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Chemical compositions and antimicrobial activity of essential oil from the rhizomes of *Curcuma singularis* growing in Vietnam

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Abstract
Curcuma singularis Gagnep., (Zingiberaceae) was found distributed in the Central Highlands of Vietnam and described as a new record for the country's flora. From the fresh rhizomes of this plant, an essential oil was obtained by hydrodistillation with 0.31% yield (w/w). By GC-FID and GC-MS analysis, a total of 68 compounds were detected, of which 60 components accounting for 90.96% of the total oil were identified. Almost all of the components are mono- and sesquiterpenes (65 compounds, 98.57% total oil), most of them are oxygenated (51 compounds, 85.46% total oil). Major components are camphor (25.83%), germacrone (8.00%), caryophyllene oxide (4.48%), terpinen-4-ol (3.84%), and germacrone-4,5-epoxide (3.84%). In the antibacterial assays, the oil exhibited significant activity against the Gram positive bacteria *Bacillus subtillis* (MIC = 100 µg/ml) and the Gram negative bacteria *Escherichia coli* (MIC = 200 µg/ml).

Keywords: *Curcuma singularis,* Zingiberaceae, new record, essential oil, antimicrobial activity

1. Introduction

The genus *Curcuma* is one of the largest genera of the Zingiberaceae family in flora of Vietnam. It consists of 27 scientifically described species, which are distributed throughout Vietnam [1-4]. The rhizomes of *Curcuma* species are the most prescribed drugs in Vietnamese traditional medicine for the treatment of digestive disorders, stomach pain, jaundice, etc [5]. Recent studies showed that many of them possess a variety of useful properties, such as antibacterial, antifungal, antiviral, anti-inflammatory, cytotoxic and anti-cancer activities [6-9]. These bioactivities explain their widespread applications in pharmaceutical products, especially in those for the treatment of dermatological diseases (itch, scabies), rheumatism, high level of cholesterol, and gastrointestinal illnesses.

Besides diarylheptanoids, for example curcumin, essential oils are known to be the active principles of *Curcuma* rhizomes [2, 6, 9]. Chemical studies on essential oils from several *Curcuma* species indicated the presence of mainly sesquiterpenes [10] and monoterpenes [11]. Compounds of other structural types were found only as minor constituents. Sesquiterpene compounds obtained from essential oils of *Curcuma* rhizomes often possess interesting characteristic structures and valuable biological activities [10, 12], and thus attracting much scientific interest.

Curcuma singularis Gagnep. (local name: *cay khoe*, which means "plant for health") was found distributed in the Central Highlands of Vietnam and described its medicinal use in folklore. Its rhizomes and roots are widely used by minority people living in this area for various medical indications. For example, the decoction of its roots and bulbils is used as a drink for male strengthening health and improving body conditions, the ethanol extract is used externally for muscle relaxation [1, 5]. Unlike other *Curcuma* species, until now *C. singularis* has not been chemically investigated. Therefore, the aim of this paper is to describe *Curcuma singularis* as a new record for the flora of the country and to report the results of our study on the chemical compositions and antibacterial properties of the essential oil obtained from the rhizomes of this plant.

2. Materials and methods
2.1 Plant material
The rhizomes of *C. singularis* were collected from the forest area of So Pai commune, KBang district, Gia Lai province, Vietnam in December 2013. A voucher specimen, SH-30, is deposited at Department of Botany, Vietnam National Museum of Nature, Vietnam Academy of Science and Technology, Hanoi, Vietnam.

2.2 Essential oil extraction
The fresh rhizomes of *C. singularis* (Fig.1) were cleaned, sliced, chopped and subjected to steam-distillation in a Clevenger-type apparatus for 4 h. The obtained essential oil was dried over anhydrous sodium sulfate and stored in a sealed vial at 10°C in the dark prior to analysis.

2.3 Essential oil analysis
GC-FID and GC-MS analysis of *C. singularis* rhizome oil was performed on an Agilent HP mode 7890A gas chromatograph (with FID detector) coupled to an Agilent 5973 mass spectrometer. Data acquisition and processing were performed using Agilent MSD productivity Chemstation Rev. E-02.02. Data interpretation was performed using the MassFinder 4.0 software. A fused-silica capillary Hewlett Packard HP5-MS (5% phenyl methyl siloxane) column (60 m x 0.25 mm i.d., 0.25 µm film thickness) was used for separation.
GC-FID operation conditions: the injector temperature was 250 °C; the detector temperature was 250 °C; the oven temperature was programmed from 60 to 240 °C at 4°C/min. Helium was used as carrier gas at a flow rate of 1.0 ml/min. A 1 µl of the oil sample was injected using split mode with a split ratio of 1:100.
GC–MS operation conditions: The mass spectrometer was operated in electron-impact (EI) mode, the ionization energy was 70 eV, the interface temperature was 280°C, the ion source temperature was 240 °C, the MS quadrupole temperature was 150°C, and the scan range was 35–450 amu. The GC operation conditions were identical to those described above for GC-FID.

2.4 Identification and quantification of essential oil constituents
Retention indices of oil constituents were determined on the HP5-MS column using standard C7–C30 straight chain hydrocarbons (Aldrich Chemical Company, USA). Individual compounds in the oil were identified by comparison of their mass spectra and retention indices with those in GC-MS libraries (NIST 08, Wiley 09 and HPCH1607) and/or with those reported in literatures. The relative percentage amounts of the separated compounds were computed from GC-FID data without the use of correction factors.

2.5 Antimicrobial activity assay
Four standardized ATCC strains from laboratory stock cultures were used in the evaluation of the antimicrobial activity of the rhizome oil of *C. singularis*. The Gram negative strains were *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 25923). The Gram positive strains were *Bacillus subtilis* (ATCC 11774) and *Staphylococcus aureus* subsp. *aureus* (ATCC 11632).
The *in vitro* antimicrobial activity assays were carried out on 96-well microtiter plate using the standard liquid dilution method described by Vanden Berghe and Vlietinck [13, 14]. For the assays, the essential oil was diluted with DMSO and loaded into the microtiter plate with each of the bacterial strains. The plate was then incubated overnight at 37ºC. The minimum inhibitory concentration (MIC) is defined as the lowest concentration of the test sample that inhibits a visible growth of a microorganism. In this study, MIC values were obtained by visual inspection of the wells after overnight incubation.

3. Results & Discussion
3.1 New record of *Curcuma singularis* species
The climate and terrain in Indochinese region have made this land be proper for the growing of numerous interesting ginger species [4].
Curcuma singularis Gagnep. species was widely distributed in the Central Highlands, South Vietnam. It is an herbaceous plant, 20-30 cm tall. *Rhizomes* small, externally ligh-yellow, fragrant; *Root* diamond-shaped or or oval-shaped at the end. *Leaf* blades elliptic or oblong, 20-27 x 6-8 cm, glabra; petiole 3-5 cm, externally reddish-brown. *Flower* ca 8.5 cm long; *calyx*, corolla white; *labellum* large obovate, ca 2.6 x 1.7 cm; white except a yellow median band by 2/3 length of labellum from the base; *lateral* staminodes obovate, ca 3.1 x 1.7 cm, white with pale yellow median band, half the length from the base. *Fruit* globose, diameter 7-8 mm, white. (Fig. 1).

(a) (b) (c)

Fig 1: Flower (a); Roots and bulbils (b); Rhizomes and roots (c) of *C. singularis*

3.2 Composition of the essential oil of *C. singularis* rhizomes
The essential oil of *C. singularis* rhizomes was obtained in 0.31% yield (w/w, fresh weight) by hydro distillation as a pleasant smelling yellow oil with a specific density of 0.88 g/mL (22°C) and a refractive index of 1.4949 (22°C). A total ion chromatogram obtained from the GC-MS analysis is presented in Fig. 2. The composition of the essential oil is presented in Table 1, whereby all peaks with less than 0.1% area and unknown peaks with less than 0.5% area were not

considered for analysis. With these criteria, a total of 68 compounds were detected, of which 60 components (accounting for 90.96% of the total oil) were identified. The identification of 58 compounds was obtained through comparison of their mass spectra and retention indices with those in GC-MS libraries, while 2 compounds (isogermacrone, and germacrone epoxide) were identified by comparison of their data with those reported in literatures [15].

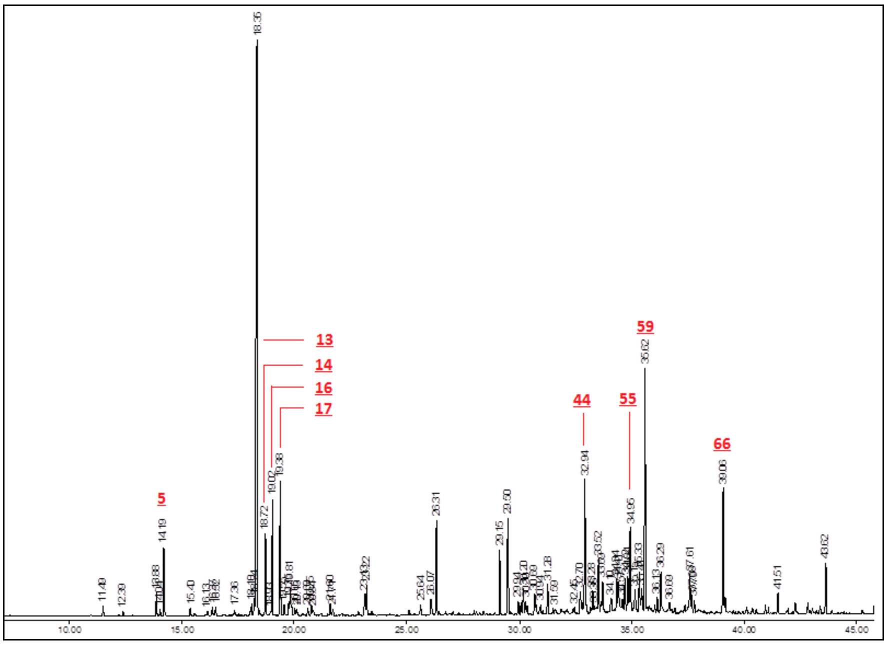

Fig 2: GC/MS total ion chromatogram of rhizome oil of *Curcuma singularis*

Table 1: Chemical compositions of rhizome oil of *Curcuma singularis*

Peak no.	Retention time (min)	Kovats index	Compounds	Molecular formula	Relative amount (%)
1	11.49	957	Camphene	$C_{10}H_{16}$	0.27
2	12.39	986	β-Pinene	$C_{10}H_{16}$	0.11
3	13.88	1030	o-Cymene	$C_{10}H_{14}$	0.61
4	14.04	1035	Limonene	$C_{10}H_{16}$	0.15
5	14.19	1039	1,8-Cineole	$C_{10}H_{16}O$	1.90
6	15.40	1074	*cis*-Sabinene hydrate	$C_{10}H_{16}O$	0.22
7	16.13	1095	*cis*-Linalool oxide (furanoid)	$C_{10}H_{20}O_2$	0.15
8	16.38	1102	Linalool	$C_{10}H_{18}O$	0.25
9	16.52	1106	*trans*-Sabinene hydrate	$C_{10}H_{16}O$	0.26
10	17.36	1130	*cis-p*-Menth-2-en-1-ol	$C_{10}H_{18}O$	0.16
11	18.10	1151	*cis*-Sabinol	$C_{10}H_{16}O$	0.50
12	18.24	1155	*trans*-Sabinol	$C_{10}H_{16}O$	0.46
13	18.35	1158	Camphor	$C_{10}H_{16}O$	25.83
14	18.72	1169	Isoborneol	$C_{10}H_{18}O$	2.45
15	18.93	1175	Pinocarvone	$C_{10}H_{14}O$	0.13
16	19.02	1177	Borneol	$C_{10}H_{18}O$	3.34
17	19.38	1187	Terpinen-4-ol	$C_{10}H_{18}O$	3.84
18	19.55	1192	*p*-Cymen-8-ol	$C_{10}H_{14}O$	0.24
19	19.75	1198	Cryptone	$C_9H_{14}O$	0.36
20	19.81	1199	α-Terpineol	$C_{10}H_{18}O$	0.77
21	20.05	1206	Myrtenol	$C_{10}H_{16}O$	0.25
22	20.13	1209	Myrtenal	$C_{10}H_{14}O$	0.23
23	20.60	1222	Verbenone	$C_{10}H_{14}O$	0.26
24	20.75	1227	*trans*-Carveol	$C_{10}H_{16}O$	0.38
25	20.84	1229	Fenchyl acetate	$C_{12}H_{20}O_2$	0.17
26	21.60	1251	Cuminaldehyde	$C_{10}H_{12}O$	0.37
27	21.71	1254	Carvone	$C_{10}H_{14}O$	0.22
28	23.13	1296	2-Undecanone	$C_{11}H_{22}O$	0.64
29	23.22	1298	Thymol	$C_{10}H_{14}O$	0.97
30	25.64	1371	(*Z*)-β-Damascone	$C_{13}H_{20}O$	0.42
31	26.07	1384	Cyclosativene	$C_{15}H_{24}$	0.58
32	26.31	1391	α-Copaene	$C_{15}H_{24}$	2.80
33	29.15	1482	9-*epi*-(*E*)-Caryophyllene	$C_{15}H_{24}$	2.04
34	29.50	1493	β-Acoradiene	$C_{15}H_{24}$	3.16
35	29.95	1507	β-Selinene	$C_{15}H_{24}$	0.43
36	30.20	1516	Cubebol	$C_{15}H_{26}O$	0.93

37	30.30	1519	β-Bisabolene	$C_{15}H_{24}$	0.44
38	30.69	1532	γ-Cadinene	$C_{15}H_{24}$	0.74
39	30.94	1540	*trans*-Calamenene	$C_{15}H_{24}$	0.49
40	31.28	1552	Caryophyllenyl alcohol	$C_{15}H_{26}O$	0.95
41	31.59	1562	α-Calacorene	$C_{15}H_{24}$	0.31
42	32.45	1591	*ar*-Turmerol	$C_{15}H_{22}O$	0.26
43	32.70	1599	Spathulenol	$C_{15}H_{24}O$	1.13
44	32.94	1608	Caryophyllene oxide	$C_{15}H_{24}O$	4.48
45	33.28	1620	Himachalene oxide	$C_{15}H_{24}O$	0.79
46	33.36	1622	Curzerenone	$C_{15}H_{18}O_2$	0.26
47	33.52	1628	Unknown sesquiterpene[1*]	$C_{15}H_{26}O$	1.77
48	33.69	1634	Humulene-1,2-epoxide	$C_{15}H_{24}O$	1.33
49	34.10	1649	1-*epi*-Cubenol	$C_{15}H_{26}O$	0.60
50	34.34	1657	Unknown sesquiterpene[2*]	$C_{15}H_{26}O$	1.08
51	34.41	1660	Unknown sesquiterpene[3*]	$C_{15}H_{26}O$	1.17
52	34.57	1665	*epi*-α-Cadinol	$C_{15}H_{26}O$	0.58
53	34.72	1671	α-Cadinol	$C_{15}H_{26}O$	1.14
54	34.84	1675	β-Eudesmol	$C_{15}H_{26}O$	1.34
55	34.95	1679	*ar*-Turmerone	$C_{15}H_{20}O$	2.90
56	35.15	1686	Germacrone isomer	$C_{15}H_{22}O$	1.06
57	35.33	1692	Eudesm-7(11)-en-4-ol	$C_{15}H_{26}O$	1.41
58	35.43	1696	Cadalene	$C_{15}H_{18}$	0.98
59	35.62	1703	Germacrone	$C_{15}H_{22}O$	8.00
60	36.13	1722	Curcumenol	$C_{15}H_{22}O_2$	0.55
61	36.29	1727	Isogermacrone	$C_{15}H_{22}O$	1.37
62	36.69	1742	Curdione	$C_{15}H_{24}O_2$	0.72
63	37.61	1776	Unknown sesquiterpene[4*]	$C_{15}H_{24}O$	1.18
64	37.68	1779	Unknown sesquiterpene[5*]	$C_{15}H_{26}O$	0.55
65	37.79	1783	Benzyl benzoate	$C_{14}H_{12}O_2$	0.37
66	39.06	1832	Germacrone-4,5-epoxide	$C_{15}H_{22}O_2$	3.84
67	41.51	1929	Unknown sesquiterpene[6*]	$C_{15}H_{22}O_2$	0.71
68	43.62	2016	Unknown sesquiterpene[7*]	$C_{17}H_{24}O_2$	1.63

Total number of constituents	68
Number (%) of constituents identified	60 (90.96%)
Number (%) of monoterpene hydrocarbons	4 (1.15%)
Number (%) of oxygenated monoterpenes	24 (43.74%)
Number (%) of sesquiterpene hydrocarbons	10 (11.97%)
Number (%) of oxygenated Sesquiterpenes	27 (41.72%)
Number (%) of others	3 (1.43%)

*The *m/z* values of major fragments of unknown compound.

As shown in Table 1, almost all components of the oil are terpenes with approximately equal amount of monoterpenes (28 compounds, 44.89% of the total oil) and sesquiterpenes (37 compounds, 53.69% of the total oil), most of them are oxygenated compounds (51 compounds, 85.46% of the total oil). The major components of the essential oils are camphor (13, 25.83%), germacrone (59, 8.00%) and caryophyllene oxide (44, 4.48%), terpinen-4-ol (17, 3.84%), and germacrone-4,5-epoxide (66, 3.84%). The contents of the remaining components are below 4%, most of them (55 compounds) even below 2%.

The sesquiterpene profile of the rhizome oil of *C. singularis* is characterized by the presence of germacrone (59, 8.00%), germacrone-4,5-epoxide (66, 3.84%), *ar*-turmerone (55, 2.90%), isogermacrone (61, 1.37%), curdione (62, 0.72%), curcumenol (60, 0.55%), curzerenone (46, 0.26%), and ar-turmerol (42, 0.26%). The monoterpene profile of the rhizome oil of *C. singularis* is characterized by the presence of camphor (13, 25.83%), terpinen-4-ol (17, 3.84%), borneol (16, 3.34%), isoborneol (14, 2.45%) and 1,8-cineol (5, 1.90%).

3.3 Antimicrobial activity

The rhizome oil of *C. singularis* did not show inhibitory effects to *Pseudomonas aeruginosa* and *Staphylococcus aureus* but exhibited significant antibacterial activity against the Gram positive bacteria *Bacillus subtilis* (MIC = 100 µg/ml) and the Gram negative bacteria *Escherichia coli* (MIC = 200 µg/ml).

The plant *Curcuma singularis* Gagnep., distributed in the Central Highlands, South Vietnam, is morphologically described in details. This is the first report regarding the chemical compositions and antimicrobial activity of the essential oil extracted from *C. singularis* rhizome. Sixty eight components accounting for 90.96% of the total oil (yield 0.31% (w/w)) from the fresh rhizomes were identified.

[1] 41(100), 55 (60), 67 (61), 81 (70), 95 (89), 109 (88), 119 (39), 135 (20), 149 (17), 161 (60), 179 (12), 189 (13), 204 (17), 220 (3), 222 (3).
[2] 41 (100), 55 (60), 69 (60), 79 (49), 91 (58), 109 (56), 125 (45), 137 (21), 149 (20), 166 (18), 177 (35), 191 (10), 206 (17).
[3] 41 (100), 55 (52), 69 (68), 79 (58), 91 (65), 105 (42), 121 (30), 138 (40), 147 (11), 161 (10), 177 (50), 193 (10), 208 (9).
[4] 43 (98), 55 (18), 79 (30), 91 (70), 105 (48), 117 (50), 131 (35), 159 (100), 220 (2)..
[5] 41 (100), 55 (65), 69 (69), 81 (85), 95 (70), 109 (37), 123 (38), 137 (85), 222 (8).
[6] 41 (100), 55 (78), 67 (72), 83 (75), 95 (42), 109 (43), 123 (60), 135 (22), 150 (23), 163 (10), 178 (8), 191 (4), 201 (23), 219 (15), 234 (40).
[7] 43 (100), 81 (78), 109 (40), 121 (52), 137 (30), 149 (22), 260 (10).

Almost all components of the oil are terpenes monoterpenes and sesquiterpenes (mostly oxygenated sesquiterpenes).
The sesquiterpene profile of the rhizome oil of *C. singularis* is different from those of other well-known *Curcuma* rhizome oils, such as oil of *C. longa* (with α-turmerone, β-turmerone, and *ar*-turmerone as main and characteristic constituents), oils of *C. zanthorrhiza* and *C. aromatica* (with xanthorrhizol and curcumene as main and characteristic constituents) [16]. Bicyclic caryophyllene sesquiterpenes (9-*epi*-(*E*)-caryophyllene (33) 2.04%, caryophyllenyl alcohol (40) 0.95%, caryophyllene oxide (44) 4.48%) were present in *C. singularis* in moderate total amount of 7.47% in compare to their presence in other essential oils such as *C. mangga* oil (12.69%), *C. aeruginosa* (1.01%) [9] and *C. aromatica* (6.33%) [17]. The sesquiterpene profile of rhizome oil of *C. singularis* is more similar to those obtained from the Chinese *C. wenyujin*, *C. kwangsiensis*, and *C. phaeocaulis* species which are used in traditional Chinese medicine as the drug Ezhu [18]. In respect of its monoterpene profile, the rhizome oil of *C. singularis* is similar to the oil obtained from one sample of the Indian *C. zedoaria* species (camphor 10.3%, isoborneol 2.7%, cineol 1.9%, and borneol 0.8%), which also has a similar sesquiterpene profile [19].. The oil of *C. singularis* is different from the above mentioned oils in its low content of curzerenone (only 0.24% as compared to 5-30% in the other oils).
In the antibacterial assays, the *C. singularis* essential oil displayed no inhibitory activity to the growth of the Gram-positive *S. aureus* and Gram-negative *P. aeruginosa*. However, the oil exhibited significant activity against the Gram positive bacteria *Bacillus subtillis* (MIC = 100 µg/ml) and the Gram negative bacteria *Escherichia coli* (MIC = 200 µg/ml). When compared with corresponding values found for rhizome oil of *C. longa*, these values are lower [2]. Interestingly, the amount of germacrone (59) in *C. singularis* essential oil was quite high (8.00%). This compound was reported by Suphrom *et al*. to possess *in vitro* and *in vivo* anti-androgenic effect by inhibition 5α-reductase activity, which converts testosterone to dihydrotestosterone and was a potential lead compound for treatment of androgen-dependent disorders [21].. This effect might explain the name of *C. singularis* as “plant for health” especially for male health in folk medicine.

4. Conclusion
Curcuma singularis is found and described as a new botanical record in Vietnam. The essential oil of *Curcuma singularis* rhizome contains variety of sesquiterpenes with camphor as major component. The high camphor content together with the antimicrobial activity of the oil may be the reasons why the ethanol tinctures/decoctions and especially the essential oil from the rhizomes of *C. singularis* are used in folk medicine to calm down nervous stress, and to treat sprains and swellings.

5. Conflict of interest statement
We declare that we have no conflict of interest.

6. Acknowledgments
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