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## Identification of constituents of essential oil of *Zanthoxylum evodiifolium* Guill. from Vietnam

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**Abstract**

The chemical compounds identified from the essential oil of the leaves of *Zanthoxylum evodiifolium* Guill, cultivated in Vietnam are being reported. The oil was obtained by hydrodistillation while the chemical constituents were analysed by means of gas chromatography-flame ionization detector (GC-FID) and gas chromatography/mass spectrometry (GC-MS). The leaf oil of *Zanthoxylum evodiifolium* had quantitative amounts of (*E*)- $\beta$ -ocimene (37.1%), 16-germacradien-5-ol (11.9%) and  $\delta$ -cadinene (6.1%). This is the first report on the volatile constituents of the plant.

**Keywords:** *Zanthoxylum evodiifolium*, essential oil composition, (*E*)- $\beta$ -ocimene, germacradien-5-ol.

**1. Introduction**

*Zanthoxylum* is the largest and most widespread genus in the Rutaceae family of plants, containing about 250 species native to warm temperate and subtropical areas worldwide. These species includes small and large trees, deciduous and evergreen trees and shrubs, most of them with spiny trunks [1]. Many species have been investigated for their non-volatile chemical constituents as well as biological and pharmacological potentials. In this paper, we report on the volatile constituents identified from the leaf oils of relatively poorly studied *Zanthoxylum* species growing in Vietnam. Our findings into the chemical volatile constituents of some Vietnamese flora have been recently published [2].

*Zanthoxylum evodiifolium* Guill, an evergreen climbing shrubs, is endemic to Vietnam. It may grow up to 8 m high. The leaves have short stalks, 1.5-2 cm, 3 leaflets bearing slender, 10 mm long, hairless. The flowers are white and fragrant. The leaves and fruits are used as seasoning and spices. It has uses in ethnomedicine for the treatment of ulcer, abdominal pain, nausea, cholera and dysentery [3]. Till now, no record of the chemical composition and biological activities of the essential oils of these plants could be found in the literature. This prompted our interest in the research into their essential oil contents as part of our continued study on the analysis of poorly studied species of Vietnamese flora [2].

**2. Materials and methods****2.1 Plant collection**

Leaves of *Z. evodiaefolium* were harvested from Pù Huống Natural Reserve, NghệAn Province, Vietnam, in August 2011. A voucher specimen, HDT 294, was deposited at the Botany Museum, Vinh University, Vietnam. Plant samples were air-dried prior to extraction.

**2.2 Extraction of essential oil**

0.5 Kg of the plant sample was shredded and the oil was obtained by hydrodistillation for 4 h at normal pressure, according to the Vietnamese Pharmacopoeia [4]. The yield content of essential oil was 0.12% (v/w; light yellow) calculated on a dry weight basis.

**2.3 Analysis of the oil**

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  $\mu$ m, Agilent Technology, Berkshire, United Kingdom). The analytical conditions were: carrier gas H<sub>2</sub> (1 mL/min), injector temperature (PTV, programmed temperature vaporisation) 250 °C, detector temperature 260 °C, column temperature programmed from 40 °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  $\mu$ L. Inlet pressure was 6.1 kPa.

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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  $\mu\text{m}$ ) 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.

#### 2.4 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 08 Libraries (on ChemStation HP) and Wiley 9<sup>th</sup> Version 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 <sup>15,61</sup>.

#### 3. Results & Discussion

The 43 identified compounds represent 94.4% of the total oil contents of *Z. evodiifolium*. Monoterpene hydrocarbons (49.7%), sesquiterpene hydrocarbons (23.4%) and oxygenated sesquiterpenes (19.9%) were the dominant class of compounds in *Z. evodiaefloium* (Table 1). The quantitatively significant

compounds were (*E*)- $\beta$ -ocimene and 1,6-germacrendien-5-ol (11.9%). Other notable compounds were  $\delta$ -cadinol (6.1%),  $\alpha$ -cadinol (3.4%),  $\beta$ -caryophyllene (3.2%) and *epi*- $\alpha$ -muurolol (3.2%). The authors are not aware of any literature citation on the volatile contents of this species, and as such the present study may represent the first of its kind.

The essential oils of few *Zanthoxylum* species from Vietnam have been studied. Monoterpenes were the major compounds of the fruit essential oil of *Zanthoxylum rhesoides* from Vietnam <sup>171</sup>. These were limonene (21.7%), *p*-cymene (15.3%),  $\beta$ -phellandrene (11.9%),  $\alpha$ -pinene (8.2%), sabinene (6.9%) and terpinen-4-ol (6.0%). The leaf of *Zanthoxylum nitidum* <sup>181</sup> revealed an abundance of  $\beta$ -caryophyllene (24.6%),  $\gamma$ -elemene (14.7%) and bicyclogermacrene (12.9%), while the seed oil <sup>191</sup> comprised mainly of limonene (44.1%), geranial (12.1%), neral (10.9%) and linalool (6.8%). The chemical compositions of *Z. evodiaefloium* oil were found to be different from those of other species already analysed from Vietnam. The major compounds found in other *Zanthoxylum* species grown in Vietnam were either present in low quantities ( $\alpha$ -pinene, limonene, *p*-cymene, geranial, neral, linalool,  $\beta$ -caryophyllene and  $\gamma$ -elemene) or completely absent (sabinene, terpinen-4-ol and bicyclogermacrene) in *Z. evodiaefloium*. In addition, (*E*)- $\beta$ -ocimene and 1,6-germacrendien-5-ol were not previously described as major constituents of *Zanthoxylum* oils from Vietnam. These variations may be due to the different parts being analysed as well as other factors such as climatic and ecological conditions.

**Table 1:** Percentage composition of Vietnamese *Zanthoxylum evodiaefloium*

Compounds <sup>a</sup>	RI <sup>b</sup>	RI <sup>c</sup>	Percent composition
$\alpha$ -Pinene	939	932	1.7
Camphene	953	946	0.1
$\beta$ -Pinene	980	974	0.3
Myrcene	990	988	1.5
$\alpha$ -Phellandrene	1006	1002	1.8
<i>p</i> -Cymene	1028	1020	0.2
Limonene	1032	1024	2.3
( <i>Z</i> )- $\beta$ -Ocimene	1042	1042	2.4
( <i>E</i> )- $\beta$ -Ocimene	1052	1044	37.1
$\gamma$ -Terpinene	1061	1054	0.4
Linalool	1100	1095	1.2
<i>allo</i> -Ocimene	1128	1128	1.9
Camphor	1143	1141	0.1
l-Menthone	1153	1148	0.2
<i>p</i> -allyl Anisole (Estragole)	1196	1196	0.2
Bornyl acetate	1287	1287	0.1
Bicycloelemene	1337	1338	1.6
$\alpha$ -Copaene	1377	1374	0.5
$\beta$ -Cubebene	1388	1387	0.3
$\beta$ -Elemene	1391	1389	0.3
$\beta$ -Caryophyllene	1419	1417	3.2
$\beta$ -Gurjunene	1431	1431	0.2
$\gamma$ -Elemene	1437	1434	0.7
$\alpha$ -Humulene	1454	1452	0.7
$\gamma$ -Gurjunene	1473	1475	0.8
$\alpha$ -Amorphene	1485	1483	2.0

Germacrene D	1485	1485	2.4
Bicyclosiquiphellandrene	1487	1487	0.4
<i>cis</i> -Cadin-1,4-diene	1496	1495	0.3
$\alpha$ -Muurolene	1500	1500	1.2
( <i>E,E</i> )- $\alpha$ -Farnesene	1510	1505	0.6
$\gamma$ -Cadinene	1514	1513	0.1
$\delta$ -Cadinene	1525	1522	6.1
Germacrene B	1557	1559	1.7
( <i>E</i> )-Nerolidol	1564	1561	0.5
Ledol	1600	1602	0.1
$\beta$ -Oplophenone	1608	1607	0.2
<i>epi</i> - $\alpha$ -Muurolol	1646	1640	3.2
$\alpha$ -Cadinol	1653	1652	3.4
1,6-Germacradien-5-ol	1661	1661	11.9
Ledene oxide II	1682	1682	0.1
( <i>Z,E</i> )-Farnesol	1723	1722	0.5
Phytol	2125	1942	0.1
<b>Total</b>			<b>94.4</b>
<b>Monoterpene hydrocarbons</b>			<b>49.7</b>
<b>Oxygenated monoterpenes</b>			<b>1.8</b>
<b>Sesquiterpene hydrocarbons</b>			<b>23.4</b>
<b>Oxygenated sesquiterpenes</b>			<b>19.9</b>
<b>Diterpenes</b>			<b>0.1</b>

<sup>a</sup> Compounds identified by RI from column, co-injection, literature MS pattern and literature retention indices; <sup>b</sup> Retention indices on HP-5 MS capillary column; <sup>c</sup> Literature retention indices

#### 4. Conclusions

For the first time, the compositions of the leaf essential oil of the Vietnamese grown *Z. evodiaefolium* were elucidated. Although, ubiquitous terpenes were identified in the sample, the composition pattern was found to be different from those of other species in the genus.

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