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In vitro Antioxidant Activity and Phytochemical Study of *Arbuscular mycorrhizal* Fungi Induced Red Ginger (*Zingiber. officinale* var. *rubrum*)

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ABSTRACT

Ginger (*Zingiber officinale*), a member of the Zingiberaceae family, has been shown to have anti-inflammatory, antioxidant, anti-nausea/antiemetic, antibacterial, cytotoxic, and antidiabetic effects. Red ginger rhizome has been used as a spice, culinary flavoring, and herbal medicine. Our research shows that gingerol has the potential to be an anti-oxidant. The Assay of Antioxidant Activity using FRAP method is used to screen potential activity. The gingerol concentration of Ginger-Ethanolic extract (MGE) was 2.8 %, and the antioxidant activity was 1.96 mmol Fe(II)/100g, to the antioxidant activity of gallic acid that is 9.34 mmol Fe (II)/100 g. The equation for the standard calibration curve for comparison (6)-gingerol obtained is Y = 23.124 X + 1509.65. It can be concluded that MGE has antioxidant potential activity.

Keywords: *Ginger, Arbuscular mycorrhizal Fungi, Chemical Content, Antioxidant Activity.*

1. INTRODUCTION

Herbal remedies are generally considered part of a dietary supplement. The long history of use and belief in the community that herbal medicines are natural and safe for consumption[1]. In some countries such as Singapore and the Republic of Korea, 76% and 86% each population still commonly uses herbal medicine[2].

Z. officinale has become a common crop in many countries, and widely grown in tropical countries and subtropics in the world. The rhizome is often used as a spice for variety of food and drink [3]. It is a rhizome that has been used for generations in various types of traditional medicine in Asia, Greece, Rome, India, Arabia, and the Mediterranean. The plant is used to treat ailments such as hypertension, dementia, fever, infectious diseases, diabetes, prevent nausea and vomiting in different digestive disorders. The content of bioactive metabolites has anti-inflammatory, anticancer, antioxidant, and antiemetic properties [4].

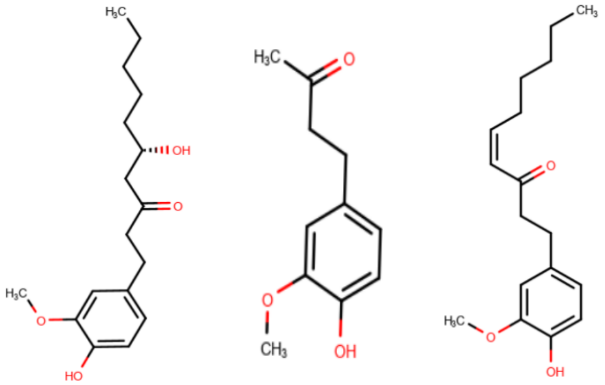

Figure 1. Chemical structures of gingerol, zingerone and shogaol[5]

In vitro studies show that ginger is beneficial for cardiovascular disease, inflammation, hyperlipidemia, thrombocytopenia aggregation, and hypertension [6].

Hydroxyl and carbonyl groups on gingerol carbon chains increase activity. glycosylation of shogaol phenolic hydroxyl groups increased melanogenesis inhibitory activity [7]. The active compounds that have potential vasorelaxant effects are 6-gingerol, 8-gingerol and 6-shogaol [8]. 6-gingerol and 6-shogaol compounds have antioxidant activity [9].

Arbuscular Mycorrhizal fungi (AMF) grow on the host plant strongly, the reciprocal relationship between the plant and this fungus increases the rate of photosynthesis and gas exchange [10]. It can be reproduced by undergoing hyphal fusion (anastomosis) and exchanging genetic material, thereby altering their genomes and resulting in genetic diversity [11]. Inoculation with *Mycorrhizal Fungi* affects the biomass production of shoots, roots and rhizomes of ginger can reduce the use of chemical fertilizers [12]. Our research aims to determine the antioxidant benefits, toxicity and physicochemical properties of Red Ginger (Zingiber. officinale var. rubrum).

2. METHODS

2.1. Materials

The rhizome of the ninth month Arbuscular Mycorrhizal fungi induced red ginger was cultivated and collected from Tarok village Padang Pariaman District Indonesia. (6)-gingerol obtain from Mark Herb Laboratory, PT.EBM Saintific and Technology Bandung-Indonesia. The chemicals and solvents used in this experiment were ethanol and methanol (Excelar reagent grade), chloroform, gallic acid, Folin-Ciocalteu reagent, sodium carbonate, ortho-phenanthroline, aquadest, iron (II) sulfate heptahydrate, sodium acetate trihydrate, and iron (III) chloride hexahydrate, concentrated acetic acid, ethyl acetate, *n*-hexane, ethanol 96%. The analytical solvents used are analytical reagents such as Dimethyl sulfoxide (DMSO), and all solvents.

2.2. Extraction

The plant material was sliced and dried for three days, then oven-dried at 40°C for 24 hours. Dried rhizomes are ground into a fine powder using a grinder. 2 kg ginger rhizome powder was macerated in 7 L 70% ethanol for three days. This process is repeated three times. The ethanol extract was evaporated and concentrated with a rotary evaporator at a temperature of 40°C. The resulting extract is stored in the refrigerator. It was dried under pressure using a rotary evaporator.

2.3 Assay of Antioxidant Activity with FRAP Method

FRAP working solution was prepared each time freshly: 0.3 m acetate buffer, 0.01 m TPTZ (2,4,6-tripyridyl-s-triazine) in 0.04 m HCl, and 0.02 m FeCl3+ were mixed and kept away from light. 0.1 ml of MGE solution (concentration 5 mg/mL) were added to 3.0 ml FRAP working solution and 0.3 ml distilled water. The mixture was vortexed and incubated at 37°c for 30 min away from light. Absorbance was measured at 510 nm using the spectrophotometer. FRAP working solution with distilled water instead of a sample was used as a blank. Antioxidant activity of the MGE samples at concentration 5 mg/mL was expressed in (Fe 2+ mM) equivalents using standard heptahydrate ferrous sulfate with a concentration of 0.3; 0.4; 0.5; 0.6; and 0.7 mmol/L.

2.4 Content Determination of Gingerol

The determination content of gingerol on MGE was carried out by TLC densitometry. The maximum wavelength of (6)-gingerol obtained is 525 nm, the r-value of the standard calibration curve equation (6)-gingerol is close to 1. The eluent that gives the best separation in MGE TLC is *n*-hexane-ethyl acetate (13:7) with an Rf value of 0.3.

3. RESULTS AND DISCUSSION

The antioxidant effect of Ginger-Ethanolic extract (MGE) was tested using the FRAP method. Totally 201.8 mg of MGE dissolved in 10 ml ethanol. FRAP solution was prepared each time freshly: 0.3 M acetate buffer, 0.01 m TPTZ (2,4,6-tripyridyl-s-triazine) in 0.04 M HCl, and 0.02 M FeCl3+were mixed and kept away from light. 0.1 ml of MGE solution were added to 3.0 ml frap working solution and 0.3 ml of deionized water. The mixture was vortexed and incubated at 37°c for 30

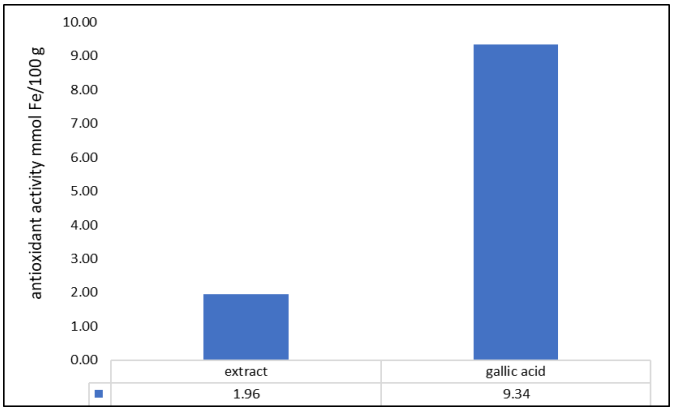

Figure 2. Graph Antioxidant activity of MGE compared to gallic acid

min away from light and obtained the results of its antioxidant activity of 1.96 mmol Fe (II)/100 g compared to the antioxidant activity of gallic acid that is

Table 1. Concentration and absorbant of iron (II) equivalents (Fe^{++} mM) using standard heptahydrate iron sulfate

No	Concentration (mmol/L)	Absorbant
1	0.3	0.215
2	0.4	0.341
3	0.5	0,504
4	0.6	0,634
5	0.7	0,781

9.34 mmol Fe (II)/100 g.

Antioxidant activity depends on the number and position of the hydroxyl groups of the aromatic ring binding site and the type of substituent of gingerol content. The amount of Fe^{+3} ions reduced to Fe^{+2} ions varies for different concentrations of phenols. Phenols with two hydroxyl groups bonded to the aromatic ring in the ortho position.

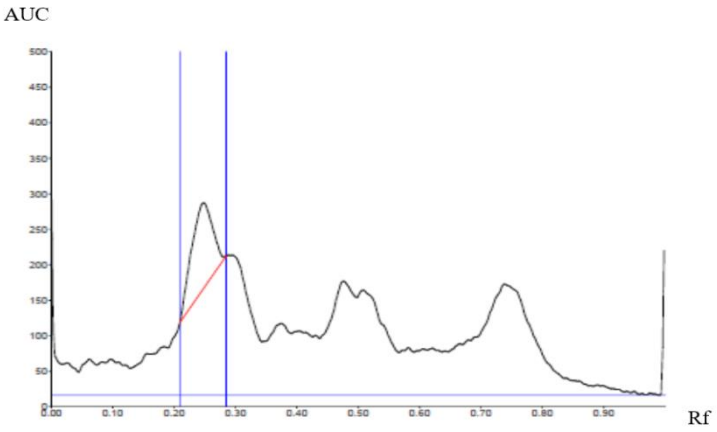

Figure 3. The histogram of 6-gingerol on MGE.

Antioxidant activity of MGE is lower than antioxidant activity of gallic acid as reference (Figure 2). The concentration-response curve shows the calculated concentration. The absorbance of the sample was measured at maximum absorption wavelength 510 nm. FRAP reagent solution with distilled water was used as a blank solution. The antioxidant activity of the samples was expressed in iron (II) equivalents (Fe^{+2} mM) using standard heptahydrate iron sulfate 0.3; 0.4; 0.5; 0.6; and 0.7 mmol/L. The equation for the standard calibration curve for comparison (6)-gingerol obtained is Y = 23.124 X + 1509.65 (Table 1). The r-value of the linear regression equation obtained is 0.99, by using (6)-gingerol as a reference with the linearity, the value is 0.995 and the gingerol content of MGE was 2.8%.

The histogram analysis (Figure 3) is performed to determine the AUC of 6-gingerol, the Rf value is obtained in the range of 0.21-0.28. There are 3 peaks of the AUC curve, namely 180, 190 and 280 (Figure 3). Identification of the content of 6-gingerol in MGE was carried out by comparing the Rf value of the sample with the standard Rf value of 6-gingerol. Two compounds are said to be similar if they both have the same Rf value measured.

4. CONCLUSION

MGE has an antioxidant activity of 1.96 mmol Fe (II) / 100g. MGE does have a lower antioxidant activity than gallic acid, which is used as a reference. The quantity and position of hydroxyl groups in the aromatic ring binding site, including the type of substituent in gingerol, can influence the antioxidant effect.

AUTHORS' CONTRIBUTIONS

N.S contributed by performing the analysis and wrote the paper. Dachriyanus, H.L, and F.S.W contributed by designed the research. P.P.P contributed by writing the paper

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