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Short Communication: Screening the potency of Zingiberaceae leaves as antioxidant and antiaging agent

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Abstract. *Zahra U, Kartika Y, Batubara I, Darusman LK, Maddu A. 2016. Short Communication: Screening the potency of Zingiberaceae leaves as antioxidant and antiaging agent. Nusantara Bioscience 8: 221-225.* Zingiberaceae is a family of plant with antioxidant, anticancer, and antiaging activities. The use of the leaves part of this family has never been explored. Therefore, the aim of this research is to evaluate the antioxidant and antiaging activities of some Zingiberaceae's leaves. The species of Zingiberaceae used in this study are *Alpinia galanga* (L), *Curcuma aeruginosa* Roxb, *Curcuma longa* L, *Curcuma zedoaria*, *Curcuma xanthorrhiza* Roxb, *Boesenbergia rotunda* (L.), *Elettaria cardamomum*, *Zingiber zerumbet*, *Zingiber officinale* Roscoe, and *Zingiber purpureum* Roscoe. Leaves were extracted with 2 different solvents sequentially: ethyl acetate and methanol. Total phenolic and flavonoid contents of the extracts were determined spectrophotometrically. Antioxidant activities were tested by using DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS (2,2'-azino-bis-3-ethyl benzothiazoline-6-sulfonic acid), whilst antiaging activity was tested by using antiglycation method. The results showed that the flavonoid content of the extracts was in the range of 0.83-25.17 g QE/100 g extract with total phenolic content ranging from 9.80-91.71 GAE mg/100 mg extract. The methanol extract of *Curcuma xanthorrhiza* displayed the highest antioxidant capacity in the DDPH (IC_{50} 282.35 mg/mL) and TEAC (10.08%) assays, followed by the ethyl acetate extract of *C. longa*, ethyl acetic extract of *C. xanthorrhiza* and methanol extract of *Z. officinale*, while the most active antiglycation is the methanol extract of *Z. officinale* (IC_{50} 203.85 mg/L).

Keywords: *Zingiber* genus, *Curcuma* genus, antiglycation, ABTS, DPPH

INTRODUCTION

Zingiberaceae is a family of subtropical and tropical plants which consist of 1400 species. Plants belonging to this family are known to have antibacterial, anticancer, antiinflammation, antiaging, and antidiabetic potency (Chompoo et al. 2012; Hartati et al. 2014; Ullah et al. 2014). The parts of Zingiberaceae plants which can be utilized are the rhizomes, leaves, stems, and roots (Hartati et al. 2014). In Indonesia, the rhizome has a wider application than other parts of the plants. On the other hand, a report from Chan et al. (2007) stated that total phenolic content in the leaves of *Alpinia galanga*, *Curcuma longa*, *Boesenbergia rotunda*, *Curcuma xanthorrhiza*, *Etlingera elatior*, and *Zingiber officinale* is higher than in the rhizomes. This indicates that the potency of Zingiberaceae leaves would be as high as the rhizomes.

The capability of a compound to capture free radicals in order to inhibit oxidation is called antioxidant. Reactive oxygen species (ROS) is one oxidant produced in the body. It leads to oxidative stress which contributes to many diseases (Ali et al. 2008). One group of antioxidant is flavonoid found in many plant organs, such as leaves, stems, roots, and rhizomes. This group has antioxidant properties that can prevent diseases caused by free radicals (Akinola et al. 2014).

Aging is defined as the accumulation of disturbances in an organism which decreases the integrity of the organism (Semba et al. 2010; Gkogkolou and Bohm 2012). One of the factors that cause aging is glycation. Glycation is a reaction between free amino groups of proteins and carbonyl groups of reducing sugars or other carbonyl compounds leading to the formation of AGEs (advanced glycation end products) (Nomoto et al. 2013). Large amount of AGEs will interact with the organs in the body and eventually, will result in dysfunctionality of those organs. In skin aging process, AGEs interact with collagen and form helix bonds resulting in skin aging, such as wrinkles, less integrity of skin, and dullness (Ichihashi et al. 2011). Further, the increasing amount of AGEs correlates with the increase in the enzymes responsible for aging process (Hori et al. 2012). Antiaging substance is a substance that inhibits glycation. Therefore, this study aims to screen antioxidant and antiaging properties of 10 kinds of Zingiberaceae leaves.

MATERIALS AND METHODS

Materials

Ten (10) species of Zingiberaceae, namely *Alpinia galanga* (L.) Willd., *Boesenbergia rotunda* (L.) Mansf., *Curcuma aeruginosa* Roxb., *Curcuma longa* L. (syn. *Curcuma domestica* Val.), *Curcuma zedoaria* (Christm.) Roscoe, *Curcuma xanthorrhiza* Roxb., *Elettaria cardamomum* (L.) Maton, *Zingiber zerumbet* (L.) J.E. Smith, *Zingiber officinale* Roscoe, and *Zingiber purpureum* Roscoe were collected from the conservation and cultivation unit of the Tropical Biopharmaca Research Center, Darmaga Campus, Bogor Agricultural University, Bogor, Indonesia. Identification of the plants was performed in the Herbarium Bogoriense, Research Center for Biology, Indonesian Institute of Sciences, Cibinong, Bogor, Indonesia, and voucher specimens were deposited in the Tropical Biopharmaca Research Center, Institut Pertanian Bogor, Bogor, Indonesia.

Procedures

Preparation of plant extracts

Fifty (50 g) of dried ground samples were extracted by increasing the polarity of the solvents (250 mL). First, *n*-hexane was used as the solvent to take out the nonpolar fraction, then extraction was continued to the residue using ethyl acetic (EtOAc), and finally using methanol (MeOH). Extracts were filtered and then vacuum-dried by using rotary evaporator. The yields of EtOAc and MeOH extracts were then weighed.

Determination of total phenolic content

Total phenolic content was determined according to the method described by Atanassova et al. (2011) with slight modifications. Briefly, 1 mL extract of different concentrations (20-100 mg/L) or standard solution (gallic acid) of different concentrations (10-125 mg/L) was transferred into a 25 mL flask. Deionized water and 1.0 mL Folin-Ciocalteu phenol reagent was then added to the flask. After incubation for 5 minutes, 7% sodium carbonate (10 mL) was added to the mixture. After being kept in total darkness for 1 h, the absorbance was measured at 750 nm using a spectrophotometer. Total phenolic content of the samples was determined by plotting the absorbance data of sample into calibration curve of gallic acid (Figure 1). The results were then expressed as gallic acid equivalents (GAE) (% (w/w)) of dried plant material

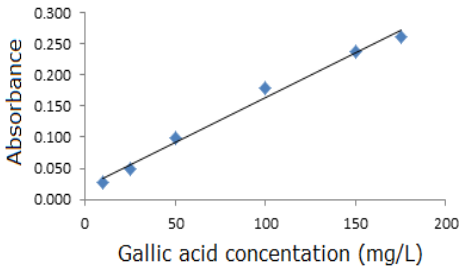

Figure 1. Calibration curve of gallic acid on total phenolic content determination

Total flavonoid

Total flavonoid was determined as described by Chang et al. (2002) with modifications. 125 µL extract or standard solution was added into a test tube containing 375 µL ethanol, 25 µL $AlCl_3$10%, 25 µL CH_3COONa 1 M, and 700 µL water. The mixture was stirred and incubated at room temperature for 30 minutes. 250 µL of the mixture was then poured into a well-plate (96-well plate) and the absorbance was measured at 415 nm. Quercetin was used as a standard solution. The results were expressed as quercetin equivalent (% (w/w)) of the extract.

Antioxidant activities

DPPH radical scavenging activity**.** The experiment was conducted as described by Salazar-Aranda et al. (2011) with some modifications. DPPH solution (100 µL) (125 µM) was reacted with 100 µL sample (500, 250, 125, 62.5 and 31.25 µg/mL). The mixture was then incubated for 30 minutes. After incubation, the absorbance was read at 517 nm. Ascorbic acid was used as positive control, while ethanol was used as solvent and negative control. Antioxidant activity was calculated with the following equation:

$$\text{Inhibition (\%)} = \frac{[(A_0 - A_1)]}{(A_0)} \times 100\%$$

Where,

A_0 = the absorbance of negative control

A_1 = test sample or positive standard

ABTS**.** The experiment was conducted as described by Re et al. (1999) and Fogarasi et al. (2015). ABTS solution (7mM) was oxidized by potassium peroxide sulfate for 12-16 hours. Further, about 180 µL ABTS radical was reacted with 20µL sample and incubated for 15 minutes at microplate 96 well. The absorbance was measured at 734 nm. The results were expressed as Trolox Equivalents Antioxidant Capacity (TEAC) %w/w.

Antiglycation

Antiglycation was measured according to the method described by Povichit (2010) with a slight modification. The reaction consisted of BSA (20 mg/mL), glucose (235 mM), fructose (235 mM) and 50 µL of sample or positive control in phosphate buffer 0.2 M (pH 7.4). The solution was incubated for 40 hours at 60ºC, and then the excitation fluorescence intensity was measured at 330 nm and the emission at 440 nm. Aminoguanidine was applied as a positive control. Sample corrected solution was prepared the same like sample but water used to replace the sample or positive control. Antiglycation activity was measured by using the following equation:

$$\text{Inhibition (\%)} = \left[1 - \frac{(A - A_0)}{(B - B_0)}\right] \times 100\%$$

Where,

A : Fluorescence intensity of sample solution

A_0 : Fluorescence intensity of sample corrected solution

B: Fluorescence intensity of control solution

B_0: Fluorescence intensity of control corrected solution

Concentration at 50% inhibition (IC_{50}) against AGE was calculated from the line regression curve.

RESULT AND DISCUSSION

The leaves of Zingiberaceae is only utilized in limited application in Indonesia (Table 1). Turmeric leaves are the only commodity that has been applied by the Indonesian as a food ingredient. Turmeric grows very well in all tropical area in Indonesia. Thus, this research can provide information about the potency of Zingiberaceae leaves, which can then be applied to the highest degree in the future.

The yield of extract from the leaves of the ten species ranges from 1.36 to 10.40%. The methanol extract of all samples gave the highest yield. This indicates that the leaves contained more polar compounds rather than non-polar compounds. The content of the extracts will directly correlate with the activity.

Total flavonoid and total phenolic contents were measured for all extracts since these indicate the characteristics of pharmacological compounds (Table 2). The highest total flavonoid content came from the ethyl acetate extract of *Boesenbergia rotunda* which is 25.17g quercetin equivalence/100 g extract, and followed by the ethyl acetate extract of *Alpinia galanga* (19.31 g quercetin equivalence/100 g extract) (Table 2). The highest total phenolic content came from the methanol extract of *Curcuma xanthorrhiza*, which is 91.71g gallic acid equivalence/100 g extract and followed, by the ethyl acetate extract of *Curcuma xanthorrhiza*, which is 80.53 g gallic acid equivalence/100 g extract (Da Gama et al. 2014). The result for the total phenolic content in the methanol extract of *Curcuma xanthorrhiza' s* leaves (91.71 gallic acid equivalence, GAE mg/100 mg extract) is lower than in the rhizomes (22.03 mg/g GAE**)** (Akinola et al. 2014). Xanthorrhizol is one of the phenolic compounds contained by *Curcuma xanthorrhiza* which act as antioxidants.

Table 1. Zingiberaceae leaves sample and traditional usage in Indonesia

Scientific name	Common name	Local name	Traditional usage in Indonesia	References
Alpinia galanga (L)	Galangal	Lengkuas		-
Boesenbergia rotunda (L)	Fingerroot	Temukunci	-	-
Curcuma aeruginosa Roxb	Blue ginger	Temuhitam	-	-
Curcuma longa L	Tumeric	Kunyit	Food herb	Said (2003)
Curcuma zedoaria	White turmeric	Temuputih	-	-
Curcuma xanthorrhiza Roxb	Javaness turmeric	Temulawak	-	-
Elettaria cardamomum	Cardamom	Kapulaga	-	-
Zingiber zerumbet	Bitter ginger	Lempuyang	-	-
Zingiber officinale Roscoe	Red ginger	Jahemerah	-	-
Zingiber purpureum Roscoe	Purple ginger	Bangle hantu	-	-

Note: (-) No information

Table 2. Extraction yield, total flavonoid and total phenolic contents of Zingiberaceae leave extracts

Sample	Solvent	Extraction yield (%)	Total flavonoid (g quercetin eq. /100 g extract)	Total phenolic (g gallic acid eq. /100 g extract)
Alpinia galanga (L)	Ethyl acetate	1.92 ± 0.30	19.31 ± 0.4 ^{b}	35.71 ± 1.1 ^{d}
	Methanol	4.23 ± 1.14	1.64 ± 0.2 ^{jk}	18.18 ± 1.8 ^{fg}
Boesenbergia rotunda (L.)	Ethyl acetate	1.36 ± 0.19	25.17 ± 1.2 ^{a}	22.79 ± 1.2 ^{fe}
	Methanol	5.86 ± 2.7	1.53 ± 0.3 ^{jk}	22.12 ± 3.2 ^{fe}
Curcuma xanthorrhiza	Ethyl acetate	2.92 ± 0.52	17.03 ± 1.0 ^{ced}	80.53 ± 0.5 ^{b}
	Methanol	8.49 ± 2.9	4.85 ± 0.2 ^{h}	91.71 ± 5.3 ^{a}
Curcuma aeruginosa	Ethyl acetate	2.78 ± 0.20	18.50 ± 0.9 ^{cb}	45.51 ± 2.8 ^{c}
	Methanol	9.72 ± 4.2	0.83 ± 0.1 ^{k}	9.80 ± 0.9 ^{h}
Curcuma longa	Ethyl acetate	1.89 ± 0.33	18.78 ± 0.5 ^{b}	79.86 ± 3.0 ^{b}
	Methanol	5.61 ± 1.7	3.41 ± 0.2 ^{i}	36.65 ± 3.4 ^{d}
Curcuma zedoaria	Ethyl acetate	2.38 ± 0.13	16.51 ± 0.6 ^{e}	47.31 ± 3.7 ^{c}
	Methanol	8.78 ± 3.5	1.91 ± 0.07 ^{jik}	36.27 ± 0.9 ^{d}
Elettaria cardamomum	Ethyl acetate	3.40 ± 0.17	13.09 ± 1.1 ^{g}	22.02 ± 2.4 ^{fe}
	Methanol	10.14 ± 4.4	0.84 ± 0.2 ^{k}	20.47 ± 2.3 ^{f}
Zingiber zerumbet	Ethyl acetate	2.03 ± 0.05	15.05 ± 1.1 ^{f}	12.27 ± 0.9 ^{hg}
	Methanol	5.12 ± 2.0	2.78 ± 0.1 ^{ji}	16.78 ± 0.5 ^{fg}
Zingiber officinale	Ethyl acetate	3.34 ± 0.03	16.71 ± 0.6 ^{ed}	79.20 ± 4.0 b
	Methanol	4.50 ± 0.99	2.99 ± 0.3 ^{ji}	28.81 ± 2.0 ^{e}
Zingiber purpureum	Ethyl acetate	1.71 ± 0.09	18.06 ± 2.5 ^{cbd}	23.17 ± 3.6 ^{fe}
	Methanol	3.81 ± 1.5	2.16 ± 0.9 ^{jik}	18.98 ± 2.4 ^{fg}

Note: Data given as mean ± standard deviation of triplicate test. Data followed by the same letter are not significantly different according to Duncan's multiple comparison test. P =0.01

Table 3. Antiglycation and antioxidant activity of Zingiberaceae leave extracts

Sample	Solvent	IC_{50} DPPH (mg/L)	TEAC (mg/g)	IC_{50} Antiglycation (mg/L)
Zingiber officinale	Methanol	516.21 ±4.2^{d}	9.60 ±0.16 ^{ba}	203.85 ± 2.7^{g}
Curcuma longa	Ethyl acetate	366.11 ± 2.1^{f}	10.24±0.17^{a}	271.79 ± 1.1^{f}
Curcuma xanthorrhiza	Methanol	282.35 ± 6.5^{g}	10.08±0.03^{a}	274.14 ± 6.5^{f}
Elettaria cardamomum	Methanol	671.37 ± 2.3^{b}	7.93 ±0.89^{fde}	285.58 ± 2.3^{e}
Zingiber purpureum	Methanol	622.69 ± 2.3^{c}	7.48 ± 0.82^{feg}	305.79 ± 2.3^{d}
Curcuma zedoaria	Methanol	544.05 ± 4.8 ^{d}	6.99 ±1.55^{fhig}	335.57 ± 4.8^{c}
Boesenbergia rotunda	Methanol	395.34 ± 6.9f^{e}	5.72 ±1.99^{ji}	342.67 ± 6.9 ^{c}
Zingiber zerumbet	Ethyl acetate	1478.36± 2.7^{a}	5.80 ±1.09^{ji}	377.95 ± 2.7^{b}
Curcuma xanthorrhiza	Ethyl acetate	417.84 ±2.1^{e}	9.78 ±0.03^{a}	383.37 ± 2.1^{b}
Alpinia galanga (L)	Ethyl acetate	614.82 ±3.1^{c}	10.07± 0.23^{a}	439.10 ± 5.6^{a}
Ascorbic acid	-	62.4	-	-
Aminoguanidine	-	-	-	18.91 ± 0.6

Note: Data given as mean ± standard deviation of triplicate test. Data followed by the same letter are not significantly different according to Duncan's multiple comparison test. P =0.01

Antioxidant activity measured by using DPPH method is expressed as IC_{50} values, the concentration of extract that will react with 50% of the DPPH Methanol extract of *C. xanthorrhiza* has the highest activity among all samples, although the activity is not as high as ascorbic acid as positive control (Table 3). The highest value for TEAC was found in the methanol extract of *C. xanthorrhiza.* However, the activity of the methanol extract of *C. xanthorrhiza* leaves toward ABTS is not significantly different from the ethyl acetate extract of *C. xanthorrhiza,* ethyl acetate extract of *C. longa,* methanol extract of *Z. officinale,* and ethyl acetate extract of *A. galangal*.

Antioxidant activities of Zingiberaceae leaves are demonstrated from their capability to neutralize free radicals. Both DPPH and ABTS methods used spectrophotometer to measure the decreasing absorbance due to radical neutralization (Shalaby and Shanab 2013). Both methods showed similar results. There was a slight difference in the results, however, which was due to the difference in the type of free radicals and the sensitivity. However, both methods proved the high antioxidant activity of *C. xanthorrhiza,* and this makes *C. xanthorrhiza* a potential to be an antiaging material. Hypothetically, dietary antioxidants should decrease oxidative stress by inhibiting peroxidation chain reaction and formation of AGEs thereby interrupt glucose metabolism. Glycation reactions depend on generation of reactive oxygen species by trace amounts of redox-active metal ions. The antioxidant in the samples can neutralize free radical like oxygen reactive species that accelerate glycation process, in conclusion, the antioxidant can be an antiaging or antiglycation (Ndlovu et al. 2013).

Antiglycation screening, as a method to see the antiaging activity, was conducted to all extracts (Table 3). IC_{50} showed that the methanol extract of *Z. officinale* had the highest activity, followed by the ethyl acetate extract of *C. longa* and the methanol extract of *C. xanthorrhiza*. However, when compared to the positive control, aminoguanidine, the extracts displayed lower activity.

Glycation produces AGEs that will interact with many kinds of tissues and organs in the body that will eventually disturb their functions (causing disturbance and damages to the tissues and organs) (Hori et al. 2012). The epidermis layer consists of collagen, elastin, and proteoglycan. AGEs interact with collagen that will manifest in skin aging. AGEs, as the end product of glycation, will also generate free radicals that may accelerate the glycation process (Ndlovu et al. 2013) and promote oxidation, which will cause oxidative stress causing many diseases (Ramasamy et al. 2005).

Taking all results together, it can be clearly seen that the methanol extract of *Z. officinale* has both antioxidant and antiaging properties. It has high trolox equivalence antioxidant capacity (TEAC) score in the ABTS test and the lowest IC_{50} in antiglycation test. The methanol extract of *Z. officinale* can inhibit aging process by inhibiting the AGEs formation. Antiglycation activity of *Z. officinale* rhizome was also reported by Kazeem et al. (2015), who stated that *Z. officinale* rhizome had an *in vivo* antiglycation potential in diabetic mice. Therefore, it is possible to continue the research to determine the specific compound responsible in the antiglycation activity.

In conclusion, the screening of leaf extracts from 10 species of Zingiberaceae showed that the methanol extract of *Zingiber officinale* leaves as the highest antioxidant and antiaging capacity. This study also showed that the leaves are potential source of antioxidant and antiglycation materials that could serve as the basis for future drugs and food supplements.

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