

## EFFECTS OF SOLVENT TYPE ON PHENOLICS AND FLAVONOIDS CONTENT AND ANTIOXIDANT ACTIVITIES OF GINGER (*ZINGIBER OFFICINALE*)

Sushila Singh<sup>\*a</sup>, Satya Shree Jangra<sup>b</sup> and V. K. Madan<sup>b</sup>

<sup>a</sup>Department of Chemistry & Biochemistry, CCS Haryana Agricultural University, Hisar

<sup>b</sup>Medicinal & Aromatic Plants Section, Old IATTE Building, CCS Haryana Agricultural University, Hisar-125004, Haryana, India

\*E-mail: singhsushila999@gmail.com

**ABSTRACT** In the present investigation, ginger (*Zingiber officinale*) extracts were assayed for their phenolics, flavonoids content and screened for their DPPH free radical scavenging activity using three different solvents namely, acetone, methanol and water. The results revealed that water extract contained the highest amount of total phenolics (789 mg GAE/g fwb) and flavonoids (2.56 mg CE/g fwb). DPPH free radical scavenging activity of the ginger extracts varied widely and it increased with increase of concentration levels. Methanol extract exhibited the highest DPPH free radical scavenging activity with IC<sub>50</sub> value (3.6 mg/mL).

**Keywords:** Ginger, phenolics, flavonoids, antioxidant activity.

### INTRODUCTION

Dietary phytochemicals are considered as an effective tool to cure various human physiological disorders. Several epidemiological studies have indicated that high intake of natural products is associated with reduced risk of a number of chronic diseases such as atherosclerosis and cancer<sup>1</sup>. During recent years consumers have been more concerned about the addition of synthetic additives to food and the two most commonly used antioxidants, butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), have shown DNA damage induction<sup>2</sup>. Therefore, an interest is growing for the search of natural antioxidants for the public perception that natural and dietary antioxidants are safer than synthetic analogues<sup>3-4</sup>. From the safety point of view, one of the important sources for the search of natural antioxidants are herbs and spices. Among them, ginger has much importance due to its versatile use as a spice as well as an herb.

Ginger (*Zingiber officinale*) belongs to family *Zingiberaceae* have been widely used as spice and flavoring agent in foods and beverages.<sup>5</sup>The rhizome of Ginger (*Zingiber officinale*) is widely consumed as common spice throughout the world and used in traditional oriental medicine<sup>6</sup>. Many herbs and spices, usually used to flavor dishes, are an

excellent source of phenolic compounds, which have been reported to show good antioxidant activity<sup>7</sup>. Several active components are present in ginger<sup>8</sup>. Among these, the major active ingredients are ginger and hexahydrocurcumin<sup>9</sup>. Atherosclerosis is the leading cause of death in modern societies. Oxidation of lipoproteins, especially low-density lipoproteins (LDL), cholesterol, play a crucial role in the initiation and progression of atherosclerosis<sup>10</sup>. Antioxidants that prevent LDL oxidation in vitro also inhibit atherosclerosis in animals<sup>11</sup>. Therefore, reducing blood lipids and inhibiting lipid oxidation are both important for the prevention and treatment of atherosclerosis in rats. Inhibition of oxidative stress improves all disorders related to diabetic nephropathy<sup>12</sup>. Consumption of antioxidants such as ginger would be a useful addition to current treatment strategies e.g. with insulin and reduce the thiobarbituric acid reactive substance<sup>13</sup>. Fresh ginger rhizome contains gingerol but it converts to zingerone, shogaol etc, after drying. Zingerone also has antioxidants and anti-inflammatory effect and can prevent the growth of cancer. Gingerol and shogaol of ginger can protect heart from blood clotting<sup>14</sup>. Ginger has been consumed since antiquity and is known to play diverse biological roles including anti-oxidation, anti-inflammation, hypolipidemia, anti-carcinogenesis, anti-nausea, antithrombosis and antibacterial properties<sup>15-18</sup>. Antioxidant compounds, present in plants, are of diverse structure and their activity in different model systems and extractability is strongly dependent on their chemical structure, so different extraction media i.e. solvent systems, may provide varying yields of extracts with selective recovery of antioxidants; depending on the structure of antioxidant compounds present in the plants<sup>19</sup>. Therefore, the present study was carried out to investigate the phenolic, flavonoid contents of ginger and to evaluate their DPPH free radical scavenging activity.

## RESULTS AND DISCUSSION

### Total phenolics content

Total phenolics content in different solvent extracts (acetone, methanol and water) of ginger is shown in Table I. On fresh weight basis, water extract of ginger contained the

highest total phenolics content (789 mg GAE/g fwb) followed by methanol (510 mg GAE/g fwb) and acetone (325 mg GAE/g fwb) extracts .

### Flavonoids content

Flavonoids content of ginger extracts in three solvents varied widely. On fresh weight basis, water extract of ginger contained the highest flavonoids content (2.56 mg CE/g fwb ) followed by methanol (0.63 mg CE/g fwb) and acetone (0.21 mg CE/g fwb) extracts (Table 1).

**Table 1: Total phenolics and flavonoids of ginger extracts prepared using different solvents**

Solvent	Total phenolics content (mg GAE/g fwb)	Flavonoids content (mg CE/g fwb)
Acetone	325 ± 0.01	0.21 ± 0.01
Methanol	510 ± 0.02	0.63 ± 0.03
Water	789 ± 0.01	2.56± 0.02

### DPPH free radical scavenging activity

DPPH free radical scavenging activity was measured by the decrease in absorbance as the DPPH radical received an electron or hydrogen radical from an antioxidant compound to become a stable diamagnetic molecule<sup>20</sup>. DPPH free radical scavenging activity (%) of the acetone, methanol and water extracts of ginger varied widely and it increased with increase of concentration levels. DPPH free radical scavenging activity (%) of ginger ranged from 39.2 to 82.3% (methanol extract), from 24.8 to 76.2% (water extract) and from 23.2 to 59.2% (acetone extract) at different concentration levels ranging from 2.5 to 10 mg/mL (Table 2). The IC<sub>50</sub> value of methanol extract was lowest i.e. 3.6 mg/mL followed by 4.6 mg/mL of water extract and 8.1 mg/mL of acetone extract thereby showing that methanol extract has highest activity followed by water and acetone extracts.

Table 2. DPPH free radical scavenging activity (%) of ginger extract.

Extracts Conc. ↓ (mg/mL)	DPPH Free Radical Scavenging Activity (%)				IC <sub>50</sub> (mg/mL)
	10	7.5	5	2.5	
Acetone	59.2±0.20	45.1±0.21	38.1±0.22	23.2±0.15	8.1
Methanol	82.3±0.02	77.3±0.21	61.4±0.32	39.2±0.21	3.6
Water	76.2±0.11	68.4±0.25	56.3±0.26	24.8±0.31	4.6

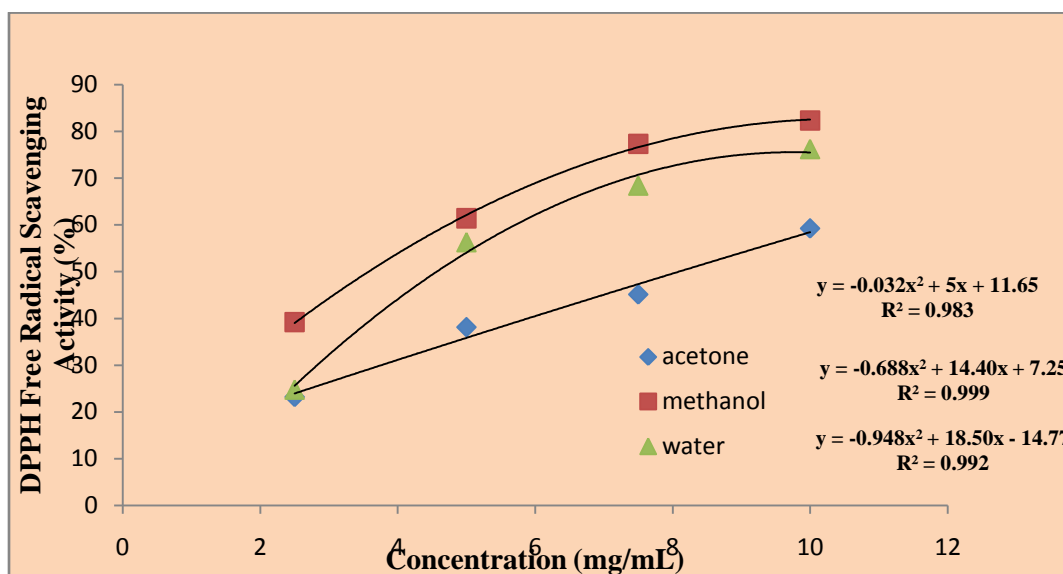


Fig. 1: Quadratic regression equations for IC<sub>50</sub> values of different extracts of ginger.

## EXPERIMENTAL

### Plant material and extraction

Fresh gingers (*Zingiber officinale*) were procured from the local market of Hisar. The extracts were prepared with ten gram ginger. Extracts were then used for estimation of total phenolics, flavonoids and DPPH free radical scavenging activity .

### Chemicals

The commercially available chemicals from Sigma-Aldrich, Qualigens, Merck and Hi-Media of highest purity, were used for various experimental procedures.

### **Estimation of total phenolics content**

Total phenolics content of extracts was determined using Folin-Ciocalteu method<sup>21</sup>. Aliquots of 0.2 ml of extracts were mixed with 1 ml of 1 mol/L Folin-Ciocalteu reagent. After that, 2.0 ml of 20% (w/v) sodium carbonate solution was added. The solutions were mixed and volume was made up to 10.0 ml with distilled water. The absorbance was measured at 730 nm using UV-VIS double beam Spectrophotometer Model 2203 (Systronics Co.). A calibration curve was prepared using gallic acid as standard. Results were expressed as mg GAE/g on fresh weight.

### **Estimation of flavonoids content**

Flavonoids content of extracts was estimated according to the colorimetric assay<sup>22</sup>. In 1.0 ml of extract, 4.0 ml of double distilled water and 0.3 ml of 5% (w/v) NaNO<sub>2</sub> were added. After 5 min., 0.3 ml of 10% (w/v) AlCl<sub>3</sub> was added. Immediately, 2.0 ml of 1 M NaOH was added and the volume was made up to 10.0 ml with double distilled water. The solution was mixed thoroughly and the absorbance was measured at 510 nm using UV-VIS double beam Spectrophotometer Model 2203 (Systronics Co.). A calibration curve was prepared using catechin as standard. Results were expressed as mg CE/g on fresh weight as well as dry weight basis.

### **DPPH free radical scavenging activity**

The antioxidant activity of the extracts was evaluated by 2,2'-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging method<sup>23</sup>. Acetone, methanol and water extracts were dried up completely and the weight of dry mass was noted. The dry mass of acetone and methanol extracts was redissolved in appropriate amount of methanol to make the stock solution (50 mg/mL). Since, the dry mass of water extract was not soluble in pure methanol, hence, it was redissolved in 50% (v/v) methanol : water to make the stock solution. From stock solution, different concentrations (2.5-10 mg/mL) were made by appropriate dilutions with respective solvents (i.e. methanol for acetone and methanol extracts and with methanol : water for water extracts). For evaluation of antioxidant activity, in 0.2 mL of extracts (various concentrations), 3.0 mL of 2,2'-diphenyl-1-picrylhydrazyl radical (DPPH; 0.1mM in 100 % methanol) was added and mixed

thoroughly for 5 min. For antioxidant activity in water extracts (various concentrations), DPPH stock solution was prepared in 50 % (v/v) methanol : water and remaining procedure was same. A control was also made containing 0.2 mL of each solvent instead of extract. The absorbance of the sample as well as control was measured at 517 nm after 30 min of incubation in dark at room temperature using the UV-VIS double beam Spectrophotometer Model 2203 (Systronics Co.) against a blank containing respective solvent. Three replications were carried out for each sample. A graph was drawn by plotting per cent DPPH free radical scavenging activity (y-axis) against extract concentration (x-axis). Then using Microsoft Excel Software, quadratic regression equation

( $y = ax^2 + bx + c$ ) was obtained and using the quadratic equation  $IC_{50}$  was calculated. The percentage of DPPH scavenged (% DPPH<sup>\*<sub>sc</sub></sup>) was calculated using:

$$\% \text{ DPPH}^*_{sc} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100$$

where,  $A_{\text{control}}$  is the absorbance of control and  $A_{\text{sample}}$  is the absorbance of the sample.

## CONCLUSION

Results of present study shows that solvent play a vital role in the extraction of the plant constituents. Water extracts of ginger contained highest amount of total phenolics and flavonoids content. Methanol extract exhibited highest antioxidant activity as compare to other solvents. Hence, methanol extracts of ginger are better source of antioxidants in comparison to other solvents.

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