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Article

Chemical Composition of Essential Oils Extracted from the Leaves and Rhizomes of *Alpinia hongiaoensis* Tagane. (Zingiberaceae) growing Wild in Vietnam

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Abstract: *Alpinia hongiaoensis* is one of the newly discovered species that belong to the Zingiberaceae family. This study's purpose is to identify the chemical constituents of the essential oils extracted from the leaves and rhizomes of *A. hongiaoensis* using the Gas Chromatography-Mass Spectrometry (GC-MS) technique. A total of sixty-nine compounds were identified in both oils. There are forty-nine components were determined in the leaf oil. Among them, β-pinene (21.2%), aristolochene (11.32%), methyl *trans*-cinnamate (10.83%), β-selinene (9.44%), and valencene (5.48%) were the main compounds. Meanwhile, fifty compounds were identified in the rhizome oil. The major constituents of the rhizome oil of *A. hongiaoensis* were β-pinene (28.4%), followed by limonene (10.85%), α-pinene (9.76%), camphene (6.59%), and methyl *trans*-cinnamate (5.78%). To the best of our knowledge, there is no information on the study of the phytochemistry and pharmacological activity of *A. hongiaoensis* in the literature. Therefore, this study provided the first glance at the chemical compositions of essential oils prepared from *A. hongiaoensis* leaves and rhizomes.

Keywords: *Alpinia hongiaoensis,* essential oil, GC-MS, monoterpene, sesquiterpene.

Introduction

The genus *Alpinia* is the largest and one of the most important members of the Zingiberaceae family includes 246 accepted species, which are distributed tropical and subtropical Asia, Australia, and the Pacific Islands [1]. Many *Alpinia* plants have been widely used as spices, natural dyes, and traditional medicines to treat many diseases such as indigestion, stomach pain, vomiting, and intestinal infections [2]. Up to 2014, there were 31 species of the *Alpinia* genus which was recorded from Vietnam [3]. Several of them are many well-known species such as *A. officinarum*, *A. zerumbet*, *A. blepharocalyx*, *A. oblongifolia*, and *A. galanga*.

Alpinia hongiaoensis Tagane. (Figure 1) (local name: Riềng núi Hòn Giao) is one of fifteen new species, which was discovered in 2020 in Bidoup - Nui Ba National Park, Southern Highlands of Vietnam by Shuichiro Tagane *al et* [4]. *A. hongiaoensis* is a perennial herb, 70 cm tall. Its rhizome is creeping, reddish brown or yellowish brown, sparsely hairy; and its scales are broadly ovate-triangular (3-4 mm long). Its leafy shoots of mature flowering individuals with 9-11 leaves. Its inflorescence is a terminal raceme, erect, to 11 cm long, 12-flowered; peduncle 5.5-7 cm long [4]. According to the description of Shuichiro Tagane *al et*, *A. hongiaoensis* is similar to *A. newmanii* N. S. Lý [5]. Till the moment no information could be found in the literature on the chemical compositions and bioactivities of essential oils extracted from the leaves and rhizomes of *A. hongiaoensis*. Therefore, the purpose of this research was to determine the chemical constituents of essential oils extracted from the leaves and rhizomes of *A. hongiaoensis* for the first time.

Materials and methods

Plant material

Fresh leaves and rhizomes of *A. hongiaoensis* were harvested from Da Chais Commune, Lac Duong District, Lam Dong Province (12°10′31.2″N, 108°41′56.1″E) in August 2022. The botanical identification was performed by Truong Quang Cuong (Botanist, Bidoup Nui Ba National Park). For future reference, a voucher specimen (No. NDD088) has been deposited at the Institute of Applied Technology, Thu Dau Mot University, Binh Duong Province, Vietnam.

Distillation of essential oils

First, fresh leaves (300 g) and rhizomes (300 g) of *A. hongiaoensis* were washed and shredded. Then, essential oils from fresh leaves and rhizomes were obtained by hydro-distillation for 3.5h using a Clevenger-type apparatus, according to the Vietnamese Pharmacopoeia [6]. Each sample was extracted in triplicate. The resulting essential oils were dried with anhydrous Na_2SO_4, filtered, and kept in the dark at 4°C until analyzed.

Figure 1. *Alpinia hongiaoensis* Tagane. Photo taken by Truong Quang Cuong

Analysis of essential oils

In this study, the chemical compositions of the essential oils extracted from the leaves and rhizomes of *A. hongiaoensis* were performed using an Agilent Technologies 7890B GC System assembled with an HP-5MS Ultral Inert column (30 m × 0.25 mm, 0.25 µm film thickness), and equipped with an Agilent 5977B MSD model mass spectrometer. Helium was used as the carrier gas with a flow rate of 1.0 mL/min. Injection volume was 1.0 µL with a split ratio of 1:25. The oven temperature was programmed from 50°C (kept for 2 min) to 150°C at a rate of 5°C/min and it was maintained for 10 min. Then, rising from 150°C to 280°C at a rate of 10°C/min (kept for 10 min). Moreover, 300°C, 150°C, 300°C, and 230°C were considered for the injector, MS Quad, transfer line temperatures, and MS source, respectively. The MS conditions were documented at 70 eV, and the mass range was 50-550 amu at 2.0 scan/s. The individual compounds were identified based on a comparison of their retention indices and their mass spectral fragmentation patterns to those listed in the libraries (NIST17 and Adams book [7]). Relative percentage amounts of the volatile content were calculated from chromatograms.

Results and discussion

Physical properties of isolated essential oils

The hydro-distillation of fresh leaves and rhizomes of *A. hongiaoensis* gave 0.18 ± 0.02% and 0.21 ± 0.01% (w/w) essential oil, respectively, calculated on a fresh weight basis. Both of the essential oils were light-yellow liquids having lower densities than water.

Chemical composition of isolated essential oils

The GC-MS analyses of both oils (Figures 2 and 3) indicated that the chemical compositions of leaf and rhizome oils showed significantly differences, however still shared some major components. As can be seen from Table 1, the rhizome oil mainly contained monoterpene hydrocarbons (68.56%), followed by oxygenated monoterpenes (14.56%), while sesquiterpene

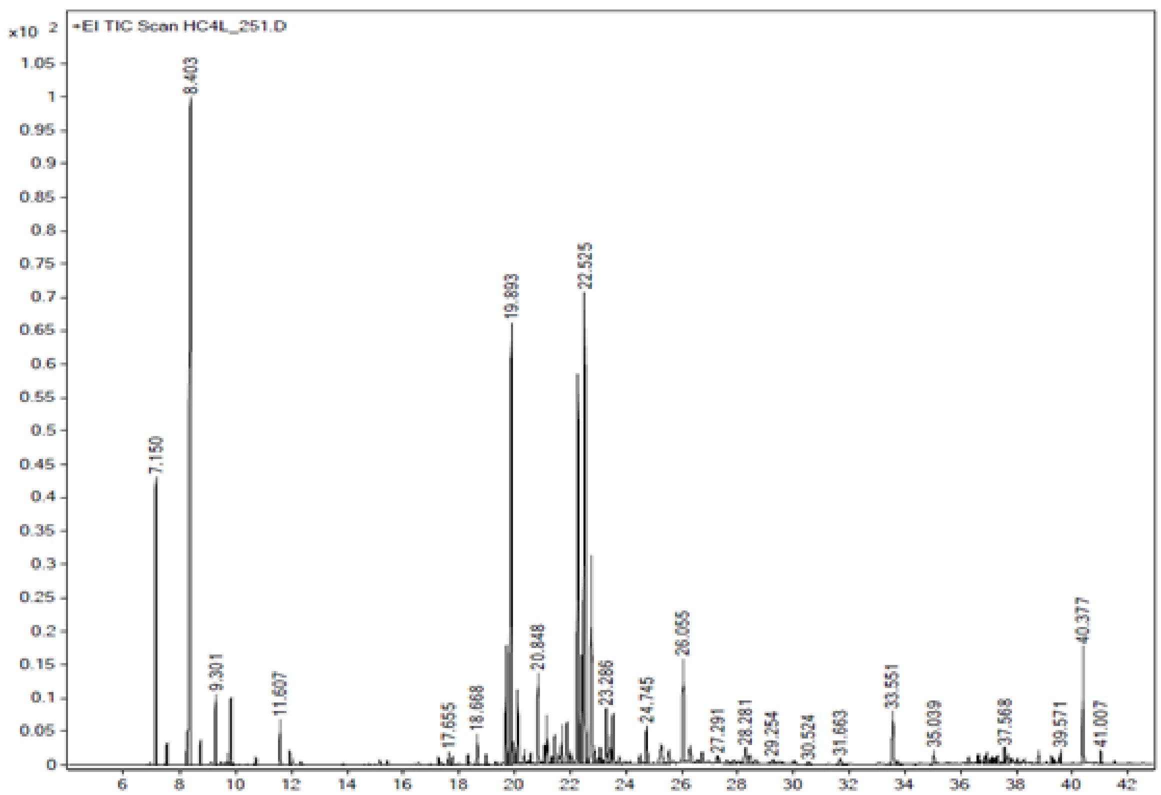

Figure 2. GC-MS chromatogram of the leaf essential oil of *A. hongiaoensis*

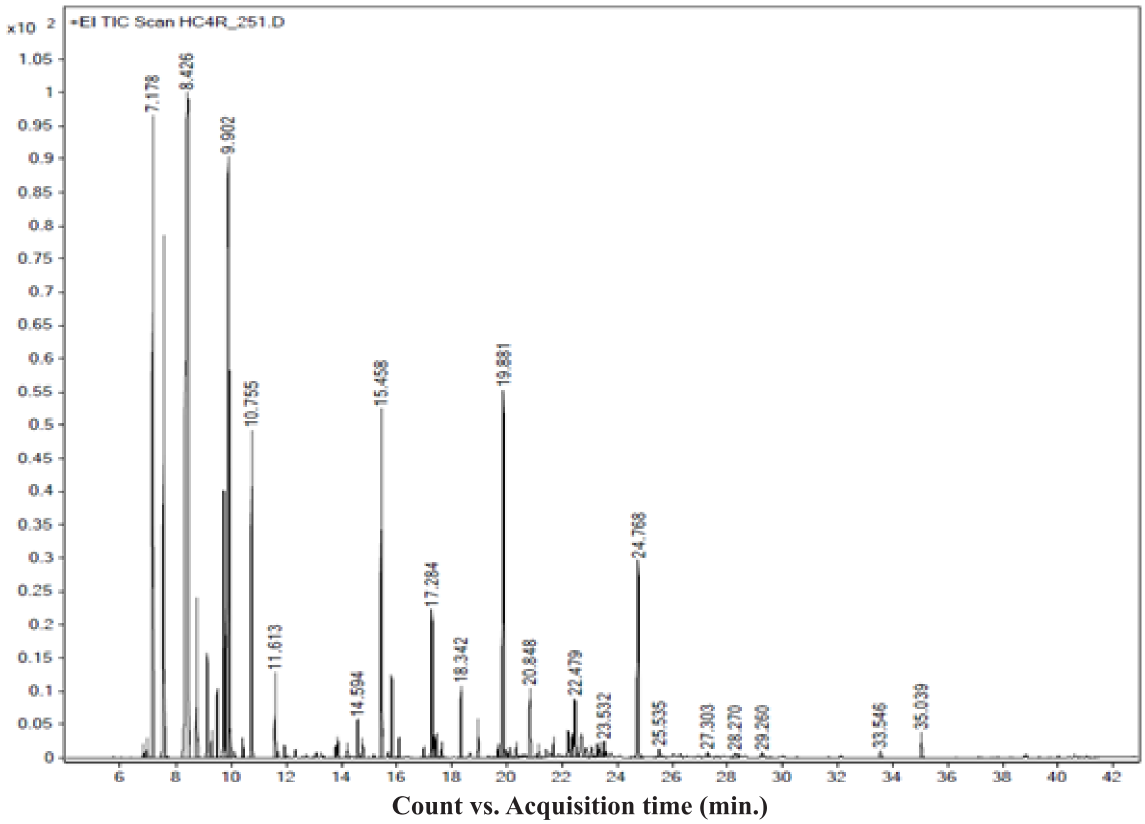

Figure 3. GC-MS chromatogram of the rhizome essential oil of *A. hongiaoensis*

Table 1. Chemical constituents of the *A. hongiaoensis* essential oils

No.	Compounds	RT (min.)	RI (Exp.)	RI (Lit.)	Area (%) Leaves	Rhizomes
1	Tricyclene	6.824	926	925	-	0.14
2	α-Thujene	6.967	932	929	-	0.22
3	α-Pinene	7.150	939	937	4.11	**9.76**
4	Camphene	7.550	954	952	0.33	**6.59**
5	4(10)-Thujene	8.282	978	974	0.61	-
6	β-Pinene	8.403	982	979	**21.20**	**28.40**
7	β-Myrcene	8.752	993	991	0.32	1.63
8	α-Phellandrene	9.141	1006	1005	-	1.21
9	3-Carene	9.301	1012	1011	1.03	0.31
10	α-Terpinene	9.496	1019	1017	-	0.75
11	p-Cymene	9.719	1028	1025	0.16	3.70
12	Limonene	9.845	1033	1030	1.05	**10.85**
13	Eucalyptol	9.959	1036	1032	-	4.00
14	α-Ocimene	10.429	1051	1047	-	0.20
15	γ-Terpinene	10.732	1063	1060	0.13	3.83
16	Terpinolene	11.607	1090	1088	0.70	0.97

table 1. (continued).

No.	Compounds	RT (min.)	RI (Exp.)	RI (Lit.)	Area (%) Leaves	Rhizomes
17	β-Linalool	11.933	1100	1099	0.23	0.16
18	Pinocarvone	13.799	1167	1164	-	0.14
19	*endo*-Borneol	13.873	1170	1167	-	0.27
20	Terpinen-4-ol	14.211	1181	1177	-	0.18
21	α-Terpineol	14.594	1193	1189	-	0.45
22	Myrtenal	14.766	1198	1193	-	0.24
23	Fenchyl acetate	15.458	1224	1223	-	4.43
24	Methyl thymyl ether	15.836	1238	1235	-	0.94
25	Methyl carvacryl ether	16.093	1248	1244	-	0.25
26	Bornyl acetate	17.278	1289	1285	0.13	1.75
27	Dihydroedulan	17.364	1292	1293	-	0.27
28	2-Undecanone	17.444	1295	1294	-	0.28
29	*trans*-Pinocarvyl acetate	17.655	1302	1297	0.20	0.21
30	Methyl *cis*-cinnamate	17.787	1307	1302	0.14	-
31	Myrtenyl acetate	18.336	1329	1327	0.16	0.83
32	δ-Elemene	18.668	1342	1338	0.52	-
33	α-Terpinyl acetate	18.966	1354	1350	-	0.46
34	α-Cubebene	18.988	1355	1351	0.24	-
35	α-Copaene	19.704	1381	1376	1.96	0.16
36	Methyl *trans*-cinnamate	19.893	1388	1379	**10.83**	**5.78**
37	β-Elemene	20.121	1396	1391	1.45	0.17
38	Cyperene	20.350	1405	1399	0.29	0.20
39	α-Gurjunene	20.591	1415	1409	0.20	-
40	Caryophyllene	20.848	1426	1419	1.73	1.01
41	β-Copaene	21.077	1435	1432	0.34	-
42	γ-Elemene	21.163	1438	1433	0.86	0.16
43	Aromandendrene	21.334	1445	1440	0.15	-
44	Guaia-6,9-diene	21.421	1449	1443	-	0.11
45	Selina-5,11-diene	21.443	1449	1447	0.58	-
46	Humulene	21.701	1460	1454	0.67	0.26
47	*allo*-Aromadendrene	21.884	1467	1461	0.87	-
48	γ-Gurjunene	22.222	1480	1473	-	0.54
49	β-Selinene	22.273	1482	1486	**9.44**	-
50	Germacrene D	22.404	1487	1481	2.26	0.42
51	Aristolochene	22.525	1491	1487	**11.32**	1.01
52	(+)-Valencene	22.754	1500	1492	**5.48**	0.75
53	α-Muurolene	22.879	1504	1499	0.33	-
54	Eremophilene	23.000	1508	1499	0.15	-
55	β-Bisabolene	23.080	1510	1509	0.36	0.18
56	γ-Cadinene	23.286	1517	1513	1.15	0.19
57	7-*epi*-α-Selinene	23.412	1521	1517	0.60	0.21
58	Cadina-1(10),4-diene	23.532	1525	1524	1.08	0.23

table 1. (continued).

No.	Compounds	RT (min.)	RI (Exp.)	RI (Lit.)	Area (%) Leaves	Rhizomes
59	*trans*-Nerolidol	24.745	1563	1564	0.88	3.51
60	(3*E*,7*E*)-4,8,12-Trimethyltrideca-1,3,7,11-tetraene	25.271	1579	1577	0.88	-
61	Caryophyllene oxide	25.535	1586	1581	-	0.17
62	*cis*-β-Elemenone	26.290	1606	1593	0.46	-
63	1,10-Diepicubenol	26.730	1616	1614	0.33	-
64	α-*epi*-Cadinol	27.875	1641	1640	0.17	-
65	*iso*-Elemicin	28.281	1649	1654	0.66	-
66	1-Pentadecanal	31.663	1720	1715	0.28	-
67	Xanthorrhizol	33.551	1764	1753	1.51	0.15
68	Ambrial	35.039	1797	1809	0.33	0.40
69	Phytol	40.377	2115	2114	1.37	-
	Total of identified compounds				90.23	99.03
	Monoterpenes hydrocarbons (Sr. No. 1-12, 14-16)				29.64	68.56
	Oxygenated monoterpenes (Sr. No. 13, 17-27, 29, 31, 33)				0.72	14.58
	Sesquiterpenes hydrocarbons (Sr. No. 32, 34, 35, 37-58)				42.03	5.60
	Oxygenated sesquiterpenes (Sr. No. 59, 61-64, 68)				2.17	4.08
	Others (Sr. No. 28, 30, 36, 60, 65-67, 69)				15.67	6.21

RT: Retention time (min)
RI (Exp.): Experimentally determined retention indices
RI (Lit.): Retention indices from literature; Area (%) in 'Bold' denotes major compounds (> 5%)
- Not identified

hydrocarbons were determined to be the major compositions of the leaf oil with the content of 42.03%, followed by monoterpene hydrocarbons (29.64%). Both leaf and rhizome essential oils were determined to contain β-pinene as the main component, specifically 21.2% for leaf oil and 28.4% for rhizome oil. In addition, methyl *trans*-cinnamate was also found abundantly in leaf and rhizome oil samples with the contents of 10.83 and 5.78%, respectively. However, it is noticeable that, each prepared essential oil contained characteristic volatile components with relatively high content. Specifically, the leaf oil was found to contain β-selinene (9.44%) and aristolochene (11.32%) while α-pinene and camphene were found to appear in the rhizome oil with noticeable amount (9.76 and 6.59%, respectively).

α- and β-pinene are two isomers of pinene (a bicyclic monoterpenoid with a double bond), which can be found abundantly in nature, especially from pine. The two compounds exhibited a wide range of pharmacological effects, especially anti-microbial, anti-viral, anti-allergic, anti-inflammatory, and anti-cancer activities [8,9]. Methyl *trans*-cinnamate is one of the main chemical components of *Alpinia* essential oils, besides α- and β-pinene [10].

Methyl *trans*-cinnamate was reported to possess potential pharmacological activities, including larvicidal activity against *Aedes aegypti* when in combination with linalool [11], suppressive effects on cell survival, migration and osteoblast differentiation [12], vasorelaxation effects in rat isolated aorta [13], and inhibitory effects on mushroom tyrosinase, as well as antimicrobial activities [14].

Conclusions

The yield and chemical constituents of the essential oils distilled from the leaves and rhizomes of *A. hongiaoensis* are demonstrated for the first time. The result revealed the predominance of sesquiterpene hydrocarbons (42.03%) and monoterpene hydrocarbons (29.64%) in the leaf oil, while monoterpene hydrocarbons (68.56%) and oxygenated monoterpenes (14.58%) were identified as the major components in the rhizome oil. Bioactivities of essential oils of the leaves and rhizomes of *A. hongiaoensis* might be studied and reported in our further investigations.

Competing interests

The authors declared no conflict of interest.

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