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Article

Chemical Composition of the Volatile Oil from the Leaves of *Kaempferia champasakensis* Picheans. & Koonterm. (Zingiberaceae)

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Abstract: The genus *Kaempferia* (Zingiberaceae) is well-known for its diverse phytochemical constituents and characteristic essential oil. *Kaempferia champasakensis* was firstly described in 2008 by Chayan Picheansoonthon and Supachai Koonterm in Champasak province of Southern Laos. To date, little is known about phytochemical components and pharmacological activities of the plant. In the present study, the extraction of essential oil from *K. champasakensis* leaves as well as Gas Chromatography - Mass Spectrometry (GC/MS) profile were investigated. The major components in essential oil extracted from *K. champasakensis* leaves were determined as β-caryophyllene (16.07%), β-elemenone (14.53%), β-pinene (13.80%), germacrone (9.85%), myrcene (9.46%) and diisooctyl phthalate (8.62%). This is the first report on chemical constituents of essential oil extracted from the leaves of *K. champasakensis*.

Keywords: β-Caryophyllene, β-elemenone, β-pinene, essential oil, *Kaempferia champasakensis.*

Introduction

The genus *Kaempferia* L. belonging to Zingiberaceae family, is widely distributed amongst India, South China, and South East Asia. It is estimated that there have been over fifty species in tropical Asian countries. More than twenty species can be found in Thailand while the reported numbers for Vietnam and China are

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only ten and six, respectively [1,2]. Phytochemical investigations on *Kaempferia* plants indicated the major constituents as diterpenoids with isopimarane, abietane, labdane, and clerodane scaffolds, phenylheptanoids, curcuminoids, tetrahydro-pyranophenolic, and steroids [3]. Crude extracts and isolated compounds from *Kaempferia* species were also found to possess a wide range of pharmacological activities, i.e anti-cancer, anti-microbial, and anti-inflammatory effects [3].

K. champasakensis (Figure 1) was firstly described in 2008 by Chayan Picheansoonthon and Supachai Koonterm in Champasak province of Southern Laos, which also explained the origin of the plant's name [4]. The species was firstly discovered in Vietnam through a ginger study (2015-2016; Binh Thuan and Ba Ria-Vung Tau provinces) with the local name of "Dia Lien hoa trang". *K. champasakensis* is a perennial herb with slender and long-creeping rhizome. It has 2-3 leaves with horizontal blades near the ground. Its flower (2-5) is white with tubular calyx (2-2.5 cm long), tubular corolla (3-4.2 cm long), and pure white and broadly obovate labellum (2-2.3×1.8-2 cm). Additionally, the fruits are fleshy and obovate (1- 1,3 cm × 5-7 mm) and the seeds are numerous with narrowly ellipsoid to deltoid (2-3 × 1-2 mm) [1,4]. The local people in Đắk Lắk Province use the young leave, especially the rolling leaves, of this species as salad, and its dry rhizome to treat stomach pain. To date, there has been no further research on phytochemical and pharmacological investigations on this species. *Kaempferia* plants in particular and Zingiberaceae plants in general are well-known for their signature essential oil, particularly *K. galanga*, *K. angustifolia*, *K. marginata*, which have been widely used in different pharmaceutical and food industries. The documented principal components were reported to contain phenylpropanoids and *trans*-ethyl cinnamate with the content varying from 16 to 35% of total essential oil components [3,5-7]. The volatile oil prepared from *K. galanga* were also shown to exhibit potential anti-microbial [8], larvicidal [9], and nematicidal [10] activities against various pathogenic microorganisms. The chemical constituents and biological activities of essential oils from some other *Kaempferia* species grown in Vietnam and other countries have been reported. For illustration, *α*-pinene (3.22%), camphene (23.63%), camphor (4.42%), borneol (4.80%), isoborneol (5.77%), ishwarane (3.29%) and 1,8-cineole (2.89%) were the major components of the essential oils of the rhizome of *K. daklakensis* from Daklak Province, Viet Nam [11]. The chemical constituents of the rhizomes of *K. galanga* from Guangxi province, China was reported to consist of *iso*-amyl *p*-methoxycinnamate (42.8-27.5%), *n*-pentadecane (21.6-32.8%), ethyl cinnamate (16.1-17.1%), cyperene (2.0-3.4%) and *p*-methoxystyrene (1.6-2.6%) [12], while the

Figure 1. *Kaempferia champasakensis* Picheans. & Koonterm

chemical composition of the rhizome essential oil of *K. rotunda* from Malaysia was dominated by pentadecane (25.4%), bornyl acetate (24.9%), benzyl benzoate (15.3%) and camphor (12.1%) [13]. Moreover, camphene (27.98%), camphor (18.73%) and α-pinene (7.42%) were the major components of the rhizome of *K. angustiflora*, while δ-3-carene (33.84%), β-pinene (12.21%) and camphene (11.51%) were the major constituents of the rhizome of *K. marginata* were collected from Thailand [9]. In this paper, an essential oil preparation method and GC/MS analytical results are described to give the first insights into the essential oil components of *K. champasakensis* leaves.

Materials and methods

Plant material

The leaves of *K. champasakensis* were obtained from Big mountain, 1 ward, Vungtau City, Ba Ria - Vung Tau province (10°21'06.8" N; 107°04'14.0" E) in July 2021. The plant materials were collected and identified by one of the authors (Assoc. Prof. PhD. Nguyen Hoang Tuan, Botanist, from Department of Pharmacognosy, Hanoi University of Pharmacy). A voucher specimen (*NHTuan 006*) has been deposited at Herbarium of Hanoi National University, Vietnam.

Isolation of the essential oil

The hydrodistillation process of fresh leaves (1.5 kg) of *K. champasakensis* was performed in the Clevenger-type apparatus until there was no significant increase in the volume of essential oil collected (4 h), according to the Vietnamese Pharmacopoeia [14]. The obtained volatile oil was separated and dried with anhydrous sodium sulfate to remove all water traces and stored at 4°C until the moment of analysis. The experiment was conducted in triplicate. The yield of essential oil obtained (%) was calculated by the following formula: The yield in fresh materials (%) = $W_1/W_2 \times 100$. Where W_1 is the mass (g) of essential oil achieved, W_2 is the mass (g) of fresh leaves used.

Chemical characterization of essential oil

The chemical components of the leaves essential oil of *K. champasakensis* were analyzed with an Agilent Technologies 7890B GC System equipped with 5977B MSD model. An HP-5MS Ultral Inert column (30 m × 0.25 mm, thin film of 0.25 μm) was used. The injection volume was 1 μL and the split ratio was 50:1. Helium carrier gas with flow rate 1.00 mL/min (7.65 psi), injector temperature of 300°C, MS Quad temperature of 150°C, transfer line temperature of 300°C and MS source was 230°C. The oven temperature program was as follows: 50°C (held for 2 min) to 150°C (held for 10 min) at 5°C/min and an increase of 10°C/min to 280°C (held for 10 min). The MS conditions were as follows: ionization voltage 70 eV, acquisitions scan mass range of 50-550 *m/z* at 2.0 scan/s. A component identification was accomplished based on their retention indices and by a comparison between their mass spectral fragmentation patterns and those stored on the MS library [15]. The relative percentage of volatile content was calculated by peak areas of integral spectrograms.

Results and discussion

The essential oil of *K. champasakensis* leaves was pale yellow in color and lighter than water. By using hydrodistillation, the yield of the essential oil was approximately 0.042% (w/w, fresh weight). The GC/MS analysis (whereby all peaks with more than 0.05% area were considered for analysis) confirmed the presence of 46 phytochemical constituents in *K. champasakensis* leaf essential oil, accounting for 93.62% of the total content, as shown in Table 1. The majority of essential oil was composed of terpenes with monoterpene hydro-carbons (29.98%), sesquiterpene hydrocarbons (24.70%) and oxygenated sesquiterpenes (30.05%). In addition, a phthalate derivative compound (diisooctyl phthalate) and oxygenated monoterpene compound were also present with a smaller proportion of 8.62% and 0.27%, respectively. Major chemical compounds were characterized by the presence of β-caryophyllene (16.07%), β-elemenone (14.53%), β-pinene (13.80%), germacrone (9.85%), myrcene (9.46%), diisooctyl phthalate (8.62%), and caryophylene oxide (2.65%). Among them, the considerable monoterpene profile of the leaf oil of *K. champasakensis* included β-pinene (13.8%), myrcene (9.46%),

Table 1. Chemical constituents of *K. champasakensis* leaves essential oil

No.	RT (min)	Components[a]	RI[b]	RI[c]	Concentration (%)
1	6.82	Tricyclene	926	926	0.10
2	7.14	α-Pinene	938	939	2.02
3	7.55	Camphene	953	946	1.32
4	8.25	Sabinene	977	975	1.92
5	**8.35**	**β-Pinene**	**980**	**979**	**13.8**
6	**8.76**	**Myrcene**	**993**	**990**	**9.46**
7	9.84	Limonene	1032	1029	0.87
8	10.42	β-Ocimene	1052	1037	0.35
9	10.73	γ-Terpinene	1063	1059	0.09
10	11.61	Terpinolene	1090	1086	0.05
11	15.67	Isobornyl formate	1232	1235	0.07
12	17.28	bBornyl acetate	1289	1288	0.11
13	18.67	α-Terpinyl acetate	1342	1349	0.09
14	19.70	α-Copaene	1381	1376	0.18
15	20.12	β-Elemene	1396	1389	1.64
16	20.34	Cyperene	1405	1398	0.50
17	20.59	α-Gurjunene	1415	1409	0.06
18	**20.86**	**β-Caryophyllene**	**1426**	**1419**	**16.07**
19	21.16	γ-Elemene	1438	1434	0.22
20	21.69	β-Farnesene	1459	1454	1.74
21	21.88	epi-β-Caryophyllene	1467	1464	0.18
22	21.95	Ishwarane	1469	1466	0.13
23	22.23	γ-Selinene	1480	1481	0.26
24	22.39	Germacrene D	1486	1485	0.36
25	22.46	2-Isopropenyl-4a,8-dimethyl-1,2,3,4,4a,5,6,7-octahydronaphthalene	1488	1492	0.18
26	22.54	β-Selinene	1491	1490	0.90
27	22.76	α-Selinene	1500	1498	1.40
28	23.03	α-Farnesene	1509	1505	0.39
29	23.16	β-Curcumene	1513	1515	0.10
30	23.28	γ-Cadinene	1517	1513	0.08
31	23.41	α-Panasinsene	1521	1327	0.10
32	23.54	δ-Cadinene	1525	1522	0.21
33	24.75	Nerolidol	1563	1563	0.15
34	25.29	Spathulenol	1579	1578	0.23
35	25.54	Caryophylene oxide	1587	1582	2.65
36	25.67	Globulol	1590	1590	0.21
37	**26.34**	**β-Elemenone**	**1608**	**1601**	**14.53**
38	26.54	Humulene epoxide II	1612	1608	0.29
39	27.63	Caryophylla-4(12),8(13)-dien-5α-ol	1636	1639	0.34

table 1. (continued).

No.	RT (min)	Components[a]	RI[b]	RI[c]	Concentration (%)
40	27.89	epi-α-Cadinol	1641	1638	0.10
41	28.46	Selin-11-en-4-α-ol	1653	1658	0.51
42	28.66	Intermedeol	1657	1658	0.58
43	**30.60**	**Germacrone**	**1696**	**1693**	**9.85**
44	35.04	γ-Bicyclohomofarnesal	1797	1809	0.11
45	36.15	cCurcumenone	1845	1844	0.50
46	**44.49**	**Diisooctyl phthalate**	**2547**	**2543**	**8.62**
	Total compounds identified (%)				**93.62**
	Monoterpene hydrocarbons (Sr. No.1-10)				29.98
	Oxygenated monoterpenes (Sr. No.11-13)				0.27
	Sesquiterpene hydrocarbons (Sr. No.14-32)				24.70
	Oxygenated sesquiterpenes (Sr. No.33-45)				30.05
	Phthalate derivative (Sr. No.46)				8.62

RT: Retention time (min) on HP-5MS Ultral Inert column; [a]Elution order on HP-5MS Ultral Inert column; [b]Retention indices on HP-5MS Ultral Inert column; [c]Literature retention indices (NIST17 and Adams book).[15]

α-pinene (2.02%), sabinene (1.92%), and camphene (1.32%). The sesquiterpene profile of the leaf essential oil of *K. champasakensis* was identified to include β-caryophyllene (16.07%), β-elemenone (14.53%), germacrone (9.85%), and caryophylene oxide (2.65%).

The major components of *K. champasakensis* leaf oil was identified as β-caryophyllene (**1**; 16.07%), β-elemenone (**2**; 14.53%), β-pinene (**3**; 13.8%) germacrone (**4**; 9.85%), myrcene (**5**; 9.46%), and diisooctyl phthalate (**6**; 8.62%) (Figure 2). β-caryophyllene (**1**) was found to exhibit a wide range of bioactivities, including neurodegenerative disorders, analgesic and anti-inflammatory effects, gastrointestinal effects, and metabolic disorder [16]. β-elemenone (**2**) was found to be effective against *Anopheles subpictus*, *Aedes albopictus*, and *Culex tritaeniorhynchus* larvae without damaging aquatic predators in the study [17]. β-pinene (**3**) was also present in many plant essential oils and exhibited various pharmacological activities, i.e anti-tumor, gastroprotective, anti-microbial, and anti-malarial activities [18]. Germacrone (**4**) showed anti-cancer effects via cell cycle arrest and activating apoptotic procedure, as well as anti-inflammatory, anti-oxidant, and anti-viral, anti-microbial, and insecticidal activities [19]. Myrcene (**5**) is also a popular flavoring additive in food industry, which showed pharmacological actions on central nervous systems, as well as anti-oxidant, anti-inflammatory, and anti-nociceptive activities [20]. It needs to be noted that, in the majority of cases, the presence of diisooctyl phthalate in the essential oil could be explained by probable pollution of the harvest area of collected plants [21].

Conclusion

The GC-MS analysis of *K. champasakensis* leaf essential oil demonstrated the total of 46 compounds with β-caryophyllene, β-elemenone, β-pinene as the main chemical components. This is the first study on chemical composition of essential oil prepared from this species. The study results have further highlighted the potential of new species from *Kaempferia* genus as resources for new essential oil, which can be used in pharmaceutical and food industry. In a further study, bioactivities of essential oil distilled from *K. champasakensis* leaves could be carried out.

Competing interests

The authors declare that no competing interest exists.

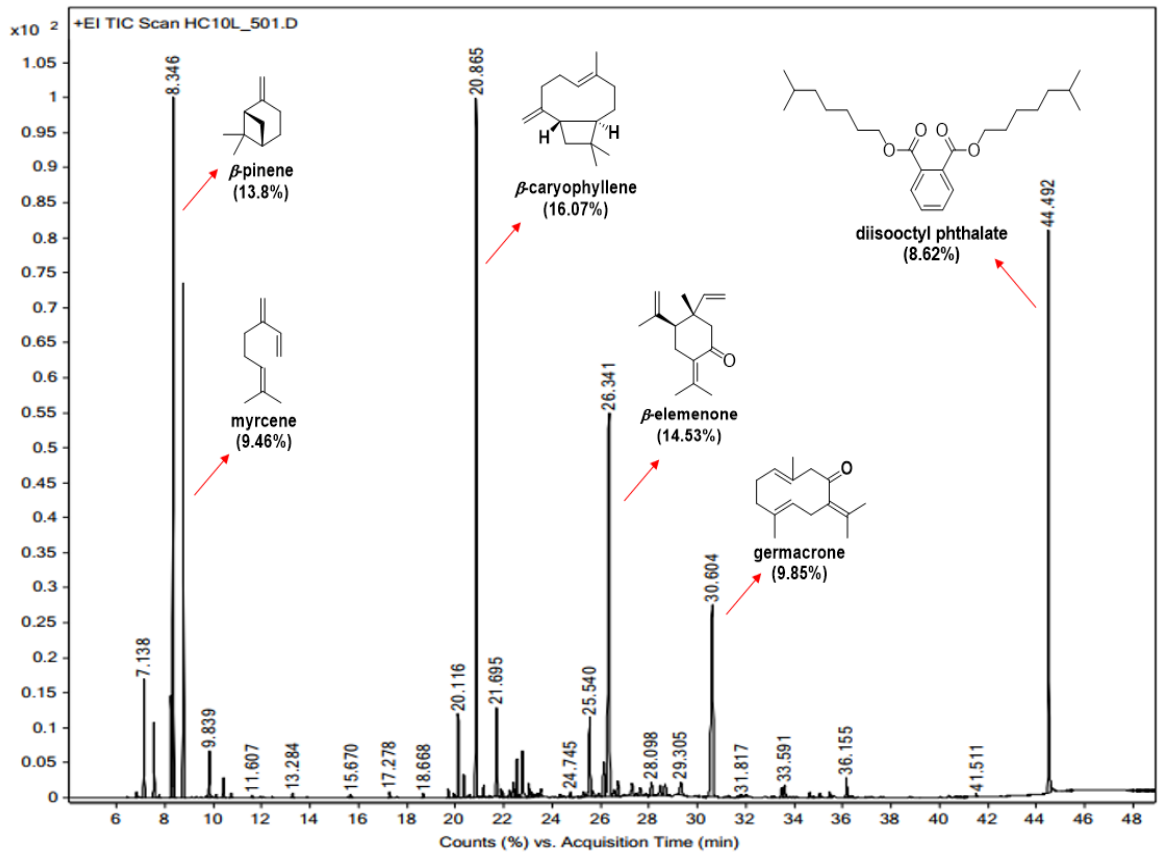

Figure 2. GC/MS profile of leaves essential oil of *K. champasakensis*

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