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Article

Antioxidant Activity of Different Parts of Ginger

Ya Li, Tong Zhou, Dong-Ping Xu, Pei Zhang, Hua-Bin Li, Jiao-Jiao Zhang *

Guangdong Provincial Key Laboratory of Food, Nutrition and Health, Department of Nutrition, School of Public Health, Sun Yat-Sen University, Guangzhou 510080, China

* Author to whom correspondence should be addressed; E-Mail: zhangjj46@mail2.sysu.edu.cn; Tel.: +86-20-87332391; Fax: +86-20-87330446.

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Abstract: Ginger (*Zingiber officinale* Roscoe) is a spice and widely consumed. It contains numerous bioactive components including phenolic and flavonoid substances. Besides, it exhibits a variety of health promoting effects. In the present study, the antioxidant activity and total phenolic and flavonoid contents of different parts (leaves, stem, young rhizome, and old rhizome) of ginger were assessed. The antioxidant activity of the old rhizome as determined by the FRAP and TEAC assays, as well as the total phenolic content were higher than those of the young rhizome, leaves and stems. Furthermore, a high positive correlation was found between the total phenolic content and antioxidant activity (R^2 = 0.994 and 0.993 for the results from the TEAC and FRAP assays, respectively), indicating the total phenolic content might be the main contributor to the antioxidant activity of four parts of ginger. On the contrary, there was a weak correlation between the total flavonoid content and antioxidant activity (R^2 = 0.202 and 0.207 for the results from the TEAC and FRAP assays, respectively), which indicated that the flavonoid components could not be the main contributor to antioxidant capacity of ginger. The results showed that the rhizome (either old or young) could be good natural resources of antioxidants.

Keywords: ginger; different parts; antioxidant activity; total phenolics; total flavonoids

1. Introduction

Free radicals can lead to various damages, including DNA mutation, alteration of gene expression, modification of cell signal transduction, cell apoptosis, lipid peroxidation and protein degradation (Guo et al., 2012). Free radicals could be neutralized by antioxidants. Thus, natural substances possessing antioxidant power have attracted increasing attention.

Ginger (*Zingiber officinale* Roscoe), depository of numerous bioactive components, is a beneficial and natural food (Saxena et al., 2016). Ginger could be used alone or in compounds as a spice or remedy in ancient recipes of Iranian traditional medicines (Khodaie and Sadeghpoor, 2015). Additionally, ginger might be effective in preventing and treating dysmenorrhea, nausea and vomiting, and cancer (Chen et al., 2016; Marx et al., 2015). Therefore, the aim of this study was to systematically evaluate the antioxidant capacities, total phenolic content and total flavonoid content of old rhizome, young rhizome, stem and leaves of ginger to supply new information for researchers, nutritionist and the general public.

2. Materials and Methods

2.1. Chemicals and Plant Material

Folin–Ciocalteu's phenol reagent (2 M), 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ), 2,2'-azinobis (3-ethylbenothiazoline-6-sulfonic acid) diammonium salt (ABTS), gallic acid, and quercetin were purchased from Sigma–Aldrich (St. Louis, MO, USA). Acetic acid, sodium acetate, iron(III) chloride hexahydrate, iron(II) sulfate heptahydrate, potassium persulphate, potassium acetate, aluminum chloride, and sodium carbonate were bought from Tianjin Chemical Factory (Tianjin, China). Ethanol, methanol and hydrochloric acid were obtained from Kelong Chemical Factory (Chengdu, China). All chemicals used in the experiments were of analytical grade, and deionized water was used throughout the experiment.

The ginger was harvested with different parts (leaves, stem, young rhizome, and old rhizome), and each part was washed with deionized water to remove the dirt on the surface, and were given an airing at room temperature. The separated parts were all kept at -23 ºC for future analysis.

2.2. Sample Preparation

The four parts of ginger were ground into fine particles/slurry with a special grinder for herbal medicines. An accurate amount of 1.00 g of the particles/slurry was extracted with 10 mL of 3 kinds of solvents (80% methanol, 50% ethanol, and hot water), respectively. The methanol and ethanol extraction were kept at room temperature for 90 min, and hot water extraction was maintained at 98 ºC for 90 min. The sample was centrifuged at 3,500 rpm for 30 min, and the supernatant was collected (Fu

et al., 2010). Another accurate amount of 1.00 g of each sample was dried to constant weights in a drying oven to determine the dry weight.

2.3. Determination of Antioxidant Activity

2.3.1. Ferric-reducing antioxidant power (FRAP) assay

The procedure of FRAP assay was carried out according to the literature with slight modifications (Benzie & Strain, 1996; Benzie & Szeto, 1999). Briefly, the FRAP reagent was prepared by sodium acetate buffer (300 mM, pH = 3.6), 10 mM TPTZ solution (using 40 mM HCl as solvent) and 20 mM iron(III) chloride solution in a volume ratio of 10:1:1, respectively. The FRAP reagent was prepared freshly daily and was warmed to 37 ºC in a water bath before use. A 100 μL of the diluted sample was mixed with 3 mL of the FRAP reagent. After 4 min, the absorbance of the reaction mixture was measured at 593 nm using a Shimadzu UV-2450 ultraviolet-visible spectrophotometer (Japan). The standard curve was drawn, and the results were expressed as μmol Fe(II)/g dry weight of each sample.

2.3.1. Trolox equivalent antioxidant capacity (TEAC) assay

The TEAC assay was carried out according to the method reported in the literature with minor modifications (Re et al., 1999). The $ABTS^{\bullet+}$ stock solution was prepared from 7 mM ABTS and 2.45 mM potassium persulfate in a volume ratio of 1:1, and then incubated in the dark for 16 h at room temperature and used within 2 days. The $ABTS^{\bullet+}$ working solution was prepared by diluting the stock solution with ethanol to reach an absorbance of 0.70 ± 0.05 at 734 nm. Before measurement, the samples were diluted to provide an approximate 20-80% inhibition of the blank absorbance. A 100 μL of the diluted sample was mixed with 3.8 mL $ABTS^{\bullet+}$ working solution. After 6 min of reaction at room temperature, the absorbance of the mixture was measured at 734 nm, and the percentage of inhibition of absorbance at 734 nm was calculated. Results were compared with the standard Trolox, and expressed as μmol Trolox/g dry weight of each sample.

2.4. Determination of Total Phenolic Content

Total phenolic content was determined according to the literature (Li et al., 2007; Singleton and Rossi, 1965). In brief, a 0.50 mL of the diluted sample was mixed with 2.5 mL of 1:10 diluted Folin–Ciocalteu reagent (0.2 M). After 4 min, 2 mL saturated sodium carbonate solution (about 75 g/L) was added. After reaction for 2 h at room temperature, the absorbance of the reaction mixture was measured at 760 nm. Gallic acid was used as standard, and the results were expressed as mg gallic acid

equivalent (mg GAE)/g dry weight of each sample.

2.5. Determination of Total Flavonoid Content

Total flavonoid content was measured according to the procedure reported in the literature (Kosalec et al., 2004). A 500 μL of the diluted sample was mixed with 1.5 mL of 95% ethanol (v/v), 0.1 mL of 10% aluminum chloride (w/v), and 0.1 mL of 1.0 M potassium acetate, and finally 2.5 mL of deionized water was added to the mixture. After 30 min of incubation in room temperature, the absorbance of the mixture was measured at 415 nm. Quercetin was used as a reference standard, and the results were expressed as mg of quercetin equivalent (QE)/g dry weight of each sample.

2.6. Statistical Analysis

All the experiments were carried out in triplicate, and the results were expressed as mean ± SD (standard deviation). Statistical analysis was performed using SPSS 20.0 and Excel 2016.

3. Results and Discussion

3.1. Antioxidant Activities of Different Part of Ginger

3.1.1. Reducing ability (FRAP assay)

The FRAP assay is a simple, inexpensive and commonly employed method to evaluate antioxidant activity (Li et al., 2008), and is based on the power of antioxidants to reduce ferric(III) ions to ferrous(II) ions (Benzie et al., 1996). The FRAP values of the different parts of ginger are given in Table 1.

Table 1. The FRAP values (μmol Fe(II)/g) of different parts of ginger

Part of ginger	FRAP values (μmol Fe(II)/g)		
	Methanol extract	Ethanol extract	Hot water extract
Leaves	21.89 ± 1.06	11.81 ± 2.48	12.31 ± 1.15
Stem	22.75 ± 1.17	16.65 ± 1.40	18.13 ± 0.23
Old rhizome	320.74 ± 11.45	312.19 ± 2.35	256.23 ± 1.83
Young rhizome	189.00 ± 1.25	132.77 ± 8.58	181.93 ± 0.54

As can be seen from Table 1, the FRAP values varied from 11.81 ± 2.48 to 320.74 ± 11.45 μmol Fe(II)/g dry weight. Among the 3 extracts of each part of ginger, the methanol extract of old rhizome showed the highest antioxidant activity with a FRAP value of 320.74 ± 11.45 μmol Fe(II)/g, while the ethanol extract of leaves had the lowest FRAP value of 11.81 ± 2.48 μmol Fe(II)/g . It could

also be seen that for the 4 parts of ginger, the methanol extract all showed higher antioxidant activity than the other two extracts. Meanwhile, for each kind of extract, the old rhizome showed the strongest antioxidant activity, followed by young rhizome, and there was no obvious difference between the leaves and stem.

The antioxidant capacities of plant samples might be influenced by a lot of factors, such as the extraction solvent (type and concentration) and test systems, and one single method could not fully describe the antioxidant activity. Most natural antioxidants are multifunctional, therefore it is necessary to perform several antioxidant activity assays to take the different mechanisms of antioxidant action into consideration. The FRAP assay could represent the reducing ability of the sample, while scavenging free radicles is also an important mechanism of antioxidant action. Therefore, the TEAC assay was carried out to evaluate the free radical scavenging activity of different parts of ginger.

3.1.2. TEAC assay

The TEAC assay is based on the ability of antioxidants to scavenge the $ABTS^{\bullet+}$ radical, and can measure antioxidant capacities of both hydrophilic and lipophilic compounds in the same sample (Re et al., 1999). The TEAC values of different parts of ginger are displayed in Table 2.

Table 2. The TEAC values (μmol Trolox/g) of different parts of ginger

Part of ginger	TEAC values (μmol Trolox/g)		
	Methanol extract	Ethanol extract	Hot water extract
Leaves	12.95 ± 0.80	8.22 ± 0.75	11.82 ± 1.77
Stem	16.22 ± 0.23	13.91 ± 0.23	16.55 ± 0.23
Old rhizome	178.78 ± 7.57	177.67 ± 8.31	146.27 ± 7.17
Young rhizome	105.53 ± 4.82	72.51 ± 5.48	105.15 ± 5.04

As can be seen from Table 2, the TEAC values varied from 8.22 ± 0.75 to 178.78 ± 7.57 μmol Trolox/g. The highest TEAC value was obtained from the methanol extract of old rhizome (178.78 ± 7.57 μmol Trolox/g), and the lowest TEAC value was obtained from ethanol extract of leaves (8.22 ± 0.75 μmol Trolox/g), which were both consistent with the results of FRAP assay. It could also be seen that for four parts of ginger, the methanol extract showed higher antioxidant activity than the other two extracts, except for the stem, of which the hot water extract showed the highest TEAC value. Meanwhile, for each kind of extract, the old rhizome showed the strongest antioxidant activity, followed by young rhizome, leaves and stem in order. The results are a bit inconsistent with the literature (Ghasemzadeh et al., 2010), where the leaves of ginger showed the highest antioxidant

activity. This might be caused by the different cultivar of the ginger material, and the difference of cultural environment, such as sunshine and rainfall.

3.2. Total Phenolic Content of Different Parts of Ginger

The total phenolic content of different parts of ginger were measured using the Folin–Ciocalteu method, which is based on the transfer of electrons from phenolic compounds to the Folin–Ciocalteu reagent in alkaline environment, and is a rapid, simple and reproducible method (Li et al., 2007; Singleton et al., 1965). The total phenolic contents of different parts of ginger are shown in Table 3.

Table 3. The total phenolic contents (mg GAE/g) of different parts of ginger

Part of ginger	Total phenolic contents (mg GAE/g)		
	Methanol extract	Ethanol extract	Hot water extract
Leaves	3.38 ± 0.44	2.41 ± 0.37	2.75 ± 0.38
Stem	2.60 ± 0.28	2.69 ± 0.24	3.08 ± 0.20
Old rhizome	15.28 ± 0.51	14.5 ± 0.68	12.83 ± 0.11
Young rhizome	9.84 ± 0.15	7.12 ± 0.35	11.33 ± 0.14

As can be seen from Table 3, the total phenolic contents varied from 2.41 ± 0.37 to 15.28 ± 0.51 mg GAE/g. The highest total phenolic content was obtained from the methanol extract of old rhizome (15.28 ± 0.51 mg GAE/g), and the lowest total phenolic content was obtained from ethanol extract of leaves (2.41 ± 0.37), which were both consistent with the results of FRAP and TEAC assays. For leaves and old rhizome of ginger, the methanol extract had the highest total phenolic content, while for the stem and young rhizome, the hot water extract had the highest total phenolic content. Meanwhile, for each kind of extract, the old rhizome showed the highest total phenolic content, followed by young rhizome, while there is no obvious difference between the leaves and stem.

3.3. Total Flavonoids Content of Different Parts of Ginger

In the present study, quercetin was used as the standard to represent the flavonoids in ginger. The total flavonoid contents of different parts of ginger are displayed in table 4.

As shown in Table 4, the total flavonoid content of different parts of ginger varied from 0.50 ± 0.09 to 1.95 ± 0.18 mg QE/g. The highest total flavonoid content was obtained from methanol extract of stem (1.95 ± 0.18 mg QE/g), and the lowest total flavonoid content was obtained from hot water extract of leaves. For all four parts, the methanol extract of each part had the higher total flavonoid content than the other two extracts. For methanol extract, the stem showed the highest total flavonoid content, while for the ethanol and hot water extract, old rhizome showed the highest value.

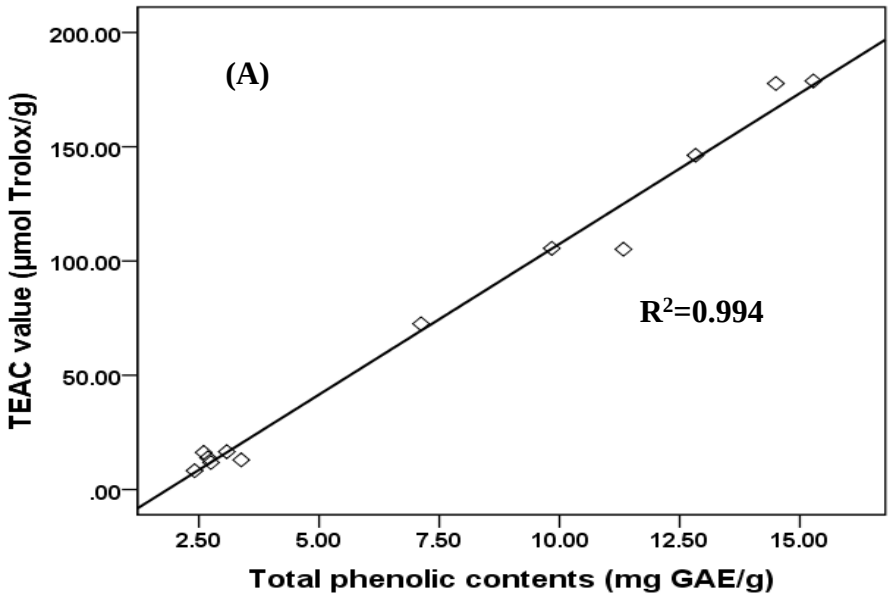
Table 4. The total flavonoid contents (mg QE/g) of different parts of ginger

Part of ginger	Total flavonoids contents (mg QE/g)		
	Methanol extract	Ethanol extract	Hot water extract
Leaves	1.79 ± 0.24	0.82 ± 0.12	0.50 ± 0.09
Stem	1.95 ± 0.18	1.14 ± 0.11	0.80 ± 0.03
Old rhizome	1.80 ± 0.07	1.32 ± 0.13	1.16 ± 0.09
Young rhizome	1.01 ± 0.01	0.67 ± 0.05	0.88 ± 0.08

3.4. Correlation between Antioxidant Activity and Total Phenolic Content

The correlation between total antioxidant capacities and the total phenolic content of the different parts of ginger is shown in Fig. 1.

As can be seen from Fig.1, the results showed a positive strong linear correlation between the antioxidant activity and total phenolic content (R^2 = 0.994 and 0.993 for the results from the TEAC and FRAP assays, respectively). These results indicated that the phenolic compounds in the four parts might be the main contributor to their antioxidant activity.



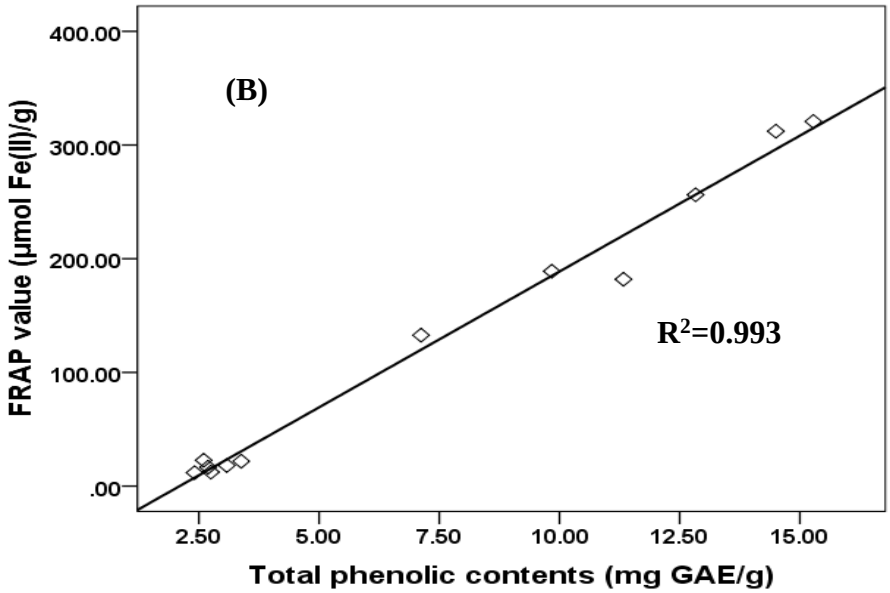
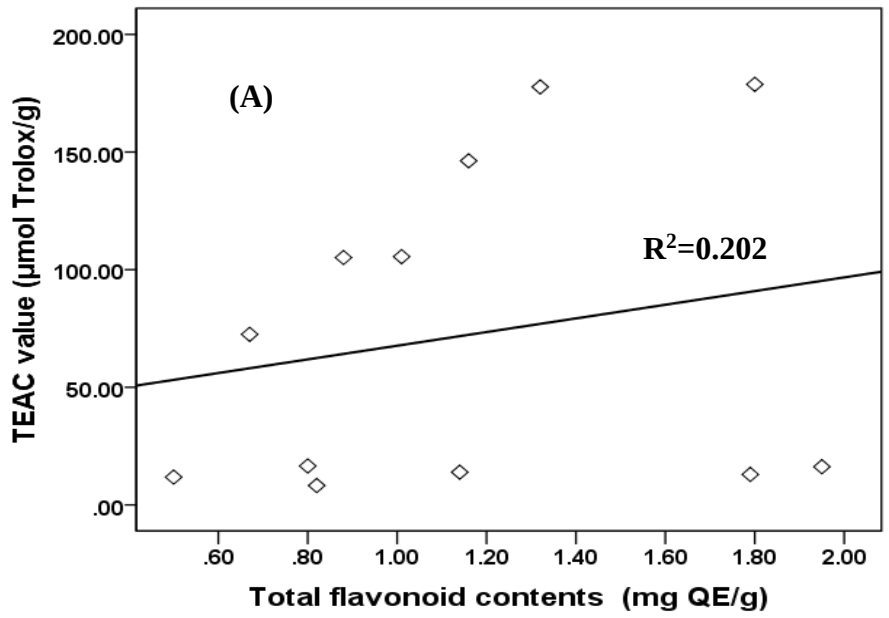


Figure 1. Correlation between the antioxidant capacities and total phenolic content. Antioxidant capacities were measured by the TEAC assay (A) and FRAP assay (B), respectively.

3.5. Correlation between Antioxidant Activity and Total Flavonoid Content

The correlation between total antioxidant capacities and the total flavonoid content of the different parts of ginger is shown in Fig. 2.

As shown in Fig. 2, the results showed a weak positive linear correlation between the antioxidant capacity and total flavonoid content (R^2 = 0.202 and 0.207 for the results from the TEAC and FRAP assays, respectively). These results indicated that the flavonoid compounds in the four parts could not be the main contributor to their antioxidant activity.



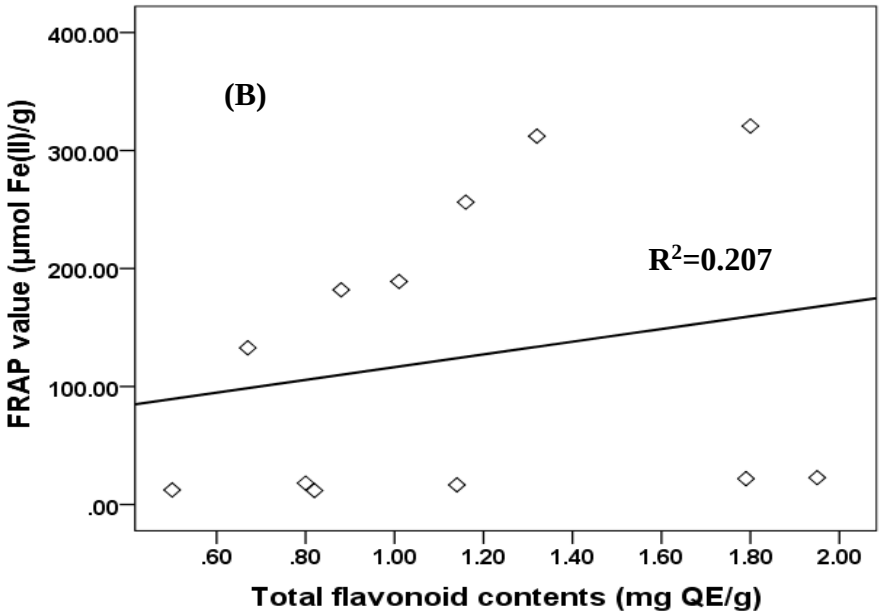


Figure 2. Correlation between the antioxidant capacities and total flavonoid content. Antioxidant capacities were measured by the TEAC assay (A) and FRAP assay (B), respectively.

4. Conclusions

Ginger is a well-known and widely used spice, which contains several interesting bioactive constituents and possesses health promoting properties. In the present study, the antioxidant activity and total phenolic and flavonoid contents of different parts of ginger were determined by various assays. The parts included the leaves, stems, old rhizome and young rhizome. The antioxidant activity of the old rhizome as determined by the FRAP and TEAC assays, as well as the total phenolic content, were higher than those of the young rhizome, leaves and stems. As for the total flavonoid content, there was no obvious difference between the four parts. Furthermore, a high positive correlation was found between the total phenolic content and antioxidant activity (R^2 = 0.994 and 0.993 for the results from the TEAC and FRAP assays, respectively), indicating the phenolic compounds might be the main contributor to the antioxidant activity of the four parts of ginger. In addition, there was a weak correlation between the total flavonoid content and antioxidant activity (R^2 = 0.202 and 0.207 for the results from the TEAC and FRAP assays, respectively), which indicated that the flavonoid components could not be the main contributor to antioxidant capacity of ginger. The results showed that the rhizome (either old or young) could be good natural resources of antioxidants. Further studies are needed for the isolation and identification of antioxidant components in ginger.

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