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Identification of Volatile Constituents of the Stem bark and Fruits of *Desmos cochinchinensis* Lour

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Abstract
The chemical constituents of essential oils obtained from the hydrodistillation of the stem bark and fruits of *Desmos cochinchinensis* Lour, were being reported. The combined techniques of gas chromatography-flame ionization detector (GC-FID) and gas chromatography-mass spectrometry (GC-MS) were employed in the analysis. The main compounds of the stem bark oil were β-caryophyllene (16.9%), bicyclogermacrene (11.6%) and benzyl benzoate (10.1%) while the fruit oil was characterized by the abundance of β-caryophyllene (20.9%), limonene (15.9%) and germacrene D (12.5%). The volatile contents of the stem bark and fruit of *Desmos cochinchinensis* have not been previously analysed and reported.

Keywords: *Desmos cochinchinensis,* essential oil, monoterpenes, sesquiterpenes.

1. Introduction
Desmos cochinchinensis Lour (family Annonaceae) is a spreading shrub that likes shady places. The flower opens between April and July [1]. Only mature flowers have strong scent that can be detected at a distance. The decoction of the dried leaf, stem or root part of the plant is used in folk medicine for the treatment of malaria [1]. Phytochemical analysis revealed the isolation of flavans with potent aromatase inhibitory activity [2], desmosinol, a cycloartane triterpenoid [3], desmosic acid, a cytotoxic fatty acid [4], 5-hydroxy-7-methoxy- 6,8-dimethylflavone, unonal, desmethoxymatteucinol and β-sitosterol [5], lawinal, isounonal and 4,7-dihydroxyl-5-methoxyl-6-methyl-8-formyl-flavan [6], cardamonin and chrysin with potent antioxidant activity [7], desmosflavans A and B, pinocembrin, and chrysin which are inhibitors of aromatase [7, 8]. Desmosflavans A inhibited lipoxygenase while desmosflavans A and B exhibited cytotoxic activity [7].
Previous analysis of the flower essential oil showed that camphor (59.1%), limonene (8.9%), α-pinene (8.6%) and camphene (6.0%) were the major constituents [9]. β-Caryophyllene (56.2%) and spathulenol (61.5%) were respectively identified as the major compound in both young and ripened flower [10]. In another report, the leaf oil comprised mainly of β-caryophyllene (26.3%), germacrene D (14.6%), α-pinene (11.5%) and bicyclogermacrene (11.4%) [11].
The present paper described the volatile constituents of the stem bark and fruits of the plant which has been lacking in the literature.

2. Materials and methods
2.1 Plants collection
Stem bark and fruit of *D. cochinchinensis* were obtained from Pù Huống Natural Resever, Nghệ an Province, Vietnam, on May 2013. Voucher specimen DND 280 was deposited at the Botany Museum, Vinh University, Vietnam. Plant samples were air-dried prior to extraction.

2.2 Extraction of the oils
0.5 Kg of each plant samples was shredded and their oils were obtained by hydrodistillation for 3h at normal pressure, according to the Vietnamese Pharmacopoeia [12]. The plant samples yielded a low content of essential oils: 0.21 and 0.33% (v/w*;* stem and fruit respectively; both light yellow) calculated on a dry weight basis.

2.3 Gas Chromatography (GC) 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 °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 μL. 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 (2 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 08 Libraries (on ChemStation HP) and Wiley 9th 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 [13, 14].

3. Results & Discussion: The identities and the percentage composition of the compounds resent in the oil samples could be seen in Table. Sesquiterpene hydrocarbons (64.9% and 61.3% respectively) were the main class of compounds present in both the stem and fruit oils. Monoterpene hydrocarbons (27.5%) are prominent in the fruit oil while oxygenated sesquiterpenes (12.5%) and aromatic ester (10.1%) were peculiar to the stem oil. 34 and 31 compounds representing 96.7% and 96.1% of the total oil contents were identified in both oils.

The main compound present in the stem oil were β-caryophyllene (16.9%), bicyclogermacrene (11.6%) and benzyl benzoate (10.1%). There were significant amounts of α-humulene (6.6%), τ-cadinol (6.3%), bicycloelemene (5.1%) and δ-cadinene (5.0%). On the other hand, β-caryophyllene (20.9%), limonene (15.9%) and germacrene D (12.5%) were the principal components of the fruit oil. It has sizeable amount of α-pinene (6.6%), β-elemene (6.3%), α-humulene (6.1%) and bicyclogermacrene (5.5%).

Literature information showed that previous analyses were focused on the volatile contents of the flower [9, 10] and leaf oils [11]. Though β-caryophyllene, bicyclogermacrene and germacrene D were present in this, several other compounds such as limonene and benzyl benzoate were not previously reported as major constituents of in previous studies on other parts of *D. cochinchinensis*.

The major constituents or synergy between the major and minor constituents present in the studied oil samples may have been responsible for the observed antimalarial potentials of the plant. β-Caryophyllene was known to have contributed to the antimalarial effects of essential oils [15, 16]. Other compounds such as limonene, α-pinene, α-humulene and germacrene D were known to possess antiprotozoal activity [16].

It is well known that there are variations between the volatile compositions of different parts of the same plant. This and other variable factors such as time of collection, age of plant, handling procedure, processing methods etc suggest the influence of external factors on volatile productions of different parts of the plant.

3.1 Tables

Table 1: Constituents of the stem bark and fruits of *Desmos cochinchinensis*

Compounds [a]	RI[b]	RI[c]	Stem	Fruits
α-Pinene	939	932	2.6	6.6
Sabinene	976	969	-	0.2
β-Pinene	982	974	1.0	1.4
β-Myrcene	990	988	0.3	1.2
δ-3-Carene	1013	1006	0.3	0.1
α-Terpinene	1016	1014	-	0.2
Limonene	1032	1024	-	15.9
(*E*)-β-Ocimene	1052	1044	2.0	0.9
γ-Terpinene	1061	1054	0.2	0.9
α-Terpinolene	1090	1084	-	0.1
Linalool	1100	1095	0.5	0.2
(E)-4,8-dimethyl-1,3,7-Nonatriene	1104	1105	0.2	-
Ethyl benzoate	1171	1175	-	0.1
Bicycloelemene	1327	1338	5.1	4.4
α-Cubebene	1351	1345	0.2	0.3
α-Copaene	1377	1374	2.2	0.9
di-epi-α-cedrene	1385	1385	-	0.2
β-Cubebene	1388	1387	-	1.5

β-Elemene	1391	1389	2.2	6.3
α-Gurjunene	1412	1409	-	0.2
β-Caryophyllene	1419	1417	16.9	20.9
γ-Elemene	1437	1435	1.3	-
Aromadendrene	1441	1439	0.9	0.2
α-Humulene	1454	1452	6.6	6.1
γ-Gurjunene	1477	1475	3.5	-
Germacrene D	1485	1484	2.3	12.5
α-Amorphene	1485	1483	2.3	-
β-Selinene	1486	1486	0.5	-
Cadina-1,4-diene	1496	1496	1.9	-
Bicyclogermacrene	1500	1500	11.6	5.5
β-Bisabolene	1506	1506	2.3	-
δ-Cadinene	1525	1522	5.0	1.9
α-Calacorene	1546	1544	0.4	-
Elemol	1550	1548	-	0.2
Germacrene B	1561	1559	-	0.4
Spathulenol	1579	1577	0.9	-
Viridiflorol	1593	1593	1.0	-
Ledol	1602	1602	0.7	0.1
τ-Cadinol	1633	1644	6.3	-
4-*allyl*-1,2-Diacetoxybenzene	1647	1647	-	0.6
α-Cadinol	1654	1652	3.6	0.3
Benzyl benzoate	1760	1759	10.1	1.3
n-Hexadecanoic acid	1982	1959	0.7	-
Octadecanoic acid	2188	200	0.2	-
(*Z*)-9-Octadecamide	2398	2398	1.3	-
TOTAL			**96.7**	**91.6**
Monoterpene hydrocarbons			**6.4**	**27.5**
Oxygenated monoterpenes			**0.5**	**0.2**
Sesquiterpene hydrocarbons			**64.9**	**61.3**
Oxygenated sesquiterpenes			**12.5**	**0.6**
Aromatic esters			**10.1**	**1.4**
Others			**1.5**	**0.6**

[a] Elution order on HP-5MS column; [b] Retention indices on HP-5MS column; [c] Literature retention indices; -Not determined

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