Antioxidant Enriched Fractions from *Zingiber Officinale* Roscoe

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Abstract: Ginger rhizome (*Zingiber officinale* Roscoe) has many diverse properties and medicinal values such as antioxidant potential combined with the properties of a spice. Dried ginger (DG) were extracted with aqueous ethanol and freeze-dried. The extract was evaluated for antioxidant potential, using 1,1'-diphenyl-2-picryl-hydrazyl radical scavenging, antioxidant capacity and reducing power assays. DG extract was further fractionated into methanol (Mfr) and water-soluble (Wfr) fractions. The Mfr exhibited higher antioxidant capacity when compared to DG extract. Higher antioxidant potential of the methanol fraction may be due to the presence higher polyphenols and [6]-gingerol content. This suggests that alcoholic soluble fraction possess enormous scope to enhance the antioxidant potential when used as a supplement in various food as well as pharmaceutical formulations / products.

Keywords: Ginger, Antioxidants enriched fraction, Polyphenols, 6-Gingerol.

Introduction

Species from Zingiberaceae family have been widely used as spices. Ginger (*Zingiber officinale* Rosc) is used in traditional oriental medicine for common cold, digestive disorders, and rheumatism^{1,2}. Fresh as well as dried forms of ginger have been used both in medicine and in culinary for flavour and pungency. India is the largest producer (380.0 thousand tonnes) and consumer of ginger and contributing 35% of the world production³.

Antioxidants from natural resources are associated with health benefits against heart diseases, malaria, neuro-degenerative diseases, AIDS, cancer and longevity⁴. Solvent extraction is the commonly used method for the extraction of bioactive components from plant sources. The selection of solvent system for the extraction will depend on the purpose of extraction, nature of the compounds, safety concerns and so on⁵.

The objective of the present study was to separate antioxidant-enriched fraction from ginger and to evaluate antioxidant potential. Ginger extract was prepared from dried ginger and antioxidants were enriched using solvent partition. Extracts and fractions were evaluated for their antioxidant potential in different *in vitro* model systems.

Experimental

Gallic acid, DPPH (1, 1-diphenyl-2-picryl-hydrazyl) were procured from Sigma Chemicals Co., USA. All other chemicals and solvents used were of analytical grade available commercially. Absorbance was measured using a UV visible spectrophotometer (Cintra 10, GBC, Australia).

Plant collection

Fresh ginger (*Zingiber officinale* Rosc) rhizomes were procured from local market, Mysore, India. A Voucher specimen (CFTRI-PPSFT-Ginger 1) was deposited in Herbarium of our department. The rhizomes were soaked in potassium metabisulphite solution, washed with water and sliced. The slices were subjected to dehydration using cross flow drier at 50 °C for 6 h. and powdered.

Preparation of extract from ginger

Ginger powder (4 kg) was loaded into stainless steel column and extracted with aqueous ethanol (50%). The material to solvent ratio was kept as 1:6 to 1:9. The combined extracts were concentrated in the solvent distillation unit. Alcohol-free extract was dried in a freeze dryer (Lyodryer, LT5B, Lyophilisation systems Inc. USA) at a temperature of 40 °C and at 0.312 millibar pressure. The crude extract (DG) was successively partitioned into methanol (Mfr) and water (Wfr) fractions. All the fractions were stored at -20 °C.

Antioxidant assays

The extracts and the fractions were evaluated for antioxidant potential through *in vitro* models such as radical scavenging activity (1,1'-diphenyl-2-picrylhydrazyl radical), reducing power (iron reducing activity) and antioxidant capacity (phosphomolybdenum complex). Also, the fractions were analysed for total phenol content and 6-gingerol.

Radical scavenging activity

The extract and the fractions were evaluated for radical scavenging ability using the stable radical DPPH• according to the method of Blois⁶. Different concentrations of the DG extract, fractions (Mfr and Wfr) and BHA solution (each 1 mL) were taken in different test tubes. Four milliliters of 0.1 mM methanolic solution of DPPH was added to these tubes, shaken vigorously and allowed to stand at 27 °C for 20 min. Absorbance of the samples was measured at 517 nm against the absorbance of the control. Radical scavenging activity was calculated using the equation,

$$\text{Radical Scavenging activity (\%)} = (1 - A_{\text{sample}} / A_{\text{control}}) \times 100.$$

Reducing power

The reducing power of the extracts was evaluated⁷. Briefly, 1 mL of various concentrations (25–200 µg) of standard or test compound in distilled water was mixed with phosphate buffer (2.5 mL, 0.2 mol/L., pH 6.6) and potassium ferricyanide (2.5 mL, 1%). The mixture was incubated at 50 °C for 20 min. Trichloroacetic acid (10%, 2.5 mL) was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. The upper layer of the solution

(2.5 mL) was mixed with distilled water (2.5 mL) and FeCl_3 (0.5 mL, 0.1%) and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicates increased reduction capability.

Antioxidant capacity

The total antioxidant capacity of DG extract and fractions (Mfr and Wfr) along with the commercial standard antioxidant propyl gallate were evaluated⁸. An aliquot of 0.01-0.2 mL solutions (containing 25-200 μg of extract) was made up to 4 mL using reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were capped and incubated in a boiling water-bath at 95 °C for 90 min. The samples were cooled to room temperature and the absorbance was measured at 695 nm against blank. The antioxidant capacity of the extracts was expressed as equivalents of ascorbic acid ($\mu\text{mol/g}$ of extract).

Total phenols

The phenolic content was determined according to the Folin-Ciocalteu (FC) method⁹. The concentrations of total phenols in extracts were measured by a UV-Vis spectrophotometer (Cintra 10, GBC, Australia), according to a colorimetric oxidation/reduction reaction. To the extracts (1 $\mu\text{g}/\mu\text{L}$), FC reagent (500 μL) and 20% saturated sodium carbonate solution (1500 μL) were added to the tubes and the mixture was made up to 10 mL with distilled water. After 2 h of incubation at room temperature, the absorbance was measured at 765 nm using a spectrophotometer. The results are expressed as gallic acid equivalent (GAE).

High performance liquid chromatographic analysis

The ginger extract were analyzed on a HPLC system consisting of a Shimadzu system controller SCL-10A VP, Shimadzu diode Array Detector (SPD-M 10A VP) and a Shimadzu liquid chromatograph LC-10AT. Chromatographic analysis was performed on a Waters Spherisorb C-18 reversed phase column (ODS 2, 4.6 mm $\times$ 250 mm, 5 μm) with acetonitrile – water (55:45, v/v) as the mobile phase. The HPLC operating parameters were as follows, injection volume, 10 μL ; flow rate, 1.0 mL/min. The eluting compounds were detected at λ_{max} of 280 nm on PDA detector^{10,11}. DG extracts and Mfr (10 mg/mL) were dissolved in methanol and injected (10 μL). Duplicate injections were performed to ensure accuracy and reproducibility. [6]-gingerol was isolated from Mfr and the structure elucidated employing NMR spectral data (unpublished). A standard calibration curve of gingerol was constructed at different concentrations using HPLC. [6]-gingerol content of the samples was determined using this calibration curve.

Statistical analysis

Experimental results were carried out in triplicate and the results are shown as mean value $\pm$ standard deviation. The obtained data were submitted to statistical analysis and means were compared by the Duncan's New Multiple Range Test ($p \leq 0.05$) and presented.

Results and Discussion

Extraction of dried ginger using aqueous ethanol resulted in 15% yield, which is higher than the result (4.01%) reported¹² using distilled ethanol. It was further fractionated into methanol (Mfr) and water (Wfr). The yields and total phenolic contents (as percent gallic acid equivalents) of extract as well as the fractions are presented in the Table 1.

Table 1. Yield and polyphenol content of extract and fractions

Sample	Yield*, %	Total polyphenols, % GAE
DG	15.0 ± 2.1	8.09 ± 0.37 ^b
Mfr	8.0 ± 2.1	10.12 ± 0.43 ^c
Wfr	7.0 ± 1.9	4.57 ± 0.75 ^a

*Values are expressed as moisture free basis; Values are mean ± SD of triplicate analyses;

^{a,b,c}-Values not having similar superscripts in the same column are significantly different ($p \leq 0.05$)

The DPPH• scavenging activity of the extracts was concentration-dependent (Figure 1). Mfr showed significantly higher radical scavenging activity (RSA) ($77.97 \pm 1.25\%$) than DG extract ($42.80 \pm 0.51\%$) and Wfr ($10.74 \pm 0.78\%$) at 50 µg/mL concentrations. This is in good agreement with the result obtained from the literature¹³⁻¹⁵ where methanol extract showed maximum activity for pomegranate peel, coffee and cinnamon fruits.

Figure 1. Radical scavenging activity of the ginger extracts and fractions at different concentrations (BHA- Butylated hydroxyl anisole)

The reducing power of extracts showing same degree of electron donation capacity in a concentration-dependent manner, but the capacities were inferior to that of ascorbic acid (AA) and BHA. The absorbance readings measured at 700 nm at 50 µg/mL indicated (Figure 2) that the Mfr showed higher reducing power compared to DG and Wfr. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity¹⁴.

Figure 2. Reducing power of extract, fractions and standard compounds. Values are mean ± SD of triplicate analyses; All values in the same group are significantly different ($p < 0.05$)

The antioxidant capacities of the dried ginger (DG) were found to be 1341 ± 122 µmol/g (ascorbic acid equivalents) and lower to that of propyl gallate (Figure 3). The Mfr exhibited higher antioxidant capacity (3343.47 ± 131.65 µmol/g) and was comparable to that of propyl gallate. The Wfr showed the least antioxidant capacity (545.46 ± 14.03 µmol/g). Higher capacity of the Mfr may be due to the presence of higher quantity of polyphenols.

Figure 3. Antioxidant capacity of the extract, fractions and standard compound (Values are mean $\pm$ SD of triplicate analyses; All values in the same group are significantly different ($p < 0.05$). (PG-Propyl gallate; BHA-Butylated hydroxyl anisole; AA-Ascorbic acid)

Correlation studies between the total polyphenols content and antioxidant activities showed a positive and significant correlation ($p < 0.05$) between the polyphenolic content and RSA. The results also confirm that the polyphenolic compounds are responsible for other activities such as reducing power and antioxidant capacity in ginger. [6]-Gingerol is the most abundant (80-90%) and important pungent compound among the other gingerols present in fresh and dry ginger products¹⁶. [6]-Gingerol was isolated from the ginger extract and the structure elucidation was carried using NMR spectral analysis¹⁷ (Table 2). [6]-Gingerol at five different concentrations was analyzed and peak area responses were noted (average of two independent injections). Calibration curve was plotted for concentration *versus* peak area response. [6]-Gingerol content was calculated using the standard curve. DG extract and Mfr possessed $6.0 \pm 1.2\%$ and $12.1 \pm 2.5\%$ [6]-gingerol content respectively (Figure 4).

Table 2. NMR data of [6]-gingerol

Carbon / Proton	[6]-Gingerol	
	¹ H NMR	¹³ C NMR
C- 1'	--	132.32
C- 2'	6.69 (d, 1.5Hz)	110.8
C- 3'	--	143.70
C- 4'	--	146.2
C- 5'	6.83 (d, 8Hz)	114.16
C-6'	6.67 (dd, 1.5Hz)	120.42
-OCH3	3.86	55.564
C- 1	2.82-2.85	28.96
C- 2	2.72-2.75	45.10
C- 3	--	199.6
C- 4	2.50-2.53 (d) 2.54-2.56 (d)	49.05
C- 5	4.01	67.40
C-6	1.23-1.52	36.14
C- 7		32.12
C- 8		24.8
C- 9		22.2
C- 10 (-CH3)	0.88 (t)	13.68

Figure 4. HPLC profiles of DG extract and methanol fraction (Mfr)* [6]-gingerol

Conclusion

Methanol soluble fraction (Mfr) of DG extract contained highest quantities polyphenols and exhibited higher RSA, antioxidant activities compared to aqueous fraction. Hence, Mfr could be used as natural antioxidant, a possible substitution of synthetic antioxidants in foods as well as pharmaceutical formulations.

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