ANTIOXIDANT ACTIVITY
AND TOTAL FLAVONOIDS CONTENT
OF CURCUMA RHIZOME EXTRACT

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ABSTRACT

The family of Zingiberaceae, including the genus Curcuma, has been used since hundreds of years ago as ingredient of traditional medicines. Various scientific studies to support its use as traditional medicine was already done. One of the most prominent biological activity possessed by the family of Zingiberaceae was the antioxidant activity. The aim of this research was to examined the antioxidant activity and flavonoid content of rhizome’s extract of Curcuma heyniana, Curcuma mangga, Curcuma aeruginosa, Curcuma phaeocaulis and Curcuma purpurascens. The antioxidant activity was determined by DPPH method and total flavonoid content was determined by colorimetric method. The antioxidant activity of Curcuma rhizome’s extract in this study ranged from very strong to weak. C. purpurascens had a very strong antioxidant activity (EC\textsubscript{50} value of 36.30 ppm) and also the highest flavonoids content measured as quercetin (14.27\%). Based on correlation analysis ($R^2 = 0.6573$), there is a positive correlation between total flavonoid content with antioxidant activity of the extract.

Keywords
Antioxidant, flavonoid, Curcuma, rhizome.
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The family of Zingiberaceae, including the genus Curcuma, has been used since hundreds of years ago as ingredient of traditional medicines. Various scientific studies to support its use as traditional medicine was already done. One of the most prominent biological activity possessed by the family of Zingiberaceae was the antioxidant activity. The aim of this research was to examined the antioxidant activity and flavonoid content of rhizome’s extract of Curcuma heyneana, Curcuma mangga, Curcuma aeruginosa, Curcuma phaeoacaulis and Curcuma purpurascens. The antioxidant activity was determined by DPPH method and total flavonoid content was determined by colorimetric method. The antioxidant activity of Curcuma rhizome’s extract in this study ranged from very strong to weak. C. purpurascens had a very strong antioxidant activity (EC50 value of 36.30 ppm) and also the highest flavonoids content measured as quercetin (14.27%). Based on correlation analysis (R² = 0.6573), there is a positive correlation between total flavonoid content with antioxidant activity of the extract.

Keywords
Antioxidant, flavonoid, Curcuma, rhizome.
INTRODUCTION

Plants of Zingiberaceae family has been used since hundreds of years ago as source of traditional medicines, including plants belong to the genus Curcuma. Various scientific studies has been done to support its use as traditional remedies. One of the most prominent biological activity of plants belong to Zingiberaceae family is the antioxidant activity (Vankar et al, 2006; Chompo et al, 2012; Kantayos and Paisooksantivatana, 2012; Sattar et al, 2013).

Antioxidants are compounds capable to either delay or inhibit the oxidation processes which occur under the influence of atmospheric oxygen or reactive oxygen species. Antioxidants are involved in the defense mechanism of the organism against pathologies associated with the attack of free radicals. (Pisoschi and Negulescu, 2011). Free radicals can be defined as any molecular species capable of independent existence that contains an unpaired electron in an atomic orbital, make them very reactive and capable of reacting with important biomolecules, such as proteins, lipids, and DNA. Free radicals damage contributes to the etiology of many chronic health problems such as cardiovascular and inflammatory diseases, cataract, and cancer (Lobo et al, 2010).

Recently, antioxidants have attracted considerable attention in relation to free radicals and oxidative stress, cancer prophylaxis and therapy, cardiovascular diseases and other degenerative diseases. Antioxidants prevent free radical induced tissue damage by preventing the formation of radicals, scavenging them, or by promoting their decomposition (Lobo et al, 2010). In addition to endogenous antioxidant defense systems which naturally present in human body, endogenous antioxidant is necessary to improve the body’s resistance against degenerative diseases.

Endogenous antioxidant can be either synthetic or natural. Synthetic antioxidants are recently reported to be dangerous to human health. Thus the search for effective, nontoxic natural compounds with antioxidative activity has been intensified in recent years. Dietary and medicinal plants are major source of natural antioxidants.

Many researches has revealed the antioxidant activity of dietary and medicinal plants, including the plants belong to Zingiberaceae family. Antioxidant activities of rhizomes of Alpinia alullahas, A. galanga, A. smithiae, A. vivatta, Hedychium coronarium, Vanoverberghia sasakiana, Zingiber cassumunar, Z. chrysanthum, Z. officinale, and Z. zerumbet had been reported (Vankar et al, 2006; Chen et al, 2008; Pal et al, 2011; Rout et al, 2011; Julie and Ernest, 2012; Sattar et al, 2013). Anget et al (2013) reported the antioxidant activity of heat stable protein isolated from aqueous extracts of rhizomes of Curcuma aeruginosa, C. amada, C. aromatica, C. brog, C. caesia, C. malabarica, C. rakthakanta and C. sylvatica. Protein extracted from C. brog, C. amada, and C. caesia had low IC₅₀ values of 0.70, 0.73, 0.80 respectively, showing high DPPH scavenging activity which were comparable with that of C. zedoaria (IC₅₀ 0.84). Antioxidant activity of ethanolic extract of Curcuma longa, C. zedoaria, C. angustifolia, C. aromatica, and C. amada had also been reported. Antioxidant activity of those species except C. angustifolia had been found to have strong correlation with curcumin and phenol content. However C. angustifolia may be active due to high aromatic oil content like eugenol, palmitic acid and camphor (Nahak and Sahu, 2011). Curcuma longa or turmeric is a famous medicinal plants, and it has strong
Some cells underwent nuclei condensation shown by the yellow color in the nucleus, indicating early events of apoptosis, while the untreated cells showed uniformly bright green colour indicating viable cells (Figure 2A). The data obtained showed that MCF-7 cells were undergoing apoptosis after incubation for 24 hours, and increasing the concentration would increase the apoptosis.

**Discussion**

Rapid and uncontrolled proliferation is main characteristic and the primary key in the progression of tumor or cancer. Therefore, the ability of a substance to inhibit or suppress the proliferation of cancer cells is an important feature or property of a potential cancer drug. In the search for new cancer drugs derived from nature, in the present study we investigated the antiproliferative activity of methanolic extract of Bangle Hantu rhizomes on human breast cancer cell line, MCF-7, and also its ability to induce apoptosis of the cells.

Figure 1 clearly showed that the cells treated with methanolic extract of Bangle Hantu rhizomes showed a decrease in viable-cell’s number from the beginning of the experiment until 72 hours incubation. At the lowest concentration used in the experiment, i.e. 20 µg/mL, the cells failed to doubled its number. It means that during the experiment some of the cells died, while proliferation did not occurred or significantly reduced.

The higher the concentration, the stronger the cytotoxicity of the extract. 24 hours incubation in 40 µg/mL extract caused cells dead with only 60% of the original number of viable cells left, while 24 hours incubation in 80 µg/mL extract only left 25% of viable cells. At the end of the experiment, i.e. at 72 hours incubation with 80 µg/mL extract, almost no viable cells could be observed (Fig. 1). These data corroborate the results of cytotoxicity assay that had previously been conducted by Sinaga et al (2011), and revealed the antiproliferative activity of methanolic extract of Bangle Hantu rhizomes. However, in this experiment, the doubling time of MCF-7 cells treated with Bangle Hantu extract could not be calculated due to the very strong toxicity of the extract. Therefore the determination of doubling time should be done with other methods, such as by AgNOR staining or by using a lower concentration of extract, i.e. below 20 µg/mL.

Apoptosis is a genetically directed process of cell self-destruction, also called programmed cell death. Malignant or cancer cells generally lack of apoptosis. Induction of apoptosis is a useful marker for screening compounds for subsequent development as possible anticancer agents. Therefore it is very interesting to know whether methanolic extract of Bangle Hantu rhizome also posses ability to induce apoptosis.

Further experiment by double staining the MCF-7 cells with acridine orange and ethidium bromide demonstrated that the dramatically growth inhibition of methanolic extract of Bangle Hantu rhizomes on MCF-7 cells was caused, at least partly, by the apoptosis induced by the extract. Figure 2B showed some of the treated cells had undergone apoptosis shown by orange-red fluorescent. Acridine orange is a vital dye that stain both live and dead cells, whereas ethidium bromide stain only those cells that have lost their membrane integrity or apoptotic cells. Viable cells will appear uniformly green, while apoptotic cells underwent membran-blebbing and would incorporate ethidium bromide and therefore stain orange (Kasibhatla et al, 2006).

Results of the experiments revealed the potentiality of substances contained in the methanolic extract of Bangle Hantu rhizomes to strongly inhibit the proliferation of human breast cancer cell lines, MCF-7, and also
antioxidant activity especially in its essential oil (Liju et al., 2011).

In attempt to search more source for natural antioxidant, in this work we evaluated the antioxidant activity of rhizomes of five species of Curcuma, i.e. *Curcuma heynaeana*, *C. mangga*, *C. aeruginosa*, *C. phaeocaulis* and *C. purpurascens*. Since antioxidant activity of plant’s extracts often related to its flavonoids content (Grassi et al., 2010; Brunetti et al., 2013), in this work we also determined the total flavonoids content of the extracts, and evaluated the correlation between antioxidant activity and flavonoids content of the extracts.

**MATERIALS AND METHODS**

**Preparation of crude rhizome extract**

Rhizomes of *Curcuma heynaeana*, *C. mangga*, *C. aeruginosa*, *C. phaeocaulis* and *C. purpurascens* were obtained from BALITTR (Balai Penelitian Tanaman Rempah dan Obat), Bogor, West Java.

The dried rhizomes were powdered using a grinder and extraction was done at room temperature. About 100 g of dried powder of the rhizomes were soaked in methanol (1 L, 98%) for 2-3 days, and then filtered through Whatman filter paper No.1. The filtrates obtained were concentrated under vacuum on a rotary evaporator at 50°C and stored at 4°C for further use. The stock solution of crude extract (5 mg/mL) was prepared by dissolving a known amount of dry extract in 98% methanol. The working solution (75, 100, 250, 500 and 750 ppm) of extracts were prepared from stock solution by suitable dilution.

**DPH Radical Scavenging Activity Assay**

The antioxidant activity of the rhizome extract was assessed on the basis of the radical scavenging effect of the stable 1,1-diphenyl-2-picryl-hydrazyl (DPPH) free radical activity as described recently (Pal et al., 2011; Chompo et al., 2012). Working solutions of the extract were prepared in methanol. Ascorbic acid was used as standard in 1-100 ppm solution. 0.002% of DPPH was prepared in methanol and 1 mL of this solution was mix with 1 mL of sample solution and standard solution separately. These solution mixtures were kept in dark for 30 minutes and optical density (OD) or absorbance (A) was measured at 517 nm using UV-Vis Spectrophotometer. Methanol (1 mL) with DPPH solution (0.002%, 1 mL) was used as blank. The optical density was recorded and % inhibition was calculated using the formula given below:

\[
\text{Ab} - \text{As} = \frac{\text{Ab}}{100} \\
\text{where: Ab} = \text{Absorbance of blank} \\
\text{As} = \text{Absorbance of sample}
\]

Linear regression analysis (Origin 6.0 version) was used to calculate the IC$_{50}$ values.

**Determination of total flavonoid**

The total flavonoid content was measured by aluminium chloride colorimetric assay as described recently (Hossain et al., 2011). An aliquot (1 mL) of extracts or standard solution of quercetin (20, 40, 60, 80 and 100 ppm) was added to 10 mL volumetric flask, containing 4 mL distilled deionized water (dd H$_2$O). To the flask was added 0.3 mL 5% NaNO$_3$. After 5 minutes, 0.3 mL 10% AlCl$_3$ was added, and after 6 minutes more, 2 mL 1 M NaOH was added and the total volume was made up to 10 mL with dd H$_2$O. The solution was mixed well and the absorbance was measured against a prepared reagent blank at 510 nm with an UV-Vis Spectrophotometer. The measurement was carried out in triplicate and the results were averaged. The data of the total flavonoid
contents of the dry rhizome extracts were expressed as % of quercetin, calculated using the formula given below:

\[
\% \text{ Flavonoids (as quercetin) } = \frac{A_q \times \text{W}}{\text{As}}
\]

where: As = Absorbance of sample  
Aq = Absorbance of quercetin  
W = sample weight

**RESULTS**

**Yield of Extraction**

Yield of extraction was expressed as weight (g) of crude extract per 100 gram of powdered-dried plant material. The yield of crude extract from Curcuma rhizomes by using methanol as solvent varied between 7.08% - 11.52%. As shown in Table 1, the highest yield generated from *C. purpurascens* and the lowest is from *C. mangga*.

**Total Flavonoid Content**

Total flavonoids content of five methanolic extract of Curcuma rhizomes measured as quercetin are presented in Table 2. The value ranged from 1.35 to 14.27%. The highest was *C. purpurascens* rhizome’s extract, while the lowest was *C. aeruginosa*.

<table>
<thead>
<tr>
<th>Species</th>
<th>Local Name</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. heynena</em></td>
<td>Temu giring</td>
<td>11.10</td>
</tr>
<tr>
<td><em>C. mangga</em></td>
<td>Temu mangga</td>
<td>7.08</td>
</tr>
<tr>
<td><em>C. aeruginosa</em></td>
<td>Temu ireng</td>
<td>7.26</td>
</tr>
<tr>
<td><em>C. phaeocaulis</em></td>
<td>Temu jingga</td>
<td>10.78</td>
</tr>
<tr>
<td><em>C. purpurascens</em></td>
<td>Temu pinggang</td>
<td>11.52</td>
</tr>
</tbody>
</table>

**Table 1. Yield of Extracts**

Table 2. Total Flavonoid Content of Curcuma species

<table>
<thead>
<tr>
<th>Species</th>
<th>Total Flavonoids as Quercetin (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td><em>C. heynena</em></td>
<td>1.94</td>
</tr>
</tbody>
</table>

**Antioxidant Activity**

Antioxidant activity of rhizomes of five Curcuma species determined using DPPH method are varied, the EC$_{50}$ values ranged from 36.30 to 199.71 ppm (Table 3). EC$_{50}$ value is concentration of sample required to scavenge 50% of DPPH radicals. The lowest EC$_{50}$ value, means the strongest antioxidant activity, was belong to rhizome’s extract of *Curcuma purpurascens* (36.30 ppm), while the highest value belong to rhizome’s extract of *Curcuma heynena* (155.68 ppm).

**Table 3. Antioxidant activity of Curcuma species**

<table>
<thead>
<tr>
<th>Species</th>
<th>Antioxidant Activity (EC$_{50}$) (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td><em>C. heynena</em></td>
<td>153.16</td>
</tr>
<tr>
<td><em>C. mangga</em></td>
<td>89.47</td>
</tr>
<tr>
<td><em>C. aeruginosa</em></td>
<td>199.57</td>
</tr>
<tr>
<td><em>C. phaeocaulis</em></td>
<td>112.96</td>
</tr>
<tr>
<td><em>C. purpurascens</em></td>
<td>36.34</td>
</tr>
</tbody>
</table>

**DISCUSSION**

In this work we used methanol as solvent for extraction of bioactive substances from the rhizomes. Methanol is a popular solvent for extraction of bioactive substances from plant material, due to its quite small molecular structure, so it can penetrate all plant tissues to pull out the active ingredient. Methanol also has the ability to dissolve almost all organic compounds, both polar and non-polar compounds. Another advantage of methanol is
that it is volatile so it easily separated from the extract. The disadvantage of methanol as solvent for extraction is due to its ability to dissolve wide range polarity of substances, so it is not selective. Yield of extraction with methanol usually higher compare to other solvent, such as ethanol, acetone, chloroform, and ethyl acetate. Extraction of Ginger (Zingiber officinale) with methanol give highest yield compare to acetone and chloroform (Ghasemzadeh et al, 2011). Similarly, the methanolic extract of A. wilkesiana and S. scabrum gave the highest yield (14.67% and 17.23%, respectively), while the ethylacetate extract gave the least yield (2.73% and 4.13% respectively) (Anokwuru et al, 2011).

Flavonoids are plant’s secondary metabolites with variable phenolic structures. More than 4000 varieties of flavonoids have been identified, most of them possess important bioactivity, such as antioxidant and anticancer activity. One of the best-described flavonoids is quercetin. Quercetin is found in abundance in onions, apples, broccoli, and berries. In Curcuma species, the most well known flavonoid is curcumin, the principal curcuminoid of turmeric (Curcuma longa). The main flavonoid content in Curcuma plants studied in this work is still unknown.

Methanolic extract of Curcuma purpurascens rhizomes had the highest total flavonoids content (measured as quercetin) among the species investigated in this work, very much higher (14.27%) than the others (1.35-5.21%). This is in line with the antioxidant activity of the extracts, as shown in Table 3. Methanolic extract of Curcuma purpurascens rhizomes showed the lowest EC50 values (antioxidant activity), i.e. 36.30 ppm, means the highest antioxidant activity, while the other four species ranged from 90.42 to 199.71 ppm (Table 3). According to Zuhra et al. (2008), a substance is said to have very strong antioxidant activity if the EC50 is less than 50 ppm, strong if EC50 ranged from 50 to 100 ppm, moderate if EC50 ranged from 100 to 150 ppm, and weak if EC50 ranged from 151 to 200 ppm. According to the criteria, C. purpurascens could be stated as having very strong antioxidant activity, followed by C.mangga (strong), C. phaeocaulis and C.heyneana (moderate) and C.aeruginosa (weak).

DPPH radical scavenging activity assay based on scavenging of DPPH through the addition of an antioxidant that decolourizes the DPPH solution. The degree of colour change is proportional to the concentration and potency of the antioxidants. A large decrease in the absorbance of the reaction mixture indicates significant free radical scavenging activity of the compound under test (Krishnaiah et al, 2011). However, to obtain a better and closer approach to its expected use, as a natural antioxidant for human use, it is necessary to conduct in vivo antioxidant assay especially for the potential one.

From five Curcuma species studied in this work, the best performance, in terms of antioxidant activity and flavonoids content was Curcuma purpurascens. Therefore, this species could be developed further as a potential source of natural antioxidant for human use.

Based on correlation analysis (R^2 = 0.6573), we revealed a positive correlation between total flavonoid content with antioxidant activity of the extract, suggested that the antioxidant activity of the extract might be due, at least partly, to the presence of flavonoids. This results in line with other works previously reported (Nahak and Sahu, 2011; Khan, 2012;Gopal, et al, 2013).

CONCLUSION
Antioxidant activity of methanolic extract of five Curcuma species; i.e. C. heyneana, C. mangga, C. aeruginosa, C. phaeocaulis and C. purpurascens varied from very strong to weak with EC50 values varied from 36.30 to 199.71 ppm. C. purpurascens had the strongest antioxidant activity in line with its highest flavonoid content (14.27%). The antioxidant activity of the extract significantly correlated with the total flavonoid content.

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