International Food Research Journal 21(5): 1931-1935 (2014)
Journal homepage: *http://www.ifrj.upm.edu.my*

Assessment of bioactive compounds, nutritional composition and antioxidant activity of Malaysian young ginger (*Zingiber officinale* Roscoe)

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Article history

Received: 27 July 2013
Received in revised form: 18 March 2014
Accepted: 21 March 2014

Keywords

Ginger rhizome
Gingerol
Total flavonoids
Total phenolic content
Antioxidant

Abstract

In current work, the nutritional composition, bioactive compounds, total phenolic contents and anti-oxidant activity of young Malaysian ginger rhizome were investigated. Proximate analysis and high performance liquid chromatography (HPLC) recruited to determine nutritional composition and bioactive compounds. The total flavonoid (TF) and total phenolic contents (TPC) of ginger rhizome were determined by aluminium chloride calorimetric assay and Folin–Ciocalteau reagent, respectively. 2,2'-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method were used to measure antioxidant capacity. The rhizome contained high moisture content and low level of carbohydrate and energy. 6-gingerol was the most abundant component in the selected ginger, and total flavonoid and phenolic content were reported to be 3.66±0.45 mg gallic acid/g and 10.22±0.87 mg quercetin/g of dry weight of rhizome, respectively. The rhizome also showed lower antioxidant activity than controls, with the IC_{50} value of 46.5 vs. 15.5 for α-tocopherol and 22 for BHT. The results of this study predicted that the young ginger rhizome originated from Malaysia may exhibit anti-oxidative and anti-inflammatory potentials due to high levels of gingerols, total flavonoid and phenolic compounds and antioxidant capacity.

Introduction

Ginger is the rhizome of the plant *Zingiber officinale* Roscoe which is commonly used as a spice around the world (Larsen *et al.*, 1999). This plant contains medicinal properties due to its bioactive compounds (Sanwal *et al.*, 2010), anti-oxidant (Singh and Gupta, 2013) and anti-inflammatory activities (Badreldin *et al.*, 2008). Pungent principles of ginger are gingerols; they are biologically active components that make a significant contribution towards medicinal applications of ginger (Sanwal *et al.*, 2010). Semi-dried ginger contains shogaols, which are more pungent than gingerols (Blaschek *et al.*, 1998). It is generally believed that ginger is a plant with considerable antioxidant content which produces protective health benefits in various diseases (Shobna and Naidu, 2000; Halvorsen *et al.*, 2002). [6]-gingerol is recognized as a strong anti-oxidant component parallel with its anti-inflammatory and anti-apoptotic action both in vivo and in vitro studies (Kim *et al.*, 2007)

According to Gruenwald (2004), food composition and active components of ginger is not uniform in all plants and may differ due to the plant varieties and regions of cultivation, hence it may change the health benefits of the rhizome. So, current work is designed to determine the nutritional composition, bioactive compounds, total flavonoids and phenolic contents and antioxidant capacity of young fresh ginger rhizome specifically originated from Selangor, Malaysia.

Materials and Methods

Sample preparation

The fresh rhizome was purchased from Pasar Borong, local market of Selangor, Malaysia. They were cleaned, washed, cut into pieces and quickly frozen in -80°C. BTK Bench Top K Manifold freeze dryer (VirTis, SP Industries, Inc., USA) was utilized to freeze-dry the samples. Ginger rhizomes were placed in glassware and dried for three or four times. The condenser temperature was approximately -55°C. Then, the samples were ground using a dry grinder and fine powders were obtained using a fine mesh sieve. The freeze-dried samples were used for determination of active components and proximate

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analysis.

Proximate analysis

Nutritional composition was performed by standard AOAC (1990) method for moisture and ash contents. Clegg Anthrone method (Clegg, 1955), Kjeldahl method (Tee *et al*., 1996) and Soxhlet method (Tee *et al*., 1996) were used to determine total available carbohydrate, total protein and total fat, respectively. Total dietary fiber was determined using total dietary fiber assay kit (Sigma-Aldrich, MO, USA).

Determination of active compounds

The major constituents of selected young ginger (6-, 8-, 10-gingerol and 6-, 8-, 10-shagaol) were analyzed using high-performance liquid chromatography procedure. The HPLC analysis of the extracts was performed on an Agilent 1100 Series HPLC system (phenomenex Luna C18 (2)-HST 100 x 2.00 mm, 2.5 um). Mobile phase A consisted of HPLC-grade acetonitrile (ACN); mobile phase B was Milli-Q water. Mobile phase pumped at 0.5 ml/min, the column temperature was 50ºC, and the injection volume was 3 µL. Data collected with quantification at 282 nm. All Standards were supplied by ChromaDex (Irvine, CA). Detection of gingerols in the sample was based on the comparisons of retention time and UV spectra with the standards. The standard was prepared by weighing 7 mg of standard into a 25 ml volumetric flask. Then, it was diluted to volume with MeOH and sonicated for 15 minutes. The sample was prepared by weighing approximately 250 mg and 2500 mg of sample into separate 50 ml volumetric flask. The flask was diluted to volume with MeOH and sonicated for 30 minutes. An aliquot of the sample solution was filtered through a 0.45 µm PTFE filter into a HPLC for analysis. The gradient program of the assay was shown in Table 1.

Determination of total phenolic content (TPC) and total flavonoid (TF)

Total phenolic content were determined using the Folin–Ciocalteau reagent (Bushra *et al.*, 2009). 100 µl of supernatant of ginger extract were mixed with 1500 µl (1/10 dilution) of Folin-Ciocalteu reagent. They incubated at room temperature for around 1 minute. 1500 µl of the 60 g/l sodium carbonate (Na_2CO_3) solution was then added. The above solution was then kept in a dark place at room temperature for 90 minutes. Absorbance was measured at 725 nm. Phenolic content of the extracts was compared against gallic acid standard calibration curve. Total phenolic content was expressed as mg of gallic acid / g of dry weight of ginger rhizome.

Table 1. Gradient program of HPLC

Time (min)	%A	%B
0	40	60
1	40	60
9	80	20
10	95	5
13	95	5
14	40	60
18	40	60

Total flavonoid of the young ginger extract was measured using aluminium chloride calorimetric assay (Quettier-Deleu *et al*., 2000). The rhizome extract (1 ml: 0.1 mg/ml) was mixed with 4 ml distilled water to dilute. 5% $NaNO_2$ solution (0.3 ml) was added to the volumetric flask. Following 5 minutes, 10% Ethanolic aluminium chloride hexahydrate ($AlCl_3.6H_2O$) was added, 1 minute later, 1.0 M NaOH (2 ml) also added. Finally, the sample and reagents mixed well with 2.4 ml distilled water. Then, absorbance was measured at 430 nm. The results were expressed in mg quercetin/g dry weight by comparison with the quercetin standard curve, prepared in similar condition.

Antioxidant activities: DPPH radical scavenging assay

Determination of antioxidant activity of ginger rhizome was done using DPPH radical scavenging assay (Mensor *et al*., 2001). Prepared samples from different concentrations (2.5 ml) were mixed with 1 ml of alcohol solution of DPPH (0.3 mM) (Sigma–Aldrich, USA) and stored in a dark area at room temperature, following 30 minutes, the absorbance was measured at 518 nm using a spectrophotometer (U-2001, Hitachi Instruments Inc., Tokyo, Japan). Following formula used to detect the anti-radical activity:

$$\frac{100 - [(Abs_{Sample} - Abs_{Empty\ sample}) \times 100]}{Abs_{Control}}$$

Empty sample prepared from 1 ml ethanol and 2.5 ml of different ginger extract concentration, and the control sample prepared from 1 ml of DPPH (0.3 mM) and 2.5 ml ethanol. BHT (butylhydroxytoluene) and α-tocopherol (Merck, India) were selected as references. A graph of scavenging activity against the concentration of sample extracts was plotted to determine IC_{50} value, which was defined as the total antioxidant necessary to decrease the initial DPPH radical concentration by 50%. Scavenging effect was calculated based on the percentage of DPPH scavenged.

Statistical analysis

All assays were done triplicate from prepared

sample and the standards. The data were presented as mean with the standard deviation.

Results and Discussion

Moisture content, yield and nutritional composition

The nutritional composition of ginger rhizome was determined by proximate analysis. After peeling and removing frills of sample around 77% of the sample used for freeze-drying. The results of this study indicated almost high moisture content of young Malaysian ginger rhizome compared to ginger sourced from Nigeria (90.9% vs. 76.9%) (Odebunmi *et al*., 2009). Moreover, this sample contained 3.8 g crude fiber in 100 g of sample, which is more than the Nigerian ginger and the value announced by the USDA Nutrient Database (31% and 90% more, respectively). Instead, it had lower carbohydrate content (6.3 g/100 g sample) than the USDA database which led to contain less energy (2.8 times less carbohydrate and almost half energy). Overall, Malaysian ginger had 0.7 g ash, 1.4 g fat, 0.7 g crude protein and total energy of 48 per 100 g of sample. To date, no previous study reported the proximate analysis of freeze-dried Malaysian young ginger, so current work tried to reveal more insight of nutritional composition.

Active components

The active components of ginger rhizome were determined by HPLC analysis. Standard sample was determined capsaicin standard following recommendation from US Pharmacopeial Convention (USP), because all the gingerols and shogaols have the same response as capsaicin. In this assay, basically each component was identified, and then quantified off of capsaicin. Figure 1 demonstrates the amount of each component, subsequent Figure shows the columns of HPLC in standard, standard sample (capsaicin) and ginger rhizome. These results suggest that following freeze-drying process, the amounts of gingerols were still higher than shogaols. It was, however, found that the concentrations of gingerols in the dry ginger were lessened slightly in comparison to the fresh one; degradation rates of gingerols to shogaols seems are mainly dependant to pH changes and thermal effects (Bhattarai *et al*., 2001; Wohlmuth *et al*., 2005). Apparently, the current study did not intend to compare the gingerols and shogaols contents before and after freeze-drying, but the results demonstrated that following freeze-drying the level of gingerols were still notable in the crude sample. This result may confirm the finding of a previous study by Zhang *et al.* (1994) that demonstrated that

Table 2. Total phenolic and flavonoid contents of the methanolic extract of ginger rhizome

	Rhizome extract
Total Flavonoids [a]	3.66±0.45
Total phenolics [b]	10.22±0.87

All analyses are the mean of triplicate measurements ± standard deviation; [a]: Expressed as mg quercetin/g of dry plant material; [b]: Expressed as mg gallic acid/g of dry plant material.

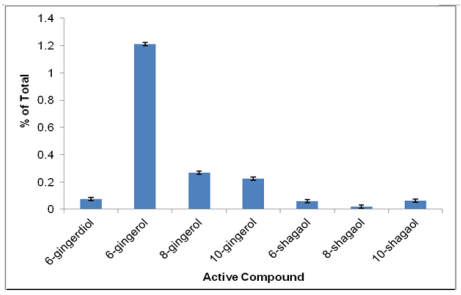

Figure 1. Percentage of active components for gingerols and shogaols

The test was done triplicate, and total amount of gingerols and shogaols was 1.92±0.0019%.

cooking and processing of fresh ginger decreased gingerol levels, but freeze-drying had no effects on them. Moreover, determination of ginger components seems to be more preferable using HPLC method than gas chromatography–mass spectrometry (GC/MS) methods, since the high temperatures associated with GC/MS analysis caused conversion of gingerols to shogaols (Chen *et al*., 1986), hence leads to over/underestimation of the components. The current study also reported that 6-gingerol was the most abundant among recognized components which re-emphasize the role of this component for probable health benefits of ginger. This result is consistent with the findings of Sanwal *et al.* (2010) and Schwertner and Rios (2007).

Total favonoids content

The total flavonoids content of ginger rhizome and total phenolic content were tested using aluminium chloride calorimetric assay and Folin–Ciocalteau reagent, respectively. The results were presented in table 2, and suggested reducing the total activity of ginger rhizome. Hence, the presence of flavonoid contents in this common herb shows ginger pharmacological capacity, which can be attributed to its health benefits.

DPPH radical scavenging activity

Methanolic extract of the rhizome of *Zingiber officinale* was used to determine DPPH radical scavenging activity and the result was compared with standardized buthylhydroxy toluene (BHT) and α-tocopherol. Extract with the highest IC_{50} value display the lowest scavenging activity. Figure 3 and Table 3 present the results for ginger rhizome

Table 3. DPPH scavenging activities of the methanolic extract of ginger rhizome at concentration of 50 µg/ml

		% Inhibition activity	IC_{50} Values
Young ginger rhizome		51.4±0.4	46.5
Controls	BHT	96.2±1.9	22
	α-tocopherol	89.6±1.7	15.5

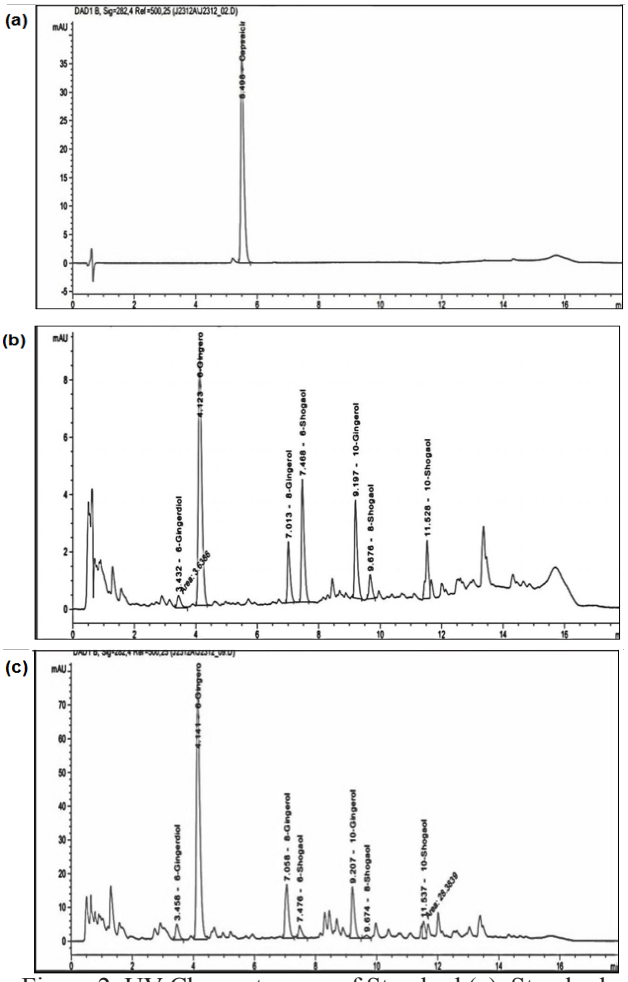

Figure 2. UV Chromatogram of Standard (a), Standard sample (b) and Ginger Rhizome (c)

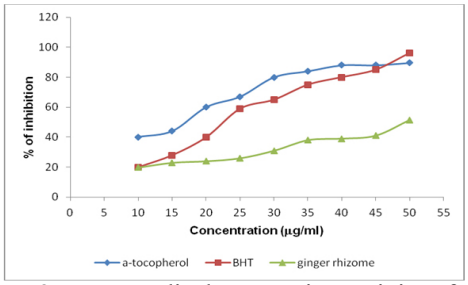

Figure 3. DPPH radical scavenging activity of the methanolic extracts of ginger rhizome compared to BHT and α-tocopherol.

compared with the controls. DPPH radical scavenging capacity was reported based on by the IC_{50} value that demonstrated the inhibitory concentration of ginger rhizome needed to scavenge 50% of 2,2-diphenyl-1-picryl hydrazyl radical (DPPH radicals). DPPH analysis showed the ability of ginger rhizome to act as donors of hydrogen atoms (Stoilova *et al.*, 2007). The results obtained in this study showed that the DPPH inhibiting from the Malaysian young ginger rhizome was 51.4%, which is almost equal to the sample cultivated in India (Singh and Gupta, 2013), but the sample from Vietnam that was tested by Stoilva *et al.* (2007) had the higher inhibitory DPPH effects by around 90%.

Conclusion

The results of this study indicated the analyzed ginger rhizome is a good source of dietary fiber, potential compounds and antioxidant properties. Additional studies are recommended to evaluate the potential benefits of this plant in animal and human trials.

Acknowledgements

The authors are thankful to the staff of Nutrition and Dietetics laboratory, Faculty of Medicine and Health Sciences for their kind helps and supports.

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