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Analyzing of 6-Gingerol and Camphene of *Zingiber Officinale* Roscoe in Lampang, Thailand by HPLC-PDA

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Zingiber Officinale Roscoe, a famous plant that has been used worldwide for the consumption and treatment of human ailments. In Thailand, ginger is grown in many areas throughout the country. Lampang is subjected to be one of the northern provinces where ginger growing had remarkably increased in recent years. This study aimed to determine the amounts of 6-gingerol and camphene from *Z. Officinale* Roscoe grown in Ban Pha-Daeng, Ngao District, Lampang Province in 2020 and 2021. Dried rhizome of *Z. Officinale* was extracted with hexane, ethyl acetate and methanol. 6-gingerol and camphene of three crude extracts were analyzed using High Performance Liquid Chromatography –Photo Diode Array (HPLC-PDA) method. The content of 6-gingerol in all extracts was found in the range of 1.95 to 13.85 *mg/g* while the content of camphene was found in the range of 1.39 to 51.97 *mg/g*. The level of 6-gingerol and camphene in ethyl acetate extracts was greater than the hexane and methanol extracts. The result indicated the amounts of 6-gingerol and camphene of *Z. Officinale* grown in 2020 was higher compared to the content in 2021.

Key words: *Zingiber Officinale* Roscoe, 6-Gingerol, Camphene, HPLC-PDA.

1. INTRODUCTION

Plants of the *Zingiberaceae* family, have been known and largely used in terms of consumption and treating human ailments for over 2000 years. Examples of famous plants in this genus are turmeric and ginger. The number of plants in this genus is approximately 56 genera and 1,300 species widely distributed in South and Southeast Asia[1,2].

Thailand is one of the Southeast Asia countries that discovered about 26 genera and more than 300 species of *Zingiberaceae* plants which are distributed throughout all areas of the country[3]. Plants are considered to be important natural resources of foods, drinks, and medicines for local people. Moreover, Thailand was reported as the fifth-highest ginger-producing country in the world amounting to 166,923 tons which accounted for 4.09% of the world's total ginger production in 2019. The main area of ginger grows in the mountainous regions of Loey, Payao, Chiang Rai, Mae Hong Sorn, Petchabun, and Pitsanulok provinces[4] which are the northern part of Thailand. Pha-Daeng Village; an agricultural village, is located in Ngao district, Lampang province the north of Thailand, where ginger is grown as an economic crop as important as rice and corn.

The literature search revealed that

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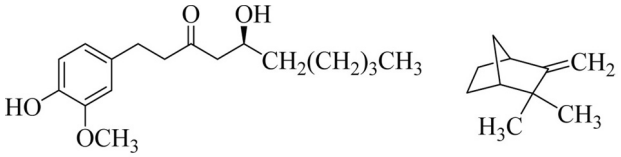
evidence of the utilization of dried ginger in medicinal terms appears in many folk medicine textbooks which were used as a main ingredient in at least 7 formulas of Thai traditional household remedies, 5 formulas in essential lists of Thai herbal Medicinal products, and 713 formulas in 21 traditional ancient medicine textbooks[5].

There were a number of reports indicated to the use of ginger to treat various ailments such as asthma, constipation catarrh, gingivitis, diabetes, nervous diseases rheumatism toothache, stroke, and gastrointestinal tract (G.I.T.) disorders[6,7]. A study of the active substances in ginger found more than 300 substances, which were divided into 3 major groups; volatile oil, gingerol, and diarylheptanoids[8]. One of the active compounds in ginger is 6-gingerol, which has the outstanding pharmacological activity of anti-cancer activity. In addition, a greater amount of 6-gingerol can be used to distinguish *Z. Officinale* Rosc from *Zingiber Ligulatum* Roxb[9,10]. Another important compound in ginger is camphene; a diterpene derivative, that has properties to reduce cholesterol and triglycerides in rats, and an analgesic effect in vivo and *in vitro* experimental models[11,12]. In addition, there are many essential substances in ginger, especially shogaol, which has shown anti-cancer activity in many cancer cells such as myeloid K562 (leukemia), NCI-60 cell line (MCF7 human breast cells), mouse ovarian cancer cell lines, HT29 cells (human colon cancer cells), A-549 (human lung cancer cells), and SK-MEL-2 (human melanoma cell)[13].

The objective of this study was to determine the amount of 6-gingerol and camphene (Fig. 1.) from gingers grown in the Ban Pha-Daeng community that was cultivated in 2020 and 2021 using HPLC-PDA. The results can be used as a database for researchers that utilized the work on a ginger cultivar in Lampang province and in other areas in the future.



6-gingerol camphene

Fig. 1. Chemical structure of 6-gingerol and camphene.

2. MATERIALS AND METHODS

2.1. Materials

Zingiber Officinale (BKF 118527; Fig. 2.) was collected from Ban Pha-Daeng (18.993000 N, 99.773341 E), Lampang province, Thailand, on May 2020 and 2021 by Dr. Narong Nuntasaen. The plants were identified and deposited in the Forest Herbarium, Department of National Park, Wildlife and Plant Conservation, Ministry of National Resources and Environment, Bangkok, Thailand. 6-Gingerol (CAS No. 23513-14-6) and camphene (CAS No. 79-92-5) were purchased from Sigma-Aldrich (USA). Methanol, acetic acid, water (HPLC grade), and nylon membrane (0.45 *μm*. 47 *mm*.) were purchased from UNION SCIENCE CO., LTD (Thailand).

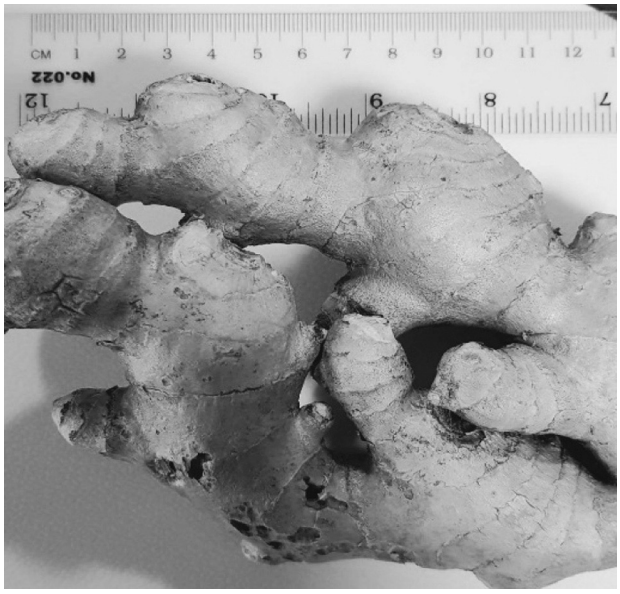


Fig. 2. Rhizome of Z. Officinale Roscoe from Ban Pha-Daeng community, Lampang.

2.2. Plant extraction

Dried rhizome of *Z. Officinale* A (2020) and B (2021) (1 *kg* each) were ground into powder and then extracted successively with three solvents; hexane, ethyl acetate, and methanol for 5 times each (5 × 3 *L*). Each extraction was completed within 72 hours. Removal of solvents from each extract under reduced pressure to give the crude extracts of *Z. Officinale*.

2.3. The crude extract preparation

The total weight of each extracted plant about 220-277 ± 0.3 *mg* was extracted with methanol (2 *mL*) under a vortex mixture for 3-5 minutes, and filtered through a nylon membrane. The filtrates were stored in a glass vial, kept under 25°*C*. The extracts were determined the 6-gingerol and camphene using HPLC.

2.4. HPLC condition

HPLC analysis was carried out on a HITACHI Chromaster equipped with a 5110 pump, a 5310 column oven, a 5430 diode array detector, and a 5210 auto sampler. Separation was undertaken on a HITACHI LaChrom C_{18} column (250 *mm* × 4.6 *mm*, 5 *μm*) at 25°*C*. The mobile phase flow rate was 1 *mL/min*. The injection volume was 10 *μL*. The quantitation wavelength was set at 282 *nm* for 6-gingerol analysis and 220 *nm* for camphene analysis. Identification of 6-gingerol and camphene was accomplished by comparing the retention times, and absorption spectra of relevant peaks to those of standard compounds. The separation condition for HPLC analysis was modified according to previous papers[14,15] as represented in Tables 1 and 2.

2.5. 6-gingerol and camphene measurement

Quantification estimation of 6-gingerol and camphene were carried out based on the calibration curve of the 6-gingerol, and camphene standard. Solution of 50-1,000 *μg/mL* 6-gingerol and 10-250 *μg/mL* camphene were prepared. The standard curves were plotted between the peak areas and concentrations. Peak areas of 6-gingerol and camphene were compared to the standard curve and calculated into their contents. The solutions were filtered through a Nylon membrane (0.45 *mm*) syringe filter and injected into HPLC in triplicate.

Table 1. The gradient system used in the chromatographic separation of 6-gingerol

Step gradient (6-gingerol)		
Time (*mins*)	Water (*%V/V*)	Methanol (*%V/V*)
0	35	65
15	35	65
20	10	90
25	0	100
35	0	100
36	35	65
40	35	65

Table 2. The isocratic system used in the chromatographic separation of camphene

Isocratic system (camphene)			
Time (*min*)	Water: Methanol (*%V/V*)	Tetrahydro-furan (*%V*)	pH 5 (adjusted with aceitc acid)
0	94:5	1	

3. RESULTE AND DISCUSSION

3.1. Extraction yield

The total weights of the relevant extracts are shown in Table 3.

The dry weight of *Z. Officinale* Roscoe A (2020) and *Z. Officinale* Roscoe B (2021) were obtained from three solvent extracts, i.e. hexane, ethyl acetate, and methanol (Table 3). The result showed that hexane provided the highest yield of the extraction for

Table 3. The yield of three crude extracts of *Z. Officinal* in 2020 (A) and 2021 (B) (*g/kg*)

Crude Extract	Dry weight (*g/kg*)	
	A	B
Hexane	29.56	26.28
EtOAc	18.64	16.38
MeOH	13.52	10. 90

A = *Z. Officinale* Roscoe in 2020.
B = *Z. Officinale* Roscoe in 2021.

both *Z. Officinale* Roscoe A and B. The amounts of the extracts (*g/kg*) were found to be 29.56 (A) 26.28 (B), 18.64 (A) 16.38 (B), and 13.52 (A) 10.9 (B) for hexane, ethyl acetate, and methanol, respectively.

From Table 3, it can be explained that the weights of the extracts decreased with increasing polarity of the solvents; hexane, ethyl acetate, and methanol. This is consistent with previous reports of the occurrence of 194 types of volatile oil (less polarity), 85 types of gingerol (moderate polarity), and 28 types of diarylheptanoids compounds[8].

3.2. Chromatography

The peak area of standard 6-gingerol and camphene were shown in Table 4.

Linearity of 6-gingerol was found in the concentration range of 50-1,000 *μg/mL* while linearity of camphene was found in the concentration range of 10-250 *μg/mL*. The calibration curves of 6-gingerol and camphene showed good linearity within the test range, and their content could be accurately determined using the regression equation (Fig. 3. and Table 5.).

Table 4. Peak area of standard 6-gingerol and camphene from HPLC chromatogram

[6-gingerol], *μg/mL*	Peak area	[Camphene], *μg/mL*	Peak area
50	90,774	10	91,582
100	154,458	25	234,346
250	394,011	50	433,877
500	906,067	100	730,474
1000	1,737,420	250	1,318,949

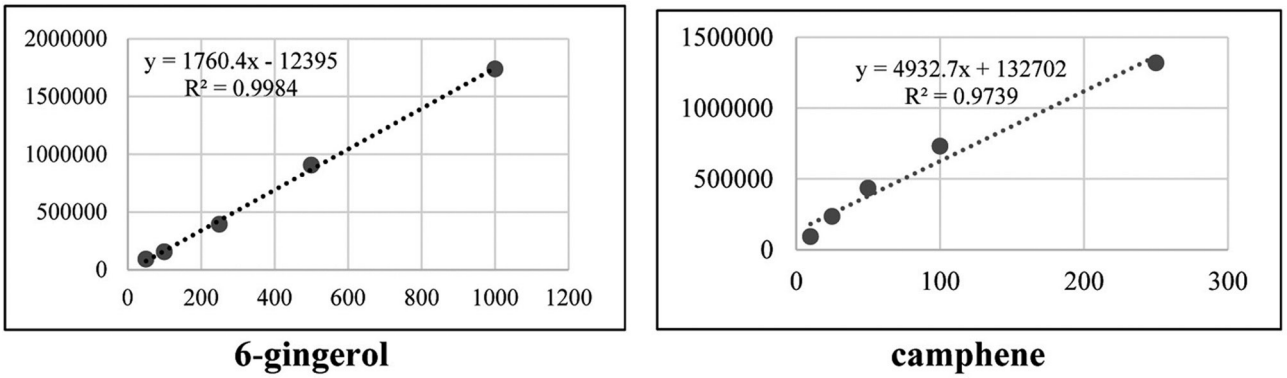


Fig. 3. Standard calibration curve of 6-gingerol and camphene.

Table 5. Linear regression equations of standard 6-gingerol and camphene contents

Standards	Regression equation (*μg/mL*)	Correlation coefficient (*r*)
6-gingerol	y = 1760.4x – 123950	0.9991
camphene	y = 4932.7x + 132702	0.9868

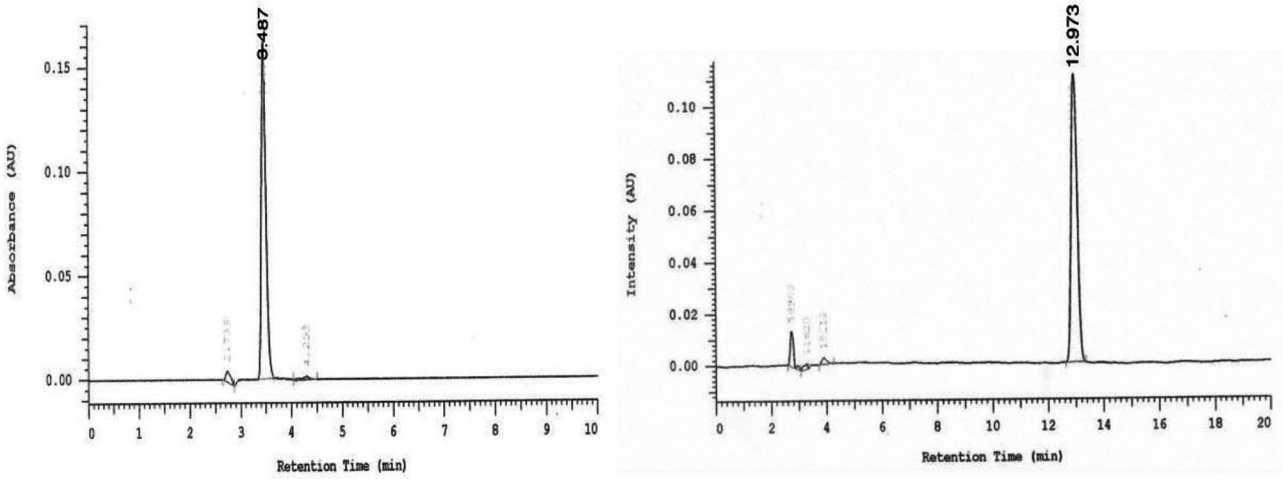


Fig. 4. HPLC Chromatogram and retention time of 6-gingerol (3.487) and camphene (12.973) standard.

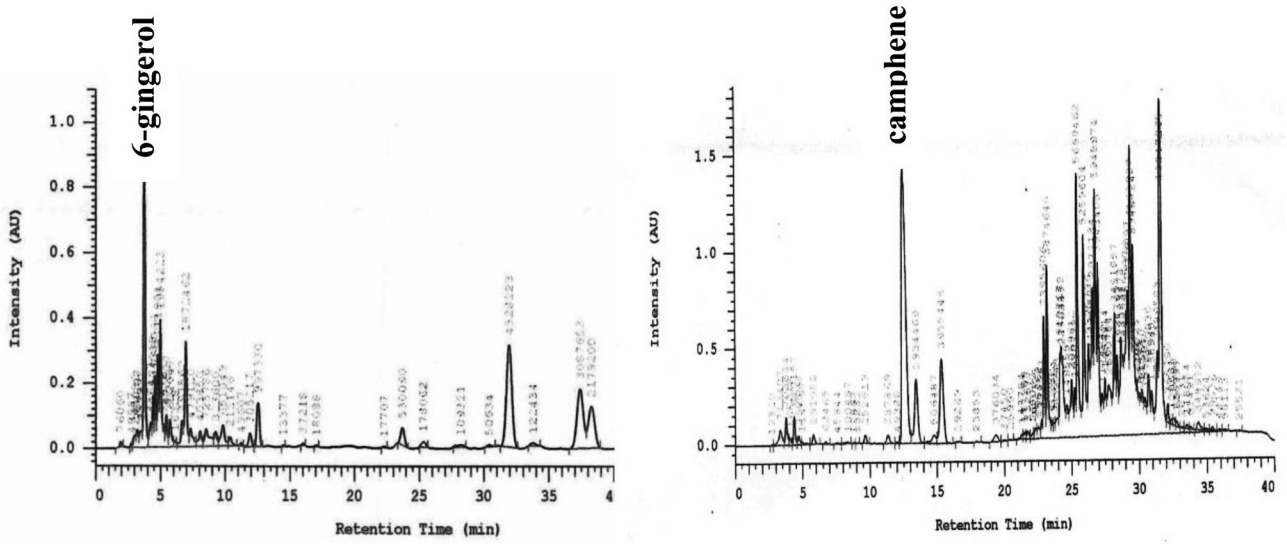


Fig. 5. HPLC Chromatogram of 6-gingerol and camphene from crude extracts.

The retention time of 6-gingerol and camphene standards were shown at 3.487 and 12.973 *mins*, respectively (Fig. 4.). HPLC chromatogram of 6-gingerol and camphene from crude extracts was shown in Fig. 5.

3.3. Analysis of 6-gingerol and camphene Contents in *Z. Officinale*

The contents of 6-gingerol and camphene in three extracts of *Z. Officinale* are listed in Table 6.

From Table 6, 6-gingerol content in all extracts was found in the range of 1.95 ±0.20 *mg/g* to 13.85±1.79 *mg/g* while the content of camphene was found in the range of 1.39±0.02 to 51.97±2.53 *mg/g*. *Z. Officinale* Roscoe A was shown to contain the content of 6-gingerol and camphene higher than *Z. Officinale* Roscoe B.

The 6-Gingerol in the ethyl acetate, hexane, and methanol extracts of *Z. Officinale* Roscoe A was found to be 13.85±1.79, 7.74 ±0.55, and 3.03±0.14 *mg/g*, respectively, while 6-gingerol in the ethyl acetate, hexane and methanol extracts of *Z. Officinale* Roscoe B was found to be 8.64±0.35, 8.04± 0.13, and 1.95±0.20 *mg/g*, respectively.

Similarly, the camphene in the ethyl acetate, hexane and methanol extracts of

Table 6. Total 6-gingerol and camphene contents of three extracts of *Z. Officinal* in 2020 (A) and 2021 (B) (*mg/g* sample).

Extract	Compound content (*mg/g*)*			
	6-Gingerol		Camphene	
	A	B	A	B
Hexane	7.74 ± 0.55^{b}	8.04 ± 0.13^{a}	36.29 ± 2.67^{b}	1.66 ± 0.01^{b}
EtOAc	13.85 ± 1.79^{a}	8.64 ± 0.35^{a}	51.97 ± 2.53^{a}	7.56 ± 0.03^{a}
MeOH	3.06 ± 0.14^{c}	1.95 ± 0.20^{b}	30.32 ± 2.13^{b}	1.39 ± 0.02^{b}

A = *Z. Officinale* Roscoe in 2020
B = *Z. Officinale* Roscoe in 2021
$^{a-c}$ = Different letters show significant difference between means in the same column ($p \leq 0.05$)
*Extraction and analysis of each sample were done in triplicate and contents were expressed as mean ± S.D.

Z. Officinale Roscoe A was found to be 51.97 ± 2.53, 36.29 ± 2.67, and 30.32 ± 2.13 *mg/g*, respectively, while camphene in the ethyl acetate, hexane and methanol extracts of *Z. Officinale* Roscoe B was found to be 7.56 ± 0.03, 1.66 ± 0.01, and 1.39 ± 0.02 *mg/g*, respectively.

The extract of ethyl acetate was found to contain the highest amount of the analyzed compound, followed by the hexane extracted and methanol extracted, respectively.

The contents of 6-gingerol and camphene in *Z. Officinale* Roscoe A and B were consistent with previous 6-gingerol research reports[16-18], and the previous camphene research report[19-21].

4. CONCLUSION

Analysis and comparison of 6-gingerol and camphene in *Z. Officinale* Roscoe grown at Ban Pha-Daeng, Ngao District, Lampang Province, found that the 6-gingerol content in ethyl acetate extracts in 2020 and 2021 was greater than 6-gingerol in the hexane and methanol extracts. The camphene content analysis revealed that camphene content in all extracts in 2020 was much higher than in the extracts in 2021. Although it is still unclear why the content of 6-gingerol and camphene in the three extracts is not the same, which is expected to arise from seasonal variation. At least the data from this study will serve as a basis data for other researchers to further in-depth studies.

Author's contributions

AK, NC, and BW collected and prepared raw materials, WP, AC, and AC designed the project, PU, and SW designed the project, conducted and concluded an experiment, and wrote the manuscript. All authors read and approved the final manuscript.

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