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## Original Article

# Comparative HPLC Analysis of 6-Gingerol and 6-Shogaol in Soil-Based and Soilless-Grown Ginger

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**ABSTRACT**

Ginger or *Zingiber officinale* Roscoe is a well-known herbal medicine and is widely used in Asian cuisine. Its major bioactive compounds, 6-gingerol and its dehydrated form, 6-shogaol, were reported to have potential medicinal properties. However, previous phytochemical studies on the compounds are limited to conventionally grown or soil-based ginger, neglecting soilless ginger grown through hydroponic techniques. This technique has been widely adopted as an alternative to circumvent soil-related complications. Therefore, this study aimed to compare both marker compounds in soil-based (SB) and soilless-grown hydroponic (HP) ginger extracted in different ethanol concentrations (95% and 100%) using high-performance liquid chromatography. The study initially found that 6-gingerol concentration in 95% SB ginger ethanolic extract (1.012%) was significantly higher ($p< 0.05$) than in 95% HP dried ginger (HP1) ethanolic extract (0.314%). The 6-gingerol content for both gingers were also significantly higher ($p< 0.05$) in 95% ethanolic extracts compared to 100% ethanolic extracts. The analysis was also performed with fresh-dried HP ginger (HP2), and it was found that the HP2 ginger (0.75%) has a significantly higher 6-gingerol concentration ($p< 0.05$) compared to HP1 ginger (0.314%), confirming that the previous results were implicated by storage conditions. The concentration of 6-gingerol in 95% SB ginger extract (1.012%) differ significantly compared to those in HP2 extract (0.75%) while both gingers have equivalent amount of low 6-shogaol concentrations (0.0004% and 0.0005% respectively). It is worth to note that HP ginger grown in soilless condition could still produce high amount of 6-gingerol. This finding encourages the usage of HP ginger in pharmacological studies considering the other economic and environmental benefits it offers.

## INTRODUCTION

Plants and herbs have been used since early civilizations for general health purposes and as traditional medicines to treat disease. Ginger (*Zingiber officinale* Roscoe, Zingiberaceae) is a well-known plant in the world of medicine and spices [1]. There are about 1,300 species in 50 genera in the Zingiberaceae family [2]. *Z. officinale* consists of various cultivars that originate from different cultivation areas throughout the country. Ginger is believed to originate from the Indo-Malaya region in Southeast Asia and India before it was introduced and cultivated in other tropical countries [3]. For example, India, Nigeria, Australia, China, and Jamaica are among the main ginger-exporting countries [2]. Ginger plants can be identified by their cone-shaped flowers and leaves that are approximately 5-30 cm long and 8-20 $mm^2$ in area. The ginger plant has a non-woody type of stem filled with leaf sheaths and can grow about one meter in height. The rhizome of ginger is yellowish and grows in branches under the soil surface [4]. In ancient times, ginger was recommended for various health conditions such as heart disease, and rheumatoid arthritis, anti-inflammatory agent, digestive aid, and others [3]. Ginger rhizomes contain various biologically active constituents consisting of phenolic compounds such as gingerol, shogaol, paradol, quercetin and zingerone, as well as terpene compounds, polysaccharides, lipids, and organic acids [5]. Phenolic ketones have various biological activities such as immunomodulatory,

anti-oxidative, anti-inflammatory, anti-emetic, antimicrobial, anti-cancer, anti-diabetic, and neuroprotective activities [5-7]. More potential medicinal benefits of ginger are being explored with an increasing number of recent studies including studies reporting ginger's anti-viral activity and potential use to treat common symptoms of SARS-COV-2 infection [8]. One of the bioactive compounds found in high levels in the ginger extract is 6-gingerol [9]. 6-gingerol is the main cause of the spicy aroma and taste of ginger and has been studied quite extensively for various pharmacological activities such as neuroprotective effects [10], antioxidant [11] and anti-emetic [12]. Nevertheless, the number of bioactive compounds in ginger, i.e., 6-gingerol and 6-shogaol (a dehydrated form of 6-gingerol) in fresh rhizomes vary according to the condition of the rhizome [3] as well as environmental temperature changes and throughout the storage period [13].

The commercial value of ginger is high, based on its use in daily nutrition as well as a health supplement. Lately, ginger plant has started to be planted using soilless methods [14,15]. Among the benefits of crops grown without soil are protection from soil pathogen attacks [15] and heavy metal traces contamination [16], better nutrient supply and more efficient use of space [17]. Plants grown without soil can also be standardized, where this factor is important to produce consistent bioactive compounds for medical applications. Therefore, there is great potential to use this soilless cultivated ginger as a source of medicinal natural products. However, the profile of its bioactive compounds needs to be well characterized so that relevant pharmacological effects can be achieved. This study reports the results comparing the level of 6-gingerol in conventionally grown ginger with that grown without soil. The results of this study can be applied to future studies that focus on 6-gingerol as a pharmaco-active compound for target diseases.

## MATERIALS AND METHODS

### Chemicals and Reagents

Analytical grade 95% ethanol and absolute ethanol (Chemiz, Malaysia) was purchased for the extraction process. Pure analytical standards of HPLC grade: 5 mg 6-gingerol (Sigma Aldrich, U.S.) and 10 mg 6-shogaol (Sigma Aldrich, U.S.) in powder form were purchased and stored at 4 °C. Other chemicals and solvents such as absolute methanol (EAM, Malaysia) and absolute acetonitrile (R&M, United Kingdom) of HPLC grade and 85% phosphoric acid of analytical grade (Sigma Aldrich, U.S) were purchased. Water from a Milli-Q RO system (Millipore Corporation, France) was used for this study.

### Plants

Hydroponic ginger (HP) supplied by the ginger farm, Millercle Berhad in Tasek Gelugor (Pulau Pinang, Malaysia). Meanwhile, soil-based (SB) ginger rhizomes were purchased from a local farm in Port Dickson (Negeri Sembilan, Malaysia). All ginger is categorized as young ginger with a harvest age of less than 6 months. Mature ginger plants (containing stems, leaves, flowers, roots, and rhizomes) from both locations were sent to the herbarium at the Forestry Research Institute of Malaysia (FRIM) (Selangor, Malaysia) for plant species identification by research officer Madihah Muhammad Nawi, from Division of Biological Resources, FRIM. Voucher specimens (SBID 024/21 and SBID 011/21) were deposited in our laboratory.

HP ginger has been supplied in two forms which are dry pieces (HP1) and fresh ginger (HP2) within 6 months of age. HP ginger is cultivated using a hydroponic system without exposure to pesticides. Fresh SB ginger has been obtained at a harvest age of around 3-4 months. Fresh SB and HP2 ginger rhizomes were thinly sliced to a thickness of 0.1-0.2 cm and dried at room temperature for two days on a sterile tray until the ginger was completely dry. Slices of ginger that have a greenish color change or are not completely dry are discarded. The dried ginger was ground using a stainless-steel heavy-duty grinder (Pensonic, Malaysia) until it became a powder. All ginger sources were stored in a refrigerator at 4 °C until extraction.

### Extraction Method

Ginger was extracted using the reflux method (Malaysian Herbal Monograph, n.d) with a 1:20 ratio of ground ginger to 95% ethanol for 30 minutes (60 °C). The same ratio of ginger to solvent was used for extraction with absolute ethanol °C oncentration. Anti-bumping glass beads are used to ensure uniform heating of the solvent in the flask. The extract was cooled, filtered using filter paper Whatman no. 1 and dried in a rotary evaporator

(BUCHI Rotavapor R-200). The extracts were then collected in glass vials, weighed, and then evaporated to dryness under a fume hood to remove excess ethanol before being stored at 4 °C.

## High Performance Liquid Chromatography (HPLC) Analysis

### Mobile Phase Preparation

The mobile phase for the estimation of marker compounds in the sample consisted of 55 volumes of acetonitrile, 44 volumes of 0.1% phosphoric acid and 1 volume of methanol in a total volume of 1 L as recommended by the Malaysian Herbal Monograph. The solvent mixture was filtered using a filtration device consisting of a cup and a filter head, lined with a 47 mm diameter filter membrane, connected by a tube to a vacuum pump that allowed the mobile phase to flow into a 2 L conical flask. The mobile phase was degassed and sonicated for 10 minutes using ultrasonic (Branson 5510) to release trapped air to prevent the formation of air bubbles and interference with chromatographic separation.

### Standard Solution Preparation

Standards: 6-gingerol and 6-shogaol (5 mg each) were weighed using an ultra-microbalance (Sartorius Cubis) and added with 5 mL of methanol to prepare a stock solution amounting to 1 mg/mL, then sonicated for 15 minutes. Both standard stock solutions were diluted to prepare a working solution with a concentration of 100 µg/mL.

### Sample Preparation for HPLC Analysis

For HPLC analysis, 10 mg of the extract was weighed using an ultra-microbalance (Sartorius Cubis) and dissolved in 10 mL of methanol into a glass vial. The solution was sonicated for 10 minutes. A working solution of 100ug/mL concentration was prepared and filtered through a 0.45 µm nylon syringe filter into a 1 mL glass vial. A 20 µL aliquot was then injected into the HPLC system. The samples were analyzed using HPLC to compare the concentration of marker compounds between the two specimens of *Z. officinale*.

HPLC analysis was carried out using a dual-wavelength absorption detector system at a wavelength of 225 nm, (Waters 2487) equipped with an isocratic HPLC pump (Waters 1515) and a Waters XBridge C-18 column (4.6 mm× 250 mm; particle size 5 µm). HPLC conditions for isocratic elution were set according to the Malaysian Herbal Monograph guidelines with an injection volume of 20 µL, a flow rate of 1 mL/min and a column oven temperature of 30 °C. Quantitative analysis of marker compounds were carried out using linear calibration curves generated using 6-gingerol and 6-shogaol standards.

### Method Validation

The method was validated using ICH guidelines [18] to monitor system suitability, linearity, precision (intra-day and inter-day), precision (recovery test), the limit of detection (LOD) and limit of quantification (LOQ).

#### i. System Suitability Test

The system suitability test (Malaysian Herbal Monograph, 2019) was carried out by performing repeated injections of the standard mixture at 100 µg/mL (n=5) and the relative standard deviation percentage (RSD), retention time (Rt), symmetry factor (As) and resolution value (Rs) between the two standards were recorded.

#### ii. Linearity

Working concentrations of standards and samples (25, 50, 75, 100 and 125 µg/mL) were made by diluting standard stock solutions with methanol, and calibration curves for reference standards were established using linear regression analysis.

#### iii. Precision

Precision was studied by analyzing three replicates of both samples and standard solutions on the same day (intra-day precision) and three consecutive days (inter-day precision) at five different concentrations (25, 50, 75, 100 and 125 µg/mL). Results from intra-day (reproducibility) and inter-day (intermediate precision) are expressed in mean ± RSD (%).

#### iv. Accuracy (Recovery test)

This test was performed by adding known amounts of standard solutions of 6-gingerol and 6-shogaol to the samples, at three different concentrations (25, 50 and 100 µg/mL, respectively) and prepared in three replicates. The average recovery is obtained with the formula [19]:

$$\text{Recovery (\%)} = \frac{(\text{amount found} - \text{original amount})}{\text{amount spiked}} \times 10$$

#### i. Sensitivity

The limit of detection (LOD) and limit of

quantification (LOQ) of the method are determined using the formula:

$$\text{LOD} = 3.3\ \frac{\alpha}{S}$$

$$\text{LOQ} = 10\ \frac{\alpha}{S}$$

where σ is the standard deviation of the intercept and S is the slope of the linear regression.

### Quantification of Marker Compounds in Ginger Extract

The concentration of 6-gingerol in 1 g of sample was calculated using the following formula [20]:

Concentration (%) of the standard in ethanolic sample extract:

$$\frac{W \times (a \times \frac{b}{c})}{\text{weight of sample} \times \text{weight of dried extract used in analysis}} \times 100\%$$

Where W is the total weight of the extract (mg), a is the standard concentration calculated from the linear regression equation (mg/mL), b is the sample preparation volume for analysis and c is the injection volume.

The concentration of 6-shogaol in 1 g of sample was calculated from the external standard method as described by Watson [21] because the area under the curve (AUC) for 6-shogaol in the ginger extract was low and the concentration calculated using linear regression was below the detection limit.

$$\text{Conc. unknown} = \frac{\text{Area unknown}}{\text{Area known}} \times \text{Conc. known}$$

### Statistical Analysis

Statistical analysis was conducted using Microsoft Excel 2016 and GraphPad Prism (Version 9.0) software. Statistical analysis for compounds in each extract was analyzed using one-way ANOVA test. The significance level was set at $p < 0.05$.

## RESULTS AND DISCUTION

### Method Development and Validation

The retention time (Rt) for 6-gingerol was 4.11 minutes while for 6-shogaol it was 10.51 minutes (Fig. 1).

The linear regression equation for 6-gingerol is y = 48311x - 58989, where y is the area under the curve (AUC) and x is the concentration of 6-gingerol. The linear regression equation for 6-shogaol is y = 7.8299x - 0.0513, where y is the AUC and x is the concentration of 6-shogaol. The $R^2$ correlation coefficient values for both standards are 0.998 and 0.999 respectively, which are within the acceptable range (Fig. 2).

The average recoveries (%) for 6-gingerol and 6-shogaol were 94.44% and 99.29%, respectively. The LOD and LOQ for 6-gingerol were 10.49 and 31.80 µg/mL and for 6-shogaol were 5.54 and 16.78 µg/mL respectively. RSD values from repeated extractions for 6-gingerol and 6-shogaol were 0.08 and 0.34, respectively. The RSD for the AUC, 1.16% and the Rt, 0.42 for 6-gingerol are exactly scattered around the same value. The RSD percentage value for Rt, 0.45% for 6-shogaol is accurate, but the AUC value is imprecise, slightly higher than the cutoff value at 2.60%. For the system compatibility test, the tailing factor obtained for 6-gingerol (1.1) and 6-shogaol (1.07) was no more than 1.5.

The RSD percentage of the retention time for 6-gingerol and 6-shogaol is not more than 2.0% while the resolution value (17.6) between the two standards is more than 1.5, indicating that all parameters for the system suitability test meet the acceptance criteria. A summary of the method validation evaluation is shown in Table 1.

### HPLC Sample Analysis

This study initially found that the concentration of 6-gingerol in 95% ethanol SB ginger extract (1.012%) was significantly higher ($p< 0.05$) than in 95% ethanol HP1 ginger extract (0.314%). The concentration of 6-gingerol in 100% ethanol SB ginger extract (0.352%) was significantly higher ($p< 0.05$) than in 100% ethanol HP1 ginger extract (0.067%). The percentage concentration of 6-gingerol in the 95% ethanol extract in both HP and SB ginger extracts was significantly higher ($p< 0.05$) than in the 100% ethanol ginger extract. The low content of 6-gingerol in ginger is likely affected by the time factor, where this HPLC analysis was conducted on HP1 ginger that was dried by the supplier, sliced, and stored for a relatively long period. According to a previous study [13], the content of 6-gingerol was found to decrease according to storage time. Therefore, the HPLC analysis was repeated with fresh HP2 ginger to confirm that the results obtained were not affected by the storage period.

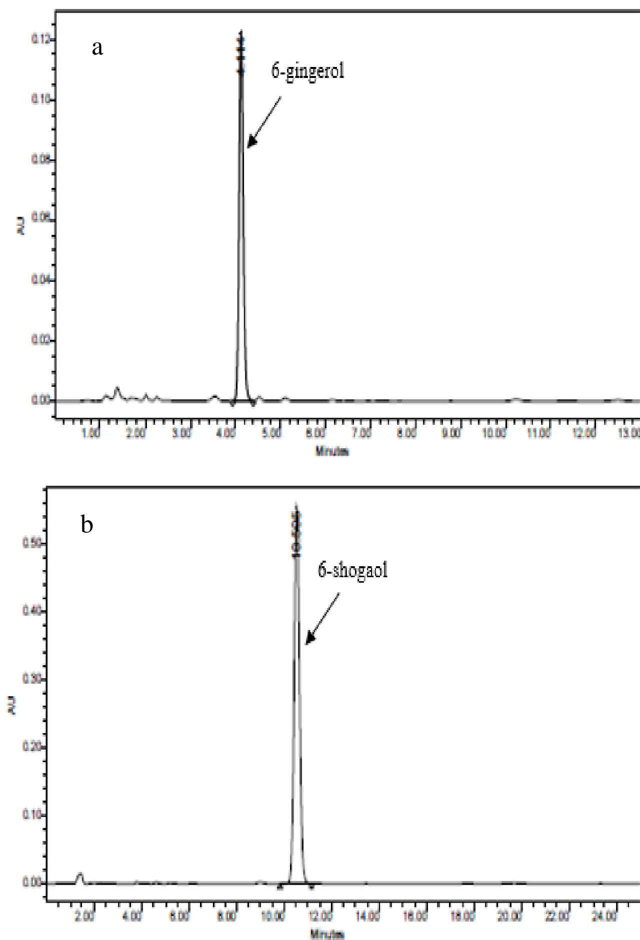


**Fig. 1** Chromatogram of [a] 6-gingerol and [b] 6-shogaol reference standards, with retention times of 4.11 minutes and 10.51 minutes respectively.

During the comparison of HPLC analysis performed between HP1 extract and HP2 extract, it was found that HP2 ginger which was supplied fresh and then dried in the laboratory, had a significantly higher concentration of 6-gingerol ($p < 0.05$) compared to HP1 ginger (0.748% vs 0.314%). The results of the new analysis confirmed that the concentration of 6-gingerol in the earlier HP1 ginger was indeed associated with a long storage period. The concentration of 6-gingerol in 95% SB ginger extract (1.012%) differ significantly compared to those in HP2 extract (0.75%) while both gingers have equivalent amount of low 6-shogaol concentrations (0.0004% and 0.0005% respectively).
The comparison between the content of 6-gingerol and 6-shogaol (Table 2) shows that all three ginger extracts have a much higher content of 6-gingerol than 6-shogaol: with respectively 1.012% and 0.0005% in SB ethanol 95% ginger extract, 0.314 % and 0.0007 % in HP1 ginger extract and 0.748% and 0.0004% in HP2 ginger extract. The compound 6-gingerol was eluted at 4.128, 4.128 and 4.060 minutes, while 6-shogaol was eluted at 10.482, 10.467 and 10.119 minutes for SB, HP1 and HP2 ginger extracts (Fig. 3). Pre-treatment methods that include drying of natural products, extraction methods and solvents used largely affect the amount of 6-gingerol and 6-shogaol in ginger. Various quantitative analyses have been carried out on ginger, regardless of its condition; fresh or dry [22]. Popular solvents to produce ginger extracts include methanol, ethanol, acetone, or water and are usually chosen based on the compatibility of the solubility of the marker compound with the solvent [23]. Ethanol was chosen as the extraction solvent in our study as it is recommended in the Malaysian Herbal Monograph [4], and because many literatures support its ability to extract bioactive compounds in ginger.

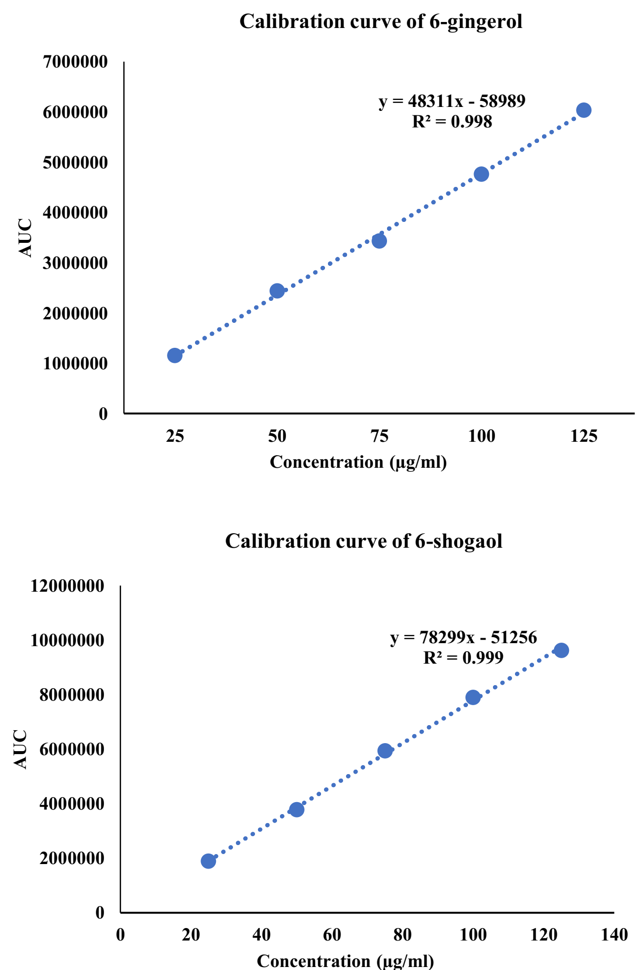


**Fig. 2** Calibration curve of [a] 6-gingerol and [b] 6-shogaol, was constructed using linear regression over five different concentrations (25, 50, 75, 100 and 125 µg/mL).

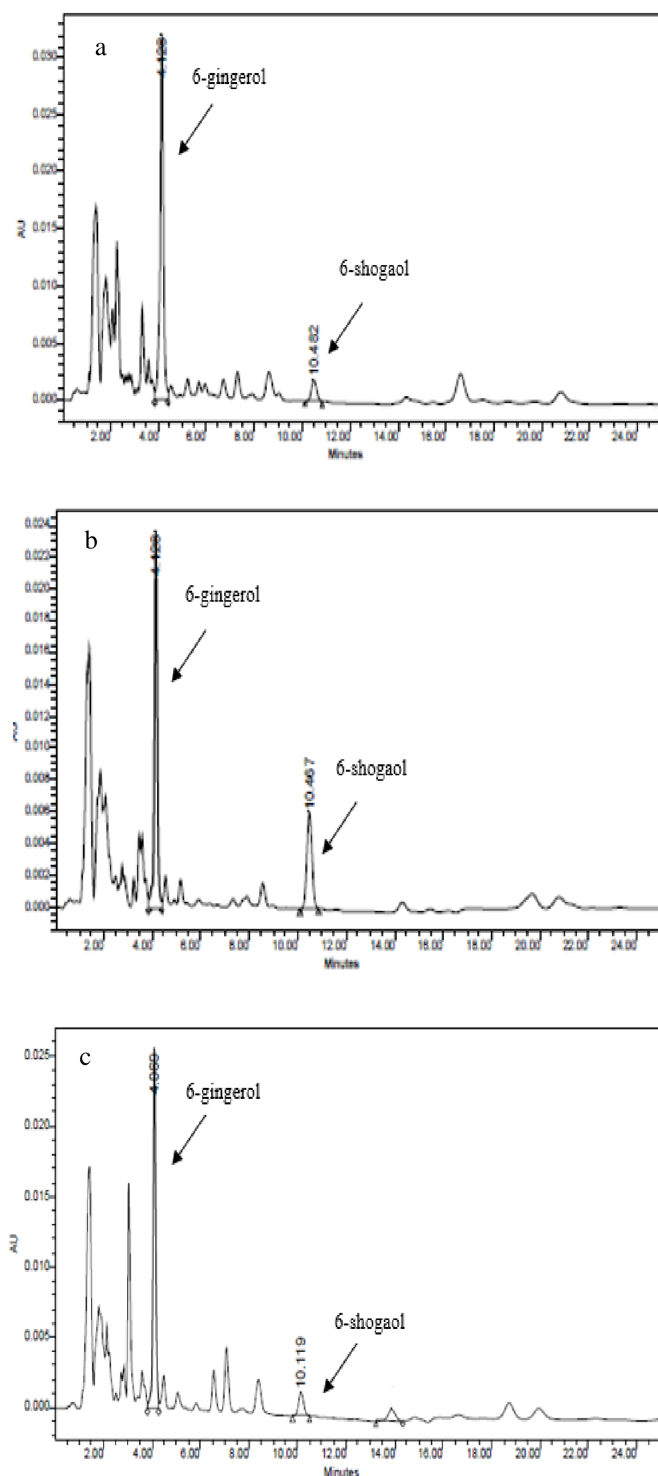


**Fig. 3** Chromatogram of [a] SB, [b] HP1 and [c] HP2 ginger extract, with 6-gingerol eluted at 4.128, 4.126 and 4.060 minutes, while 6-shogaol eluted at 10.482, 10.467 and 10.119 minutes.

A comparison of the extraction of 6-gingerol in petroleum ether, hexane, ethanol, ethyl acetate, and acetone using microwave-assisted extraction (MAE) found that ethanol as a solvent produced the highest amount of 6-gingerol [7]. In addition, Teng *et al*. [7] also tested extraction methods with different concentrations of ethanol and concluded that the variable did indeed affect the results obtained. They suggested that at a fixed extraction time, the optimum concentration of ethanol for a better yield of 6-gingerol is between 70-100%. The study also discussed that solvents that contain little water allow the diffusion of water into the cellular matrix, helping the heating process and the transport of compounds into the solvent at a faster rate [7]. This explains the analytical results obtained from our study, where the 95% ethanol extract of both SB and HP gingers produced more 6-gingerol content than ginger extracted using absolute ethanol. Some of the methods that have been optimally developed for extracting ginger include reflux, sonication, high-pressure Soxhlet extraction and microwave-assisted extraction. The reflux method was used in our study because it is the most effective method for the extraction of gingerol compounds [22]. Ginger plant specimens are usually pre-treated or dried before extraction. There are many drying techniques for plant samples including conventional air drying, modified atmosphere heat pump drying, freeze drying, and vacuum drying. A previous study [24] found that vacuum drying maintained the highest 6-gingerol content compared to other drying techniques. On the other hand, air drying showed the lowest retention of 6-gingerol due to the unstable nature of 6-gingerol compounds [24]. Gingerol generally undergoes oxidation in the presence of oxygen in the air and is easily dehydrated to shogaol in different pH or temperature settings [24]. In our study, ginger was air-dried due to the ease of the method and cost-effectiveness. In terms of storage, the gingerol content shows different changing trends according to the storage period [13]. Studies have found that gingerol compounds in cooked ginger and processed ginger products undergo gradual degradation during storage [13,25]. In our study, samples of HP1 ginger in the form of dry flakes were stored at 4 °C for about 5 months. Although this method of preservation and storage is generally considered stable and does not affect the quality of the sample [13, 26] it turns out that it is not the best method to protect the bioactive compounds of ginger. The results of these findings are reported so that they can be a reference for other related studies in the future.

**Table 1** The summarized results of HPLC method validation.

| Parameters | Results | | |
|---|---|---|---|
| System suitability test | | 6-Gingerol | 6-Shogaol |
| | Tailing Factor | 1.1 | 1.07 |
| | RSD Rt (%) | 0.077 | 0.335 |
| | Resolution | 17.6 | |
| | Conclusion: Tailing factor, RSD Rt (%) and resolution for both standards meet the acceptance criteria | | |
| Specificity | Conclusion: No peak was observed near the retention time of interest peaks and the resolution value between the peak of interest. A high degree of specificity. | | |
| Linearity | Slope | 48311 | 78299.3 |
| | Intercept | -58989 | -51256.1 |
| | $R^2$ | 0.998 | 0.999 |
| | Conclusion: Linearity of both standards produced satisfactory results | | |
| Determination of LOD and LOQ | LOD | 10.494 | LOD 5.537 |
| | LOQ | 31.8 | LOQ 16.779 |
| | Conclusion: LOD and LOQ below the specified range | | |
| Accuracy | Mean recovery (%) | 94.44 | 99.29 |
| | Conclusion: The accuracy of both standards produced satisfactory reproducibility results. | | |
| Precision | | | |
| (Intra-day/ Repeatability) | RSD (%) | 0.077 | 0.335 |
| (Intermediate precision) | RSD (%) | AUC 1.161 | AUC 2.601 |
| | | Rt 0.422 | Rt 0.451 |
| | Conclusion: 6-Gingerol AUC and Rt were precisely scattered around the same values. 6-Shogaol Rt value was precise, but the AUC value was imprecise. | | |

**Table 2** The percentage concentration of 6-gingerol and 6-shogaol compounds in ginger extracts.

| | The concentration of compounds in ginger extracts (%) | | |
|---|---|---|---|
| | SB | HP1 | HP2 |
| 6-gingerol | | | |
| 95% ethanolic extract | 1.012 ± 0.058 a | 0.314 ± 0.013 b | 0.748 ± 0.003 c |
| 100% ethanolic extract | 0.352 ± 0.019 b | 0.067 ± 0.003 d | - |
| 6-shogaol | | | |
| 95% ethanolic extract | 0.0005 ± 0 b | 0.0007 ± 0 a | 0.0004 ± 0 bc |
| 100% ethanolic extract | 0.0002 ± 0 bc | 0.0001 ± 0 c | - |

Values are expressed as means of percentage + SEM (n = 3). Different alphabets (a-d) indicate the values were significantly different at $p < 0.05$ using one-way ANOVA test. Comparisons were made separately for each compound.

The results of our study found that 6-gingerol represents the main active compound of ginger rhizome, in line with many previous studies by [27-30]. The quantity of phenolic compounds found in ginger varies according to the origin of the ginger or the cultivar, most likely influenced by environmental factors that affect the biosynthesis and concentration of compounds [27]. Asamenew *et al*. [27] reported that Ethiopian ginger had a slightly higher concentration of total phenolic content than Korean ginger. Ahui *et al*. [31] identified that 8-gingerol was found to be the main compound contained in Nigerian ginger. Meanwhile, research was conducted on black ginger or *Kaempferia parviflora* Wall. reported that methoxyflavone is the main phenolic compound of black ginger [27]. The concentration of the compound that produces the pungent smell of ginger, especially 6-gingerol varies in each part of the ginger plant itself. For example, an analysis by Tanweer and colleagues [32] showed that the leaves of the ginger plant contained significantly higher amounts of 6-gingerol than the rhizomes and flowers. Ko, Nam, & Chung [33] reported that ginger pulp extracted in methanol contained higher levels of 6-gingerol and 6-shogaol than ginger peel extract.

In terms of cultivation, commercialized ginger can be harvested young, 3 to 4 months after planting or when it has reached full maturity within 8 to 10 months [34]. The level of maturity of ginger during harvest affects the weight of ginger rhizomes, water and nutrient content and compounds in ginger [35]. HPLC analysis in ginger rhizome samples harvested at 9 months showed higher 6-gingerol content than in 6-month ginger samples[35]. Vedashree, Asha, Roopavati, & Naidu [36] also reported the results of a similar study, where a significant difference was reported in the content of 6-gingerol found in ginger rhizomes aged 5 months and 9 months, while a significant increase could be observed at the end of 9 months. However, various studies have found that the amount of 6-gingerol is affected by the effects of long-term cultivation and the environment [36].

Ginger has traditionally been cultivated using crop rotation, intercropping, or shifting cultivation systems, most of which face challenges for long-term implementation as they pose many economic and environmental concerns. In shifting cultivation techniques, land depleted of nutrients will be abandoned and new land for agriculture obtained, resulting in deforestation for more land, soil infertility, and soil erosion problems [14]. Plants grown in soil are susceptible to soil-borne diseases caused by bacterial or fungal infections. These plants can also be exposed to heavy metal traces pollution in the soil resulting from the bioaccumulation of metals from various sources such as natural sources (such as rock weathering), agricultural sources (such as pesticides and inorganic fertilizers), industrial sources (such as mining) and domestic sources (such as combustion, transport, aerosol) [16]. Therefore, landless agriculture is increasingly becoming the focus as an alternative or solution to these problems [37]. Soilless farming includes hydroponics, aeroponics or substrate culture where the soil is replaced with growing media or substrates such as rockwool, hemp, sawdust, husks, or plant fibers [14,37]. Soilless cultivation techniques produce higher crop yields using less time and less space. Gingers have been planted in soilless cultivation, but studies reporting the profile of bioactive compounds in soilless ginger are limited. This is important to allow ginger grown without soil to be used as a source of natural products for medical applications, considering the benefits of this cultivation method as mentioned above. This study focuses on 6-gingerol and 6-shogaol, as the main bioactive compounds that have been proven to have various pharmacological effects, based on previous studies. It is worth to note that HP ginger grown in soilless condition could still produce a considerably high amount of 6-gingerol, therefore it has the potential to be used for further pharmacological research and developed as a medicinal natural product. Nevertheless, it is important for further studies to also characterize the profile of other bioactive compounds in ginger grown without soil, because all of them may have chemical interactions and provide integrative pharmacological effects.

## CONCLUSION

Overall, our study found that both gingers have different amount of 6-gingerol and 6-shogaol with factors such as storage conditions and extraction solvent affecting their quantity. Nevertheless, it is quite interesting to note that HP ginger grown in pesticide-free and soil-free conditions can still produce high levels of 6-gingerol compounds comparable to conventionally grown SB ginger. Further studies in the future should prioritize a detailed comparison between HP and SB ginger for good quality control and safety assurance as a commercial ginger product. In the meantime, more research is needed for a comprehensive understanding of hydroponic plants and the benefits for the economic and environmental sectors that this agricultural system promises.

## Conflict of Interest

The authors have not declared any conflict of interests.

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## REFERENCES

1. Nafi A., *et al*. Partial Characterization of an Enzymatic Extract from Bentong Ginger (*Zingiber officinale* var. Bentong). Molecules. 2014; 19: p. 12336-12348.
2. Kubra I.R, Rao L.J. An impression on current

developments in the technology, chemistry, and biological activities of ginger (*Zingiber officinale* Roscoe). Crit Rev Food Sci Nutr. 2012; 52(8): p. 651-88.

3. Kizhakkayil J., Sasikumar B. Diversity, characterization and utilization of ginger: a review. Plant Genetic Resources. 2011; 9(3): p. 464-477.
4. Committee M.H.M. Malaysian Herbal Monograph. Forest Res Institute Malaysia. 2015; 517-531.
5. Mao Q.Q., *et al*. Bioactive Compounds and Bioactivities of Ginger (*Zingiber officinale* Roscoe). Foods. 2019; 8(6).
6. Jafarzadeh A., Nemati M. Therapeutic potentials of ginger for treatment of Multiple sclerosis: A review with emphasis on its immunomodulatory, anti-inflammatory and anti-oxidative properties. J Neuroimmunol. 2018; 324: p. 54-75.
7. Teng H., *et al*. Comparing the effects of microwave radiation on 6-gingerol and 6-shogaol from ginger rhizomes (*Zingiber officinale* Rosc). PLoS One. 2019; 14(6): p. e0214893.
8. Jafarzadeh H., *et al*., Making sense of COVID-19 over time in New Zealand: Assessing the public conversation using Twitter. PLoS One. 2021; 16(12): p. e0259882.
9. Ali B.H., *et al*. Some phytochemical, pharmacological and toxicological properties of ginger (*Zingiber officinale* Roscoe): a review of recent research. Food Chem Toxicol. 2008; 46(2): p. 409-20.
10. Kim C.Y., *et al*. Neuroprotective Effect and Molecular Mechanism of [6]-Gingerol against Scopolamine-Induced Amnesia in C57BL/6 Mice. Evid Based Complement Alternat Med. 2018; 2018: p. 8941564.
11. Kim J.K., *et al.* [6]-Gingerol prevents UVB-induced ROS production and COX-2 expression in vitro and in vivo. Free Radic Res. 2007; 41(5): p. 603-14.
12. Qian Q.H., *et al*. Effect of gingerol on substance P and NK1 receptor expression in a vomiting model of mink. Chin Med J (Engl). 2010; 123(4): p. 478-84.
13. Shukor N.I.A., *et al*. The changes of chemical quality of ginger during postharvest storage at chilling temperature. Food Res. 2020; 4(5): p. 1653-1662.
14. Yaseer S.M, Mohamad A.M. Commercial ginger production using fertigation system. Bulletin Teknol MARDI. 2012; (1): p. 97-105.
15. Yaseer S.M., *et al.* Effects of Organic Substrates on Growth and Yield of Ginger Cultivated Using Soilless Culture. Malays. Appl. Bio. 2015; 44(3): p. 63-68.
16. Srivastava V., *et al*. Agroecological Responses of Heavy Metal Pollution with Special Emphasis on Soil Health and Plant Performances. Frontiers in Environmental Sci. 2017; 5.
17. Sambo P., *et al*. Hydroponic Solutions for Soilless Production Systems: Issues and Opportunities in a Smart Agriculture Perspective. Front Plant Sci. 2019; 10: p. 923.
18. Guideline I.H.T. Validation of analytical procedures: Text and Methodology. Q2 (R1). 2005; 1(20): p. 05.
19. Kajsongkram T., *et al*. Development and Validation of a HPLC Method for 6-Gingerol and 6-Shogaol in Joint Pain Relief Gel Containing Ginger (*Zingiber officinale*). Int J Med and Health Sci. 2015; 9(12).
20. Khalib S.C. Development and Validation of a HPLC-UV Method for Standardization of *Zingiber Cassumunar* Roxb. Based on Selected Chemical Markers, in FACULTY OF PHARMACY. UNIVERSITI KEBANGSAAN MALAYSIA. p. 84.
21. Watson D.G. Pharmaceutical Analysis: A Textbook for Pharmacy Students and Pharmaceutical Chemists. 2 ed. 2012; London: Churchill Livingstone.
22. Ok S., Jeong W.S. Optimization of Extraction Conditions for the 6-Shogaol-rich Extract from Ginger (*Zingiber officinale* Roscoe. Prev Nutr Food Sci. 2012; 17: p. 166-171.
23. Sharif M.F., Bennett M.T. The effect of different methods and solvents on the extraction of polyphenols in ginger (*Zingiber officinale*). J Teknol (Sci Engin). 2016; 78(11-2): p. 49-54.
24. Hawlader M.N.A., Perera C.O, Tian M. Comparison of the Retention of 6-Gingerol in Drying of Ginger Under Modified Atmosphere Heat Pump Drying and other Drying Methods. Drying Technol. 2006; 24(1): p. 51-56.
25. Zhang X., *et al*. Gingerol Decreases after Processing and Storage of Ginger. J Food Sci. 1994; 59(6).
26. Wills R., *et al*. Physiology and biochemistry. Postharvest: An introduction to the physiology and handling of fruit, vegetables and ornamentals. 2007; p. 28-51.
27. Asamenew G., *et al*. Characterization of phenolic compounds from normal ginger (*Zingiber officinale* Rosc.) and black ginger (Kaempferia parviflora Wall.) using UPLC–DAD–QToF–MS. European Food Res Technol. 2019; 245: p. 653-665.
28. Bailey-Shaw Y.A., *et al*. Changes in the contents of oleoresin and pungent bioactive principles of Jamaican ginger (*Zingiber officinale* Roscoe.) during maturation. J Agric Food Chem. 2008; 56(14): p. 5564-5571.
29. Jiang H., *et al*. Characterization of gingerol-related compounds in ginger rhizome (*Zingiber officinale* Rosc.) by high-performance liquid chromatography/electrospray ionization mass spectrometry. Rapid Communications in Mass Spectrometry: An Int J Devoted to the Rapid Dissemination of Up-to-the-Minute Res in Mass Spectrometry. 2005; 19(20): p. 2957-2964.
30. Sanwal S.K., *et al*. Antioxidant phytochemicals and gingerol content in diploid and tetraploid clones of ginger (*Zingiber officinale* Roscoe). Scientia Horticulturae. 2010; 124(2): p. 280-285.
31. Ahui M.L.B., *et al*. Identification of gingerols in ginger (*Zingiber officinale* Rosc.) by high-performance liquid chromatography-tandem mass spectrometry and pharmacologic studies of its aqueous extract on the rabbit

isolated duodenum contractility. J Phys Pharm Adv. 2013; 3: p. 16-26.

32. Tanweer S., *et al.* Comparison and HPLC quantification of antioxidant profiling of ginger rhizome, leaves and flower extracts. Clinical Phytoscience. 2020; 6(12).
33. Ko M.J., Nam H.H, Chung M.S. Conversion of 6-gingerol to 6-shogaol in ginger (*Zingiber officinale*) pulp and peel during subcritical water extraction. Food Chem. 2019; 270: p. 149-155.
34. Yaseer S.M., Adzemi M.A, Zaliha W. Effect of Organic Substrates On Ginger Growth, Yield and [6]-Gingerol Content Cultivated Using Soilless Culture System. Agrofor. 2018; 3.
35. Sida S., Samakradhamrongthai R., Utama-ang N. Influence of maturity and drying temperature on antioxidant activity and chemical compositions in ginger. Current Applied Sci and Technol. 2019; 19: p. 28-42.
36. Vedashree M., *et al*. Characterization of volatile components from ginger plant at maturity and its value addition to ice cream. J Food Sci Technol. 2020; 57(9): p. 3371-3380.
37. El-Kazzaz A. Soilless Agriculture a New and Advanced Method for Agriculture Development: an Introduction. Agric Res Technol: Open Access J. 2017; 3(2).