ESTABLISHMENT OF THE 6-SHOGAOL EXTRACTION PROCESS FROM 6-SHOGAOL-ENRICHED GINGER FOR CANCER TREATMENT

Ho Ba Ngoc Minh[1], Nguyen Trong Diep[1], Pham Van Hien[1], Vu Binh Duong[1]*

Abstract

Objectives: To optimally extract the potential phytochemical "6-shogaol" from fermented shogaol-enriched ginger. ***Methods:*** Extraction process parameters were investigated using 6-shogaol as a marker. ***Results:*** The suitable parameters of the 6-shogaol extraction process: Hot extraction using reflux equipment, solvent of 90% ethanol, solvent/herb ratio of 15/1, temperature of 70ºC, duration of 90 minutes, and two-time extraction. The 6-shogaol extraction efficiency was 89.61 ± 0.68%. ***Conclusion:*** The optimal process for 6-shogaol extraction from fermented shogaol-enriched ginger has been studied.

Keywords: 6-shogaol; Extraction process; 6-shogaol-enriched ginger.

INTRODUCTION

Ginger (*Zingiber officinale* Rosc.), a member of the Zingiberaceae family, has been found to possess various biological activities, including antioxidant, anti-inflammatory, antibacterial, anti-diabetic, and anti-cancer effects, etc. [1, 2, 3]. These biological effects relate to some active ingredients such as 6-gingerol, zingiberol, etc. Many studies have shown that 6-shogaol has more potent therapeutic effects in anti-inflammatory, antioxidant, and cancer-preventing than 6-gingerol [4, 5]. Moreover, 6-shogaol inhibited the growth of several cancer lines, such as lung, breast, and cervical cancer etc. [6]. However, the 6-shogaol content in dried ginger (Can Khuong) is very low, while in fresh ginger, it is almost undetectable [7]. To solve the problem, some studies have been conducted to enrich 6-shogaol by dehydrating 6-gingerol. The conversion of 6-gingerol to 6-shogaol is accelerated in high-temperature and acidic environments [4, 8]; this process is referred to as ginger fermentation.

[1]Vietnam Military Medical University

[*]Corresponding author: Vu Binh Duong (vbduong2978@gmail.com)

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Currently, we have been studying the enrichment of 6-shogaol in ginger by steaming it in an acidic environment [9, 10] to create raw materials with potential treatment in pharmaceutical production. To effectively incorporate these herbal materials into practical production, it is crucial to thoroughly research and develop a process for extracting 6-shogaol from fermented ginger. Therefore, this study aims to: *Establish the necessary process parameters for extracting 6-shogaol from shogaol-enriched ginger.*

MATERIALS AND METHODS

1. Materials

Including 6-shogaol-enriched ginger (fermented ginger) provided by the Drug Research and Development Center, Vietnam Military Medical University meeting institutional standards.

* *Standard substance:* 6-shogaol 98% from ChemFaces, China; chemicals and solvents: Acetonitrile, glacial acetic acid, methanol, ethanol 98%, etc., qualified analytical purity standards and pharmacopeia.

* *Equipment:* HPLC system e2695 and PDA Detector 2998, Waters, USA; C_{18} column (150 x 4.6mm; 5µm), InertSustain AQ, Japan; Reflux extractor, China; Rotary vacuum evaporator N1200B, EYELA, Japan.

2. Methods

* *Extraction method:* To develop the extraction process, the following investigations were conducted:

Extraction method: Hot extraction using reflux equipment, ultrasonic extraction, microwave extraction;

Solvent: Ethanol (50%, 70%, 90%, 96%);

Ratio of solvent/herb: 5/1, 10/1, 15/1, 20/1, 25/1 (mL/g);

Temperature: 40, 50, 60, 70, and 80ºC;

Duration: 30, 60, 90, 120 minutes per time;

Extraction time: 1 time, 2 times, 3 times.

* *The quantitative method of 6-shogaol by high-performance liquid chromatography (HPLC):*

Standard sample: 50mg of 6-shogaol was accurately weighed and dissolved in methanol in a 25mL volumetric flask to obtain a stock standard solution with a concentration of about 2000 µg/mL. Working concentrations of standards and samples (about 10, 50, 100, 200, and 500 µg/mL) were made by diluting standard stock solutions with methanol. Samples were filtered through a 0.45µm membrane prior to HPLC quantification.

Material sample: About 2g of fermented ginger was accurately weighed into a round-bottom flask, added 30mL of 90% ethanol, and refluxed for 90

minutes at 60ºC. Extracting 3 times. Combine all extracts and volumetrically measure in a 100mL flask. Centrifuge the extract at 5500rpm for 10 minutes. Collecting the clear solution, then diluting it twofold to obtain a suitable concentration. Samples were filtered through a 0.45µm membrane prior to HPLC quantification.

Extract sample: Fermented ginger was extracted according to the investigated conditions. Centrifuge the extract samples at 5500rpm for 10 minutes. Collecting the clear solution, then diluting it to a suitable concentration. Samples were filtered through a 0.45µm membrane prior to HPLC quantification.

Chromatographic conditions*: InertSustain AQ C_{18} column (150 x 4.6mm; 5µm); wavelength: 280nm; mobile phase: Acetonitrile (C) and 0.4% acetic acid (A) (60/40; v/v); flow rate: 1 mL/min; injection volume: 5µL.

The 6-shogaol content was calculated by the following formula:

$$\text{The 6-shogaol content (mg/g)} = \frac{C \times V \times n}{m \times 1000 \times (1 - h/100)}$$

C: Concentration of 6-shogaol in the extract (µg/mL) calculated from the calibration curve; V: Volume of the herb extract (mL); n: Dilution factor; m: Weight of herb (g); h: Moisture content of herb (%).

*This chromatographic condition has been validated using ICH guidelines. The calibration curves (y = 13462x + 190171) showed an acceptable correlation ($r^2 > 0.99$). The 6-shogaol average recoveries (%) for LQC, MQC, and HQC were 107.91%, 107.92%, and 101.50%, respectively, and RSD for each recovery varied from 0.58% to 1.38%. The precision of 6-shogaol on the same day (intra-day precision) and three consecutive days (inter-day precision) had RSD < 2.00%. LOD and LOQ. The LOD was found to be 0.012 µg/mL, and the LOQ was determined to be 0.040 µg/mL under the specified experimental conditions. These results indicate that the method is capable of detecting and quantifying low concentrations of 6-shogaol with a high degree of accuracy.

3. Ethics

The study has been approved by the Ethics Committee according to decision No. 3934/QĐ-HVQY, dated 19/9/2023. The Institute of Pharmaceutical Education, Vietnam Military Medical University granted permission for the use and publication of the research data. The authors declare to have no conflicts of interest.

RESULTS AND DISCUSSION

1. Results of determination of 6-shogaol content in fermented ginger

Table 1. 6-shogaol content in fermented ginger (n = 3).

m (g)	h (%)	V (mL)	n	S peak (µAU.s)	C (µg/mL)	6-shogaol content (mg/g)
2.012	18.16	100	2.5	3372834	235.01	35.68
2.009	18.16	100	2.5	3352110	237.52	36.11
2.013	18.16	100	2.5	3287472	230.49	34.97
Mean ± SD						35.59 ± 0.57

From table 1, the content of 6-shogaol in fermented ginger is 35.59 ± 0.57 mg/g calculated on the dry material. When examining the extraction process parameters, the extraction efficiency was calculated based on the content of 6-shogaol in the fermented ginger.

* *Investigation of the effect of the extraction method:*

The fermented ginger was extracted under the same conditions using 90% ethanol solvent, solvent/herb ratio of 10/1, temperature of 60ºC, duration of 90 minutes, and one-time extraction by different methods: Ultrasonic extraction, hot extraction, and microwave extraction. The results are shown in table 2.

Table 2. The effect of extraction method on 6-shogaol extraction from fermented ginger (n = 3).

Method	6-shogaol content (mg/g)	Extraction efficiency (%)
Ultrasonication	19.01 ± 0.13	53.41 ± 3.56
Hot extraction	22.20 ± 0.12	62.38 ± 3.35
Microwave	21.92 ± 0,14	61.59 ± 3.91

The results in table 2 showed that hot extraction using reflux equipment gave the highest extraction efficiency of 62.38%. The ultrasonic extraction method yielded only 53.41%, and the microwave extraction method yielded 61.59%. During the hot extraction process, the herb was exposed to the solvent at a high

temperature, which increased the diffusion coefficient, thereby increasing the amount of extracted active ingredients, leading to an increase in extraction yield. Therefore, the hot extraction using the reflux method with simple and easy-to-operate equipment was chosen for further research.

** Investigation of the effect of extraction solvent:*

The fermented ginger was extracted through hot extraction using reflux equipment at 60ºC, solvent/herb ratio of 10/1, duration of 90 minutes, and one-time extraction with different solvents, including ethanol (50, 70, 90, 96%). The results are shown in table 3.

Table 3. The effect of extraction solvent on 6-shogaol extraction from fermented ginger (n = 3).

Solvent	6-shogaol content (mg/g)	Extraction efficiency (%)
EtOH 50%	19.07 ± 0.42	53.59 ± 3.37
EtOH 70%	20.48 ± 0.11	57.54 ± 2.96
EtOH 90%	22.20 ± 0.14	62.38 ± 3.90
EtOH 96%	23.38 ± 0.12	65.70 ± 3.24

Table 3 shows that when the EtOH concentration increased from 50% to 96%, the extraction efficiency of 6-shogaol also gradually increased from 53.59% to 65.70%. This is due to the poor solubility in water of 6-shogaol, with a solvent which is low alcohol concentrations and high-water content, the ability to dissolve the active ingredient is lower, so the content and extraction efficiency of 6-shogaol is lower. However, the extraction efficiency of active ingredients when extracted with 90% ethanol (62.38%) was slightly lower than when extracted with 96% ethanol (65.70%). Therefore, 90% ethanol is the most suitable solvent for extracting fermented ginger and conducted for further research.

** Investigation of the effect of the herb/solvent ratio:*

The fermented ginger was extracted through hot extraction using reflux equipment with the solvent of 90% ethanol, temperature of 60ºC, duration of 90 minutes, and one-time extraction with different solvent/herb ratios, including 5/1, 10/1, 15/1, 20/1, 25/1. The results are shown in table 4.

Table 4. The effect of the herb/solvent ratio on 6-shogaol extraction from fermented ginger (n = 3).

Ratio	6-shogaol content (mg/g)	Extraction efficiency (%)
5/1	20.96 ± 0.05	58.90 ± 1.41
10/1	22.20 ± 0.13	62.38 ± 3.68
15/1	24.89 ± 0.11	69.94 ± 2.93
20/1	25.74 ± 0.13	72.33 ± 3.51
25/1	25.68 ± 0.32	72.15 ± 0.90

Table 4 shows that the solvent/herb ratio has an influence on extraction efficiency. When the solvent ratio increased, the content and extraction efficiency of 6-shogaol also increased. At the solvent/herbal material ratio of 15/1, the extraction efficiency nearly reached 70%; however, when the solvent ratio increased to 20/1, the extraction efficiency increased insignificantly. When extracting at a solvent/ herbal material ratio of 20/1, the extraction efficiency reached 72.33%. The extraction efficiency of 6-shogaol increased slightly when the ratio of active ingredients and solvent reached saturation. Therefore, the study chose the solvent/herb ratio of 15/1 to conduct further tests.

* *Investigation of the effect of extraction temperature:*

The fermented ginger was extracted through hot extraction using reflux equipment with the solvent of 90% ethanol, solvent/herb ratio of 15/1, duration of 90 minutes, and one-time extraction with different temperatures of 40, 50, 60, 70, and 80ºC, respectively. The results are shown in table 5.

Table 5. The effect of temperature on 6-shogaol extraction from fermented ginger (n = 3).

Temperature (ºC)	6-shogaol content (mg/g)	Extraction efficiency (%)
40	15.42 ± 0.12	43.32 ± 3.29
50	20.30 ± 0.02	57.03 ± 0.48
60	24.89 ± 0.05	69.94 ± 1.36
70	26.80 ± 0.05	75.31 ± 1.49
80	26.61 ± 0.12	74.77 ± 3.40

Table 5 shows that when increasing the temperature from 40ºC to 70ºC, the extraction efficiency also increased gradually, reaching its highest at 70ºC (75.31%). However, when the extraction temperature increased to 80ºC, the extraction efficiency decreased slightly (from 75.31% to 74.77%), which may be due to the decomposition of 6-shogaol when extracted at high temperature for a long time (90 minutes), leading to a decrease in extraction efficiency. Therefore, the study chose the temperature of 70ºC to conduct the following parameter research.

** Investigation of the effect of extraction duration:*

The fermented ginger was extracted through hot extraction using reflux equipment with a solvent of 90% ethanol, solvent/herb ratio of 15/1, temperature of 70ºC, and one-time extraction with different durations of 30, 60, 90, and 120 minutes, respectively. The results are presented in table 6.

Table 6. The effect of duration on 6-shogaol extraction from fermented ginger (n = 3).

Duration (minute)	6-shogaol content (mg/g)	Extraction efficiency (%)
30	21.02 ± 0.14	59.07 ± 3.96
60	23.80 ± 0.10	66.88 ± 2.88
90	26.80 ± 0.08	75.31 ± 2.29
120	25.17 ± 0.15	70.73 ± 4.03

Table 6 shows that extraction duration also has a significant effect on extraction efficiency. 30-minute extraction gave the lowest efficiency. When increasing the extraction duration to 90 minutes, the efficiency increased significantly (75.31%). However, if the duration is extended, specifically to 120 minutes, the extraction efficiency decreases slightly (from 75.31% to 70.73%). Therefore, the study chose the duration of 90 minutes to continue the following research.

** Investigation of the extraction times:*

The fermented ginger was extracted through hot extraction using reflux equipment with a solvent of 90% ethanol, solvent/herb ratio of 15/1, temperature of 70ºC, and duration of 90 minutes. The samples were extracted three times, and the extract was quantified separately each time. The results are presented in table 7.

Table 7. The effect of the extraction time on 6-shogaol extraction from fermented ginger (n = 3).

Times	6-shogaol content (mg/g)	Extraction efficiency (%)
1	26.80 ± 0.11	75.31 ± 0.57
2	5.09 ± 0.02	14.30 ± 0.11
3	2.45 ± 0.01	6.90 ± 0.04

The results of table 7 show that after one-time extraction, the extraction efficiency of 6-shogaol is quite high (75.31%). After two-time extractions, the extraction efficiency of 6-shogaol is nearly 90%. Therefore, to save time and solvent, the study chose the number of extractions to be 2 times, with the extraction efficiency of 6-shogaol reaching 89.61 ± 0.68%.

With the goal of investigating and selecting the optimal parameters to extract the fermented ginger rich in 6-shogaol for application in the preparation of dry powder and other pharmaceutical products, ethanol - water solvent is the most suitable, although organic solvents may extract 6-shogaol better. Increasing the amount of solvent can also increase the extraction efficiency of 6-shogaol. However, it will waste solvent, and the extract will be diluted when concentrated, so it will waste energy and prolong the concentration duration, so there will be a risk of reducing the active ingredient content. According to Seon Ok and Woo-Sik Jeong, the higher the extraction temperature, the greater the 6-shogaol content obtained. At 60ºC, the extracted 6-shogaol content was about 10 mg/g, but at 80ºC, it reached about 22 mg/g [3]. The difference in the extracted 6-shogaol content between our research and the study of Seon Ok and Woo-Sik Jeong may be related to the herb/solvent ratio as well as the raw herb used, but they all have a common trend of relatively high extraction temperature, about 70 - 80ºC.

CONCLUSION

The process parameters for extracting 6-shogaol from 6-shogaol-enriched ginger were investigated, and the most suitable extraction condition was selected: Hot extraction using reflux equipment, solvent of 90% ethanol, solvent/herb ratio of 15/1, temperature of 70ºC, duration of 90 minutes per extraction, 2-time extractions. The extraction efficiency of 6-shogaol was 89.61 ± 0.68%. With the obtained results, this extraction process can be applied in the study of modernizing 6-shogaol-enriched ginger.

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