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Full Length Research Paper

Chemical composition and antioxidant properties of ginger root (*Zingiber officinale*)

Shirin Adel P. R.* and Jamuna Prakash

Department of Food Science and Nutrition, University of Mysore, Manasagangotri, Mysore 570 006, India.

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The chemical composition and antioxidant activity (in aqueous and solvent extracts) of Ginger root *(Zingiber officinale)* were determined. The antioxidant components analysed were polyphenols, vitamin C, β carotene, flavonoids and tannins. Antioxidant assays such as free radical scavenging activity, reducing power and total antioxidant activity were carried out for ethanol, methanol, acetone, 80% methanol and 80% ethanolic extracts. Protein and fat of sample were 5.08 and 3.72 g/100 g respectively. Ash, minerals namely iron, calcium, phosphorous, zinc, copper, chromium and manganese) and vitamin C were 3.85 (g), 8.0 (mg), 88.4 (mg), 174 (mg), 0.92 (mg), 0.545 (mg), 70 (µg), 9.13 (mg) and 9.33 (mg) per 100 g of sample, respectively. Antioxidant components (polyphenols, flavonoids and total tannin) were higher in hot water (100°C) extract than other solvent extracts and 30°C water extract. Antioxidant activity by 3 different methods showed higher activity in solvent extract than water extract. Order of antioxidant activity by reducing power and free radical scavenging activity by DPPH was as follows, 80% methanolic > 80%ethanolic > methanolic > ethanolic > 30°C water >100°C water > acetonic extract.

Key words: Flavonoids, medicinal plant, polyphenol, spice, tannin.

INTRODUCTION

Until synthetic drugs were developed in nineteenth century, herbs were the basis for nearly all medicinal therapy. Today, herbs are still found in 40% of prescriptions, and the interest for use of herbal remedies instead of chemical drugs is increasing because of lesser side effects (Craig, 1999). A remarkable increase in the use of medicinal plant products has been observed in the past decade. Due to their properties, medicinal plants are used as primary health care aid among 80% of the world's population in the form of plant extracts or their active components (Word Health Organisation, 2008).

In the last decade, studies have also focused on new group of bioactive components in some foods which have protective effects against cell oxidation. These food groups have been classified as functional foods. The value of functional foods has been recognised for their health benefits (Klein et al., 2000). Heart diseases continue to be a major cause of death; cancer, osteoporosis and arthritis remain highly prevalent. Though, genetics play a major role in the progress of the diseases mentioned, by and large most are considered preventable or could be minimized by activity, a proper diet, physical activity, weight management and a healthy lifestyle. Functional foods can prevent or delay the onset of chronic diseases and also provide basic nutritional requirements (Medoua et al., 2009).

Ginger has been used as a spice and as natural additives for more than 2000 years (Bartley and Jacobs, 2000). Also, ginger has many medicinal properties. Studies have shown that, the long term dietary intake of ginger has hypoglycaemic and hypolipidaemic effect (Ahmed and Sharma, 1997). Ginger has been identified as an herbal medicinal product with pharmacological effect. Ginger suppresses prostaglandin synthesis through inhibition of cyclooxygenase- 1 and cyclooxygenase- 2. In traditional Chinese and Indian medicine, ginger has been used to treat a wide range of ailments including stomach aches, diarrhea, nausea, asthma, respiratory disorders (Grzanna et al., 2005). As

*Corresponding author. E-mail: Shirin59s@yahoo.com. Tel: 00919739692912.

ginger is widely used both as a spice and for its medicinal properties, the present study was undertaken to determine the nutritional composition of dry ginger as well as its antioxidant activity and components. An attempt was also made to investigate association between the antioxidant activities and components of dry ginger extracted in different solvents.

MATERIALS AND METHODS

Plant material

Fresh ginger root *(Zingiber officinale)* were procured from local market, washed with distilled water and dried in oven at 40°C, then ground and stored in air tight container under refrigeration.

Chemical reagents and solvents

The chemicals used for the study were procured from Qualigen Company Mumbai, India, Himedia Company, Mumbai, India and Sigma Company, USA. They were all of analytical grade. Double distilled water, methanol, ethanol, acetone, 80% methanol and 80% ethanol were used for extraction.

Extraction of the sample

One gram of sample was weighed accurately and suspended in 100 ml of solvent. It was shaken for 3 h in an electronic shaker at room temperature, unless otherwise specified, centrifuged at 4000 rpm for 20 min and filtered with Whatman No. 1 filter paper. For all experiments fresh extracts were used.

Sample preparation for determination of antioxidant activity

250 mg of sample were mixed with 25 ml of extracting solvent and extracted for 3 h, centrifuged at 4000 rpm for 20 min, and passed through filter paper (Whatman No.1) to get clear extract. Water extracts were taken at 30 and 100°C.

Proximate composition and trace element estimation

Nutritional composition -Moisture, total protein, fat, ash, calcium, phosphorus, iron, vitamin C (Horwitz and Latimer, 2005), dietary fibre (Asp et al., 1983) - of all the samples were estimated with standard techniques. The ash solutions were prepared with wet digestion (Ranganna, 1986) and analyzed for zinc, copper, chromium and manganese using atomic absorption spectrophotometer (AAS) (GBC Scientific equipment, Australia). Instrument parameters such as resonant wavelength, slit width and air-acetylene flow rate that are appropriate for each element were selected (AOAC, 2000). The instrument was set up and calibrated as per the guidelines in the manual provided by the manufacturer. A calibration curve (Concentration Vs absorbance) for each mineral to be determined was prepared using a range of working standards. The flame parameters were optimized in accordance with the instrument manufacturer's instructions.

The standard solutions were read before estimation of each mineral. The burner was flushed with water between samples and zero was re-established each time. Suitable dilutions of the ash solutions were made to read the contents of the minerals in the ash solution. The concentration of metals in the ash solutions of samples as well as in the blank solutions were read from the calibration curve and the concentration in the test samples was calculated taking into account the dilutions and the weight of the samples taken.

Antioxidant components estimation

Total phenolic compound analysis

Total polyphenol content was estimated using Folin-Ciocalteu (FC) assay which is widely used in routine analysis (Wright et al., 2000; Atoui et al., 2005). A known amount of extract (10 mg/ml) was mixed with 1.0 ml of FC reagent and 0.8 ml of 2% Na2Co3 was added and the volume was made up to 10 ml using water- methanol (4:6) as diluting fluid. Absorbance was read at 740 nm after 30 min using spectrophotometer. Tannic acid (0 - 800 mg/L) was used to produce standard calibration curve. The total phenolic content was expressed in mg of Tannic acid equivalents (TAE) /100 g of sample (Matthaus, 2002).

Determination of total flavonoids

The total flavonoid content was determined using the Dowd method as adapted by (Arvouet-Grand et al., 1994). A 5.0 ml of 2% aluminium trichloride ($AlCl_3$) in methanol was mixed with the same volume of the extract solution (10 mg/ml). Absorption readings at 415 nm using Perkin Elmer UV-VIS spectrophotometer were taken after 10 min against a blank sample consisting of extract solution with 5.0 ml methanol without $AlCl_3$. The total flavonoid content was determined using a standard curve with quercetin. Total flavonoid content is expressed as g of quercetin equivalents / 100 g of sample.

Total tannin estimation

Colorimetric estimation of tannins is based on the measurement of blue colour formed by the reduction of phosphotungstomolybdic acid by tannin like compounds in alkaline solution (Ranganna, 1986). A known amount of extract was mixed with 5.0 ml of Folin- Denis reagent (FD) and Na_2Co_3 solution and made up to 100 ml, mixed well and absorbance was read at 760 nm after 30 min using spectrophotometer. Total tannin content as expressed as mg tannic acid equivalent /100 g of sample.

Determination of antioxidant activity of different extract

Radical scavenging activity by DPPH (2, 2-diphenyl-1-picrylhydrazyl)

Effect of different extracts on DPPH free radical was measured according to Lee (Lee et al., 1996). Positive control (standard) was prepared by mixing 4.0 ml of ascorbic acid (0.05 mg/ml) and 1.0 ml of DPPH (0.4 mg/ml) for water extract, and negative control (blank) was prepared by mixing extract base (water/methanol/ethanol/acetone) with 1.0 ml of DPPH. Four different concentrations of extract was mixed with 4.0 ml of DPPH, the volume made up to known volume, mixed well and left to stand at room temperature in a dark place for 30 min. Absorbance was read using a spectrophotometer at 520 nm. The ability of extract to scavenge DPPH was calculated using the following equation:

$$\text{Radical scavenging activity \%} = \frac{\text{Control OD} - \text{Sample OD}}{\text{Control OD}}$$

Reducing power

A spectrophotometric method was used for the measurement of reducing power. Different concentrations of extracts were mixed with 2.5 ml phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of 1% potassium ferricyanide (10 mg ml^{-1}). The mixture was incubated at 50°C for 20 min, then rapidly cooled, mixed with 2.5 ml of 10% trichloroactic acid and centrifuged at 6500 rpm for 10 min. The supernatant (2.5 ml) was mixed with distilled water (2.5 ml) and then ferric chloride (0.5 ml, 0.1%) was added and allowed to stand for 10 min. The absorbance was read spectrophotometrically at 700 nm (Oyaizu, 1986).

Table 1. Nutritional composition of ginger (per 100g).

Constituent	Value	Constituent	Value
Moisture	15.02 ± 0.04	Ash (g)	3.85 ± 0.61 (4.53)
Protein (g)	5.087 ± 0.09(5.98)	Calcium (mg)	88.4 ± 0.97 (104.02)
Fat (g)	3.72 ± 0.03 (4.37)	Phosphorous (mg)	174±1.2 (204.75)
Insoluble fibre (%)	23.5 ± 0.06 (27.65)	Iron (mg)	8.0 ± 0.2 (9.41)
Soluble fibre (%)	25.5 ± 0.04 (30.0)	Zinc (mg)	0.92 ± 0 (1.08)
Carbohydrate (g)	38.35 ± 0.1	Copper (mg)	0.545 ± 0.002 (0.641)
Vitamin C (mg)	9.33 ± 0.08 (10.97)	Manganese (mg)	9.13 ± 001 (10.74)
Total carotenoids (mg)	79 ± 0.2 (9296)	Chromium (µg)	70 ± 0 (83.37)

All value in this table represent the mean ± SD (n = 4). Figures in the parenthesis represent the dry weight values.

Total antioxidant activity by phosphomolybdenum method

The extract (0.1 ml) (10 mg/ml) was mixed with reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate in 100 ml). The tubes were capped and incubated in boiling water bath at 95°C for 90 min, then cooled to room temperature and absorbance was read at 695 nm with spectrophotometer against blank. Water soluble antioxidant capacity expressed as equivalent of ascorbic acid (µmol/g of sample) (Prieto et al., 1999).

Statistical analysis

Data were expressed as mean ± SD. (n = 4) in all the experiments. To determine the extent of association between antioxidant activity and antioxidant components in different extracts, data were subjected to correlation coefficient in Excel 2007.

RESULTS AND DISCUSSION

Nutritional composition

The nutritional composition of dry ginger was determined with standard techniques and results are shown in (Table 1). Protein and fat content was found to be 5.98 and 4.37 g /100 g DW. The reported values for composition of ginger by various authors are in the following range; for protein, 7.2 to 8.7, fat, 5.5 to 7.3 and ash, 2.5 to 5.7 g/100 g DW (Nwinuka et al., 2005; Hussain et al., 2009; Odebunmi et al., 2010). In our study, ash, iron, calcium and phosphorous contents were 4.53 g, 9.41 mg, 104.02 mg, 204.75 mg/100 g DW, respectively. Ash content was in the range of reported values and calcium content, that, 104.02 mg/100 g DW was very close to the value reported for Indian foods (Gopalan et al., 2004).

Trace minerals namely zinc, copper, manganese and total chromium were estimated with atomic absorption spectrophotometer and found to be 1.08 mg, 0.641 mg, 10.74 mg and total chromium was 83.37 µg/100g DW, respectively. Vitamin C and total carotenoids content were found to be 10.97 and 92.96 mg/100 g, respectively. Soluble and insoluble fibre of sample was determined and as shown in Table 1, soluble fibre was slightly higher than insoluble fibre. Antioxidant components were estimated in seven extracting media and results are shown in Table 2. Total polyphenols, tannin and flavonoids were found to be more in 100°C water extract than other extracts. It can be because of more solubility of these components in hot water than other solvents.

Total polyphenols

Total polyphenols were highest in aqueous extract with almost similar amounts at different temperatures (840 and 830 mg/g). Least polyphenols were seen in acetonic extract. Antioxidant activities of plant extracts were usually linked to their phenolic content. Hydrogen donating characteristics of the phenolic compounds is responsible for the inhibition of free radical induced lipid ability to scavenge free radicals and give oxygen species such as singlet oxygen, superoxide free radicals and hydroxyl radicals (Hall, 1997), though, it is well accepted that non phenolic antioxidants might also contribute to the antioxidant activity of plant extract (Hassimotto et al., 2005; Harish and Shivanandappa, 2006). In a study, researchers estimated total polyphenol content of 35 different herbs and medicinal plants in 80% methanolic extract. The polyphenol content was between 0.8 to 42.1 mg of gallic acid equivalent /g dry weight (DW) (Kahkonen et al., 1999). Hinneburg et al. (2006) found the total phenolic content of aqueous ginger extract to be 23.5 mg gallic acid/g of sample. Another researcher (Rababah et al., 2004) estimated the total phenolic content of 60% ethanolic extract of ginger to be 39.9 mg of chlorogenic acid equivalent/g DW. In our study, when we calculated total polyphenols content in 80% methanolic extract/g of DW ginger, it showed 780 mg of TAE/ 100 g of sample.

Flavonoids

Flavonoids were estimated in all the extracts and data is shown in Table 2. Highest flavonoid content was reported in 30 and 100°C aqueous extract at 1.37 and 2.98 g/100 g of sample, respectively. Flavonoid content of 80% methanolic extract and 80% ethanolic extracts were

Table 2. Antioxidant components and total antioxidant activity of ginger in different solvent extracts.

Antioxidant components	Water		Methanol	Ethanol	Methanol (80%)	Ethanol (80%)	Acetone
	(0.060)	(0.010)					
Total polyphenols mg/100 g	840 ± 2.1	838 ± 3.0	510 ± 2.2	565 ± 4.1	780 ± 5	800 ± 4.3	325 ± 1.9
Tannin g/100 g	1.51 ± 0.05	1.34 ± 0.08	1.12 ± 0.05	0.98 ± 0.03	1.28 ± 0.01	1.15 ± 0.1	0.67 ± 0.08
Flavonoids g/100 g	2.98 ± 0.06	1.371 ± 0.01	0.685 ± 0.005	0.278 ± 0.003	0.404 ± 0.002	0.352 ± 0.002	0.249 ± 0.002
Total antioxidant activity µmol/ g of sample	73529.4 ± 121	79400 ± 88	98822.5 ± 74	91176.25 ± 66	85294 ± 47	80000 ± 38	32056 ± 27

All values in this table represent the mean ± SD (n = 4).

Table 3. Correlation between antioxidant activity and antioxidant component of sample in different solvent extracts.

Correlation (R^2 values)	Water extract			Solvent extract		
Antioxidant components	Method of assay			Method of assay		
	DPPH	Reducing power	Total antioxidant	DPPH	Reducing power	Total antioxidant
Flavonoids	-1	-1	-1	0.493	0.505	0.613
Polyphenols	1	1	1	0.901	0.847	0.579
Total tannin	-1	-1	-1	0.985	0.887	0.885

For solvent extract in DPPH method, 80% methanolic extract was not included for statistical analysis since the concentration did not match with other extracts.

found to be more than methanolic and ethanolic extracts,respectively, but lesser than aqueous extract. It can be due to higher solubility of ginger flavonoids in water than other solvents.

Tannin estimation

Tannin was also estimated in all seven different extracts (Table 2). As was observed for the other antioxidant components, 30 and 100°C aqueous extract had the highest tannin (1.34 and 1.51 g/100 g of sample, respectively) and acetonic extract showed the least content (0.67 g/100 g). As shown in Tables 2 and 3, tannin content did not show any correlation with antioxidant activity in aqueous extract, but high correlation was seen with solvent extract.

Estimation of antioxidant activity

Antioxidant activity of dry ginger was estimated with three different methods.

Total antioxidant activity

Total antioxidant activity (Table 2) was highest in methanolic extract at 98822 µmol/g followed by ethanolic extract at 91176 µmol/g. Least total antioxidant activity was found in acetonic extract.

Free radical scavenging activity

DPPH is a stable free radical in methanol or aqueous solution and accepts an electron or hydrogen radical to turn into stable diamagnetic molecule. It is usually used as a substrate to evaluate the antioxidative activity of antioxidants (Duh et al., 1999), thus we have estimated the antioxidant activity through free radical scavenging of ginger. Free radical scavenging potency of sample is shown in Figure 1 which showed the highest DPPH radical scavenging activity in 80% methanolic extract followed by 80% ethanolic extract. In a study, by Chen et al. (2008), DPPH radical scavenging activity of methanolic extract was found to be in a range of

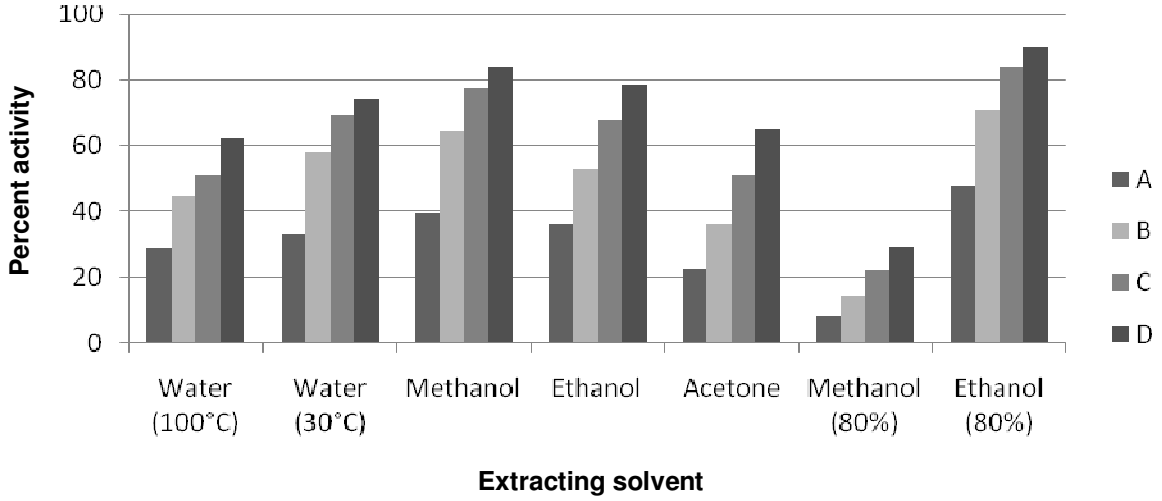

Figure 1. Free Radical scavenging activity of ginger in different solvent extracts. Concentration of sample (in mg): In 80% methanolic extract, A: 0.4, B: 0.6, C: 0.8 and D: 1.0. For all other extracts, A: 2.5, B: 5.0, C: 7.5, D: 10.

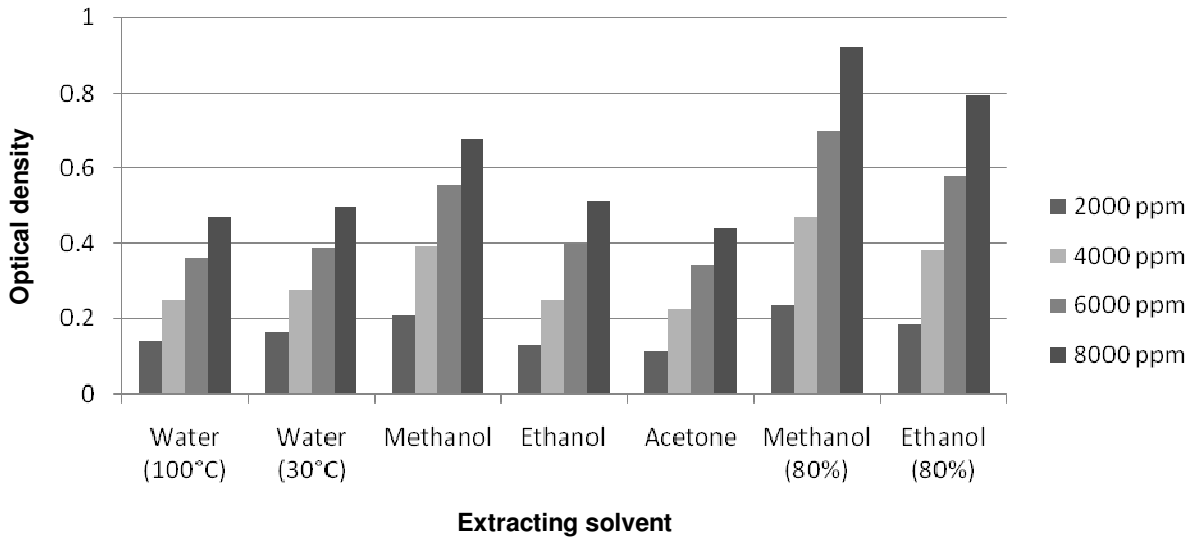

Figure 2. Reducing power of ginger in different solvent extracts.

32 to 90.1% in 100 mg of 18 different ginger species. In the present study, DPPH free radical scavenging activity of methanolic extract was 39.6, 64.7, 77.6 and 84.4% in 0.25, 0.5, 0.75 and 1.0 mg of sample, respectively, which is higher than the reported values.

Reducing power

It has been reported that the reducing power of bioactive compounds is associated with antioxidant activity (Yen et al., 1993; Siddhuraju et al., 2002). Hence, it is essential to determine the reducing power of phenolic constituents to explain the relationship between their antioxidant effect and their reducing power. The reducing power of different solvent extracts of ginger was estimated. Highest reducing power was also in 80% methanolic extract followed by 80% ethanolic extract (Figure 2). As reported by Chen et al. (2008), the reducing power of methanolic extract of 18 different species of ginger ranged from 0.34 to 1.6 nm in 100 mg of sample. In our study, methanolic extract of sample showed much higher activity of 0.208, 0.393, 0.558, 0.681 nm for 2.0, 4.0, 6.0 and 8.0 mg of sample.

Antioxidant components and activity are highly dependent on extracting solvent and concentration of solvent (Turkmen et al., 2006), but they also vary within the samples. Many researchers have reported the

relationship between phenolic content and antioxidant activity. In some studies, they found a correlation between the phenolic content and antioxidant activity (Velioglu et al., 1998), whereas others found no relationship (Kahkonen et al., 1999). As it is shown in Table 3, in this study we also found high correlation between polyphenol content and antioxidant activity in both water extract (R^2 = 1) and solvent extract (DPPH, R^2 = 0.901, reducing power, R^2 = 0.847 and total antioxidant activity R^2 = 0.579). Total tannin and flavonoids did not show any correlation with antioxidant activity in aqueous extract. In solvent extract, total tannin showed high correlation with reducing activity (R^2 = 0.887), total antioxidant activity (R^2 = 0.885) and free radical scavenging activity (R^2 = 0.985). Flavonoids showed correlation with reducing power (R^2 = 0.505), total antioxidant (R^2 = 0.613) and DPPH (R^2 = 0.493). Since the antioxidant activity was higher in alcoholic extract than aqueous extract, it is advisable to use the extracting media capable of extracting the lipophilic antioxidant compounds from ginger.

Conclusion

It can be concluded that ginger is a good source of antioxidant and most of the antioxidant components exhibit higher activities in alcoholic media as determined by different assays. Hence, apart from its medicinal properties, ginger can also be used as an antioxidant supplement.

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