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

Do Ngoc Dai, Tran Dinh Thang, AbdulRazaq Ogunmoye, Olanrewaju Isola Eresanya, Isiaka Ajani Ogunwande

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* Thumb. (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], cerebroside [6], 6′-O-β-d- apiofuranosyl-trichocarpin and 1-heptadec-4,6-diyne-3,10,16,17-tetraol-3-O-β-d-glucopyranoside [7] isocoumarin [8], troitundrin 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 bicyclogermaencne (8.0%) characterized the oil of the flower [13]. Another report identified an abundance of α-pinene (60.9 -75.7 %) and (E,E)-α-farnesene (34.42%) in the leaves of *T. diversifolia*. Another analysis [15] reported an abundance of α-pinene (34.42%), β-caryophyllene (22.34%), (Z)-β-ocimene (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* Thumb (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 quercitin, querectin and hyperoside from this plant have shown strong antioxidant effect [19]. Essential oil from *H. cordata* was reported to exhibit anti-inflammatory activity [25, 26]. 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].

### 2.1 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 H₂ (1 mL/min), injector temperature (PTV) 250 °C, detector temperature 260 °C, column temperature programmed from 60 °C (2 min hold) to 220 °C (10 min hold) at 4 °C/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. The identification of compounds 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.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>RI</th>
<th>RI</th>
<th>T.d</th>
<th>H.c</th>
<th>A.g</th>
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<tbody>
<tr>
<td>α-Pinene</td>
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<td>30.7</td>
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<td>Sabine</td>
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<tr>
<td>β-Pinene</td>
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<td>4.6</td>
<td>1.6</td>
<td>2.1</td>
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<tr>
<td>β-Myrcene</td>
<td>990</td>
<td>988</td>
<td>1.8</td>
<td>30.8</td>
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<td>1002</td>
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<tr>
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<td>1024</td>
<td>4.7</td>
<td>1.8</td>
<td>1.2</td>
</tr>
</tbody>
</table>

### 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] 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, sabine, 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, decanol 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 major components of the oil were safrole (46.6%) and apiole (17.0%). There were significant amounts of croweacin (46.6%) 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 isoegenol and α-aroseone 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 caulescens* [51] presented a compositional pattern dominated by ubiquitous terpene components.
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.

### 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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