

Research Article

Effects of Solvents on Total Phenolic Content and Antioxidant Activity of Ginger Extracts

Dessie Ezez¹ and Molla Tefera² 

¹Department of Chemistry, Arba Minch University, P. O. Box 21, Arba Minch, Ethiopia

²Department of Chemistry, University of Gondar, P. O. Box 196, Gondar, Ethiopia

Correspondence should be addressed to Molla Tefera; mollatef2001@gmail.com

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Ginger (*Zingiber officinale*) is a popular spice which is used for the treatment of different gastrointestinal and inflammatory discomfort. In the present study, the total phenolic content (TPC) and antioxidant activity of ginger extract using four solvents (ethanol, methanol, acetone, and ethyl acetate) were determined. Among the four solvents, methanol extract showed the maximum phenolic content (1183.813 mg GAE/100 g at Ayikel and 1022.409 mg GAE/100 g at Mandura) and the least phenolic content was found in acetone extract (748.865 mg GAE/100 g at Ayikel and 690.152 mg GAE/100 g at Mandura). In addition, the highest DPPH radical scavenging activity (84.868% at Ayikel and 82.883% at Mandura) was observed in methanol. However, acetone showed the least DPPH radical scavenging activity (73.864% at Ayikel and 70.597% at Mandura). Antioxidant activities of ginger extracts were also expressed as IC₅₀ values, and acetone extract has maximum IC₅₀ value (0.654 and 0.812 mg/mL) followed by ethyl acetate and ethanol, while being the lowest for methanol (0.481 and 0.525 mg/mL). The result of this study showed that extraction solvents significantly affected the total phenolic content and antioxidant activities of ginger. Thus, ginger can be regarded as promising candidates for natural sources of antioxidants with a high value of phenolic contents.

1. Introduction

Natural bioactive compounds especially plant sources have been investigated for their characteristics and health effects [1]. Many spices such as cardamom, long pepper, black cumin, ginger, bishops weed, and coriander are highly cultivated in Ethiopia over many years. However, ginger (*Zingiber officinale Roscoe*) is cultivated in many places of the country than any other spices [2]. The refreshing aroma and pungent taste makes ginger an essential ingredient in most world cuisine and food processing industry [3]. Besides, ginger has been employed as an alternative medicine around the world for antiarthritic activity [4], protects against gastrointestinal ulcers, improves blood circulation, lowers blood glucose in the treatment of diabetes [5], and diarrhea [6].

Numerous active ingredients are present in ginger such as terpenes (sesquiterpene hydrocarbons), alkaloids, and polyphenols [7].

Phenolic compounds are associated with a high number of biological activities and one with special interest is their antioxidant capacity [8] and may help to protect the cells against the oxidative damage caused by free radicals [9, 10]. Antioxidant activities of ginger have been identified by many researchers [11, 12]. Several studies revealed that ginger has showed antioxidant activity against lipid oxidation and oxidative stress [13, 14].

There are many techniques to extract total polyphenols from plants, such as Soxhlet extraction, maceration, supercritical fluid extraction, subcritical water extraction, and ultrasound-assisted extraction. Due to simplicity and low economic outlay, classical extraction methods are most commonly used for isolating these compounds in many samples. For successful separation and determination of biologically active compounds from plant material, it mainly depends on the type of solvent used in the extraction procedure. Extraction with water alone was not as effective

as extraction with aqueous solution of organic solvents such as ethanol, methanol, diethyl ether, chloroform, ethyl acetate, and *n*-butanol [15–17].

Taking into account all these aspects, the present study was undertaken with the purpose of determining the effects of aqueous solution of different solvents (ethanol, methanol, acetone, and ethyl acetate) on the total polyphenol and antioxidant capacity of ginger extracts collected from local markets in Ayikel and Mandura towns, Ethiopia.

2. Materials and Methods

2.1. Chemicals. DPPH (2,2-diphenyl-1-picrylhydrazyl), ascorbic acid, gallic acid, sodium carbonate, Folin–Ciocalteu reagent were purchased from Sigma-Aldrich (Mumbai, India). The solvents acetone, ethanol, methanol, and ethyl acetate were obtained from Merck (Darmstadt, Germany). All the reagents and chemicals used were of analytical reagent grade and were acquired from commercial sources. Deionized water was used for sample preparation, dilution, and rinsing apparatus prior to analysis.

2.2. Instruments. Microprocessor UV-Vis double beam spectrophotometer (Abron, India), refrigerator (LR 1602, England), vortex mixer (Abron, India), scalpel, grinder, magnetic stirrer, measuring cylinder, Whatman filter paper (No. 42) micropipettes, electronic balance (CTG 1200), separatory funnel, and aluminum foil.

2.3. Sample Collection and Preparation. Three kilograms of fresh ginger rhizome samples ($n=6$) with no apparent physical or microbial damages were collected randomly from local markets of Ayikel and Mandura town, Ethiopia. The collected samples from each study area were pooled together and mixed well to have one bulk sample from each site. The ginger samples were washed with tap water and distilled water and finally were peeled. The peeled samples were then sliced separately into pieces using scalpel and dried at room temperature for several days. Finally, the dried samples were ground to a fine powder using grinder and then sieved using mesh and stored in until required for extraction.

2.4. Extraction of Ginger Samples. Solvent extractions are the most commonly used procedures to extract polyphenol from plant materials due to their ease of use, efficiency, and wide applicability. For the present study, an aqueous solution of methanol, acetone, ethanol, and ethyl acetate (1:4, water, solvent, v, v) was used as solvent to extract total polyphenol contents from the samples. One gram (1.0 g) of ginger was weighed and mixed with 20 mL of organic solvents (acetone, methanol, ethanol, and ethyl acetate) into different 100 mL conical flasks and covered with aluminum foil. The solution was magnetically stirred at 900 rpm for 24 h at room temperature. The supernatant was collected, filtered, and finally kept in the refrigerator at 4°C until further analysis.

2.5. Total Phenolic Content. The concentration of total phenol present in ginger extracts was determined by Folin–Ciocalteu (FC) reagent method described by Munro et al. [18]. In brief, 0.5 mL of solvent extracts of each ginger sample was mixed with 2.5 mL of 10% Folin–Ciocalteu reagent. After 5 min in the dark, 2 mL of 7.5% sodium carbonate was added. The solution was agitated with a vortex mixer for a min before incubation in the dark for 1 h at room temperature. The absorbance was determined using a UV-Vis spectrophotometer at 760 nm. The calibration curve was established using gallic acid (5–150 mg/L). The phenolic content was expressed as milligram gallic acid equivalents per 100 g dry extract (mg GAE/100 g). All determinations were performed in triplicate.

2.6. Antioxidant Capacity. The antioxidant activity of ginger extracts was evaluated using the method described by Koleva et al. [19]. In brief, 1.0 mL of sample extracts at various concentrations (0.2–1.5 mg/mL) was added to 2 mL of 0.040 g/L DPPH in methanol solution. The test tube was incubated in the dark for about 30 min at room temperature. Ascorbic acid was used as positive control. Different concentrations ranging from 0.2 to 1.5 mg/mL of ascorbic acid were used for constructing calibration curve, and IC_{50} values were calculated.

The antioxidant activity was recorded spectrophotometrically at an absorbance of 517 nm, and the percentage inhibition of radicals was calculated using the following formula:

$$\% \text{ inhibition} = \left[\frac{A_{bl} - A_{sa}}{A_{bl}} \right] \times 100, \quad (1)$$

where A_{bl} is the absorbance of the blank DPPH solution without ginger extract and A_{sa} is the absorbance of sample extracts with DPPH. All antioxidant determinations were performed in triplicate.

2.7. Statistical Analysis. All the experiments were carried out in triplicate, and the values were expressed as mean \pm standard deviation, and the data were analyzed statistically using the IBM SPSS software (version 20). An analysis of variance was performed by one-way ANOVA, and significant differences between the means due to composition of extraction solvent were determined by Tukey's HSD (homogeneous subset difference) test at the significance level $p = 0.05$.

3. Results and Discussion

3.1. Total Phenolic Content. The total phenolic contents were determined by plotting the standard calibration curve of different concentrations of gallic acid using a spectrophotometer at 760 nm. The values of TPC were calculated as gallic acid equivalents (GAE) per 100 gram of dry weight.

The amount of total phenolic content in ginger samples collected from Ayikel and Mandura was influenced significantly by extracting solvent ($p < 0.05$), and the contents

were varied within the range of 690.152 to 1183.813 mg of GAE/100 g of dry weight for acetone and methanol, respectively. Among the solvents, methanol was the most efficient extracting solvent for TPC, followed by ethanol, ethyl acetate, and acetone, indicating that the TPC extracted in ginger was higher in polar solvents compared with less polar solvents (Table 1). The variations in the extract yields from ginger using different solvents might be explained by the difference in polarity of different compounds in the samples [20, 21].

Between the two study areas, the higher TPC was found in a ginger sample collected from Ayikel in all extracts. The difference in the quantity of TPC may be attributable to different intrinsic and extrinsic factors, including cultivars, type of soil and growing conditions, maturity state, and harvest conditions [22, 23].

3.2. Antioxidant Activity. Antioxidant activity of ginger extract was evaluated using ascorbic acid as standard. It is one of the greatest antioxidant compound known by scavenging the stable radical of 1, 1-diphenyl-2-picryl-hydrazyl (DPPH).

The antioxidant values obtained are presented in Table 2. From the data, it is evident the Ayikel ginger samples showed higher value of DPPH (% inhibition) and TPC as compared to Mandura. The value for DPPH (% inhibition) activity of the extracts can be ranked as methanol extract > ethanol extract > ethyl acetate extract > acetone extract. In addition, the current finding revealed that antioxidant activity was significantly correlated with the phenolic content. Except acetone and ethyl acetate extracts, there were significant differences ($p < 0.05$) of DPPH radical scavenging abilities between all extracts. As with TPC value, it was observed that methanol extract owned the highest DPPH radical scavenging ability, followed by ethanol, ethyl acetate, and acetone. The radical scavenging activities of ginger were close to the positive control, i.e., ascorbic acid with % inhibition of 89.75 ± 0.361 .

The result was in agreement with those findings reported in literature, where the phenolic content and antioxidant activity were influenced by extracting solvents. In addition, the current finding revealed that a highest DPPH radical scavenging activity of ginger extract was obtained when methanol was used for extracting solvent [1, 24, 25].

The half maximal inhibitory concentration (IC_{50}) is defined as the amount of antioxidant that causes decrease in the DPPH concentration by 50% [26]. The IC_{50} value was calculated from the linear regression plots of percentage inhibition (% DPPH scavenging activity) against concentration of ginger extracts. As depicted in Table 3, the IC_{50} values of Ayikel ranged from 0.481 to 0.654 mg/mL for methanol and acetone extracts, respectively. Similarly, it was found that acetone extracts owned the highest IC_{50} value followed by ethyl acetate, ethanol, and methanol extracts in Mandura ginger. This implies that the concentration of acetone extract required to decrease the initial concentration of DPPH solution by 50% is 0.654 mg/mL, whereas for methanol extract, it is 0.481 mg/mL. The result showed that the IC_{50} value is inversely related to its antioxidant capacity.

TABLE 1: Total phenolic content of ginger from Ayikel and Mandura locations extracted with different solvents.

Solvents	TPC (mg GAE/100g)*	
	Ayikel	Mandura
Ethanol	$1009.917 \pm 0.140^{a,x}$	$941.847 \pm 0.177^{c,y}$
Ethyl acetate	$899.041 \pm 0.121^{b,x}$	$778.806 \pm 0.253^{f,y}$
Methanol	$1183.813 \pm 0.418^{c,x}$	$1022.409 \pm 0.265^{b,y}$
Acetone	$748.865 \pm 0.210^{d,x}$	$690.152 \pm 0.214^{h,y}$

Values represented mean \pm S.D. of three parallel measurements ($p < 0.05$). For each solvent extracts, values in the same column for each sample followed by different letters (a–h) are significantly different ($p < 0.05$). For each plant sample, values in same row for each solvent followed by a different letter (x, y) are significantly different ($p < 0.05$) by Tukey's multiple range tests.

It was elucidated that the methanol extracts showed highest antioxidant activities than the other solvents. However, the IC_{50} values regarding different solvents used for extraction were as follows: acetone > ethyl acetate > ethanol > methanol. The results are similar to those reported by [17, 27], where a lowest DPPH radical scavenging activity of a plant extract had the highest IC_{50} .

In our study, ascorbic acid was used as the positive control; with an IC_{50} value estimated at 0.239 mg/mL, while the IC_{50} values of the ginger extracts ranged from 0.481 to 0.812 mg/mL. This indicates that the extracts are slightly potent inhibitors in comparison with ascorbic acid.

3.3. Comparison of the Current Study with Results from Other Countries. There are some reports from different countries on the analysis of the phenolic contents and antioxidants activities of ginger. It is important to compare the results obtained in this study with the values reported in other countries. This comparison helps to identify the differences in composition of samples between countries.

As shown in Table 4, the total phenol contents of ginger extract obtained in this study are higher than those reported by Sharif and Bennett [28] and Adel and Prakash [29].

However, methanol extract reported by Ghorab et al. [30] was found to be higher than the results of this study. The total polyphenol of methanol extract was found to be comparable with the results reported by Mohd and Muhd [31]. Besides, total phenol content of acetone and ethyl acetate extract reported by Mohd and Muhd [31] and Ghasemzadeh et al. [32], respectively, was found to be higher than the results obtained in this study at both study sites.

The antioxidant properties of ginger were also compared with the reports from other countries. As shown in Table 5, the antioxidant activities were found to be slightly higher than those reported by Ghasemzadeh et al. [1, 32] and Mohd and Muhd [31]. However, the results of present study were in agreement with the reported values by Ghorab et al. [30] and Sharif and Bennett [28]. The differences in the total phenol contents and antioxidant activity of this study with previously reported values were attributed to several factors such as the difference in plant variety, the method and conditions of extraction (temperature and time), environmental conditions, degree of ripeness, plant variety, and sun exposure

TABLE 2: % inhibition of ginger extract.

Solvents	Samples (% inhibition \pm SD)	
	Ayikel	Mandura
Ethanol	82.108 \pm 0.416 ^{a,x}	77.975 \pm 0.297 ^{d,y}
Ethyl acetate	81.398 \pm 0.297 ^{b,x}	75.967 \pm 0.391 ^{e,y}
Methanol	84.868 \pm 0.293 ^{c,x}	82.883 \pm 0.216 ^{f,y}
Acetone	73.864 \pm 0.418 ^{b,x}	70.597 \pm 0.332 ^{e,y}

For each solvent extracts, values in the same column for each sample followed by a different letter (a–f) are significantly different ($p < 0.05$). For each plant sample, values in same row for each solvent followed by a different letter (x, y) are significantly different ($p < 0.05$) by Tukey's multiple range tests.

TABLE 3: IC₅₀ (mg/mL) values of ginger by different solvent extracts.

Solvents	IC ₅₀ (mg/mL)*	
	Ayikel	Mandura
Ethanol	0.499 \pm 0.021	0.548 \pm 0.045
Ethyl acetate	0.501 \pm 0.034	0.653 \pm 0.028
Methanol	0.481 \pm 0.015	0.525 \pm 0.017
Acetone	0.654 \pm 0.054	0.812 \pm 0.06

*Values represented as mean \pm S.D.

TABLE 4: Comparison of total phenol content of ginger with that reported in the rest of the world (mg GAE/100 g).

Ethanol	Methanol	Ethyl acetate	Acetone	References
263	148	NA	216	Sharif and Bennett, 2016
NA	NA	1022	NA	Ghasemzadeh et al., 2010
NA	565	510	325	Adel and Prakash, 2010
NA	9520	NA	NA	Ghorab et al., 2010
NA	1340	NA	1110	Mohd and Muhd, 2016
1009.917	1183.813	899.041	748.865	This study (Ayikel)
941.847	1022.409	778.806	690.152	This study (Mandura)

TABLE 5: Comparison of % inhibition of ginger with that reported in the rest of the world.

Ethanol	Methanol	Ethyl acetate	Acetone	References
93	82.2	NG	87.1	Sharif and bennett, 2016
NA	51.48	NG	49.22	Ghasemzadeh et al., 2011
NG	51.41	NG	NG	Ghasemzadeh et al., 2010
NG	58.21	NG	56.18	Mohd and Muhd, 2016
NG	87.66	NG	NG	Ghorab et al. 2010
82.883	84.868	81.398	73.864	This study (Ayikel)
77.975	82.883	75.967	70.597	This study (Mandura)

[17, 33, 34]. For instance, the ginger studied by [28, 29] was extracted at 8 h and 3 h, respectively.

4. Conclusion

According to the results, the yield and efficiency of the phenolic content extraction depend on the type and kind of the solvent which is being isolated. The highest concentration of phenolic compounds in the extracts was obtained using solvents of high polarity relative to the other solvents, and methanol extract manifested greater power of extraction for phenolic compounds from ginger rhizome.

The highest total phenolic content is 1183.813 \pm 0.418 mg GAE/100 g DW for methanol extract, 1009.917 \pm 0.140 mg GAE/100 g DW for ethanol extract for Ayikel samples, and 1022.409 \pm 0.265 mg GAE/100 g DW for methanol extract, followed by 941.847 \pm 0.177 mg GAE/100 g DW for ethanol extract for Mandura samples. For total phenolic extraction from ginger, methanol was more efficient than ethanol, ethyl acetate, and acetone. Methanol extract has maximum antioxidant activity than all other solvents followed by ethanol extract.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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