern of essential oils from the *Schisandra* species.

The aim of the present study is to report for the first time the chemical constituents and antimicrobial activity of essential oils from the leaves and stems of *S. henryi* subsp. *hoatii* collected in Vietnam. In previous reports, the non-volatile extracts of *S. sphenanthera*, *S. henryi*, and *Schisandra rubriflora* are known to contain lignans such as 4-aryltetralin, aryltetralone, tetrahydrofuran, and butane-type lignans [6]. These compounds are responsible for the hepatoprotective, cytotoxic, and anti-HIV-1 activities of these plants. Extracts from *S. chinensis* and their compounds are known to exhibit an anti-oxidant effect [7].

The leaves and stems of *S. henryi* subsp. *hoatii* were obtained from plants growing in Kon Tum Province: Tu Mo Rong District, Ngoc Lay Commune, Moya Village (GPS: 14°58′30.42′′N 107°59′13.33′′E), Vietnam. The samples were collected in April 2020, at an elevation of 1341 m and were identified by Assoc. Prof. Le Thi Huong and a voucher specimen, LTH 906, was deposited in the plant specimen room at Vinh University, Vietnam.

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TABLE 1. Chemical Constituents of Essential Oils from the Leaves and Stems of *Schisandra henryi* subsp. *hoatii*

Compound [a]	RI[b]	Leaves	Stems	Compound [a]	RI[b]	Leaves	Stems
α-Thujene	930	–	4.5	*ar*-Curcumene	1492	5.4	3.1
α-Pinene	939	1.6	7.4	2-Tridecanone	1497	1.5	2.5
Camphene	955	0.2	2.2	Germacrene D	1498	0.8	0.7
Sabinene	978	0.5	8.5	α-Zingiberene	1503	–	0.4
β-Pinene	984	0.6	2.0	β-Selinene	1505	0.3	–
Myrcene	991	0.2	2.4	*trans*-Muurola-4(14),5-diene	1511	0.8	0.2
δ-3-Carene	1019	0.1	0.5	α-Muurolene	1514	1.4	2.0
α-Terpinene	1021	–	0.4	β-Bisabolene	1517	0.2	–
o-Cymene	1029	0.9	4.6	α-Chamigrene	1523	–	0.1
Limonene	1034	0.6	1.0	Cuparene	1525	–	0.1
β-Phellandrene	1035	0.2	0.7	γ-Cadinene	1530	1.1	0.1
1,8-Cineole	1038	0.4	0.2	β-Sesquiphellandrene	1533	–	0.3
(*E*)-β-Ocimene	1049	–	0.1	δ-Cadinene	1536	1.7	2.5
γ-Terpinene	1063	–	0.2	*cis*-Calamenene	1538	0.7	0.6
2-Nonanone	1092	–	0.1	α-Cadinene	1553	0.3	–
Terpinolene	1094	–	0.2	β-Calacorene	1560	0.2	–
Linalool	1101	0.9	0.1	(*E*)-Nerolidol	1574	20.7	0.2
4-Hydroxy-4-methylhex-2-en-one	1124	0.2	–	Germacrene B	1578	0.5	–
cis-*p*-Menth-2-en-1-ol	1128	0.2	–	*ar*-Turmerol	1590	0.2	–
trans-*p*-Menth-2-en-1-ol	1145	0.4	–	Spathulenol	1599	1.2	0.3
Terpinen-4-ol	1183	3.0	2.2	Caryophyllene oxide	1607	11.2	5.1
p-Cymen-8-ol	1190	0.6	0.1	Humulene epoxide I	1621	0.2	–
α-Terpineol	1197	0.4	–	Zingiberenol	1625	0.2	–
Methyl chavicol	1204	3.2	0.8	Torilenol	1630	0.5	–
Thymol methyl ether	1238	–	0.2	Humulene epoxide II	1632	1.3	0.6
Geraniol	1255	0.2	–	1-*epi*-Cubenol	1646	0.7	–
Bornyl acetate	1294	1.4	1.1	Caryophylla-3(15),7(14)-dien-6-ol	1657	–	0.4
2-Undecanone	1295	–	1.3	*epi*-α-Cadinol	1658	1.0	–
α-Cubebene	1360	–	0.4	*epi*-α-Muurolol	1660	1.5	0.2
Cyclosativene	1383	–	0.4	α-Muurolol	1663	1.0	–
α-Copaene	1388	0.8	3.3	α-Cadinol	1674	3.4	0.1
7-*epi*-Sesquithujene	1397	–	0.2	*cis*-Calamenen-10-ol	1677	0.5	–
β-Bourbonene	1400	0.4	–	*trans*-Calamenen-10-ol	1686	0.7	–
cis-β-Elemene	1404	1.3	1.2	14-Hydroxy-9-*epi*-(*E*)-caryophyllene	1689	0.4	–
Sativene	1407	–	0.3	α-Bisabolol	1699	0.4	–
α-Santalene	1432	–	0.8	Eudesma-4(15),7-dien-1β-ol	1706	0.4	–
β-Caryophyllene	1435	8.6	21.2	10-*nor*-Calamenen-10-one	1724	0.3	–
β-Gurjunene	1445	0.2	–	Oplopanone	1759	0.7	–
trans-α-Bergamotene	1446	0.2	0.3	*cis*-5-Hydroxycalamenene	1797	0.4	0.8
(*Z*)-β-Farnesene	1460	0.2	–	Total		92.0	98.3
(*E*)-β-Farnesene	1465	0.2	0.2	Monoterpene hydrocarbons		4.9	34.7
α-Humulene	1470	2.0	2.9	Oxygenated monoterpenes		10.7	4.7
α-Acoradiene	1475	0.5	5.1	Sesquiterpene hydrocarbons		29.3	49.8
β-Acoradiene	1483	–	0.3	Oxygenated sesquiterpenes		46.9	7.7
γ-Curcumene	1490	–	0.3	Non-terpenes		0.2	1.4

[a] Elution order on HP-5MS column; [b] Retention indices on HP-5MS column; – not identified.

The amount of the collected leaves sampled reduced to 2.0 kg after separation from debris, and other substances. In effect, 2.0 kg of each plant were subjected to separate hydrodistillation using a Clevenger-type apparatus as described previously [8–12].

TABLE 2. Antimicrobial Activity of the Essential Oils from the Leaves and Stems of *Schisandra henryi* subsp. *hoatii*

Microorganism	MIC, μg/mL[a]		IC_{50}, μg/mL[a]	
	Leaves	Stems	Leaves	Stems
Enterococcus faecalis ATCC299212	128.0 ± 0.00	128.0 ± 0.00	25.56 ± 0.00	47.34±0.10
Staphylococcus aureus ATCC25923	256.0 ± 0.10	128.0 ± 0.00	98.98 ± 0.50	35.67±0.00
Bacillus cereus ATCC14579	128.0 ± 0.05	64.0 ± 0.00	34.56 ± 0.10	21.66± 0.10

[a] Mean value of three replicate assays. MIC: streptomycin 1.2–1.5 μm/mL, nystatin – 1.8 μM/mL [8].

All experimental procedures used in this study were similar to those described earlier in our previous published studies [8–12]. The instrumental analysis of the essential oils was as in [6–12]. The identification of constituents from essential oils was done as described earlier [8–12, 13]. Additionally, the minimum inhibitory concentration (MIC) and median inhibitory concentration (IC_{50}) values were measured by the microdilution broth susceptibility assay as described earlier [8–12].

The colours of essential oils obtained from the leaves and stems of *S. henryi* subsp. *hoatii* were light-yellow. The yields of both essential oils were 0.28% (leaves) and 0.30% (stems). The classes of compounds identified in the essential oil of the leaves of *S. henryi* subsp. *hoatii* (Table 1) were monoterpene hydrocarbons (4.9%), oxygenated monoterpenes (10.7%), sesquiterpene hydrocarbons (29.3%), oxygenated sesquiterpenes (46.9%), diterpenes (1.2%), and non-terpenes (0.2%). The major compounds of the essential oil consists mainly of sesquiterpenes represented by (*E*)-nerolidol (20.7%), caryophyllene oxide (11.2%), *β*-caryophyllene (8.6%), and *ar*-curcumene (5.4%). Additionally, there are significant quantities of *α*-cadinol (3.4%), methyl chavicol (3.2%), terpinen-4-ol (3.0%), and *α*-humulene (2.0%). However, monoterpene hydrocarbons (34.7%), oxygenated monoterpenes (4.7%), sesquiterpene hydrocarbons (49.8%), oxygenated sesquiterpenes (7.7%), and nonterpenes (1.4%) were the classes of compounds identified in the stem essential oil of *S. henryi* subsp. *hoatii*. The main constituents of the essential oil consist of mixtures of monoterpenes and sesquiterpene compounds – *β*-caryophyllene (21.2%), sabinene (8.5%), *α*-pinene (7.4%), *α*-acoradiene (5.1%), and caryophyllene oxide (5.1%).

Terpenes compounds were identified in the essential oils in tandem with compositions of essential oils from other *Schisandra* plants. The identities of these terpenes differ from species to species – for example, *γ*-ylangene, *cis*-*α*-bergamotene and *di*-*epi*-*α*-cedrene, the major compounds of *S. chinesis* [2, 3], were conspicuously absent in *S. henryi* subsp. *hoatii* essential oils. Moreover, *α*-bulnesene and *β*-chamigrene, the main compounds of *S. rubriflora* [5] and *S. propinqua*, respectively, were not identified in *S. henryi* subsp. *hoatii*. In addition, the amounts of *δ*-cadinene and *α*-chamigrene found in *S. henryi* subsp. *hoatii* was much lower than present in *S. sphenanthera* [4]. The high contents of *β*-caryophyllene in *S. henryi* subsp. *hoatii* confers compositional similarity with *S. grandiflora* [5], which is an indication of chemical variability in the compositional patterns of essential oils from *Schisandra* plants.

The antimicrobial activity of essential oils of *S. henryi* subsp. *hoatii* are shown in Table 2. Both essential oils displayed similar antimicrobial activity against *Enterococcus faecalis* ATCC299212, with a MIC value of 128.0 μg/mL. The IC_{50} value of 25.56 μg/mL and 47.34 μg/mL, were obtained, respectively. The stem essential oil exhibited stronger antimicrobial activity than the leaf oil towards *Staphylococcus aureus* ATCC25923 and *Bacillus cereus* ATCC14579 with MIC values of 128.0 and 64.0 μg/mL, respectively, as well as IC_{50} values of 35.67 μg/mL and 21.66 μg/mL, respectively. The essential oils neither displayed antimicrobial activity towards the Gram-negative microorganisms of *Pseudomonas aeruginosa* ATCC27853, *Escherichia coli* ATCC25922, and *Salmonella enterica* ATCC13076 nor showed noticeable anticandidal activity towards *Candida albican*s ATCC10231.

It could be observed that the studied essential oils of *S. henryi* subsp. *hoatii* only displayed antimicrobial activity towards the Gram-positive pathogens, which was in accordance with the results obtained in the testing of the antimicrobial activity of *S. sphenanthera* [4]. However, the fruit essential oil of *S. chinensis* isolated by simultaneous distillation extraction (SDE) showed good antibacterial activity against both Gram-positive (*S. epidermidis*, *S. aureus*, *B. subtilis*) and Gram-negative (*E. coli*, *P. aeruginosa*, *Proteus vulgaris*) bacteria [3]. Also, the pulp and seed essential oils of *S. chinensis* showed antibacterial activity towards Gram-positive *S. aureus* and *B. subtilis* and Gram-negative *P. aeruginosa* and *E. coli* [14]. This shows *Schisandra* essential oils selectively inhibited the growth of different microorganisms. Overall, the studied essential oils exhibited good antimicrobial activity against the tested microorganisms with MIC values < 200 μg/mL.

It is believed that the constituents present in the studied essential oils might have influenced the observed antimicrobial activity of *S. henryi* subsp. *hoatii*. A number of compounds identified in the essential oils of *S. henryi* subsp. *hoatii* were reported previously to possess antimicrobial activity [8–12, 15].

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