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Chemical constituents of essential oils from the leaves of *Tithonia diversifolia*, *Houttuynia cordata* and *Asarum glabrum* grown in Vietnam

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Abstract
The essential oil constituents of three medicinal plants grown in Vietnam were analyzed by gas chromatography-flame ionization detector (GC-FID) and gas chromatography-mass spectrometry (GC-MS) techniques. The main constituents of *Tithonia diversifolia* (Hemsl.) A. Gray (Asteraceae) were α-pinene (30.7%) along with (*E,E*)-α-farnesene (6.1%) and β-caryophyllene (5.1%). *Houttuynia cordata* Thunb. (Saururaceae) gave oil whose major components were β-myrcene (30.8%), 2-undecanone (19.7%) and (*Z*)-β-ocimene (10.2%). *Asarum glabrum* Merr. (Aristolochiaceae) consists mainly of safrole (46.6%) and apiole (17.0%).

Keywords: *Asarum glabrum*, essential oil composition, *Houttuynia* cordata, phenylpropanoids, terpenes, *Tithonia diversifolia*

1. Introduction
In this paper, the volatile constituents identified in three plants growing in Vietnam are being described, as part of our continued interest on the analysis of chemical compounds of Vietnamese flora [1]. *Tithonia diversifolia* (Hemsl.) A. Gray (Asteraceae) is a widespread plant in Vietnam, and the species of *Tithonia* are known as plants containing many biologically active compounds. Extracts of the plant displayed larvicidal activity against *Aedes aegypti* [2] as well as antimicrobial [3] and antimalarial [4] effects. Anti-hyperglycemic compounds [5], cerebrosides [6], 6″-O-*β*-d-apiofuranosyl-trichocarpin and 1-heptade-4,6-diyne-3,10,16,17-tetraol-3-O-*β*-d-glucopyranoside [7] isocoumarin [8], tirotundin and tagitinin A which serves as peroxisome proliferator-activated receptor agonists [9] and anti-inflammatory chlorogenic acid [10] were isolated from the aerial parts of *T. diversifolia*. The main constituents of its essential oils were α-pinene (50.8–61.0%), (*Z*)-β-ocimene (15.5–21.4%) from the flowers [11] and (*Z*)-β-ocimene (40.2%) from the leaves [12]. The composition of leaf oil of another sample comprised mainly of α-pinene (32.9%), β-caryophyllene (20.8%), germacrene D (12.6%), β-pinene (10.9%) and 1, 8-cineole (9.1%), while germacrene D (20.3%), β-caryophyllene (20.1%) and bicyclogermacrene (8.0%) characterized the oil of the flower [13]. Another report identified an abundance of α-pinene (60.9 -75.7 %) and δ-pinene (7.2–11.0 %) in all the plant parts [14]. Another analysis [15] reported an abundance of α-pinene (34.42%), β-caryophyllene (22.34%), β-pinene (11.14%), germacrene D (11.13%) and 1,8-cineole (8.76%). The composition of the volatile oils of the plant from Vietnam has not been reported previously.
Houttuynia cordata Thunb (Saururaceae) is a flowering plant native to Japan, southern China and Southeast Asia, where it grows in moist shady places. The shoots are eaten as a vegetable and aerial parts are used in traditional Chinese medicine. *H. cordata* possess a number of medicinally important activities such as antihyperglycemic [16], anti-cancer [17], wound-healing [18], hepatoprotective [19], anti-leukemic [20], protective against liver-injury [21], anthelmintic [22], inhibit dengue fever [23], anti-obesity [24] among others [25]. Moreover, compounds isolated from *H. cordata* have also been utilized for the treatment of herpes simplex virus type 1 (HSV-1), influenza virus [26], human immunodeficiency virus type 1, radical-scavenging property and exhibited strong tyrosinase inhibitory activity [27], while quercitrin, quercetin and hyperoside from this plant have shown strong antioxidant effect [19]. Essential oil from *H. cordata* was reported to exhibit anti-inflammatory activity [25, 28]. Terpenes, fatty acids, aldehydes, ketones and acids compounds were previously identified in the essential oil from *H. cordata* growing

in China [29]. Several other biologically active compounds of diverse structural patterns were characterized from the plant [25].

Asarum glabrum Merr. is a species of flowering plant in the family Aristolochiaceae. The whole plant is used in ethnomedicine for the treatment of stomach pain, pneumonia, whooping cough, malaria and toothache. Extract of *A. glabrum* are known to possess anti-inflammatory effect [30]. The major constituents found in the essential oil from aerial part [31] were safrole (42.24%), apiole and (27.11%) while safrole (41.9%) and phenylpropanoids were contained in the sample from another investigation [32].

2. Materials and methods

2.1 Plant collections

Leaves of *T. diversifolia*, *H. cordata*, and *A. glabrum* were collected from Huong Son district, Ha Tinh Province, Vietnam, in July 2011. Voucher specimens DND 231, DND 235 and DND 262, respectively have been deposited at the Botany Museum, Vinh University, Vietnam. Plant samples were air-dried prior to extraction.

2.2 Extraction of the essential oils

0.5 Kg of air-dried leaves of each plant samples was shredded and their oils were obtained by hydrodistillation for 3h at normal pressure, according to the Vietnamese Pharmacopoeia [33]. The yields of essential oils were 0.12% (v/w, *T. diversifolia*), 0.12% (v/w, *H. cordata*), and 0.21% (v/w, *A. glabrum*), calculated on a dry weight basis. Oil samples were leaf light yellow in coloration.

2.3 Analysis of the oils

Gas chromatography (GC) analysis was performed on an Agilent Technologies HP 6890 Plus Gas chromatograph equipped with a FID and fitted with HP-5MS column (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 ºC, detector temperature 260 ºC, column temperature programmed from 60 ºC (2 min hold) to 220 ºC (10 min hold) at 4 ºC/min. Samples were injected by splitting and the split ratio was 10:1. The volume injected was 1.0 µL. Inlet pressure was 6.1 kPa.

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 70eV; emission current 40 mA; acquisitions scan mass range of 35-350 amu at a sampling rate of 1.0 scan/s.

2.4 Identification of the constituents

The identification of constituents was performed on the basis of retention indices (RI) determined with reference to a homologous series of *n*-alkanes, under identical experimental conditions, co-injection with standards (Sigma-Aldrich, St. Louis, MO, USA) or known essential oil constituents, MS library search (NIST 08 and Wiley 9th Version), and by comparing with MS literature data [34, 35]. The relative amounts of individual components were calculated based on the GC peak area (FID response) without using correction factors.

3. Results & Discussion

Table 1 showed the percentage compositions as well as the identities of compounds present in the oil samples. The major classes of compounds present in *T. diversifolia* were the monoterpene hydrocarbons (52.0%), sesquiterpene hydrocarbons (22.7%) and oxygenated sesquiterpenes (11.8%). The main constituents include α-pinene (30.7%) along with (*E, E*)-α-farnesene (6.1%) and β-caryophyllene (5.1%). Other notable monoterpenes were limonene (4.7%), β-pinene (4.6%) and *p*-cymene (4.6%). The high content of α-pinene in *T. diversifolia* makes the oil similar to previously reported results [11-15] but differs due to its relatively much lower contents of (*Z*)-β-ocimene, β-caryophyllene, germacrene D and bicyclogermacrene and the absence of δ-pinene.

Monoterpene hydrocarbons (49.9%) and aliphatic ketones (25.5%) constitute the main classes of compounds present in *H. cordata* (Table 1). The major components were β-myrcene (30.8%), 2-undecanone (19.7%) and (*Z*)-β-ocimene (10.2%). β-Myrcene and 2-undecanone (methyl-n-nonyl ketone) were also previously reported from other analysis on *H. cordata* oil [36-42]. The quantitative amount of (*Z*)-β-ocimene in this oil is noteworthy, since it has not been previously reported to be a major compound of *H. cordata.* The contents of some compounds such as α-pinene, β-pinene, sabinene, limonene, bornyl acetate and decanoic acid were too low when compared with previously analysed samples from other parts of the world. Also, some compounds such as decanal, dodecanal, decanoyl acetaldehyde, dodecanaldehyde, ethyl caprate, houttuyninum, methyl linolenate, hexadecanoic acid, capric acid and capric acid ethyl ester [29, 37-40, 43-47] were not identified in the oil under investigation.

Phenylpropanoids (70.8%) along with monoterpene hydrocarbons (7.0%) and sesquiterpene hydrocarbons (9.9%) represent the abundant class of compounds identified in *A. glabrum*. The main constituents of the oil were safrole (46.6%) and apiole (17.0%). There were significant amounts of croweacin (6.4%) and myristicin (4.1%). The amount of safrole and apiole in this result on *A. glabrum* competes favourably with previous reports [31,32] suggesting a homogeneity in the oil composition of *A. glabrum*. Phenylpropanoids have been identified as the main class of compounds in several *Asarum* oils but the identities of the major compounds vary from one species to another. For example, methyl isoeugenol and α-asarone were the main constituents of the leaf oil from *Asarum forbesii* while elemicin was the major component of *Asarum cordifolium* with the oil of *Asarum heterotropoides* comprising mainly of methyl eugenol and safrole [48, 49]. However, only the oils of *Asarum insigne* [50] and *Asarum caulescene* [51] presented a compositional pattern dominated by ubiquitous terpene components.

Table 1: Compounds identified in the studied oil samples from Vietnam

Compounds [a]	RI [b]	RI [c]	*T.d*	*H.c*	*A.g*
α-Pinene	939	932	30.7	1.9	0.6
Camphene	953	946	0.8	0.9	0.1
Sabinene	976	969	0.6	0.4	-
β-Pinene	980	974	4.6	1.6	2.1
β-Myrcene	990	988	1.8	30.8	-
α-Phellandrene	1006	1002	3.9	-	-
α-Terpinene	1017	1014	-	-	1.8
p-Cymene	1026	1020	4.6	0.8	0.1
Limonene	1032	1024	4.7	1.8	1.2

(*Z*)-β-Ocimene	1042	1032	0.1	10.2	-
(*E*)-β-Ocimene	1053	1044	0.2	1.4	0.6
γ-Terpinene	1061	1054	-	0.1	0.1
α-Terpinolene	1090	1086	-	-	0.1
Rosefuran	1099	1095	-	0.3	-
Linalool	1100	1095	0.3	-	0.8
allo-Ocimene	1128	1128	-	-	0.3
Octyl acetate	1132	1132	-	0.1	-
trans-Pinocarveol	1139	1135	0.1	-	-
trans-Verbenol	1145	1140	0.2	-	-
Borneol	1167	1165	-	-	0.2
Terpinen-4-ol	1177	1174	0.2	0.3	0.3
α-Terpineol	1189	1186	0.2	-	-
2-Decanone	1191	1190	-	0.1	-
Decanal	1209	1200	-	0.2	-
Piperitone	1240	1249	-	0.3	-
Geraniol	1249	1249	0.2	-	-
Safrole	1287	1285	-	-	46.6
Bornyl acetate	1289	1287	-	2.5	-
2-Undecanone	1291	1293	-	19.7	-
Linalyl propanoate	1323	1334	-	-	0.6
α-Cubebene	1343	1345	0.1	-	
Eugenol	1356	1356	-	-	0.2
n-Decanoic acid	1366	1364	-	0.1	-
Isoledene	1372	1374	1.7	-	-
α-Copaene	1376	1374	0.6	-	-
Geranyl acetate	1378	1379	-	3.6	-
2-Dodecanone	1380	1381	-	0.9	-
β-Bourbonene	1388	1387	0.9	-	-
β-Elemene	1391	1389	0.5	-	-
Methyl eugenol	1407	1403	0.6	-	1.2
α-Cederene	1412	1410	-	-	0.3
α-Gurjunene	1412	1409	-	-	0.2
β-Caryophyllene	1419	1417	5.1	1.9	-
β-Gurjunene	1428	1431	0.5	-	-
trans-α-Bergamotene	1435	1432	-	-	0.4
Aromadendrene	1441	1439	0.2	-	-
Pentyl octanoate	1450	1450	-	0.2	-
α-Humulene	1454	1452	2.7	0.3	-
(*E*)-β-Farnesene	1454	1454	0.2	1.3	-
β-Santalene	1456	1457	-	-	0.1
Croweacin	1460	1457	-	-	6.4
1-Dodecanol	1469	1469	-	1.6	-
γ-Curcumene	1480	1481	-	-	1.3
Germacrene D	1480	1484	0.2	-	-
β-Selinene	1486	1489	0.5	0.9	-
epi-Bicyclosesquiphellandrene	1490	1490	-	0.2	-
δ-Selinene	1494	1492	0.8	-	-
2-Tridecanone	1497	1495	-	4.8	-
Bicyclogermacrene	1499	1500	0.1	-	-
Epizonarene	1503	1501	0.3	-	-
(*E,E*)-α-Farnesene	1506	1505	6.1	0.7	-
β-Bisabolene	1509	1505	0.1	-	0.2
γ-Cadinene	1514	1513	0.6	-	-
Myristicin	1515	1517	-	-	4.1
7-epi-α-Selinene	1518	1520	-	0.2	-
δ-Cadinene	1525	1522	1.5	-	-
Elemicin	1550	1555	-	-	0.7
(*E*)-Nerolidol	1564	1561	0.3	0.2	0.3
Spathulenol	1577	1577	3.3	0.2	0.4
Caryophyllene oxide	1581	1582	1.2	0.6	-
Viridiflorol	1593	1592	0.3	-	-
α-Cedrol	1601	1600	-	-	0.3
α-Guaiol	1602	1600	0.2	-	-
Humulene epooxide II	1608	1608	0.5	-	-
(*Z*)- Asarone	1617	1616	-	-	1.7
epi-α-Cadinol	1640	1638	2.0	-	-
β-Eudesmol	1649	1649	0.2	-	-
α-Cadinol	1653	1652	1.8	-	-
δ-Cadinol	1657	1655	-	3.0	-
Apiole	1674	1677	-	-	17.0
Ledene oxide I	1680	1682	0.7	-	-
α-Bisabolol	1685	1685	-	0.2	-
Farnesol [d]	1717	1714	1.3	-	-
Platambin	1846	1842	0.2	-	-
Phytol	1958	1942	0.9	0.5	-
Hexadecanoic acid	1960	1959	0.5	-	-
Kaur-16-ene	2056	2043	0.8	-	-
1-Octadecanol	2074	2077	0.3	-	-
(*Z*)-9-Octadecamide	2398	2398	0.8	-	-
Total			91.8	94.9	90.6
Monoterpene hydrocarbons			52.0	49.9	7.0
Oxygenated monoterpenes			1.2	3.5	1.9
Sesquiterpene hydrocarbons			22.7	5.5	9.9
Oxygenated sesquiterpenes			11.8	4.1	1.0
Diterpenes			1.9	0.5	-
Phenylpropanoids			0.6	0.1	70.8
Aliphatic ketones			-	25.5	-
Non-terpenes			1.6	5.8	-

[a] Elution order on HP-5MS column; [b] Retention indices on HP-5 MS column; [c] Literature retention indices; [d] Correct isomer not identified; - Not identified; *T.d*, *Tithonia diversifolia*; *H.c*, *Houttuynia cordata*; *A.g*, *Asarum glabrum*

4. Conclusions

In this report, major differences were observed between the oil compositions of *T. diversifolia, H. cordata* and *A. glabrum* growing in Vietnam and previous studies from other parts of the world. This may be attributed to differences in the ecological and climatic conditions between Vietnam and other parts of the world as well as the age and nature of the plant, handling procedure etc.

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