Study on Polyphenol Content and Antioxidant Activity of Ginger Rhizome from Local Market

Hla Thidar Aung
Meiktila University
aunghlathidar@gmail.com

Tun Zaw Moe
Meiktila University

Ni Ni Aung
Meiktila University

Abstract

According to literature report, ginger rhizomes (Zingiber officinale Rosc.) has been known to use in Myanmar traditional medicine system in connection with antioxidant, antimicrobial and anticancer activities and therefore locally grown ginger (Zingiber officinale Rosc.) has been chosen for this study. This research aims to investigate the polyphenol content and antioxidant activity of the selected sample. The total phenolic content was determined by Folin-Ciocalteau assay method and it was found to be 14.572 and 3.64 EAE/mg respectively for ethanol and watery extract. Antioxidant activity of the sample was determined by DPPH method at Meiktila University and it possessed IC_{50} values of 12.21µg/mL for ethanol extract and 48.34µg/mL for watery extract of Zingiber officinale Rosc.

***Keyword** : ginger. polyphenol, antioxidant, Folin-Ciocalteau assay*

1. Introduction

Ginger is well known spice and flavoring agent which has also been used in traditional medicine in many countries. This large seasonal plant is cultivated in Southeast Asia and China, India and some parts of Africa.

Ginger a source of valuable phytonutrients, is characterized as having an aromatic odor and a pungent taste [2]. The part of the ginger plant that is used is the root, which is botanically the rhizome. The flat surfaces of the rhizome are removed, leaving the remains of the underground stem [7]. Ginger contains essential oils including gingerol and zingiberene. It also contains pungent principles such as zingerone, gingerol and shogaol [7].

Botanical Aspect of Ginger Rhizomes (*Zingiber officinale* Rosc.)

Botanical name	:	*Zingiber officinale*
Family	:	Zingiberaceae
Genus	:	*Zingiber*
Species	:	*Z.officinale*
Common name	:	Ginger
Myanmar name	:	Gin
Part used	:	Rhizome

Figure.1 Ginger Rhizomes (*Zingiber officinale* Rosc.)

Antioxidants

Antioxidants are the substances that may protect cells from the damage caused by free radicals. Antioxidants interact with and stabilize free radicals might otherwise cause. The antioxidants may be exogenous or endogenous in nature. The endogenous antioxidants can be classified as enzymatic and non-enzymatic.

2. Materials and Methods

Sample Collection

The samples of the rhizomes of *Zingiber officinale* Rosc. (Ginger) were collected from Meiktila Central Market, Meiktila Township, Mandalay Region, Myanmar.

Determination of Total Phenol Content by FCR Method

One of the anti-oxidative factors, total phenolic content (TPC) was measured spectrophotometrically according to the Folin-Ciocateu method [9].

Procedure
Construction of gallic acid standard curve

At first, 1mL of different concentration of gallic acid solution (20, 10, 5,2.5,1.25, and 0.625µg/mL) was mixed with 5mL of FC reagent sodium carbonate solution was added and the tubes were kept at room temperature for 2 hours and the absorbance of reaction mixture was measured at λ_{max} 765 nm. A standard curve was constructed by plotting the absorbing against concentration of gallic acid [6]. The data is shown in Table 1.

Determination of Total Phenol contents as Gallic acid Equivalent in Sample

Each extract solution (1mL) was mixed with 5 mL of FC reagent (1:10) and incubated for about 5 min. To each test tube, 4 mL of 1M sodium carbonate was added and the test tubes were kept at room temperature for 2hrs and absorbance of reaction mixtures was measured at λ_{max} 765 nm. The blank solution was prepared as the above procedure by using distilled water instead of sample solution. Total phenol content was estimated as microgram gallic acid equivalent per milligram (µg/ GAE/mg) of extract [6]. The TPC contents of tested samples are shown in Table 2.

Determination of Antioxidant Activity of Crude Extracts of Ginger Rhizomes by DPPH Free Radical Scavenging Assay

The free radical scavenging activity of crude of ginger rhizomes was measured using free radical scavenging assay [4].

Procedure

DPPH radical scavenging activity of ethanol and watery extracts of ginger rhizomes was determined by UV-visible spectrophotometer [4]. The absorbance of these solutions were measured at 517 nm and the percentage of radical scavenging activity (% RSA) was calculated by the following equation. % RSA of crude extract of ginger rhizomes results are shown in Table 3 and Figure 2. IC_{50} (50% inhibition concentration) values were calculated by linear regressive excel program. The standard deviation was also calculated by the following equation [8][5][3].

$$\% \text{ inhibition} = \frac{\text{DPPH alone} - (\text{sample} - \text{blank})}{\text{DPPH alone}} \times 100\%$$

Where
% inhibition = percent inhibition of test sample
DPPH alone = absorbance of DPPH solution
Sample= absorbance of sample solution
Blank = absorbance of blank solution

3. Results and Discussion

Total Phenolic Content of Crude Extracts of Ginger Rhizomes (*Zingiber officinale* Rosc.)

The phenolic compounds are plants metabolism characterized by the presence of several phenol groups. Some of them are very reactive in neutralizing free radical by donating a hydrogen atom or an electron [1]. Phenolic compounds have antioxidant properties of protective against degenerative disease like heart disease and cancer.

The total phenolic contents of EtOH and watery extracts of ginger rhizomes were evaluated with spectrophotometric method using Folin-Ciocalteau reagent. The principle of this method is reduction ability of phenol functional group. Phenols react with an oxidizing agent phosphomolybdate in Folin-Ciocalteu reagent under alkaline conditions and resulted in the formation of blue colored complex [9]. The reaction of complex will increase when the extracts contain more phenolic compounds. Thus, the colour will be darker and the absorbance will be higher. The absorbance can be measured at UV 765nm. Gallic acid (3,4,5-trihydroxybenzoic acid) was used to construct standard calibration curve. Total phenolic content was expressed as microgram gallic acid equivalent per milligram (µg GAE/ mg) of crude extract. In this study, high phenolic contents have been found to extract high antioxidant potential. The study has shown a direct relation between antioxidant activity and total phenolic contents. According to the results, the higher TPC ((µg GAE/mg) was detected in ethanol (14.57 µg GAE/mg) than water (3.64 µg GAE/mg) extracts of ginger rhizomes. This means that phenolic compounds were more soluble in ethanol.

Table 1. Absorbance of gallicacid standard solution at λ_{max} 765nm

No.	Concentration (µg/mL)	Absorbance
1.	0.625	0.024
2.	1.25	0.042
3.	2.5	0.075
4.	5	0.148
5.	10	0.286
6.	20	0.573

Table 2. Total phenolic content (TPC) in EtOH and watery extracts of ginger rhizomes (*Zingiber officinale* Rosc.)

No.	Extracts	TPC (µg GAE/mg)
1.	EtOH	14.57
2.	Watery	3.64

GAE = Gallic Acid equivalent

Screening of Radical Scavenging Activity of Crude Extracts from Ginger Rhizomes Sample

By solvent expression of ginger rhizomes sample with various solvents, two extracts namely EtOH and watery were available for screening of radical scavenging activity by DPPH method.

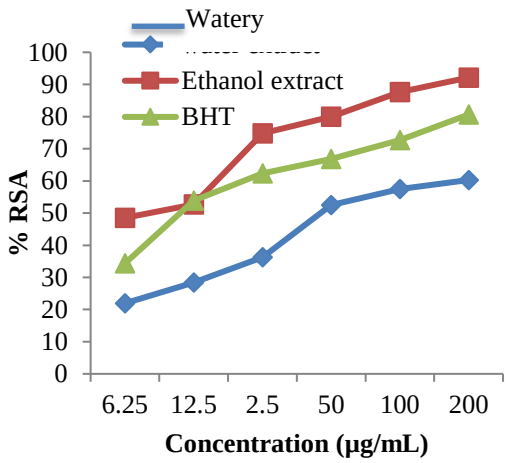

Figure 2. A plot of radical scavenging activity (%RSA) Vs (µg/mL) of EtOH and watery extract from *Zingiber officinale* Rosc. and standard BHT

Determination of radical scavenging activity by DPPH method depends on the change in absorbance of crude extracts solution in various concentrations. Six kinds of concentration namely 6.25, 12.5, 25, 50, 100 and 200 µg/mL were prepared by dilution with ethanol, BHT (Butylated Hydroxyl Toluene, synthetic antioxidant) was used standard sample and ethanol without crude extract was employed as control. Blank solution was also prepared by mixing sample and ethanol only, not including DPPH. Sample solution was prepared by mixingsample and DPPH solution. Determination of absorbance was carried out at wavelength 517 nm using spectrophotometer. Decrease in absorbance indicates increase in radical scavenging activity.

The absorbance value of two extracts and BHT were described in Table 3 and plot of concentration versus average absorbance was shown in Figure 2. From the average values of percent inhibition IC_{50} values in µg/mL (50% inhibition concentration) were calculated by computer program called linear regressive Excel Program. From these results, it was also observed that increase in concentration showed increase in percent inhibition, i.e., increase free radical scavenging activities.

IC_{50} values of two extracts indicated that free radical scavenging activity of ethanol extract (IC_{50}= 12.21 µg/mL) is greater than that of watery extract (IC_{50} = 48.34 µg/mL). It was found that radical scavenging activities of EtOH extract nearly equal to that of BHT (IC_{50} = 11.25 µg/mL).

The high radical scavenging activity of both extracts may be probably due to the presence of relatively more polar constituents present in these extracts. Therefore it indicated that the higher activity was used to obtain the active

Table 3. Radical scavenging activity (%RSA) and IC_{50} value of EtOH and watery extract from *ZingiberofficinaleRosc*. and standard BHT

Sample	% Radical Scavenging activity						IC_{50} µg/mL
	6.25 µg/mL	12.5 µg/mL	25 µg/mL	50 µg/mL	100 µg/mL	200 µg/mL	
Watery extract	21.95	28.37	36.24	52.46	57.51	60.29	48.34
EtOH extract	48.52	50.67	74.83	79.98	87.64	92.16	12.21
BHT	34.36	53.87	62.38	66.87	72.75	80.65	11.25

4. Conclusion

In the present work, total phenol contents and antioxidant activity of ginger rhizomes (*Zingiber officinale* Rosc.) were tabulated.

The total phenolic content was determined by Folin-Ciocalteau assay. The total phenolic content of EtOH and watery extracts were found to be14.57 and 3.64µg GAE/mg respectively. The total phenolic content of ethanol extract was higher than that of watery extract.

The antioxidant activity of ethanol and watery extracts of ginger rhizomes was evaluated by DPPH free radical scavenging assay. These two crude extracts were found to possess antioxidant properties. The EtOH extract (IC_{50}=12.21µg/mL) was found to be the more potent than watery extract (IC_{50}=48.34µg/mL) in antioxidant activity. Standard BHT showed the highest antioxidant activity (ID_{50}=11.25 µg/mL) compared to that of EtOH and watery extracts of ginger rhizomes.

The findings from the present work will contribute to the scientific development of Myanmar traditional medicine. According to the above mentioned scientific results from the investigation of some bioactivities, it may be concluded ginger rhizomes can be used with the advantage for the treatment of the diseases related to the ageing process problems.

Acknowledgement

We would like to express our deepest thanks to Dr Ba Han, Rector Meiktila University, for his kind permission and research facilities to carry out this paper.

References

[1] A.A. Almey, C.A.J. Khan, J.S. Zahir, K.M. Suleiman, M. R. Aisyah, and K. K. Rahim, "Total Phenolic Content and Primary Antioxidant Activity of Methanolic and Ethanolic Extracts of Aromatic Plants Leaves". *International Food Research Journal*, 2010, 17, pp.1077-1084

[2] Bernard, A. and M. Ferda, *Biochemical Engineering and Biotechnology Handbook*, New York: 1st Ed., The Nature Press, 1983, pp.475-487

[3] Costantino, L., A. Albasini, G. Rastelli, and S. Benvenuti, *Activity of Polyphenolic Crude Extracts as Scavengers of Superoxide Radicals and Inhibitors of Xanthine Oxidase*, Planta Medica, 1992, 58, pp.342-344.

[4] G. Marinova, and V. Batchvarov, "Evaluation of the Methods for Determination of the Free Radical Scavenging Activity by DPPH". *BulgarianJournal of Agricultural Science*, 2011, 17, pp.11-24

[5] Imadia, K., S. Fukushima, T. Shirai, M. Ohtani, K. Nakanish, and N. Ito, *Promoting Activities of Butylated Hydroxytoluene on 2-stage Urinary Bladed Carcinogenesis and Inhibition of c-glutamylTranspep-tidase- Positive Foci Development in the Liver of Rats*, Carcinogenesis, 1983, 4, pp.895-899

[6] M. Maizura, A. Aminah, and W.M.W. Aida, "Total Phenolic Content and Antioxidant Activity of Kesum (Polygonum minus), Ginger (Zingiberofficinale) and Turmeric (Curcuma longa) Extract".*International Food Research Journal*, 2011, 18, pp.529-534

[7] M. Majeed, Ginger (*Zingiberofficinale*): product write up. Product Insight Paper 1-7, 2007.

[8] P. Kiran, S. Chagani, S.R. Gundala, P.C.G Rida, G. Asif, V. Sharma, M. V. Gupta, and R. Aneja, "Benefits of Whole Ginger Extract in Prostate Cancer". *British Journal of Nutrition*, 2012, 107, pp.473-484.

[9] S. Dudonnen, X. Vitrac, P. Coutiere, M. Woillez, and J.M. Merillon, "Comperative Study of Antioxidant Properties and Total Phenolic Contents of 30 Plant Extracts of Industria Interest Using DPPH, ABTS, FRAP, SOD and ORAC Assays". *J. Agric. Food Chem*, 2009, 57, pp.1768-1770 compound from the isolation of these crude extracts.