

Potency of Radical Scavenging Activity and Determination of Total Phenolic Content of Five Ethanolic Extract of Rhizome Zingiberaceae Family

Muhammad Da'i*, Didiek Setiawan, Rosita Melannisa

Faculty of Pharmacy, Universitas Muhammadiyah Surakarta, Jalan Achmad Yani, Sukoharjo, Indonesia

Abstract

Several studies show that some plants and fruits could protect human body from free radical danger exposure. Zingiberaceae family has some chemical substances that have antiradical activity, such as phenolic compound and flavonoid. The aim of this study is to determine the correlation of total phenolic compound towards its antiradical activity. Antiradical activity assay was determined by DPPH method (1,1 difenil-2-pikrilhidrazil). The total phenolic compound is determined by spectrofotometry using Folin-Ciocalteu reagent and counted as GAE (Gallic Acid Equivalent). Based on this study, the total phenolic concentration of ethanolic extract of jahe (*Zingiber officinale* (L.) Rosc), kencur (*Kaemferia galanga* L.), lengkuas (*Languas galanga*), lempuyang gajah (*Zingiber littorale*) and lempuyang pahit (*Zingiber americana*) is 73.99 mg/g; 23.848 mg/g; 37.841 mg/g; 49.725 mg/g and 40.802 mg/g respectively. The radical scavenging activity (IC₅₀) of jahe (27.38 µg/mL), lengkuas (93,27 µg/mL), lempuyang pahit (105,08 µg/mL), meanwhile lempuyang gajah and kencur show low activity of radical scavenging.

Keywords: Zingiberaceae, total phenolic compound, antiradical, DPPH

INTRODUCTION

Free radical is an atom or atomic group having one or more uncoupled electron (Fessenden and Fessenden, 1986). Free radical in human body is very reactive and can interact with some parts of body or cells. This condition can happen continuously and causing the damage to the human tissue. Approximately as many as 40 diseases including atherosclerosis, hypertension, ischemy, alzheimer, parkinson, cancer, and the inflammation can be caused by free radical damage (Niki, *et al.*, 1995; Khanom, *et al.*, 2000; Tripathy, *et al.*, 2001; Toda, *et al.*, 1991; Behera, *et al.*, 2004).

Antioxidant is needed in order to inhibit the free radical activity. Some antioxidant compounds contained in plants and fruits such as ascaroten, flavonoid, and others phenolic compounds (Teow, *et al.*, 2006). Other compound such as vitamin C and E (Windono, 2001) showed some benefits in the body protection of free radical (Rohman and Riyanto, 2006).

Family of Zingiberaceae is known as antioxidant source. The member of

Zingiberaceae family such as jahe (*Zingiber officinale* (L.) Rosc), kencur (*Kaemferia galanga* L.), lengkuas (*Languas galanga*), lempuyang gajah (*Zingiber littorale*), and lempuyang pahit (*Zingiber americana*) have been used in traditional medication. Jahe (*Zingiber officinale* (L.) Rosc) has been reported to have antioxidant, anticancer, antiinflammation, antiangiogenesis, antiatherosclerotic, and inhibition ability towards carcinogenic agents (Shukla, 2007; Surh, 2003; Kim, 2004; Shafina, 2008). Kencur (*Kaemferia galanga* L.) extract has antibactery (Tewtrakul, *et al.*, 1983), anticancer, antihypertensi, larvasidal activity and can also be used as skin disease therapy, rheumatic, and mellitus diabetic (Tara, *et al.*, 2006).

Lengkuas (*Languas galanga* L.) contain 1'-acetoxychavicolacetate (ACA) and 1'-acetoxyeugenolacetate as antitumor compound (Zaeoung, *et al.*, 2004). Lempuyang gajah (*Zingiber littorale*) reported to have antiulcer, antioxidant, antimicrobia (Bhuiyan, *et al.*, 2009), antitumor and antiinflammation (Murakmi, *et al.*, 2002; Sakinah, *et al.*, 2007; Abdul, *et al.*, 2008). Some compounds

contained in *Zingiberaceae* family are phenolic substances such as gingerol, shogaol, gingeron (Ravindran, et al., 2005), bidescurcuminoid (Wulandari, 1996), kaempferol (Usia, et al., 2004), flavonoid, terpenoid and non polar constituents such as curcuminoid, kava piron and gingerol (Sirat, et al., 1996). Those substances have been known to have antiradical (Sirat, et al., 1996). The study about the comparison of radical scavenging activity *Zingiberaceae* (jahe, kencur, lengkuas, lempuyang gajah and lempuyang pahit) and the concentration of total phenolic constituent that probably responsible as antiradical compound needs to be done and to be further analyzed in the correlation between phenolic compound concentration and the radical scavenging activity by DPPH method.

MATERIALS AND METHODS

Materials

Five ethanolic extracts from jahe (*Zingiber officinale* (L.) Rosc), kencur (*Kaempferia galanga* L.), lengkuas (*Languas galanga*), lempuyang gajah (*Zingiber zerumbet*) and lempuyang pahit (*Zingiber americana*) were obtained from Faculty of Pharmacy Universitas Muhammadiyah Surakarta, DPPH (2,2-diphenyl-1-picrylhydrazyl), etanol *p.a.*, technical ethanol, aqua bidestilata, vitamin E, asam galat, *Folin Ciocalteu* reagent, Na_2CO_3 , *aluminium foil*, TLC plates.

Determination of Total Phenolic Concentration

Based on the modified method from Chun, et al. (2003), the determination of total phenolic compound of the extract was done by Folin Ciocalteu method with Galic acid as the reference. The value obtained was the equivalence of miligram of galic acid per gram of extract Gallic Acid Equivalent (GAE).

Antioxidant Activity Assay (DPPH method)

Sample from the stock solution of ethanolic extract from jahe (*Zingiber officinale* (L.) Rosc), kencur (*Kaempferia galanga* L.), lengkuas (*Languas galanga*), lempuyang gajah (*Zingiber zerumbet*), lempuyang pahit (*Zingiber americana*) and vitamin E were prepared in five different concentrations. Sampel added by 0.7 mL DPPH 0.4 mM and ethanol added up to 5.0 mL. This mixture was homogenized by mxing for 30 seconds and incubated for 30 minutes.

The sampel absorbance were measured by UV-Vis spectrofotometer (Shimadzu) with λ_{max} 517.6 nm. The sample absorbance also compared with the control solution containing 0.7 mL DPPH 0.4 mM diluted in ethanol. The percentage (%) of antiradical activity were measured. The linier regretion between concentration curve versus antiradical activity precentage were obtained. Then, the linier regretion formula and sample concentration at 50% activity were determined.

RESULT AND DISCUSSION

Total Phenolic Concentration

Total phenolic concentration was determined by using Folin-Ciocalteu reagent. The mechanism of this reagent is based on the hydroxy-fenolic group's reducing ability. The existence of aromatic groups in phenolic compounds (hydroxy phenolic groups) can turn phosphomolybdate and phosphotungstate into molybdenum which have blue colour (Sudjadi and Rohman, 2004).

The total phenolic concentration of rhizomes ethanolic extract from the highest to the lowest are jahe (73.992 mg/g), lempuyang gajah (49.725 mg/g), lempuyang pahit (40.802 mg/g), lengkuas (37.841 mg/g) and kencur (23.848 mg/g) (Fig. 1).

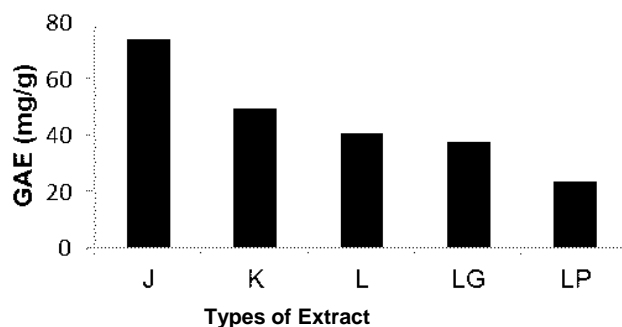


Figure 1. Total phenolic concentration from J (Jahe), K (Kencur), L (Lengkuas), LG (Lempuyang gajah) dan LP (Lempuyang pahit). Total phenolic concentration were determined by using Folin-Ciocalteu reagent. Galic acid was used as the reference. The value obtained was the equivalence of miligram of galic acid per gram of extract Gallic Acid Equivalent (GAE).

Radical Scavenging Activity

Antiradical activity determined by the ability of a compound contained in extract to reduce the purple colour intensity of DPPH radical in its maximum wavelenght (Rohman and Riyanto, 2006). The reduction of purple colour intensity of DPPH radical is caused by the decrease of chromophore or conjugated double bond in DPPH compound. This caused by the existence of extracts compounds which scavenging the radical by donating hydrogen atom to DPPH structure so that become reduced DPPH-H. DPPH-H is a compound

which have yellow colour (Huang, *et al.*, 2005).

Based on Reynertson, *et al.* (2007), a compound can be determined as an active antioxidant if its inhibitory concentration 50% (IC_{50}) value is lower than 50 $\mu\text{g/mL}$. The medium antioxidant activity is determined if the IC_{50} value of a compound is between 50-100 $\mu\text{g/mL}$. The IC_{50} value between 100-200 $\mu\text{g/mL}$ showed that the compound is less active in antioxidant activity, meanwhile IC_{50} value more than 200 $\mu\text{g/mL}$ showed that the compound is not active as antioxidant.

Table 1. The comparison of the antiradical activity of extracts

Extract	Concentration ($\mu\text{g/mL}$)	Antiradical activity(%)
Jahe	100	80.03 \pm 0.593
Kencur	100	35.58 \pm 4.284
Lengkuas	100	46.04 \pm 3.531
Lempuyang gajah	100	38.61 \pm 1.678
Lempuyang pahit	100	55.31 \pm 5.785

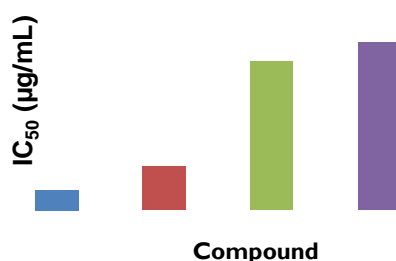


Figure 2. The IC_{50} values of ethanolic extract of Jahe, lengkuas, lempuyang pahit and vitamin E. The sampel absorbance were measured by UV-Vis spectrofotometer with λ_{max} 517.6 nm.

The parameter used in the interpretation of antiradical activity is the value of IC_{50} . IC_{50} is the sample concentration having 50% antiradical activity compared to control which obtained by linier regrestion between the concentration versus the percentage of antiradical activity (Rohman and Riyanto, 2006). The results on radical scavenging activity of ethanolic extract of jahe, lengkuas, lempuyang pahit are compared to on radical scavenging activity of vitamin E.

The radical scavenging activity respectively from the lowest to the highest activity are vitamin E (12.55 $\mu\text{g/mL}$); extract jahe (27.38 $\mu\text{g/mL}$); lengkuas (93.27 $\mu\text{g/mL}$); lempuyang pahit (105.08 $\mu\text{g/mL}$) (Fig. 2).

Correlation between Radical Scavenging Activity and Total Phenolic Concentration

This study shows that radical scavenging activity (IC_{50}) and total phenolic concentration (GAE) of ethanolic extract of jahe (27.38 $\mu\text{g/mL}$; 73.992 mg/g), lengkuas (93.27 $\mu\text{g/mL}$; 37.841 mg/g), and lempuyang pahit (105.08 $\mu\text{g/mL}$; 40.802 mg/g).

The higher concentration of total phenolic concentration in the extract that means the higher antioxidant activity. The correlation between phenolic concentration and antioxidant activity of the rhizom is known by the correlation coefficient (R^2) from linier regrestion equation between antioxidant activity and total phenolic concentration.

Based on the correlation between total phenolic concentration and the antiradical activity (IC_{50}) by using linier regrestion equation is obtained the value of $R^2=0.954$ (Fig. 3). This mean 95.4% antiradical activities of Zingiberaceae family extracts were donated by phenolic compounds and as many as 4.6% activities were donated by other compounds. Phenolic compound contained in Zingiberaceae family are gingerol, shogaol, gingeron (Ravindran, et al., 2005), bisdescurcuminoid (Wulandari, 1996), kaempferol (Usia, et al., 2004), flavonoid and non polar constituents such as curcuminoid, kavapiron and gingerol (Sirat, et al, 1996), galangin, kaempferid and pinobanksin flavononol (Hisashi, et al., 2005), 3-O-metil galangin (Tao, et al., 2006).

In the lempuyang gajah and lempuyang pahit are found kaempferol as a flavonol substance that in the 3-O position bind eter and glucoside (Prakash, et al., 2011, Subehan, et al., 2005). Those compound have been known to have antiradical activity (Sirat, et al., 1996) because of their ability in donating the hidrogen atom (H), so that H could be bind to DPPH radical (hidrazil) to become the neutral form (hidrazin). Beside the phenolic compound, other compounds contained in Zingiberaceae family such as volatile oil, including terpenoid, carotenoid (Habsah, et al., 2000; Mau, et al., 2003; Suhaj, 2006; Chen, et al., 2008), 1'-acetoxychavicolasetat (ACA) (Mahae, et al., 2009) are known to have important roles in antiradical activity.

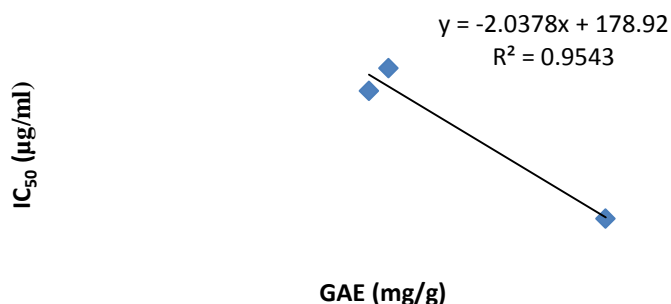


Figure 2. The correlation of GAE and IC_{50} . The correlation between phenolic concentration and antioxidant activity of the rhizom is known by the correlation coefficient (R^2) from linier regrestion equation between antioxidant activity and total phenolic concentration. The higher concentration of total phenolic concentration in the extract that means the higher antioxidant activity.

Antiradical activity of Jahe is known to have the highest activity than the other rhizome used in this study IC₅₀ value of jahe = 27.38 µg/mL). Also the total fenolic concentration of jahe is 73.99 mg/g and based on Zaeoung, *et al.* (2004) reported that gingerol compound in jahe is the compound responsible to the antiradical activity, meanwhile the main compound from the extracts used in this study that is responsible in the antiradical activity are not been known yet.

ACKNOWLEDGEMENT

We would like to thank to Rector of Universitas Muhammadiyah Surakarta for granting of this research through PINPRU program.

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