

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

Dessie Ezez

Arba Minch University

Molla Tefera (✉ molla.tefera@uog.edu.et)

University of Gondar College of Natural and Computational Sciences <https://orcid.org/0000-0003-4469-5388>

Research article

Keywords: Ginger, solvents, total polyphenol, antioxidant, IC50

Posted Date: June 8th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-30897/v1>

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Version of Record: A version of this preprint was published at Journal of Chemistry on May 3rd, 2021. See the published version at <https://doi.org/10.1155/2021/6635199>.

Abstract

Ginger (*Zingiber officinale*) is a popular spice which 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 that the maximum phenolic (1183.813 mg GAE/100 g at Ayikel and 1022.409 mg GAE/100 g at Mandura) and the least were 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 the lowest for methanol extracts (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 high value of phenolic contents.

Introduction

Natural bioactive compounds especially plant sources have been investigated for their characteristics and health effects [1]. Many spices like 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 it has been extensively used as spices in food and drinks, ginger has been employed as an alternative medicine around the world for anti-arthritis [4], anti-migraine, hypocholesterolemic [5], temporary relief and protection against gastrointestinal ulcers, improves blood circulation, lowers blood glucose in the treatment of diabetes [6], treat cancer [3] and diarrhea [7].

Numerous active ingredients are present in ginger such as terpenes (sesquiterpene hydrocarbons), alkaloids and polyphenols [8].

Phenolic compounds are associated with a high number of biological activities and one with special interest is their antioxidant capacity [9] and may help to protect the cells against the oxidative damage caused by free radicals [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].

Taking into account all these aspects, the present study was undertaken with the purpose of determining the effects of different solvents on the total polyphenol and antioxidant capacity of ginger extracts collected from local market in Avikel and Mandura town, Ethiopia.

Materials And Methods

Chemicals

DPPH (2,2-diphenyl-1-picrylhydrazyl), ascorbic acid, gallic acid, sodium carbonate, Folin-Ciocalteu reagent, acetone, ethanol, methanol and ethyl acetate were used in this study. 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.

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, aluminum foil.

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 was 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.

Extraction of ginger samples

Solvent extractions are 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, v, v) were 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 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.

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.* [15]. Briefly, 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 incubated in the dark for 1 h at room temperature. The absorbance was determined using 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.

Antioxidant capacity

The antioxidant activity of ginger extracts were evaluated using the method described by Koleva, *et al.* [16]. Briefly, 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 range from 0.2–1.5 mg/mL of ascorbic acid were used for constructing calibration curve and IC₅₀ 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:

$$\% \text{inhibition} = [(A_{bl} - A_{sa}) / A_{bl}] \times 100$$

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.

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 $p = 0.05$.

Results And Discussion

Total Phenolic Content

The total phenolic contents were determined by plotting standard calibration curve of different concentration of gallic acid using 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 were 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 were 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 [17–19].

Table 1
The effects of solvent extracts on total phenolic contents of ginger in Ayikel and Mandura

Solvents	TPC (mg GAE/100 g)*	
	Ayikel	Mandura
Ethanol	1009.917 ± 0.140 ^{a,x}	941.847 ± 0.177 ^{e,y}
Ethyl acetate	899.041 ± 0.121 ^{b,x}	778.806 ± 0.253 ^{f,y}
Methanol	1183.813 ± 0.418 ^{c,x}	1022.409 ± 0.265 ^{g,y}
Acetone	748.865 ± 0.210 ^{d,x}	690.152 ± 0.214 ^{h,y}
Values represented mean ± S.D. of three parallel measurements (P < 0.05).		
For each solvent extracts, values in the same column for each sample followed by a different letter (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.		

Between the two study areas, the higher TPC were 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 [20, 21].

Table 1.

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 ethanol 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).

Table 2
% inhibition of ginger extract

Solvents	Samples (mean±SD)	
	Ayikel	Mandura
Ethanol	82.108 ± 0.416 ^{a,x}	77.975 ± 0.297 ^{d,y}
Ethyl acetate	81.398 ± 0.297 ^{b,x}	75.967 ± 0.391 ^{e,y}
Methanol	84.868 ± 0.293 ^{c,x}	82.883 ± 0.216 ^{f,y}
Acetone	73.864 ± 0.418 ^{b,x}	70.597 ± 0.332 ^{e,y}
AA(control)	89.75 ± 0.361	
AA = ascorbic acid		
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.		

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 highest DPPH radical scavenging activities of ginger extract was obtained when methanol was used for extracting solvent [1, 22, 23].

Table 2

The half maximal inhibitory concentration (IC₅₀) is defined as the amount of antioxidant that causes decrease the DPPH concentration by 50%, [24]. IC₅₀ 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 IC₅₀ values of Ayikel were 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 IC₅₀ value followed by ethyl acetate, ethanol and methanol extracts in Mandura ginger. This implies that the concentration of acetone extract required decreasing the initial concentration of DPPH solution by 50% is 0.654 mg/mL, whereas for methanol extract is 0.481 mg/mL. The result showed that IC₅₀ value is inversely related to its antioxidant capacity.

Table 3
 IC_{50} (mg/mL) values of ginger by different solvent extract

Solvents	Sample site*	
	Ayikel	Mandura
Ethanol	0.499±0.021	0.548±0.045
Ethyl acetate	0.501±0.034	0.653±0.028
Methanol	0.481±0.015	0.525±0.017
Acetone	0.654±0.054	0.812±0.06
AA	0.239±0.015	
*Values represented mean ± S.D. of three parallel measurements.		

It was elucidated that the methanol extracts showed highest antioxidant activities than the other solvents. However, the IC_{50} values with regards to different solvents used for extraction was as follows: acetone > ethyl acetate > ethanol > methanol. The results are similar to that reported by [18, 25], where by 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.

Table 3

Comparison of 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 is higher than that of the results reported by Sharif and Bennett [26] and Adel and Prakash [27]. However, methanol extract reported by Ghorab *et al.* [28] 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 [29]. Besides, total phenol content of acetone and ethyl acetate extract reported by Mohd and Muhd [29] and Ghasemzadeh *et al.* [30], respectively were found to be higher than the results obtained in this study at both study sites.

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	[26]
NA	565	510	325	[27]
NA	9520	NA	NA	[28]
NA	1340	NA	1110	[29]
NA	NA	1022	NA	[30]
1009.917	1183.813	899.041	748.865	This study (Ayikel)
941.847	1022.409	778.806	690.152	This study (Mandura)

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 reported by Ghasemzadeh *et al*, [1, 30], Mohd and Muhd, [29]. However, the results of present study were in agreement with the reported values by Ghorab *et al*. [28] and Sharif and Bennett, [26].

Table 5
Comparison of % inhibition of ginger in this study with that reported in the rest of the world

Ethanol	Methanol	Ethyl acetate	Acetone	References
NA	51.48	NA	49.22	[1]
93	82.2	NG	87.1	[26]
NG	87.66	NG	NG	[28]
NG	58.21	NG	56.18	[29]
NG	51.41	NG	NG	[30]
82.883	84.868	81.398	73.864	This study (Ayikel)
77.975	82.883	75.967	70.597	This study (Mandura)

Table 4

Table 5

Conclusion

According to the results, the yield and efficiency of the phenolic content extraction depends on the type of solvent used. The highest concentration of phenolic compounds in the

extracts were obtained using solvents of high polarity relative to the other solvents, methanol extract manifested greater power of extraction for phenolic compounds from ginger rhizome.

The highest total phenolic content is 1183.813 ± 0.418 mg GAE/100 g DW for methanol extract, and 1009.917 ± 0.140 mg GAE/100 g DW for ethanol extract for Ayikel samples and 1022.409 ± 0.265 mg GAE/100 g DW for methanol extract, followed by 941.847 ± 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 others followed by ethanol extract in terms of both maximum inhibition effect and IC_{50} value.

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Declarations

Authors' contributions

MT designed the experiment, read and approved the final manuscript. DE has conducted the experimental work and analyzed the results

Declaration

The authors declare that there is no conflict of interest regarding the publication of the paper.

Funding

This work only funded by Authors

Availability of data and materials

All data and materials are all provided

References

1. Ghasemzadeh A, Jaafar H, Rahmat A (2011) Effects of solvent type on phenolics and flavonoids content and antioxidant activities in two varieties of young ginger (*Zingiber officinale* Roscoe) extracts. *J Med Plants Res* 5:1147–1154
2. Hegde V, Hegde S (2012) An Economic Overview of Ginger Production in Ethiopia. *Int J Sci Res* 2012:2052–2054
3. Sanwal S, Rai N, Singh J, Buragohain J (2010) Antioxidant phytochemicals and gingerol content in diploid and tetraploid clones of ginger (*Zingiber officinale* Roscoe). *Sci Hortic* 124:280–285
4. Mangprayool T, Kupittayanant S, Chudapongse N (2013) Participation of citral in the bronchodilatory effect of ginger oil and possible mechanism of action. *Fitoterapia* 89:68–73

5. Breemen R, Tao Y, Li W (2011) Cyclooxygenase-2 inhibitors in ginger (*Zingiber officinale*). *Fitoterapia* 82:38–43
6. Oboh G, Akinyemi A, Ademiluyi A, Adefegha S (2010) Inhibitory effects of aqueous extract of two varieties of ginger on some key enzymes linked to type-2 diabetes in vitro. *J Food Nutr Res* 49:14–20
7. An K, Zhao D, Wang Z, Wu J, Xu Y, Xiao G (2016) Comparison of different drying methods on Chinese ginger (*Zingiber officinale* Roscoe): Changes in volatiles, chemical profile, antioxidant properties, and microstructure. *Food Chem* 197:1292–1300
8. Bonilla J, Poloni T, Lourenco R, Sobral P (2018) Antioxidant potential of eugenol and ginger essential oils with gelatin/chitosan films. *Food Biosci* 23:107–114
9. Gouveia S, Paula C, Castilho P (2011) Antioxidant potential of *Artemisia argentea* L'Hér alcoholic extract and its relation with the phenolic composition. *Food Res Int* 44:1620–1631
10. Auddy B, Ferreira M, Blasina F, Mukherjee B (2003) Screening of antioxidant activity of three Indian medicinal plants. *J Ethnopharmacol* 84:131–138
11. Stoilova I, Krastanov A, Stoyanova A, Denev P, Gargova S (2007) Antioxidant activity of a ginger extract (*Zingiber officinale*). *Food Chem* 102:764–770
12. Maizura M, Aminah A, Aida W (2011) Total phenolic content and antioxidant activity of kesum (*Polygonum minus*), ginger (*Zingiber officinale*) and turmeric (*Curcuma longa*) extract. *Int Food Res J* 18:526–531
13. Singh S, Patel J, Bachle D (2014) A review on *Zingiber officinale* a natural gift. *Int J Pharm Biol Sci* 5:508–525
14. Rababah T, Hettiarachchy N, Horax R (2004) Total phenolic and antioxidant activities of fenugreek, green tea and tert-Butelhydroquinone. *J Agric Food Chem* 52:5183–5186
15. Munro B, Vuong Q, Chalmers A, Goldsmith C, Bowyer M, Scarlett C (2015) Phytochemical, antioxidant and anti-cancer properties of *Euphorbia tirucalli* methanolic and aqueous extracts. *Antioxidants* 4:647–661
16. Koleva I, Van Beek T, Linssen J, Groot A, Evstatieva L (2002) Screening of plant extract for antioxidant activity: a comparative study on three testing methods. *Phytochem Anal* 13:8–17
17. Babbar N, Oberoi H, Sandhu S, Bhargav V (2014) Influence of different solvents in extraction of phenolic compounds from vegetable residues and their evaluation as natural sources of antioxidants. *J Food Sci Tech* 51:2568–2575
18. Do Q, Angkawijaya A, Tran-Nguyen P, Huynh L, Soetaredjo F, Ismadji S, Ju Y (2014) Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of *Limnophila aromatic*. *J Food Drug Anal* 22:296–302
19. Denardin C, Hirsch G, da Rocha R, Vizzotto M, Henriques A, Moreira J, Guma F, Emanuelli T (2015) Antioxidant capacity and bioactive compounds of four Brazilian native fruits. *J Food Drug anal* 23:387–398

20. Phang C, Malek S, Ibrahim H, Wahab N (2011) Antioxidant properties of crude and fractionated extracts of *Alpinia mutica* rhizomes and their total phenolic content. *Afr J Pharm Pharmacol* 5:842–852
21. Jaffery E, Brown A, Matusheski N (2003) Antioxidant activity of ginger and identification of its active components. *J Food Compos Anal* 16:323–330
22. Dailey A, Vuong Q (2015) Effect of extraction solvents on recovery of bioactive compounds and antioxidant properties from macadamia (*Macadamia tetraphylla*) skin waste. *Cogent food Agric* 1:1–10
23. Zazouli S, Chigr M, Jouaiti A (2016) Effect of polar and nonpolar solvent on total phenolic and antioxidant activity of roots extracts of *Caralluma europaea*. *Der Pharma Chemica* 8:191–196
24. Tauheeda R, Muhammad A, Tayyaba S (2012) Antioxidant activity and phenolic content of *Dodonaea viscosa*. *J Serbian Chem Soci* 77:423–435
25. Thouri A, Chahdoura H, El Arem A, Omri H, Hassin B, Achour L (2017) Effect of solvents extraction on phytochemical components and biological activities of Tunisian date seeds (var. Korkobbi and Arehti). *BMC Complem Altern Med* 17:1–10
26. Sharif M, Bennett M (2016) The effect of different methods and solvents on the extraction of polyphenols in *Zingiber officinale*. *J Sci Engin* 78:49–54
27. Adel S, Prakash J (2010) Chemical composition and antioxidant properties of ginger root (*Zingiber officinale*). *J Med Plants Res* 4:2674–2679
28. Ghorab A, Nauman M, Anjum F, Hussain S, Nadeem M (2014) Comparative Study on Chemical Composition and Antioxidant Activity of Ginger (*Zingiber officinale*) and Cumin (*Cuminum cyminum*). *J Agri Food Chem* 58:8231–8237
29. Mohd F, Muhd T (2016) The effect of different methods and solvents on the extraction of polyphenols in *Zingiber officinale*. *J Sci Technol* 78:49–54
30. Ghasemzadeh A, Hawa Z, Asmah R (2010) Antioxidant Activities, Total Phenolics and Flavonoids Content in Two Varieties of Malaysia Young Ginger (*Zingiber officinale* Roscoe). *Molecules* 15:4324–4333