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Composition of Stem Bark Essential Oils of Three Vietnamese Essential Oils of Three Vietnamese Species of *Kadsura* (Schisandraceae)

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Abstract: The chemical composition of volatiles from the stem barks of three different *Kadsura* species has been studied. The essential oils were obtained by hydrodistillation and analyzed by GC and GC-MS. The components were identified by MS libraries and their LRIs. The essential oils content varied between 0.15% and 0.20% (v/w), calculated on a dry weight basis. Sesquiterpene hydrocarbons (25.2% - 57.9%) and oxygenated sesquiterpenes (27.1%- 64.4%) are the main oil fractions. *Kadsura coccinea* (Lemaire) A. C. Smith., afforded oil whose major compounds were β-caryophylene (23.6%), δ-cadinene (8.6%)**,** caryophyllene oxide (7.8%), *epi*-α-bisabolol (7.5%) and α-copaene (6.6%). On the other hand, α-muurolol (43.5%) was the most singly abundant constituent of *Kadsura longipeduculata* Finet & Gagnepain., with minor amounts of α-cadinol (5.4%), β-caryophylene (5.4%) and δ-cadinene (5.0%). However, we have identified α-acorenol (10.1%), δ-cadinene (9.6%), γ-cadinene (8.1%) and δ-elemene (6.8%) as the major constituents of *Kadsura induta* A. C. Smith.

Keywords: *Kadsura;* essential oil composition; terpenes.

1. Introduction

The Schisandraceae family constitute a small ''primitive''angiosperm family with only two species: Schisandra (25 species) and Kadsura (22 species). *Kadsura coccinea* (Lemaire) A. C. Smith, is a glabrous plants in which the leaf blade is elliptic to rarely ovate and papery to leathery. The flowers tepals are whitish red, purplish red or occasionally yellowish while the fruit peduncle have the apocarps as red to purplish red with 1 or 2 seeds. Flowering is from May through July while fruiting takes place between October and December [1]. *K. coccinea* fruit is a good source of nutrition [2] and is known to contain natural antioxidant polyphenols and anthocyanin [2, 3].

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Phytochemical investigations revealed the characterisation of dibenzocyclooctadiene lignans kadsuralignan G and kadsuralignan L which moderate NO production inhibitory activities, arylnaphthalene lignin [4-6], kadsuralignan H, kadsuralignans I, J and K [7], kadsulignans L-N, lanostane-type triterpenoids, coccinone A, coccinone B, coccinone C, coccinone D and coccinilactone B [8], seco-coccinic acids A-F and coccinilactone A with exhibited antiproliferative effects [9], kadcoccilactones A−J, kadsuphilactone A and micrandilactone B [10], kadcoccilactones K–R, triterpenoids [11], kadcoccilactone O with cytotoxicity against K562, Bel-7402, and A549 cell lines [10], 3-hydroxy-12-acetoxycoccinic acid [12, 13], seco-cycloartanes triterpenes [14], kadsuracoccinic acids A-C, kadsuric acid and micranoic acid A [15], 3-hydroxy-12-hydroxyl coccinic acid and 3-hydroxy-neokadsuranic acid A with antiproliferative effects against four human tumor cell lines, A549, HCT116, HL-60 and HepG2. Lignans such as kadangustin L and kadcoccilignan which has inhibitory activities on LPS-induced NO production [16, 17] as well as ascorbic acid [18] were also isolated from the plant. A known record of its root essential oil identified β-caryophyllene (52.17%) as the major compound while β-himachalene (5.95%), α-humulene (5.04%), β-pinene (4.38%), α-copaene (3.47%) and δ-cadinene (3.47%) were the minor compounds [19]. Another investigation identified the main compounds of the oil as iso-caryophyllene, δ-elemene, bornyl acetate, δ-cubebene, β-gurjunene and γ-muurolene [20].

Kadsura induta A. C. Smith., is a plant with young shoots. The petiole 1.7–2.6 cm long, are pubescent-tomentose while the leaf baldes are elliptic. Fruit peduncle, 4 cm wide, are apocarps red and bears about 3 or 4 seeds. Flowering is in July while fruiting occurs in November [1]. The non-volatile constituents of this species includes dibenzocyclooctane lignans, kadsurindutins A and B, and structurally related lignans schisantherin L , schisantherin P, kadsulignan L, and neokadsuranin. Both kadsurindutins A, kadsulignan L, and neokadsuranin showed *in vitro* antiviral effects on hepatitis B virus [21]. In addition, lignans, kadsurindutins C and H as well 18(13→12)-*abeo*-lanostane triterpenoid acid and kadindutic acid, were isolated from the stems of *Kadsura induta* [22]. The authors are not aware of any report of its essential oil composition.

Kadsura longipedunculata Finet & Gagnepain, is an evergreen twining vine. The leaf baldes are elliptic to rarely ovate-elliptic or obovate-elliptic, 5.5–12(–15) × 2–4.5(–6.5) cm, papery to leathery. The flower tepals are pale yellow, yellow, or occasionally reddish, while the fruit peduncles are apocarps red, purple, or rarely black, which bears 1-3 seeds. The flowering stages occur between June and September, with fruiting taking place between September and December. This species is used medicinally. The fruit is edible [1]. The total triterpenic acid (containing kadsuric acid and nigranoic acid) from this plant prevented gastric mucosal lesions induced by indomethacin, absolute alcohol, as well stress [1]. The plant also possesses free radical scavenging activity [23]. Phytochemical studies revealed the characterisation of dibenzocyclooctadiene lignans [24], tetrahydrofuran lignans and cadinane-type sesquiterpenoid [25], anti HIV-1 protease lignans, longipedunins A, B and C and triterpene dilactones [26-28]. The chemical compositions of its root essential oil, which also possess antimicrobial, antioxidant activities and cytotoxicities activities [29] were cadinane type compounds and their derivatives (54.2%) and especially δ-cadinene (13.8%). Another author [30] identified δ-cadinene (21.79%), camphene (7.27%), borneol (6.05%), cubenol (5.12%) and δ-cadinol (5.11%) as the major components of the oil.

The essential oil profiles of these plant species have received very little attention and this arouses our interest on researches into the volatile compositions of these relatively unexploited floras of Vietnam. In this paper, we report herein the compounds identified in the essential oils from the stem bark of *K. coccinea*, *K. induta* and *K. longipedunculata*.

2. Materials and Methods

2.1. Plants collection

Stem bark of *K. induta* were obtained from Lào Cai Province, Vietnam, on September 2011, while those of *K. coccinea* were harvested from Tam Đảo National Park, Vĩnh Phúc Province, Vietnam, on September 2011. However, sample of *K. longipedunculata* were collected from Kon Tum province, Vietnam, on July 2010. Voucher specimens BVT 16, BVT 21 and BVT 15, respectively were deposited at the Botany Museum, Vinh University, Vietnam. Plant samples were air-dried prior to extraction.

2.2 Extraction of the oils

Aliquots of 0.5 kg of each of pulverised plant samples was used for the experiment and their oils were obtained by hydrodistillation for 3h at normal pressure, according to the Vietnamese Pharmacopoeia [31]. The plant samples yielded a low content of essential oils: 0.20% (v/w; *K. induta*; light yellow); 0.15% (v/w; *K. coccinea*; light yellow); and 0.20% (v/w; *K. longipedunculata*; light yellow), calculated on a dry weight basis.

2.3 Gas Chromatography (GC) analysis of the oils

Gas chromatography analysis was performed on an Agilent Technologies HP 6890 Plus Gas chromatograph equipped with a FID and fitted with HP-5MS column (both 30 m x 0.25 mm, film thickness 0.25 μm, Agilent Technology). The analytical conditions were: carrier gas H_2 (1 mL/min), injector temperature (PTV) 250^{o}C, detector temperature 260^{o}C, column temperature programmed from 40^{o}C (2 min hold) to 220^{o}C (10 min hold) at 4^{o}C/min. Samples were injected by splitting and the split ratio was 10:1. The volume injected was 1.0 μL (diluted oil in hexane). Inlet pressure was 6.1 kPa. Each sample was analyzed thrice.

2.4 Gas Chromatography-Mass spectrometry (GC-MS) analysis

An Agilent Technologies HP 6890N Plus Chromatograph fitted with a fused silica capillary HP-5 MS column (30 m x 0.25 mm, film thickness 0.25 μm) and interfaced with a mass spectrometer HP 5973 MSD was used for the GC/MS analysis, under the same conditions as those used for GC analysis. The conditions were the same as described above with He (1 mL/min) as carrier gas. The MS conditions were as follows: ionization voltage 70 eV; emission current 40 mA; acquisitions scan mass range of 35-350 amu at a sampling rate of 1.0 scan/s.

2.5 Identification of the constituents

The identification of constituents was performed on the basis of retention indices (RI) determined by co-injection with reference to a homologous series of *n*-alkanes, under identical experimental conditions. Further identification was performed by comparison of their mass spectra with those from NIST [32] and and the home-made MS library built up from pure substances and components of known essential oils, as well as by comparison of their retention indices with literature values [33, 34].

3. Results and Discussion

Table 1 indicates the list of compounds identified from the studied oil samples. Sesuqiterpene hydrocarbons (57.9%, 25.2% and 55.1% respectively) and oxygenated sesquiterpenes (33.8%, 64.4% and 27.1% respectively) are the main oil fractions of *K. coccinea*, *K. longipedunculata* and *K. induta* respectively. Monoterpenes are less common, 0.5%, 0.2% and 14.5% respectively.

The main sesquiterpene constituents of *K. coccinea* were β-caryophyllene (23.6%), δ-cadinene (8.6%), caryophyllene oxide (7.8%), *epi*-α-bisabolol (7.5%), α-copaene (6.6%) and τ-muurolol (6.1%). The quantitative amount of caryophyllene is in agreement with previous reports [19, 20], but differ in the fact that compounds such as β-pinene, bornyl acetate, δ-elemene, β-himachalene and β-gurjunene, found in the said reports, were conspicuously absent in the present investigation.

The major sesquiterpene of *K. longipedunculata* was α-muurolol (43.5%) along with minor ones such as α-cadinol (5.4%), β-caryophyllene (5.4%) and δ-cadinene (5.0%). Cadinane type compounds and their derivatives, especially δ-cadinene were characteristics of previous report [29, 30] as against α-muurolol identified in this oil sample. The sesquiterpenes of *K. induta* were represented by α-acorenol (10.1%), δ-cadinene (9.6%), γ-cadinene (8.1%), δ-elemene (6.8%), γ-amorphene (5.4%) and τ-muurolol (5.2%), while terpinen-4-ol (6.5%) was the only monoterpene of quantitative significant.

No record of its essential oil composition could be found in literature as such this may represent the first of its kind.

Table 1. Volatile compounds of three *Kadsura* species from Vietnam.

			Percent composition (± SD) [a]			
Compounds [b]	**RI[c]**	**RI[d]**	***K.c***	***K.l***	***K.i***	**MI**
α-Thujene	924	924	-	-	2.5	MS, RI
α-Pinene	931	932	-	-	0.3	MS, RI
Sabinene	970	964	-	-	1.8	MS, RI
β-Pinene	974	974	-	-	0.3	MS, RI
β-Myrcene	988	988	-	-	0.5	MS, RI
α-Terpinene	1015	1014	-	-	0.3	MS, RI
ρ-Cymene	1024	1020	-	-	0.7	MS, RI
β-Phellandrene	1027	1025	-	-	0.2	MS, RI
(*E*)-β-Ocimene	1045	1044	-	-	0.2	MS, RI
γ-Terpinene	1056	1054	-	-	0.5	MS, RI
Terpinolene	1086	1086	-	-	0.1	MS, RI
Terpinen-4-ol	1177	1174	0.2	-	6.5	MS, RI
α-Terpineol	1191	1186	0.5	0.2	0.2	MS, RI
Bornyl acetate	1284	1287	-	-	0.3	MS, RI
δ-Elemene	1337	1335	-	-	6.8	MS, RI
α-Cubebene	1351	1345	0.7	-	1.3	MS, RI
Neryl acetate	1363	1359	-	-	0.1	MS, RI
α-Copaene	1377	1374	6.6	0.7	4.3	MS, RI
Daucene	1378	1380	-	-	0.2	MS, RI
β-Cubebene	1389	1387	0.3	-	2.7	MS, RI
β-Elemene	1391	1389	0.2	0.3	0.7	MS, RI
Sesquithujene	1404	1405	0.2	-	-	MS, RI
cis-α-Bergamotene	1414	1411	-	0.6	-	MS, RI
β-Caryophylene	1419	1417	23.6	5.4	0.7	MS, RI
trans-α-Bergamotene	1434	1432	2.0	0.4	0.2	MS, RI, Co
Aromadendrene	1441	1439	0.7	-	-	MS, RI
cis-Muurola-3,5-diene	1449	1448	0.2	0.2	0.8	MS, RI
α-Humulene	1454	1452	4.8	1.0	1.3	MS, RI
9-*epi*-(*E*)-Caryophyllene	1460	1464	0.4	-	0.8	MS, RI
cis-Muurola-4(14),5-diene	1462	1465	-	0.7	-	MS, RI
γ-Muurolene	1476	1478	0.8	0.5	1.0	MS, RI
Germacrene D	1480	1484	0.5	0.9	1.1	MS, RI
β-Selinene	1486	1489	1.0	0.3	0.3	MS, RI
trans-Muurola-4(14),5-diene	1491	1493	0.5	-	2.0	MS, RI
Viridiflorene	1494	1496	1.6	0.8	-	MS, RI
γ-Amorphene	1495	1495	-	-	5.4	MS, RI, Co
cis-Cadina-1,4-diene	1498	1496	0.6	0.1	0.6	MS, RI
α-Muurolene	1499	1500	1.3	-	2.1	MS, RI
β-Bisabolene	1507	1505	1.3	0.3	-	MS, RI
γ-Cadinene	1510	1507	0.2	-	-	MS, RI
δ-Amorphene	1513	1511	0.6	-	-	MS, RI

Zonarene	1521	1521	-	1.4	-	MS, RI
δ-Cadinene	1525	1522	8.6	5.0	9.6	MS, RI, Co
trans-Cadina-1(6), 4-diene	1535	1533	0.7	0.3	1.0	MS, RI, Co
α-Cadinene	1537	1537	-	1.6	-	MS, RI, Co
Elemol	1550	1548	0.1	-	-	MS, RI, Co
Germacrene B	1556	1559	-	-	0.4	MS, RI, Co
β-Calacorene	1563	1564	0.2	-	0.2	MS, RI, Co
Caryophyllenyl alcohol	1571	1570	0.1	0.3	-	MS, RI, Co
Scapanol	1572	1580	-	-	0.5	MS, RI, Co
Spathulenol	1579	1577	2.2	3.2	0.6	MS, RI, Co
Caryophyllene oxide	1584	1582	7.8	4.3	-	MS, RI, Co
Gleenol	1585	1586	-	-	0.8	MS,RI
Champacol	1593	1602	0.4	-	0.4	MS,RI
Humulene Epoxide II	1609	1608	1.2	0.6	0.4	MS,RI
10-*diepi*-Cubenol	1616	1618	-	2.3	-	MS,RI
epi-Cubenol	1629	1627	2.2	0.9	4.6	MS,RI
α-Acorenol	1632	1632	-	-	10.1	MS,RI, Co
γ-Eudesmol	1633	1630	0.6	-	-	MS,RI
Caryophylla-3(15), (7(14)-dien-6-ol [e]	1637	-	0.6	0.6	-	MS,RI
τ-Muurolol	1643	1640	1.0	-	5.2	MS,RI
α-Muurolol	1647	1644	1.0	43.5	1.5	MS,RI, Co
1(10)-Spirovetivene-7-ol [e]	1648	-	-	1.5	-	MS,RI, Co
β-Eudesmol	1652	1649	0.7	-	-	MS,RI
α-Cadinol	1656	1652	2.6	5.4	1.5	MS,RI
Cadalene	1676	1675	0.5	0.5	-	MS,RI
epi-α-Bisabolol	1685	1683	7.5	0.3	-	MS,RI,Co
Eudesma-4(15), 7-dien-1-ol	1686	1687	-	1.0	0.2	MS,RI
(*E*, *E*)-Farnesol [f]	1720	-	-	-	0.9	MS,RI
cis-5-Hydroxycalamenene [e]	1785	-	-	-	0.4	MS,RI
1,2-Benzenedicarboxylic acid	1917	1917	-	0.3	-	MS,RI
(*Z*)-9-Octadecenamide	2398	2398	0.2	-	-	MS,RI
	Total		**92.6**	**90.1**	**96.7**	
Monoterpene hydrocarbons			-	-	7.4	
Oxygenated monoterpenes			0.7	0.2	7.1	
Sesquiterpene hydrocarbons			57.9	25.2	55.1	
Oxygenated sesquiterpenes			33.8	64.4	27.1	
Non-terpenes			0.2	0.3	-	

[a] SD= Standard deviation were recorded, values were insignificant and omitted from the Table to avoid congestion; [b] Elution order on HP-5MS capillary column; [c] Retention indices on HP-5MS capillary column; [d] Literature Retention indices (see Experimental); [e] Tentative assignment; [f] Correct isomer not identified; - Not identified and not present in Literature; *K.c =Kadsura coccinea; K.l= Kadusra longipenduculata; K. i= Kadusra induta*; MI Mode of identification; MS, Mass spectrum; RI, Retention indices; Co, Co-injection with authentic samples.

Few literature reports are available on the oil contents of *Kadsura* plants. The main components of *K. heteroclita* stem oil were α-eudesmol (17.56%), 4-terpineol (9.74%) and δ-cadinene (9.27%) and essential oil showed potential to be developed as a possible natural insecticide/nematicide for control of stored product insects/nematodes [35]. However, δ-cadinene (22.59%), δ-cadinol (17.64%) and calarene (7.63%) were the main compounds identified in another investigation [36]. Li et al. [37] demonstrated that the supercritical carbon dioxide fluid extraction and steam distillated oils of *K. heteroclita* contained δ-cadinene (14.42% and 19.46%), δ-cadinol (9.94% and 11.14%) and calarene (6.50% and 8.00%) respectively, as major compounds.

The predominance of sesquiterpene compounds in the present investigation is in agreement with previous reports on the compositions of some *Kadsura* species [19, 20, 29, 30, 35-37]. Cadinane type compounds such as *cis*-cadina-1-4-diene, γ-cadinene, δ-adinene and α-cadinol are also prominent among the sesquiterpene volatiles of these *Kadsura* oils, as previously found in *K. longipedunculata* [29, 30], *K. coccinea* [19, 20], *K. heteroclita* [35-37] and *K. oblongifolia* [38]. However, it was noted that β-caryophyllene, the major compound of the stem oil of *K. coccinea* was also the significant compound of the leaf oil of *K. oblongifolia* [38] from Vietnam.

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