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Extraction and characterization of 6-shogaol and 6-gingerol from *Zingiber officinale* var.Bentong

[1,2]Sharizan, A. and [2,*]Sahilah A.M.

[1]*Food Science and Technology Research Center, Malaysia Agricultural Research and Development Institute (MARDI), Ibu Pejabat MARDI, Persiaran MARDI-UPM, 43400 Serdang, Selangor, Malaysia.*
[2]*Department of Food Science, Faculty of Science and Technology, 43600 Bangi, Selangor, Malaysia*

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Abstract

Zingiber officinale Roscoe var. Bentong or locally known as Bentong ginger is exclusively planted and harvested in the district of Bentong, Pahang, Malaysia. The demand for this ginger species has dramatically increased due to its high food and medicinal values, owing to the presence of 6-shogaol and 6-gingerol as active compounds. This study aimed to measure the concentrations of those active compounds with respect to their one-year plantation duration (January - December 2018). The proximate analysis, heavy metals and antioxidant activity were simultaneously determined during the plant growth. 6-gingerol was present in both fresh and dried samples whilst 6-shogaol could only be found in dried samples. Fresh ginger recorded the highest 6-gingerol content (2.09 mg/g) in the seventh month of harvesting time. On the other hand, in the sixth month of harvesting time, dried ginger had the highest concentration of 6-gingerol (0.66 mg/g) and 6-shogaol (1.85 mg/g). Notably, the accumulation of heavy metals such as As, Pb, Cd and Hg in Bentong ginger was relatively low and within the permissible limit. Meanwhile, the total polyphenol and phenolic content of Bentong ginger extract solution were observed to decrease as the ginger was maturing. In conclusion, *Z. officinale* Roscoe var. Bentong yielded different amounts of active compounds with respect to its harvesting time. Nevertheless, it generally exhibits good values in terms of chemical compositions that can be potentially used as nutraceutical food.

1. Introduction

Ginger is widely used as a spice or flavouring agent in the food and beverage industry that includes daily consumption of dietary meals (Shivraj and Se-Won, 2015). In the medicinal aspect, ginger is one of the essential ingredients in folk medicine such as Chinese, Ayurveda and Greek since decades ago. It is traditionally used to treat flu fever, arthritis, gingivitis, stomachache, diarrhoea, asthma, constipation and diabetes (Malek *et al*., 2005; Arshad *et al.,* 2014). Studies on the chemical composition of ginger disclose that ginger has many active compounds that possess antioxidant, anti-inflammatory and potentially, antitumor properties (Shukla and Singh, 2007; Stoilova *et al.,* 2007). Particularly, the rhizomes are reported to give significant anticancer, antimicrobial, antidiabetic and analgesic effects (Prabhakaran-Nair, 2013; Semwal *et al.,* 2015; Srinivasan, 2017).

In Malaysia, *Zingibe rofficinale* Roscoe var. Bentong or commonly known as Bentong ginger is exclusive ginger that can only be found in the district of Bentong, Pahang. It is rich with amazing nutraceutical values that are characterized by the presence of bioactive groups of gingerol, zingiberine and shogaol. The amounts of secondary active compounds in Bentong ginger are mostly higher than local ginger, hence explaining its higher demand by many sectors. Generally, two main components in ginger are volatile compounds and non-volatile oleoresin compounds. Volatile compounds consist of essential oils that give a pleasant odour to ginger. They are made up of monoterpenoids and hydrocarbon intermediates such as α-zingiberene, ar-curcumene, α-farnesene, β-farnesene, linalool, gin-gerol, β-sesquiphellandrene, zingerone, dehydrogingerol and hexahydrocurcumin (Prabhakaran-Nair, 2013). Meanwhile, non-volatile compounds or oleoresins usually consist of phenolic groups such as gingerol, shogaol and zingerone that exhibit the spice flavour of ginger (Huang *et al.,* 2012). Once extracted, the

*Corresponding author.
Email: *sahilah@ukm.edu.my*

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composition of oleoresins is usually in the range of 3-11%, depending on the extraction method, location of plantation and state of rhizomes. In addition, other typical organic and inorganic groups can also be found in ginger, for example, ascorbic acid, manganese, sodium, chlorine, fatty acids and carbohydrate (Prabhakaran-Nair, 2013).

Gingerol group has been identified as the main active compound in fresh ginger. Its derivative, shogaol group can be detected in dried ginger, due to the degradation of gingerol from the thermal or acidic environment (Hans *et al.,* 2005; Kubra and Rao, 2012). Reportedly, shogaol has a more distinct spice sensation and antioxidant activity than gingerol (Swarnalatha *et al.,* 2010; Guo *et al.*, 2014). In ginger rhizomes, there are several types of gingerol such as 4-gingerol, 8-gingerol, 10-gingerol and 12- gingerol. These compounds are thermally dependent as they form into shogaol structure that is less spicy but more aromatic than gingerol when exposed to high temperature. Progressively, they will be absent if the rhizomes are being dried or grilled (Semwal *et al.*, 2015).

A number of analytical methods have been proposed to determine the concentration of 6-gingerol and 6-shogaol including high-performance liquid chromatography (HPLC) and gas chromatography-mass spectrometry (GCMS) (El-Ghorab *et al.,* 2010; Cheng *et al.,*2011; Yeah *et al.,* 2014). HPLC analysis is preferred over GCMS to analyse both gingerol and shogaol groups as it is more thermal sensitive to hinder the conversion of gingerol into shogaol as a result of high temperature during GCMS analysis (Chen, 1986). The detection of these two compounds in ginger rhizomes has been successfully reported using HPLC analysis (Bailey-Shaw *et al.,* 2008; Hans *et al.,* 2015; Semwal *et al.,* 2015).

The Malaysian government has prioritized the plantation project of Bentong ginger in the National Key Economic Area (NKEA #EPP1) to promote economic growth in the local community. A few studies on Bentong ginger have been conducted but they are limited to the plantation practice, harvesting and selected phenolic compounds in matured species only (Ali *et al.,* 2010; Ahmad *et al.,* 2014; Tan *et al.,* 2017; Lim and Wong, 2018). However, studies on the concentration of 6 -gingerol and 6-shogaol as active compounds in Bentong ginger and its optimum harvesting time have yet to be reported. Once identified, the findings are beneficial to farmers to plan the plantation and harvesting time according to the growth phase of Bentong ginger, while the consumers can expect the quality of Bentong ginger based on its chemical compositions that are time dependent. Besides, the pathway of secondary metabolites production in Bentong ginger can be better understood by recognizing the accumulation of 6-gingerol and 6-shogaol in fresh and dried samples.

2. Materials and methods

2.1 Sampling

Raw samples of Bentong ginger (rhizomes) were collected from Kampong Janda Baik, Pahang, Malaysia according to pre-determined harvesting time in the third, sixth, seventh, eighth, ninth and twelfth months from January to December 2018. The samples were brought back to the laboratory to be cleaned by removal of soils, washing with distilled water, drying, grinding, and being stored in a closed vial at room temperature (25^{o}C) before further analysis.

2.2 Preliminary analysis

2.2.1 Proximate analysis

The proximate composition of Bentong ginger (i.e., moisture, ash, fat, protein, carbohydrate) was performed according to AOAC (1995). The moisture was determined by weighing 5 g of fresh rhizomes that had been ground beforehand, followed by drying at 105^{o}C for 3 hrs. The dried sample was weighed. The moisture percentage was calculated based on the difference in sample weight before and after drying. To determine the ash content, about 5-10 g of rhizomes was placed in a crucible and heated up to 550^{o}C to produce ash. After that, the ash was weighed to determine the ash percentage. The amount of fat and protein in the rhizome was calculated using the Soxhlet apparatus in petroleum ether solvent and Kjedahl micro method, respectively. Lastly, the carbohydrate content was obtained by calculating the total dried sample minus the weight of ash, fat and protein.

2.2.2 Heavy metals content

About 5 g of fresh sample was ground and weighed in a 250 mL conical flask. Next, 10 mL of concentrated nitric acid was added. The mixture was left at room temperature for 24 hrs before being heated at 80^{o}C for 5 hrs. The sample was left to cool at room temperature and later being diluted into 50 mL of solution. The concentrations of As, Cd, Hg and Pb were determined using inductively coupled plasma (ICP) (Perkin Elmer Optima, USA) (Tokalıoğlu *et al.,* 2018).

2.2.3 Total phenolic content

The estimation of total phenolic content was performed using the Folin-Ciocalteu method (Shabanian *et al.,* 2017). About 125 µL of the ginger extract was added into a test tube with 500 µL distilled water. After that, about 125 µL Folin-Ciocalteu reagent was added and left for 6 mins. About 1.25 mL of 7% sodium

carbonate was added with distilled water to obtain 3 mL of solution and left for another 90 mins. The absorbance value was determined using a UV-vis spectrometer at 750 nm with gallic acid as a standard solution (0-450 µg/mL). The standard curve of gallic acid was used as a reference to calculate the total phenolic content in the sample. The procedures were conducted thrice to get the mean value for each sample.

2.2.4 Free radical scavenging activity (DPPH)

The free radical scavenging activity of ginger extract solution was measured using UV-vis spectrophotometer (Spectro UV-VIS Double Beam PC Scanning Spectrophotometer UVD 2950, Labomed Inc) at 517 nm absorbance (Ab) (Rosli- *et al.*, 2018). Methanol solution containing DPPH was prepared prior to the analysis. A few ginger extract solutions were prepared at different concentrations (40, 80, 120. 160, 200 and 240 µg/mL). About 1 mL of DPPH solution was added to each solution. The solution was then shaken using an orbital shaker continuously for 30 min at room temperature. Butylated hydroxyl toluene (BHT) acted as an antioxidant standard. This experiment was conducted thrice. The free radical scavenging activity (DPPH) was calculated using the formula as follows:

$$\text{Free radical scavenging activity (DPPH)} = \text{Ab}_{\text{standard}} - \frac{\text{Ab}_{\text{sample}}}{\text{Ab}_{\text{standard}}} \times 100$$

where $\text{Ab}_{\text{standard}}$ refers to the absorbance of standard solution and $\text{Ab}_{\text{sample}}$ is the absorbance of sample solution.

2.2.5 Ferric ion reducing antioxidant power assay

The antioxidant activity can also be determined using the ferric ion reducing antioxidant power assay method (Mahdavi *et al.*, 2017). About 1 mL of ginger extract solution with concentrations ranging from 40-40 µg/mL was mixed with 25 mL of phosphate solution (0.2 M, pH 6.6) and 2.5 mL of 1% potassium hexacyanoferate. The mixture was incubated for 20 mins at 25°C. Next, about 2.5 mL of 10% trichloroacetic acid was added and the mixture was centrifuged for 20 mins at 3000 rpm. About 1 mL of the upper aliquot was used to mix with 2.5 mL of distilled water and 0.5 mL of 0.1% $FeCl_2$. The adsorption effect was measured using a UV-vis spectrophotometer at 700 nm with butylated hydroxyanisole (BHA) acted as a standard solution. All readings were performed thrice.

2.3 Extraction of active compounds

The analysis of 6-shogaol content was performed on the Bentong ginger samples that had been dried at <50 °C and the fresh samples according to Cho *et al.* (2015) with some modifications. All samples were ground into a fine powder and added to a 250 mL conical flask. About 100 mL of methanol solution was added and shook for 2-5 hrs using an orbital shaker. The extract solution was filtered using Whatman membrane filter 0.4 um prior to HPLC analysis.

2.3.1 Standard solution preparation

About 1 mg of standard 6-gingerol and 6-shogaol (Sigma Aldrich) was dissolved in methanol HPLC grade solution in two separate flasks to prepare the standard solution with a known concentration of 2500 ppm. These standard solutions were then diluted into a series of solutions with concentrations of 150, 200, 250, 300 and 400 ppm for 6-gingerol and 25, 50, 75, 100 and 150 ppm for 6-shogaol.

2.3.2 Chromatography and determination of 6-gingerol and 6-shogaol content

HPLC (Shimadzu model Class VP, Japan) with a mobile phase of water (A) and acetonitrile (B) and stationary phase consisting of Waters Spherisorb 5 µm ODS2 (4.6 × 150 mm) separating column was employed to determine the 6-gingerol and 6-shogaol content in Bentong ginger. Photo iodide detector array was used at 230 nm. The protocols for the mobile phase were as follows: from 0-20 mins, linear exchange of solution A from 70% to 10%, followed by constant water content in stationary phase for the remaining 20-30 mins of analysis. The flow rate was fixed at 1 mL/min. About 10 µL pf sample was injected. Table 1 shows the composition of the mobile phase programme during analysis.

Table 1. Mobile phase composition in HPLC

Time (min)	Flow rate (mL/min)	Percentage of water (%)	Percentage of acetonitrile (%)
0	1	70	30
20	1	10	90
30	1	10	90

3. Results and discussion

3.1 Physical appearance of Bentong ginger

Normally by practice, matured Bentong ginger is harvested in the fifth to sixth month after plantation. Commercially, in the seventh to the ninth month of the plantation, Bentong ginger is harvested to be marketed for its high fibre content and weight (1.8-3 kg). Figure 1 illustrates the appearances of Bentong ginger pertaining to harvesting time. During the first to the sixth month, the rhizomes had a small structure and were yellowish. Gradually, the size would be bigger, more fibrous and darker in yellow colour. However, in the eleventh and twelfth months of harvesting, the size shrunk to be smaller in size than previous progress. Usually, these kinds of rhizomes will be used for replanting purposes

Figure 1. Physical appearances of Bentong ginger harvested in third (a) sixth (b) ninth (c) and twelfth month (d)

(League *et al.,* 2006).

The cross-sectional area of Bentong ginger displayed a dark ring appearance that can be used to distinguish Bentong ginger from other species of ginger (Figure 2). This appearance was only visible earliest in the seventh month of harvesting time. Alternatively, this visual identification can be used to identify Bentong ginger

Figure 2. Dark ring appearance at the cross sectional area of Bentong ginger

other than spectrophotometry methods that list out the fingerprint chemicals for each ginger species.

3.2 Proximate analysis

Rhizomes in the seventh month of harvesting time were selected as samples for proximate analysis of Bentong ginger, as tabulated in Table 2. The content for ash, fat, moisture, protein and carbohydrate in Bentong ginger was 1.4, 0.54, 1.74, 88.57 and 7.71% respectively. Similarly, El Ghorab *et al.* (2010) reported 88.5% of moisture in their South Australia ginger. The relative moisture in ginger is commonly in the range of 79-90% that is typically influenced by weather and its storage manner.

Table 2. Proximate analysis of Bentong ginger

Ash content (%)	Fat content (%)	Protein content (%)	Moisture content (%)	Carbohydrate content (%)
1.45	0.54	1.74	88.57	7.71

3.3 Heavy metals content

Table 3. Heavy metals content in Bentong ginger

Heavy metals	Concentration (mg/kg)
As	undetected
Pb	1.55
Cd	0.22
Hg	0.003

Table 3 lists the heavy metals content (As, Pb, Cd and Hg) in Bentong ginger. Heavy metals analysis detected 1.55, 0.22 and 0.003 mg/kg of Pb, Cd, and Hg respectively. The accumulation of these heavy metals was relatively low and considered safe to consume according to the Food and Drug Administration (FDA) agency. The presence of selected heavy metals might originate from the fertilizing activity on the soils (Saod *et al.,* 2019).

3.4 Total phenolic content and antioxidant activity

Selected Bentong ginger samples from the seventh, eighth and ninth of harvesting time were tested for their total phenolic content and antioxidant activity as can be seen in Table 4. Interestingly, the total phenolic content showed a synergistic effect with the antioxidant activity, as a higher amount of total phenolic content would promote higher antioxidant activity. Similar findings were reported by Maizura *et al.* (2011) that found a significant correlation between total phenolic content and antioxidant activity in all of their samples, namely kesum, ginger and turmeric. The reduction of those compounds with harvesting time can be associated with the maturity of ginger that contained less phenolic content (Ghasemzadeh *et al.,* 2010).

3.6 Determination of 6-gingerol and 6-shogaol

Table 4. Total phenolic content and antioxidant activity of Bentong ginger

Harvesting time (month)	Polyphenol content (mg GAE/100 g)	Total phenolic content (mg CE/100 g)	DPPH scavenging activity (% inhibition)
7	940.87	204.49	75.99
8	871.78	191.41	77.23
9	803.38	170.05	73.33

The content of 6-gingerol and 6-shogaol in all samples was determined using HPLC-PDA spectrophotometry based on the comparison of chromatograms of samples with standards of 6-gingerol and 6-shogaol, as can be seen in Figure 3. As a result, 6-gingerol and 6-shogaol were detected at retention time of 11.82 min and 16.36 min, respectively. As illustrated, 6-shogaol was detectable only in dried Bentong ginger. Meanwhile, 6-gingerol could be found in both fresh and dried Bentong ginger. Previous studies confirm that 6-shogaol is present when 6-gingerol undergoes dehydration process from thermal effect or long period of storage (Ko *et al.,* 2019).

Figure 4 shows the UV-vis spectra for the identification of active compounds in the range of 200-800 nm. Two wavelength peaks can be used as a reference to determine the active compounds in ginger (226 and 282 nm). The 230 nm wavelength was set as a reference in this study. This absorbance pattern was in agreement with the findings by Kubra *et al.,* (2012) that investigated the absorbance spectra of active compounds of the gingerol group.

Meanwhile, Figure 5 displays the calibration curve for the standard solution of 6-gingerol and 6-shogaol. The correlation coefficients for both data exceeded 0.99, thus signifying good linear regression between HPLC data and concentration of a standard solution. On the other hand, Figure 6 compares the 6-gingerol content in fresh rhizomes of ginger varieties. Bentong ginger recorded the highest 6-gingerol (2.4660 mg) when compared with Chinese ginger (0.2021 mg), Tambunan ginger (0.4963 mg), fertigated ginger (0.7404 mg/g),

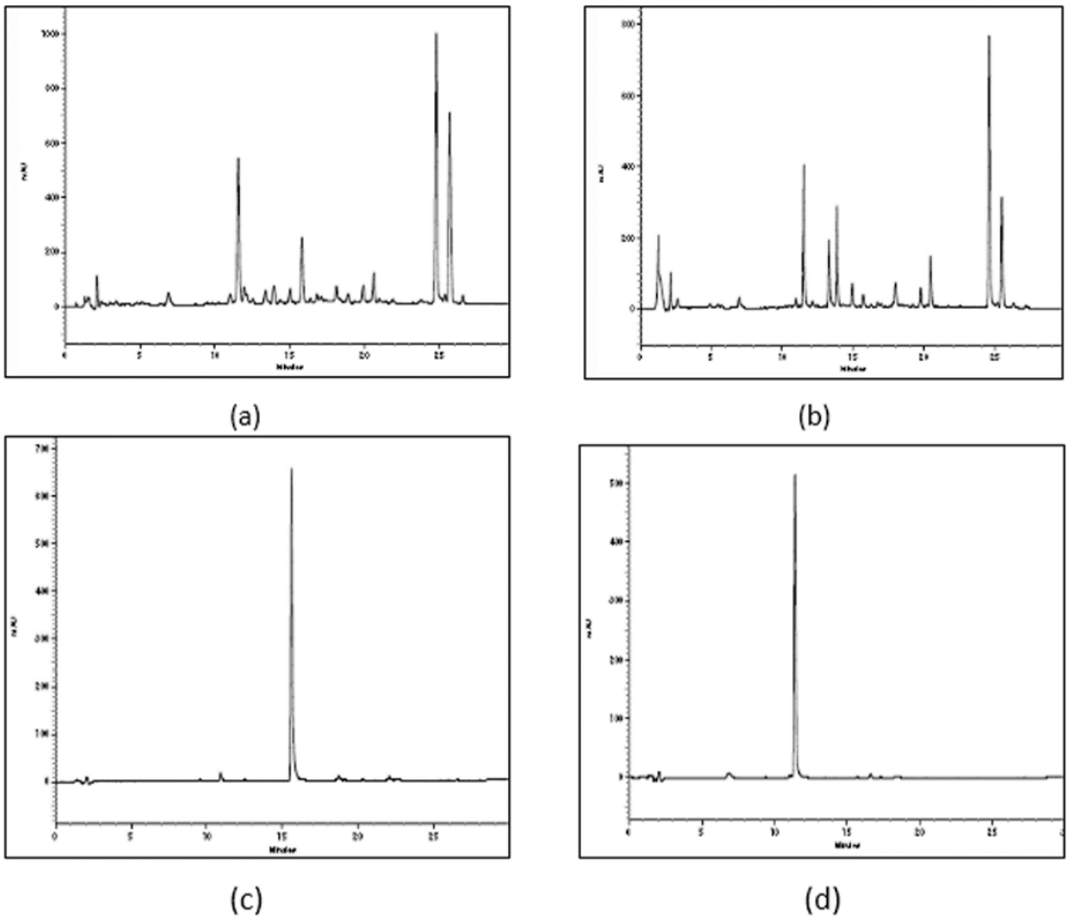

Figure 3. Chromatograms of Fresh Rhizomes (a) Dried Rhizomes (b) Standard 6-gingerol and (c) Standard 6-shogaol (d)

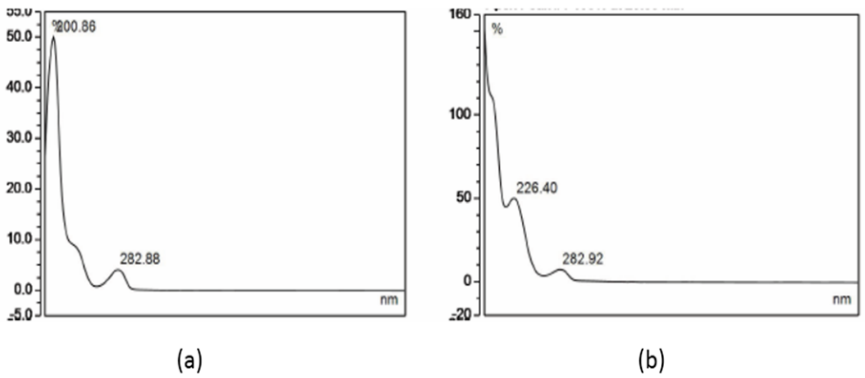

Figure 4. UV-vis Spectra for (a) 6-gingerol and (b) 6-shogaol

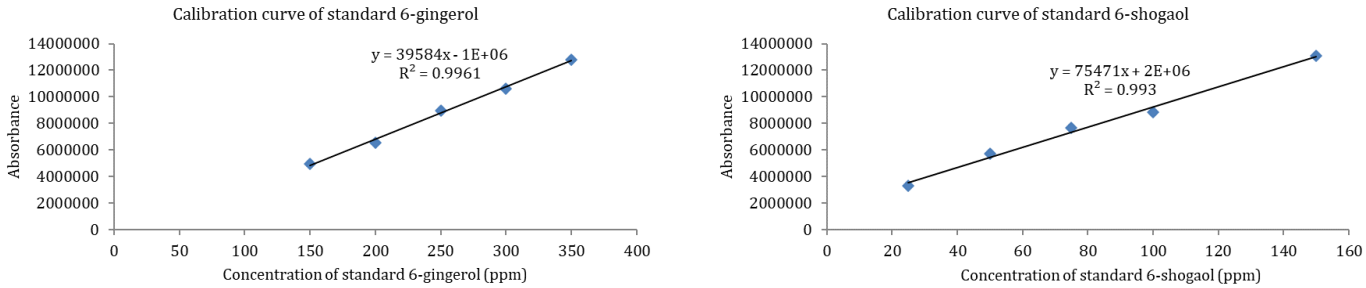

Figure 5. Calibration Curve for 6-gingerol and 6-shogaol

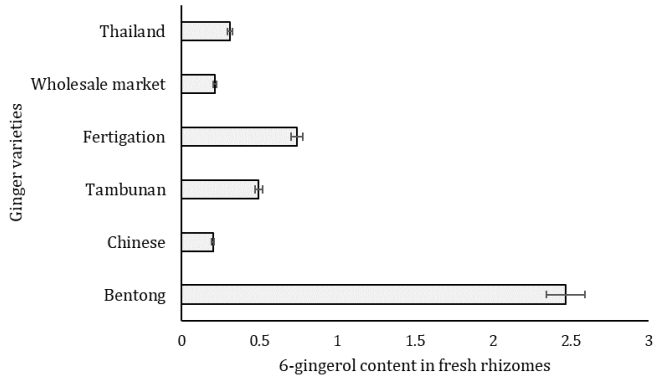

Figure 6. Comparison of 6-gingerol content among fresh ginger varieties

wholesale market ginger (0.2163 mg/g) and Thailand ginger (0.3102 mg/g). This information has further highlighted the speciality of Bentong ginger that is rich in 6-gingerol for nutraceutical value.

The content of 6-gingerol and 6-shogaol in fresh and dried Bentong ginger is compared in Table 5. In fresh rhizomes, the highest amount of 6-gingerol was 2.0833 mg/g in the seventh month of harvesting time. Meanwhile, the highest amount of 6-gingerol and 6-shogaol in dried Bentong ginger was 0.6653 and 1.8504 mg/g, respectively in the sixth month of harvesting time. Srinivasan *et al.* (2019) reported similar trend of 6-gingerol and 6-shogaol concentrations in their ginger. The decrease in the amount of those active compounds can be explained by the maturity of ginger that forms more fibrous structure with increasing harvesting time.

4. Conclusion

The amount of 6-gingerol and 6-shogaol as active compounds in Bentong ginger has been successfully determined using HPLC method. Our findings discovered that Bentong ginger had the highest 6-gingerol content among other varieties of ginger that demonstrates the speciality of Bentong ginger. In addition, 6-shogaol was only found in dried samples. The active compounds were found to be time-dependent as their optimum content was in the sixth and seventh month of harvesting time, for fresh and dried Bentong ginger, respectively. Future studies on Bentong ginger should highlight another parameter that influences the concentrations of these active compounds, for instance, extraction methods. Besides, other active compounds in the volatile component of ginger can be further explored.

Conflict of interest

The authors declare no conflict of interest.

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Table 5. Comparison of 6-gingerol and 6-shogaol content in fresh and dried rhizomes of Bentong ginger

Harvesting time (month)	Fresh rhizomes		Dried rhizomes	
	6-gingerol (mg/g)	6-shogaol (mg/g)	6-gingerol (mg/g)	6-shogaol (mg/g)
3	0.1355 ± 0.0205	ND	0.2378±0.0181	0.9120±0.0936
6	1.9263±0.0775	ND	0.6653±0.1467	1.8504±0.2030
7	2.0883±0.1811	ND	0.3417±0.0380	1.5456±0.1077
8	1.6126±0.0295	ND	0.2956±0.0325	1.5021±0.1494
9	0.8611±0.0257	ND	0.4960±0.0203	0.9631±0.0931
12	0.5188±0.1534	ND	0.3185±0.3979	0.7704±0.0386

ND: Not Detected

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