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Full Paper

THE EFFECT OF DIFFERENT METHODS AND SOLVENTS ON THE EXTRACTION OF POLYPHENOLS IN GINGER *(Zingiber officinale)*

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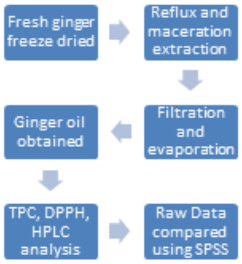


Abstract

Food ingredients derived from plants are a rich source of polyphenols. In recent times, polyphenols have become popular for their therapeutic properties as antioxidants. 6-gingerol, the main bioactive phenolic compound in *Zingiber officinale*, is well known for its free radical scavenging capabilities in treating multiple ailments. The most common method for extracting polyphenols from plant materials is through the use of solvents. The extraction of polyphenols from plant matrix is largely dependent on the type of solvent used and the extraction method employed. However, due to the complexity of plant chemistry there is no obvious choice in solvent or extraction method. In this study we made use of three different solvents; acetone, ethanol, and methanol. Variations of two different methods were used. The reflux was run for 30 minutes at 95 ℃ and maceration was done at room temperature for 8 hours. Between the different methods, maceration extracts generally showed better TPC, 6-gingerol content and antioxidant activity. Ethanol was significantly the best extracting solvent due to its higher polarity index. While the maceration ethanol extracts significantly had the highest TPC and 6-gingerol content, its antioxidant activity was not significantly different from the ethanol reflux extracts. A significantly positive correlation was determined between TPC and antioxidant activity, with reflux extracts having a better correlation than the maceration extracts. A significant positive correlation was also drawn between TPC and 6-gingerol content.

Keywords: Ginger, TPC, DPPH, anti-oxidant activity, IC_{50}, HPLC, 6-gingerol

1.0 INTRODUCTION

Polyphenols have gained much attention for being the main bioactive plant constituents with therapeutic properties. Fruits and vegetables are considered natural sources of polyphenolic compounds which can be divided mainly into phenolic acids, flavanoids, and tannins [1]. High amounts of polyphenols can be found in plant leaves, stems, and roots. As a result they frequently become a part of our diet [2]. Researchers and food engineers are especially interested in the anti-oxidant properties of plant polyphenols [3, 4].
Ginger (*Zingiber officinale*) is one of the most heavily consumed dietary substances in the world, rich in polyphenols and available at low prices. Apart from diet, the rhizome or root part of ginger has been used in traditional medicine for centuries for their anti-inflammatory, anti-nausea properties, and for treating migraines and breathing problems [5]. In most recent times they have been widely studied for exhibiting anti-microbial, anti-tumour, anti-diabetic, and anti-oxidant activities [6]. In fresh ginger rhizome, gingerols are identified as the major active component, with 6-gingerol being the most abundant constituent in the gingerol series [7, 8, 9].

Solvent extractions are the most commonly used procedures to obtain polyphenol extracts from plant materials due to their ease of use, efficiency, and

wide applicability. The most commonly used solvents are hot or cold aqueous mixtures containing acetone, ethanol, methanol, and ethyl acetate [10]. Since plant chemistry has a complex array of different bioactive compounds there is no clear cut solvent that can be recommended for extraction since optimum recovery varies from one sample to another and depends on the plant matrix, what phenolic constituents you want to extract, and how soluble the phenolic constituents are in the chosen solvent [11].

Several other parameters may influence the yield of polyphenols including extraction time, temperature, pH, and solvent polarity [12]. From the literature review, the efficiency of polyphenol extraction yield is strongly dependent on the plant matrix and solvent choice [13, 14, 15] as well as the method of extraction used [16, 17]. The aim of the current study was to determine the effect of 2 different methods and 3 different solvents on the extraction of polyphenols in ginger and to draw a relationship between total phenolic content (TPC), antioxidant activity, and 6-gingerol content.

2.0 METHODOLOGY

2.1 Sample Preparation

Fresh ginger rhizomes were washed thoroughly to remove any trace amount of soil. Then the rhizomes were cut into thinly sliced pieces before being stored in a deep freezer at – 80 ℃ overnight. The ginger samples were freeze-dried on the following day for 1 week. The dried ginger was then ground to a fine powder using a blender.

2.2 Extraction

Extraction methods used in this experiment were reflux and maceration. In order to maximize extraction yield while minimizing the extraction cost, a solvent to sample ration of 1g per 20 mL was adopted (1).

For maceration, 10 g of ginger were each weighed and placed into 3 different Schott bottles before adding 200 mL of appropriate organic solvent (acetone, ethanol, and methanol independently). Schott bottle tops were covered with aluminium foil before being placed on an orbital shaker at 200 revolutions per minute for 8 hours. The heating coils were turned on for 10 minutes before running reflux. Then, 2.5 g of ginger and 50 ml of appropriate organic solvent were added into a round bottle flask. The sample was then refluxed at 90℃ for 30 minutes. Each of the samples was run in triplicate.

After solvent extraction, the ginger for each extract were then filtered through a cotton wrapped muslin cloth with an additional 100 ml of same fresh solvent into a conical flask. The extracts were then passed through a filter paper (Whatman No.1). The extracts of ginger were then evaporated using a rotary evaporator under reduced pressure at 40 °C. The extracts were removed and placed into falcon tubes. Trace amounts of solvent were evaporated by blowing nitrogen gas over the extracts.

2.3 Total Phenolic Content (TPC) Analysis

The total phenolic content was expressed as Gallic acid equivalent (GAE) in mg/100g of material. First, 10% Folin Ciocalteu (FC) reagent was prepared by diluting 10 mL of FC reagent into 100 mL of distilled water. Next, 7.5% sodium carbonate was prepared by diluting 7.5 g of sodium carbonate into 100 mL of distilled water. A stock solution of Gallic acid at a concentration of 0.1 mg/mL was prepared by dissolving 10 mg of Gallic acid into 100 mL methanol. Then the stock solution was diluted into 6 different concentrations ranging from 0.0031 to 0.1 mg/mL [18].

A pipette was used to transfer 1 mL of each extract into a falcon test tube. Then, 5 mL of 10% Folin-Ciocalteu reagent was added. After 5 minutes in the dark, 4 mL of 7.5% sodium carbonate was added. The solution was agitated with a vortex mixer for a minute before incubation at room temperature for an hour in the dark. The absorbance of the extracts and prepared blank were measured at 760 nm using a UV-visible spectrophotometer.

2.4 DPPH (1, 1-Diphenyl-2-Picryl-Hydrazyl) Radical Scavenging Assay

The free radical scavenging activity of *Z. officinale* extracts were measured using the DPPH assay based on the method by Tomsone *et al.* [19] with some modifications. A fresh stock of DPPH solution was prepared by dissolving 4 mg of DPPH with 100 mL of 70% ethanol in order to obtain a concentration of 0.004%. The DPPH was added to the extracts and the absorbance was determined at 515 nm using UV/VIS spectrophotometer after 30 minutes of incubation at room temperature in the dark. The colour changes were observed for the six extracts. Synthetic antioxidant ascorbic acid was used as a positive control. It was prepared by dissolving 100 mg of ascorbic acid in 100 mL 70% ethanol. The ascorbic acid was diluted in different concentrations of 0.0031 to 0.1 mg/mL. The same procedure was applied to each extract to measure the absorbance. The capability to scavenge the DPPH radical or known as inhibition percentage was calculated using the following formula:

$$I\ \% = [(A_{DPPH} - A_{sample})/ A_{DPPH}] \times 100$$

Where A_{DPPH} is the absorbance of the blank DPPH solution, and A_{sample} is the absorbance of the extracts.

2.5 High Performance Liquid Chromatography (HPLC)

A stock solution of 6-gingerol was prepared by dissolving 5 mg of 6-gingerol in 10 mL of 80% methanol then a series of dilutions were prepared ranging from

40 to 500 ppm. Then, 20 μL from each ginger extract was diluted in 4 ml of 80% methanol. Dilution extracts were passed through a filter using a 0.45 μm syringe filter into 2 mL vials prior to running the HPLC. The parameters for HPLC running conditions were adopted from Aly *et al.* [20] with some variations (Table 1).

Table 1: HPLC running conditions

Mobile phase	Acetonitrile:Water (50:50)
Flow rate	1.0 mL/min
Injection volume	20μL
Wavelength detection	282 nm
Run time	5 min
Temperature	50°C

2.6 Statistical Analysis

Triplicate analytical replications were carried out for both TPC and DPPH analysis while duplicate analytical replications were done for HPLC. Tabulated data was represented as the mean ± SD. A statistical analysis was carried out using the IBM SPSS Statistics 22 software.

An analysis of variance was performed by one-way ANOVA and significant differences between the means were determined by the Duncan's multiple range analysis ($p \leq 0.05$) and Pearson correlation ($p \leq 0.01$) to draw a relationship between TPC, DPPH, and 6-gingerol content [21].

3.0 RESULTS AND DISCUSSION

3.1 Determination of Total Phenolic Content (TPC)

For ethanol, the mean GAE values for the maceration and reflux methods were 263 and 205.4 mg/100g respectively. For acetone, the mean GAE for the maceration and reflux methods were 216 and 184 mg/100g respectively. For methanol, the mean GAE for the maceration and reflux methods were 148 and 95 mg/100g, respectively (Figure 1).

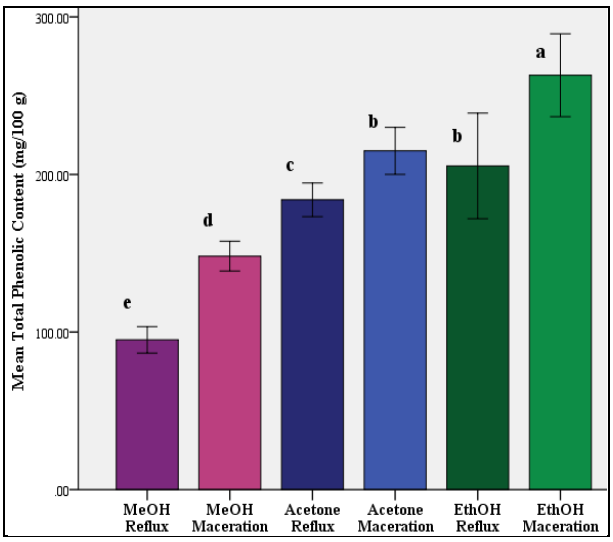


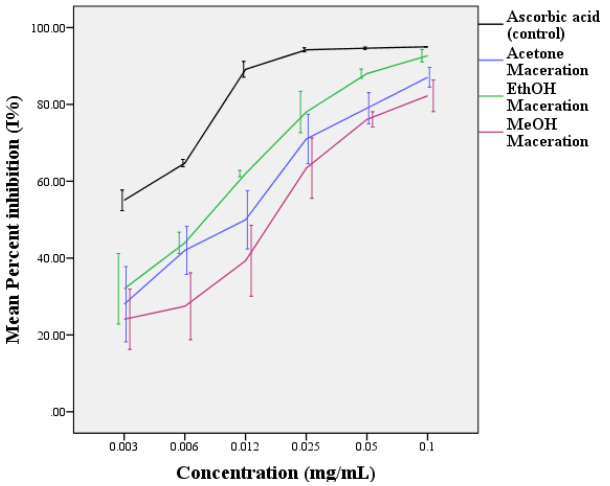
Figure 1 Mean GAE of ginger extracts. Different letters are significantly different at $p \leq 0.05$

All maceration extracts were significantly better when compared to the reflux extracts for all equivalent solvents. Ethanol maceration extracts significantly gave the highest TPC readings. However, the phenolic content between ethanol reflux extracts and acetone maceration extracts were not significantly different. True to its polarity index, ethanol proved to be the best extracting solvent [19].

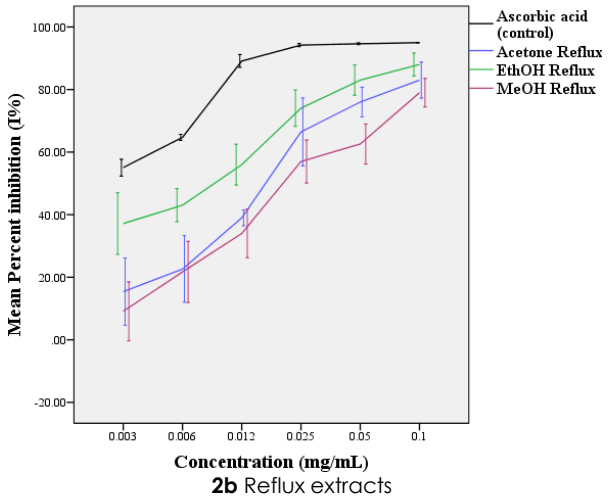
In contrast, acetone was significantly a better solvent at extracting polyphenols than methanol despite having a similar polarity index [22]. As a result, the plant matrix played a more important role than polarity index in polyphenol recovery [23]. The results also disagree with the findings of Sultana *et al.* [11] who found that reflux extracts would all yield higher phenolic content.

3.2 Determination Of DPPH· Radical Scavenging Acitivity

The antioxidant activity of maceration extracts was better than the reflux extracts for all equivalent solvents. The antioxidant activity was highest in ethanol followed by acetone and methanol. A percentage inhibition graph against concentration was plotted to determine antioxidant activity of reflux and maceration ginger extracts (Figure 2 a, b).



2a Maceration extracts



2b Reflux extracts

Figure 2 a, b Percent inhibition of ginger extracts.

Since percent inhibition reflects scavenged DPPH radicals, a higher percent inhibition will mean better antioxidant activity. For maceration extracts, ethanol showed the highest antioxidant activity with a mean inhibition of 93% followed by acetone with 87.1% and methanol with 82.2%. For reflux samples, ethanol showed the highest antioxidant activity with a mean inhibition of 88% followed by acetone with 83% and methanol with 69%.

A simpler way of representing the antioxidant activity of ginger extracts is depicted in Figure 3. The abbreviation IC_{50} is the concentration of the ginger extract (μg/mL) needed to scavenge 50% of the initial DPPH radicals. Since the IC_{50} value is inversely proportional to percent inhibition, a lower IC_{50} value correlates to better antioxidant activity [24].

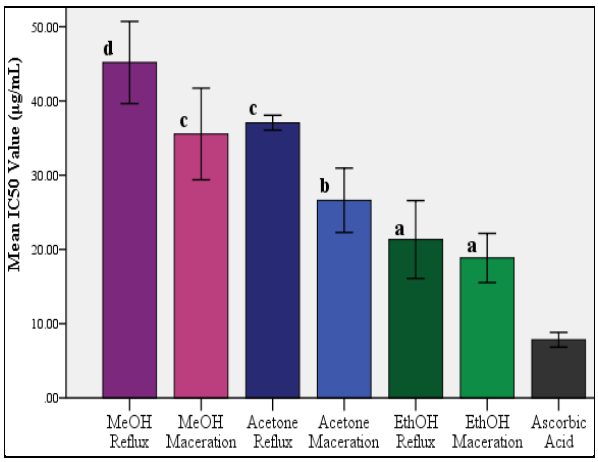
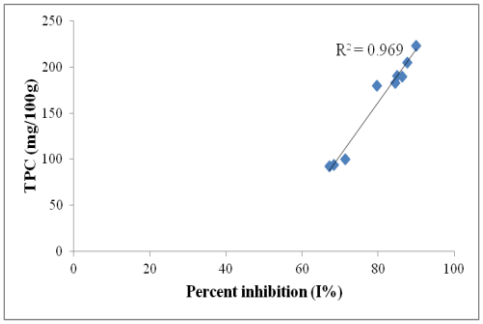


Figure 3 Mean IC_{50} value of ginger extracts. Different letters are significantly different at $p \leq 0.05$

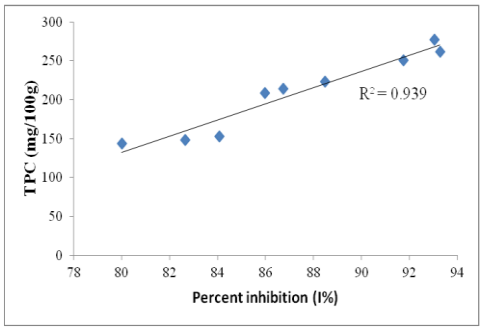
The ethanol extracts significantly yielded the best antioxidant activity. However, there was no significant difference in antioxidant activity between ethanol extracts using different methods. In contrast, a significant difference in antioxidant activity can be seen between methanol extracts using different methods and between acetone extracts using different methods. Polyphenol solubility plays an important role in antioxidant activity. As a result, an increase in total phenolic content is a direct correlation to an increase in antioxidant activity [19].

3.3 Correlation Between TPC And DPPH Radical Inhibition

Pearson correlation was done to compare the mean total phenolic content and mean DPPH radical inhibition (I%) and a graph was plotted (Figure 4). From the current study, regardless of the method or solvent used, a significant positive correlation between TPC and antioxidant activity was drawn. The reflux extracts showed better correlation than the maceration extracts with a steeper curve. It can be concluded that temperature played a more important role than time for antioxidant recovery due to its effectiveness in disrupting the cell wall.



4a Reflux extracts



4b Maceration extracts

Figure 4 Mean correlation between TPC and Percent inhibition (I%) of ginger extracts

3.4 HPLC Analysis Of 6-Gingerol Content

UV-HPLC was run to determine the 6-gingerol concentration in the ginger extracts **(Figure 6).** The maceration extracts yielded higher 6-gingerol content for all equivalent solvents. For maceration, the ethanol extracts yielded the highest 6-gingerol content (258.4 mg/L) followed by acetone (208.6 mg/L) and methanol (183.7 mg/L). Similarly, for reflux extracts, ethanol yielded the highest 6-gingerol content (226.3 mg/L) followed by acetone (189.7 mg/L) and methanol (159.2 mg/L).

Comparing different methods, the 6-gingerol content of ethanol extracts were significantly different. The 6-gingerol from the methanol extracts for different methods were also significantly different. In contrast, the 6-gingerol content between acetone extracts using different methods were not significantly different. Within each method, acetone extracts significantly yielded better 6-gingerol content than methanol extracts, agreeing with a finding by Usman *et al.* [25].

A significant positive correlation between TPC and 6-gingerol content can be seen in Table 2. Since 6-gingerol was the most abundant bioactive polyphenol constituent in *Z. officinale*, it was understandable that better polyphenol extractions yielded higher 6-gingerol content.

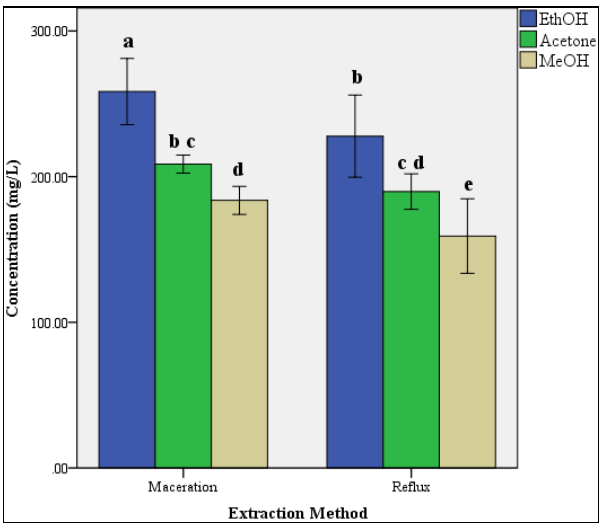


Figure 6 Concentration of 6-gingerol. Letters (a to e) indicate significant different at p ≤0.05

Table 2 Correlation of 6-gingerol and TPC

		TPC	6-gingerol
TPC	Pearson Correlation	1	.950*
	Sig. (2-tailed)		.004
	N	6	6
6-gingerol	Pearson Correlation	.950*	1
	Sig. (2-tailed)	.004	
	N	6	6

* Correlation is significant at p ≤ 0.01

4.0 CONCLUSION

Different solvents and different methods affect the yield of polyphenol extraction. Two different methods of extraction, reflux and maceration, were carried out using 3 different solvents to determine their effect on the TPC, antioxidant activity, and 6-gingerol content present in *Zingiber officinale*. Between the different methods, maceration extracts generally did better than reflux for all 3 categories.

Between the solvents, ethanol was significantly the best followed by acetone and methanol respectively. Among the different methods and solvents the ethanol maceration extracts significantly yielded the best TPC and 6-gingerol. However, in terms of antioxidant activity, ethanol maceration extracts did not prove to be significantly better than the ethanol reflux extracts. Regardless of the method, acetone extracts showed significantly better TPC than methanol extracts. However, the antioxidant activity and 6-gingerol content of the acetone reflux extracts were not significantly different from the methanol maceration extracts. The similarities between acetone and methanol extracts can be explained in terms of their similar polarity index.

A significant positive correlation could be made between total phenolic content, 6-gingerol content and antioxidant activity for both extraction methods.

Reflux extracts showed better correlation between TPC and antioxidant activity than the maceration extracts.

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