



## Chemical Composition of Essential Oils from the Stem Barks of Three *Cinnamomum* Species

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### Authors' contributions

This work was carried out in collaboration between all authors. Authors TDT and DND designed the study and performed both the hydrodistillation of the essential oils and the GC/MS analysis. Author LCS collected the samples assisted by author DDH. Authors TOO and AO managed the literature searches while author IAO wrote the first draft of the manuscript. All authors read and approved the final manuscript.

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### ABSTRACT

The stem barks essential oils of three Vietnamese species of *Cinnamomum* grown in Vietnam were analyzed by gas chromatography (GC) and gas chromatography-mass spectrometry (GC/MS). The main monoterpene compounds of *Cinnamomum kunstleri* Rild., were 1, 8-cineole (25.3%),  $\alpha$ -terpineol (10.7%) and terpinen-4-ol (6.7%). However, linalool (27.0%), limonene (23.4%), terpinen-

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4-ol (9.8%) and  $\alpha$ -phellandrene (9.5%) were the principal monoterpene components of *Cinnamomum cambodianum* Lecomte. Sesquiterpenes represented mainly by aromadendrene (26.0%),  $\beta$ -caryophyllene (17.2%) and  $\alpha$ -copaene (5.7%) were the dominant compounds in the bark oil of *Cinnamomum rigidifolium* Kosterm.

**Aims:** The aim of this study was to isolate essential oils from the stem barks of *Cinnamomum kunstleri*, *Cinnamomum cambodianum* and *Cinnamomum rigidifolium* and investigate the volatile constituents present therein.

**Study Design:** Distillation of essential oils from the plant materials and analysis of their chemical composition.

**Place and Duration of Study:** The stem barks of *C. kunstleri*, *C. cambodianum* and *C. rigidifolium* were collected from Bạch Mã National Park, Thừa Thiên-Huế Province, Vietnam, in August 2012.

**Methodology:** About 500 g of air-dried plant samples was shredded and their oils were obtained by separate hydrodistillation for 4 h at normal pressure, according to the Vietnamese Pharmacopoeia. The chemical constituents of the distilled oils were analyzed by means of gas chromatography-flame ionization detector (GC-FID) and gas chromatography coupled with mass spectrometry (GC-MS).

**Results:** The major constituents of *C. kunstleri* were identified as 1, 8-cineole (25.3%),  $\alpha$ -terpineol (10.7%) and terpinen-4-ol (6.7%) while linalool (27.0%), limonene (23.4%), terpinen-4-ol (9.8%) and  $\alpha$ -phellandrene (9.5%) were the principal components of *C. cambodianum*. However, aromadendrene (26.0%),  $\beta$ -caryophyllene (17.2%) and  $\alpha$ -copaene (5.7%) were the dominant compounds in *C. rigidifolium*.

**Conclusion:** The present oil compositions were found to be different from the results obtained previously from the essential oils of *Cinnamomum* species grown in Vietnam and other parts of the world.

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

## 1. INTRODUCTION

Vietnam is blessed with many medicinal plants. Literature information has shown that the volatile oils of these plants have received little chemical analysis. In view of this, we have developed interest in the analysis of essential oil constituents of plant species from Vietnam [1,2]. *Cinnamomum* is a genus of evergreen trees and shrubs belonging to the Laurel family, Lauraceae. *Cinnamomum* species have aromatic oils in their leaves and bark. The genus contains over 300 species, distributed in tropical and subtropical regions of North America, Central America, South America, Asia, Oceania and Australasia. The genus is a source of various biologically active compounds [3,4].

Majority of *Cinnamomum* species are aromatic trees with a characteristic smell and the reports on their volatile components are readily available in the literature. Recent findings revealed that the volatile oils of *Cinnamomum* species possess variety of biological activities such as cytokine modulatory effect [5], antioxidant and antibacterial [6], toxicity and insecticidal activity [7,8], anti-inflammatory [9], xanthine oxidase inhibitor [10] and cytotoxicity [11]. The volatile compounds are usually mono- and

sesquiterpene compounds, though (*E*)-cinnamaldehyde featured prominently as the major compound of some species which included *C. paciflorum* [12], *C. pubescens* [6], *C. aromaticum* [8] and *C. zeylanicum* [13]. On the other hand, compounds such as linalool in *C. camphora* var. *linaloolifera* [14] safrole and benzyl benzoate in *C. parthenoxylon* [15] as well as 2-methylene-3-buten-1-yl-benzoate in *Cinnamomum* sp [16]. A high content of eugenol and 1, 8-cineole were reported in *C. albiflorum* [17] while spathulenol, caryophyllene oxide and  $\beta$ -caryophyllene constituted the major compounds in *C. sericans* [1]. However, the significant compounds of *C. durifolium* were *p*-cymene, limonene and  $\alpha$ -phellandrene while bicyclogermacrene,  $\beta$ -caryophyllene were present in *C. magnificum* [1]. On the other hand  $\beta$ -caryophyllene, caryophyllene oxide and spathulenol were identified previously in *C. iners* [1].

The volatile oil content of several species of *Cinnamomum* growing in Vietnam has been characterized with low content of (*E*)-cinnamaldehyde [1,2,14-19]. Only the oil of *C. tonkinensis* [20] contained an appreciable amount of (*E*)-cinnamaldehyde. In this paper, we report the volatile constituents identified from the

stem barks of *Cinnamomum kunstleri* Rild., *Cinnamomum rigidifolium* Kosterm and *Cinnamomum cambodianum* Lecomte, endemics to Vietnam. *Cinnamomum cambodianum* was known to possessed anti-allergic effect [21]. Although, the chemical composition of essential oils from the leaves of these *Cinnamomum* plants have been characterized and reported [2], information is scanty on the volatile and non-volatile contents of other parts of these plant species. In addition, *C. cambodianum* oil was reported to contain  $\alpha$ -terpineol (33.4%), linalool (22.4%) and terpinen-4-ol (13.3%) as the main constituents [18]. The lack of readily available information on the chemical constituents of these plants arouses our interest to investigate the volatile oils of these poorly studied Vietnamese grown floras.

## 2. MATERIALS AND METHODS

### 2.1 Plant Materials

Stem bark samples of *C. kunstleri*, *C. cambodianum* and *C. rigidifolium* were collected from Bạch Mã National Park, Thừa Thiên - Huế Province, Vietnam, in August 2012. Botanical identification was performed by curators at the Herbarium Botany Museum, Vinh University, Vietnam. Voucher specimens LCS 241, LCS 254 and LCS 260 respectively, were deposited at the Botany Museum, Vinh University, Vietnam. Plant samples were air-dried prior to extraction.

### 2.2 Distillation of the Essential Oils

About 0.5 kg of air-dried plant samples was shredded and their oils were obtained by separate hydrodistillation for 4 h at normal pressure, according to the Vietnamese Pharmacopoeia [22].

### 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  $\mu$ m, Agilent Technology). The analytical conditions were: carrier gas  $H_2$  (1 mL/min), injector temperature (PTV-programmed temperature vaporization injection) 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  $\mu$ L. 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  $\mu$ m) and interfaced with a mass spectrometer HP 5973 MSD was used for the GC/MS 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. The MS fragmentation patterns were checked with those of other essential oils of known composition with Wiley (Wiley 9<sup>th</sup> Version), NIST 08 Libraries (on ChemStation HP), with those in the literature, and also with standard substances.

### 2.4 Identification Procedure

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 ( $C_5$ - $C_{30}$ ), under identical experimental conditions. Further identification was performed by comparison of their mass spectra with those from NIST 08 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 [23,24].

## 3. RESULTS AND DISCUSSION

The plant samples afforded low content of essential oils: 0.2% (v/w, *C. kunstleri*), 0.35% (v/w, *C. cambodianum*) and 0.25% (v/w, *C. rigidifolium*), calculated on a dry weight basis. All the oil samples were light yellow colored. The percentage of each constituent identified in the oil samples as well as the experimental and literature retention indices are summarized in Table 1, according to their elution order on an HP-5MS column. Monoterpenes (74.1%) and sesquiterpenes (23.4%) were the main constituent groupings identified in the bark oil of *C. kunstleri*. The main monoterpene compounds were 1, 8-cineole (25.3%),  $\alpha$ -terpineol (10.7%), terpinen-4-ol (6.7%),  $\alpha$ -pinene (5.3%), sabinene (4.4%),  $\beta$ -pinene (4.0%) and borneol (3.5%). The sesquiterpenes were repressed by  $\alpha$ -amorphene (4.3%),  $\delta$ -cadinene (3.7%) and  $\alpha$ -copaene (2.5%). However, methyl eugenol, the main

compound present in the leaf oil [2] was not identified in the stem oil.

Monoterpene hydrocarbons (53.0%) and oxygenated monoterpenes (39.9%) were the main class of compounds identified in the bark oil of *C. cambodianum*. The main constituents featured significant amounts of linalool (27.0%), limonene (23.4%), terpinen-4-ol (9.8%) and  $\alpha$ -phellandrene (9.5%). Other significant compound includes (*E*)- $\beta$ -ocimene (5.9%), *o*-cymene (5.8%)

and eugenol (5.6%). Although, linalool and terpinen-4-ol constitutes larger portion of the leaf oil [2], the content of limonene in the stem is higher than that of the leaf. The chemical pattern of this oil resemble those of *C. camphora* var. *linaloolifera* [14] and previous analysis on *C. cambodianum* [18], only in the general prevalence of linalool and terpinen-4-ol, but the identity of most other prominent compounds differed from each other.

**Table 1. Chemical composition of the stem barks essential oils of *Cinnamomum* species**

Compounds <sup>b</sup>	RI <sup>c</sup>	RI <sup>d</sup>	Percentage composition ( $\pm$ SD) <sup>a</sup>		
			<i>C. kunstleri</i>	<i>C. cambodianum</i>	<i>C. rigidifolium</i>
$\alpha$ -Thujene	930	924	1.7	-	-
$\alpha$ -Pinene	939	932	5.3	1.7	-
Sabinene	976	969	4.4	2.1	-
$\beta$ -Pinene	980	974	4.0	-	-
$\beta$ -Myrcene	990	988	1.5	1.8	-
$\alpha$ -Phellandrene	1006	1002	2.7	9.5	-
$\alpha$ -Terpinene	1017	1014	-	1.4	-
<i>o</i> -Cymene	1026	1022	1.9	5.8	-
Limonene	1032	1024	-	23.4	0.8
1,8-Cineole	1034	1026	25.3	-	0.5
( <i>E</i> )- $\beta$ -Ocimene	1052	1044	-	5.9	0.5
$\gamma$ -Terpinene	1061	1054	1.8	0.8	-
<i>cis</i> -Sabinene hydrate	1075	1065	0.1	-	-
$\alpha$ -Terpinolene	1090	1086	0.7	0.6	-
Linalool	1100	1095	0.2	27.0	1.4
<i>trans-p</i> -Menth-2-en-1-ol	1136	1136	0.2	-	-
<i>trans</i> -Sabinol	1137	1137	0.2	-	-
<i>trans</i> -Pinocarveol	1138	1135	0.1	-	-
Borneol	1167	1165	3.5	-	-
Linalool oxide (pyranoid)	1173	1170	0.2	-	-
Terpinen-4-ol	1177	1174	6.7	9.8	1.3
$\alpha$ -Terpineol	1189	1186	10.7	-	-
Nerol	1227	1227	0.2	-	-
( <i>E</i> )-Cinnamaldehyde	1270	1270	0.1	1.0	0.8
Bornyl acetate	1289	1287	0.1	-	-
Carvacrol	1300	1298	0.1	-	-
( <i>Z</i> )-Dimethoxy citral	1318	1316	0.1	2.1	-
Bicycloelemene	1337	1338	-	-	2.1
$\delta$ -Elemene	1340	1335	0.4	-	-
$\alpha$ -Cubebene	1351	1345	0.6	-	0.8
Eugenol	1356	1356	-	5.6	4.1
Cyclosativene	1371	1369	0.6	-	-
$\alpha$ -Ylangene	1375	1373	0.2	-	-
$\alpha$ -Copaene	1377	1374	2.5	-	5.7
$\beta$ -Cubebene	1388	1387	-	-	1.7
$\beta$ -Elemene	1397	1398	1.0	-	1.1
Dodecanal	1408	1408	0.4	-	-
$\beta$ -Caryophyllene	1419	1417	2.1	-	17.2
$\gamma$ -Elemene	1437	1434	0.4	-	-

Compounds <sup>b</sup>	RI <sup>c</sup>	RI <sup>d</sup>	Percentage composition ( $\pm$ SD) <sup>a</sup>		
			<i>C. kunstleri</i>	<i>C. cambodianum</i>	<i>C. rigidifolium</i>
Aromadendrene	1441	1439	0.1	-	26.0
$\alpha$ -Humulene	1454	1452	0.5	-	3.0
$\alpha$ -Amorphene	1485	1483	4.3	-	-
$\beta$ -Selinene	1489	1489	0.4	-	-
$\delta$ -Selinene	1493	1492	0.1	-	-
<i>cis</i> -Cadin-1,4-diene	1496	1495	1.4	-	1.1
$\alpha$ -Muurolene	1500	1500	0.6	-	-
Bicyclogermacrene	1501	1500	-	-	2.9
$\gamma$ -Cadinene	1513	1513	0.8	-	-
<i>cis</i> -Z- $\alpha$ -Bisabolene epoxide	1515	1515	0.5	-	1.7
$\delta$ -Cadinene	1526	1522	3.7	-	-
$\alpha$ -Calacorene	1546	1544	0.3	-	-
Spathulenol	1578	1577	0.4	-	3.6
Caryophyllene oxide	1583	1582	0.6	-	5.6
Viridiflorol	1593	1592	-	-	0.7
<i>trans</i> - $\beta$ -Elemenone	1601	1601	0.7	-	-
Methoxy eugenol	1610	1609	-	-	4.2
Tetradecanal	1611	1611	0.5	-	-
Fonenol	1621	1621	0.1	-	-
5- <i>epi</i> -Neointermedeol	1639	1639	-	1.1	-
$\tau$ -Muurolol ( <i>epi</i> - $\alpha$ -Muurolol)	1646	1640	-	-	3.2
$\alpha$ -Cadinol	1654	1653	1.0	-	1.2
Valerianol	1655	1656	0.1	-	-
Phenylethyl benzoate	1842	1841	-	-	2.3
1,2-Benzenedicarboxylic acid	1917	1917	1.0	-	3.1
Hexadecanoic acid methyl ester	1928	1926	-	-	3.8
Hexadecanoic acid	1960	1959	0.1	-	-
(Z)-13-Docosenamide	2499	2499	0.1	0.1	0.3
TOTAL			99.6	99.7	98.7
Monoterpene hydrocarbons			26.3	53.0	1.3
Oxygenated monoterpenes			47.8	39.9	6.2
Sesquiterpene hydrocarbon			21.2	-	61.6
Oxygenated sesquiterpenes			2.2	1.1	16.0
Phenylpropanoids			-	5.6	4.1
Aromatic esters			-	-	2.3
Fatty acids			0.6	-	3.8
Non-terpenes			1.5	0.1	3.4

<sup>a</sup>SD Standard deviation, values were insignificant and excluded from the table to avoid congestion; <sup>b</sup> Elution order on HP-5MS capillary column; <sup>c</sup> Retention indices on HP-5MS capillary column; <sup>d</sup> Literature Retention indices; - Not identified

Sesquiterpene hydrocarbons (61.6%) and oxygenated sesquiterpenes (16.0%) represent the major fractions of the bark oil of *C. rigidifolium*. The main constituents of the oil were aromadendrene (26.0%),  $\beta$ -caryophyllene (17.2%) and  $\alpha$ -copaene (5.7%). Monoterpenes (7.5%) were less common and are represented mainly by linalool (1.4%) and terpinen-4-ol (1.3%). It should be noted that  $\alpha$ -selinene and germacrene D the main constituents of the leaf oil [2] were conspicuously absent in the stem oil.

It is well known that the biological activities of an essential oil may depend on the major constituents or a synergy between the major and some minor compounds. Referring to literature, essential oils containing large amounts of the major compounds found in the studied *Cinnamomum* species such as 1, 8-cineole,  $\alpha$ -terpineol, terpinen-4-ol, linalool, limonene and  $\beta$ -caryophyllene have shown antimicrobial, antioxidant, anti-inflammatory, cytotoxicity, larvicidal and insecticidal potentials [25-27]. This

may as well contribute to the biological potentials of the plant samples.

The authors have no literature citation on the volatile contents of the bark oils of these plant species and such the present results may represent the first of its kind. Nevertheless ubiquitous terpenes were the main compounds present in the oil samples. These findings provided additional information on the chemical constituents of essential oils from different parts of the studied plant samples and may be of chemotaxonomic importance.

The low content of (*E*)-cinnamaldehyde (0.1-1.0%) in the studied oil samples is in agreement with previous results for majority of *Cinnamomum* species already reported from Vietnam. It could be seen that each species has its own compositional pattern quite different from the leaves oils. It is well known that different parts of plant accumulate different phytochemicals [1,2]. These differences in accumulated phytochemicals may be attributed to differences in ethnomedical uses and biological potentials of the different parts of the same plant. Other factors such as the nature of the plant, geographical areas, time of collection, method of extraction, plant parts and maturation of the harvested plant may be responsible for the varying compositional pattern between the studied samples and other *Cinnamomum* plants grown in Vietnam.

#### 4. CONCLUSION

For the first time, the chemical constituents of essential oils from the stem barks *C. kunstleri*, *C. rigidifolium* and *C. cambodianum* endemics to Vietnam are being reported. The compositional patterns were found to be different from those reported previously for other *Cinnamomum* plants grow in Vietnam and other parts of the world. In addition, the compounds present in the oil maybe responsible for the observed biological potentials of the plant samples.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist

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