Curcuminoid Contents, Antioxidant and Anti-Inflammatory Activities of Curcuma xanthorrhiza RoxB. and Curcuma domestica Val. Promising Lines From Sukabumi of Indonesia

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ABSTRACT

The main bioactive substances in the rhizomes of Curcuma xanthorrhiza and Curcuma domestica that have efficacy as antioxidant and anti-inflammatory activities are curcuminoids. In this study, ethanol extracts of C. xanthorrhiza and C. domestica promising lines from Sukabumi of Indonesia were investigated for the presence of curcuminoids, antioxidant and anti-inflammatory activities. HPLC method was used to determine curcuminoids content. The antioxidant (radical scavenging) potential of the samples was evaluated using 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical method. While for the anti-inflammatory activity, the in vitro cyclooxygenase 2 (COX2) inhibition method was used. The curcuminoid content of C. xanthorrhiza and C. domestica were 31.27 and 66.32 mg/g, respectively. IC₅₀ values for DPPH radical scavenging activity were 81.99 and 73.31 µg/mL, with C. domestica having lowest value and most potent than C. xanthorrhiza. Percent inhibition values for COX2 inhibitor activity were 74.84 and 67.96 %, with C. domestica having the highest value. In this study, the ethanol extracts of C. domestica promising line from Sukabumi of Indonesia exhibited most in curcuminoids content, antioxidants properties and anti-inflammatory activity than C. xanthorrhiza promising line.

Keywords: Curcuminoid, Antioxidant, Anti-Inflammatory, Curcuma xanthorrhiza, Curcuma domestica, Promosing line

INTRODUCTION

Curcuma xanthorrhiza Roxb., also known as “temulawak” in Indonesia, and Curcuma domestica Val., known in Indonesia as “kunyit”, are a medicinal plant from the family Zingiberaceae distributed in Indonesia. Traditionally, C. xanthorrhiza rhizomes have been used to treat stomach diseases, liver disorders, constipation, bloody diarrhea, dysentery, children’s fevers, hemorrhoids, and skin eruptions (Hwang et al., 2000). Pharmacologically is has been reported that C. xanthorrhiza has the antimicrobial (Hwang et al., 2000), anti-metastatic (Choi et al., 2004), anti-cancer (Huang et al., 1998), anti-candidal (Rukayadi et al., 2006), anti-oxidant (Masuda et al., 1992) and hypolipidemic activities (Yasni et al., 1993). The rhizomes of C.
domestica are commonly used as a flavoring, coloring agent and preservative (Thaikert & Paisooksantivatana, 2009). The traditional Indian medicine claims the use of C. domestica powder against biliary disorders, anorexia, coryza, cough, diabetic wounds, hepatic disorder, rheumatism and sinusitis (Ammon et al., 1992). Pharmacological research has demonstrated that C. domestica possesses anti-inflammatory (Mukophadhyay et al. 1982; Arora et al., 1971; Chandra and Gupta, 1972), antioxidant (Unnikrishnan and Rao, 1995; Pulla Reddy & Lokesh, 1992), anti/protozoal (Rasmussen et al., 2000), nematocidal (Kiuchi et al., 1993), anti-bacterial (Chopra et al., 1941; Bhavani Shankar & Murthy, 1979), antivenom (Ferreira et al., 1992), anti-HIV (Mazumber et al., 1995; Eigner and Scholz 1999), and anti-tumor activities (Huang et al., 1988).

The main yellow bioactive substances in the rhizomes of C. xanthorrhiza and C. domestica are curcuminoid (Duke, 2002; Chainani-Wu, 2003). The pharmacological effect of curcuminoids has been investigated, such as radical scavenging (Sreejayan & Rao, 1996), the inhibition of nitric oxide (NO) (Pan et al., 2000; Onoda et al., 2000), anti-inflammatory (Banerjee et al., 2003), anti-tumor (Khar et al., 1999), anti-allergy (Ram et al., 2003) and anti-dementia (Lim et al., 2001). This study was undertaken to verify the variation C. xanthorrhiza and C. domestica promising lines from Sukabumi of Indonesia for their total curcuminoid, antioxidant and anti-inflammatory activities.

**MATERIALS AND METHODS**

**Materials**

The rhizomes of C. xanthorrhiza and C. domestica promising lines were collected during July 2011 from the area of Sukabumi, West Java, Indonesia. The authentic chemical standards of curcuminoid (curcumin, demethoxycurcumin, and bisdemethoxycurcumin) and 2,2-diphenyl-1-picrylhydrazyl were obtained from Sigma-Aldrich, USA. The bioassay kit COX was purchased from Cayman Chemicals. All the chemicals and solvents used were analytical grade.

**Extraction of plans**

Fresh rhizomes of plant materials were washed with water, cut into small pieces and dried for 5 days in the sun (the moisture: < 10%). They were then ground in a grinder to be obtained in a powder form (the size: 100 mesh). One kilogram of the powder of plants were macerated using 1 x 10 L ethanol 70% in a tightly closed round bottom flask at room temperature for a period of 24 h and filtered with Whatman filter paper (type 4). The whole process was repeated one times and the filtrate was concentrated under reduced pressure on rotavapor (BUCHI, R-250, Switzerland) at 50 °C temperature. The concentrated extracts were then used for the experiments.

**Determination of curcuminoid content**

The curcuminoid content of ethanol extract of C. xanthorrhiza and C. domestica promising lines
from Sukabumi was carried out according to the principle and protocol previously described by Jayaprakasha et al., (2002) using high performance liquid chromatography (HPLC), with modification. The systems condition of HPLC are C18 column, UV-Vis detector, 10 µL volum injection, and 48 °C temperature column. It’s used methanol as an additional mobile phase, which included solvents A: methanol; B: 2% acetic acid; and C: acetonitrile.

Antioxidant activity through measurement of free radical scavenging capacity by DPPH assay

Free radical scavenging capacity was investigated on the basis of the scavenging activity of DPPH by measuring the reduction of absorbance at 517 nm. The method was carried out as described by Udenigwe et al., (2009) with a modification. The different concentrations of the plant extracts (12.5–200 µg/ml; total volume of 40 µL) in 96-well plates were mixed with 160 µL of 100 mM DPPH in ethanol and then incubated in the dark for 30 min at room temperature prior to reading the absorbance at 517 nm in a micro plate reader. A negative control, containing water instead of the sample and blank samples, using the same volume of ethanol only in place of the DPPH solution in ethanol, were all evaluated at the same time per micro titre plate.

The percentage of radical scavenging was calculated as follows:

\[
\text{% radical scavenging} = \frac{(Ac - Acb) - (As - Asb)}{(Ac - Acb)} \times 100
\]

where Ac is the absorbance of water plus DPPH (in ethanol), Acb is the absorbance of the blank (water plus ethanol without DPPH), As is the absorbance of the sample plus DPPH (in ethanol) and Asb is the absorbance of the sample plus ethanol without DPPH. Different sample concentrations were used in order to obtain calibration curves and to calculate the IC\text{50} values (IC\text{50}: concentration required to obtain a 50% radical scavenging activity). All test samples were conducted in triplicate (n = 3).

Determination of anti-inflammatory activity

The ethanol extracts of \textit{C. xanthorrhiza} and \textit{C. domestica} promising lines from Sukabumi was evaluated as anti-inflammatory with cyclooxygenase 2 (COX2) inhibitory activities. COX2 inhibition activity was measured using the Cayman Chemical Colorimetric COX (ovine) Inhibitor Screening Assay Kit. All procedures were performed as indicated in the assay kit instructions. The ethanol extracts were dissolved in DMSO and added to the enzyme reaction mixture.

RESULTS AND DISCUSSION

Curcuminoids content

Curcuminoids compound are commonly in Curcuma species and have been reported to have several biological activities including antioxidant and anti-inflammatory properties (Itokawa et al., 2008). The results of curcuminoids content on
the ethanol extract of *C. xanthorrhiza* and *C. domestica* promising lines from Sukabumi are shown in Figure 1A. The curcuminoids content of *C. domestica* (value, 66.32 mg/g) most highest than *C. xanthorrhiza* with value 31.27 mg/g. The rhizomes of *C. xanthorrhiza* and *C. domestica* show different profiles in the three major curcuminoids. The curcuminoids in *C. domestica* are mainly curcumin, demethoxy-curcumin, and bisdemethoxy-curcumin (Figure 1B) (Lechtenberg et al., 2004; Thaikert & Paisookantivatana, 2009). While for the curcuminoids in *C. xanthorrhiza* are curcumin and demethoxy-curcumin (Figure 1C) (Lechtenberg et al., 2004).

**Antioxidant activity**

The measures of antioxidant activity were obtained using DPPH (2,2-diphenyl-1-picrylhydrazyl) method. Figure 2 shows the DPPH radical scavenging activity of the different successive extracts of *C. xanthorrhiza* and *C. domestica*. This activity was increased by increasing the concentration of sample extracts. DPPH antioxidant assay is based on the ability of DPPH, a stable free radical, to decolorize in the presence of antioxidants. The DPPH radical contains an odd electron, which is responsible for the absorbance at 517 nm and also for a visible deep purple color. When DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolorized, which can be quantitatively measured from the changes in absorbance. Thus, the DPPH radicals were widely used to investigate the scavenging activity of some natural compounds. DPPH is widely used to evaluate the antioxidant activity of natural compounds (Udenigwe et al., 2009). However, DPPH’s scavenging activity indicates the ability of the antioxidant compound to donate electrons or hydrogen, thereby converting the radical to a more stable species (Bougatef et al., 2009).

![Figure 1](image.png)

Figure 1. (A). Curcuminoids content of *C. xanthorrhiza* and *C. domestica* promising lines from Sukabumi, West Java, Indonesia. (B). the curcuminoids component of *C. domestica*: curcumin, demethoxy-curcumin, and bisdemethoxy-curcumin. (C). the curcuminoids component of *C. xanthorrhiza*: curcumin and demethoxy-curcumin.
Antioxidant activity using DPPH radical scavenging assay reported with IC$_{50}$ value is show in Figure 3. The lower the IC$_{50}$ is the higher the antioxidant activity of the Curcuma promising lines.

The results showed that the ethanol extracts of *C. xanthorrhiza* and *C. domestica* have highest of antioxidant activity (with IC$_{50}$ value < 200 µg/mL, Blois, 1958). IC$_{50}$ values for DPPH radical scavenging activity were 81.99 and 73.31 µg/mL, with *C. domestica* having lowest value and most potent than *C. xanthorrhiza*. Curcuminoids compound are the major *C. xanthorrhiza* and *C. domestica* compounds with antioxidant activity (Jayaprakasha et al., 2006; Masuda et al., 1992). This study, we used ethanol crude extract of *C. xanthorrhiza* and *C. domestica*, so it’s possible has pure compound most stronger as DPPH free radical scavenging activity than that extracts.

Anti-inflammatory activity

Anti-inflammatory activity of *C. xanthorrhiza* and *C. domestica* promising lines from Sukabumi was evaluated through the percentages of
cyclooxygenase 2 (COX2) inhibition. Results are given in Figure 4. Percent inhibition values for COX2 inhibitor activity of C. domestica and C. xanthorrhiza were 74.84 and 67.96 %, with C. domestica having the highest value. Curcuminoids compound are the major C. xanthorrhiza and C. domestica compounds with anti-inflammatory properties (Chainani-Wu, 2003; Tohda et al., 2006; Ozaki, 1990). This study, we used ethanol crude extract of C. xanthorrhiza and C. domestica, so it’s possible has pure compound most stronger as anti-inflammatory activity than that extracts.

![Graph](image)

Figure 4. Anti-inflammatory activity of C. xanthorrhiza and C. domestica promising lines from Sukabumi, West Java, Indonesia on cyclooxygenase 2 (COX2) inhibition.

CONCLUSION

Curcuma domestica promising line from Sukabumi, West Java, Indonesia is the highest curcuminoinds content compared to Curcuma xanthorrhiza, which are also known to have antioxidant and anti-inflammatory activities.

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REFERENCES


Itokawa H, Shi Q, Akiyama T, Morris-Natschke SL, Lee KH. 2008. Recent advances in the investigation of
Pulla Reddy Ach, Lokesh BR. 1992. Studies on spice principles as


