Comparison of antioxidant activity between decoction of dried Curcuma longa L., and Curcuma xanthorrhiza Roxb. rhizomes

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ABSTRACT

Free radical can cause the number of diseases. Almost plants produce antioxidant compounds, which have function to neutralize or terminate the chain reaction of free radical. Curcuma longa and Curcuma xanthorrhiza are medicinal plants, that the main constituent in the rhizomes is curcuminoid, which has antioxidant activity. The objective of the research is to compare the antioxidant activity between the dried decoction rhizomes. The rhizomes were screened of the phytochemical constituents. The decoctions were made by boiled of 3.98 g dried rhizomes at 90°C during 30 minutes, volume to be kept at constant, and then were filtered and the supernatants were evaporated on the water bath. Curcumin were isolated with 50 ml methanol, and antioxidant activity were determined with 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. Phytochemicals screening of both showed of positive results for the presence of alkaloid, saponin, quinon, and steroid/triterfenoid. Antioxidant activity of decoction of dried Curcuma longa is 18.4 μg/ml and Curcuma xanthorrhiza is 10.5 μg/ml. In conclusion, the antioxidant activity of dried decoction Curcuma xanthorrhiza is higher than Curcuma longa, and both of decoctions have very strong antioxidant activity.

Keywords: Antioxidant activity; Curcuma longa; Curcuma xanthorrhiza; decoction; DPPH assay

INTRODUCTION

Free radicals are produced continuously in the cells as part of normal cellular function, however excess production might play a role in pathophysiology of many disease conditions. Free radicals and other ROS are derived either from the endogenous metabolic processes in the human body includes mitochondrial respiration, peroxisomal metabolism, and others or from external sources include exposure to radiation, ozone, cigarette smoke, and others. Free radicals can attack a number of macro-molecules including lipids, proteins and DNA resulting in the cellular damage. In human, oxidative damage and free radicals are associated with a number of diseases including atherosclerosis, Alzheimer’s disease, cancer, ocular disease, diabetes, rheumatoid arthritis, and motor neuron disease. (Hajhashemi et al., 2010, Young et al., 2001).

Phytochemicals or plant constituents are the major source of antioxidants. Plants produce an extremely impressive array of antioxidant compounds such as carotenoids, flavonoids, cinnamic acids, benzoic acids, folic acid, ascorbic acid, tocopherols and tocotrienols to prevent oxidation of the susceptible substrate. Recent researches have shown that plant extracts, phytoconstituents and food from plant sources have effective inhibitors of lipid peroxidation and oxidative stress. Antioxidants of plant origin with free-radical scavenging properties could have great importance as prophylactic and therapeutic agents in several diseases caused due to oxidative stress (Sen et al., 2011).

Curcumin is extracted from the dried root of the rhizome Curcuma longa. The process of extraction requires the raw material to be ground into powder, and washed with a suitable solvent that selectively extracts coloring matter. The selection of solvents is done with care to meet extractability and regulatory criteria. The following solvents are considered suitable: isopropanol, in the curcumin manufacturing process isopropyl alcohol is used as a processing aid for purifying curcumin. Ethyl acetate, with a restriction placed on the use of chlorinated solvents, such as dichloroethane, it is found that ethyl acetate, owing to its polarity, is a reasonable replacement providing acceptable quality of product and commercially viable yields. Acetone, this is used as a solvent in the curcumin manufacturing process. Carbon dioxide, this is not currently used in commercial production. However, it is listed in EC Directive 95/45/EC and has potential as a substitute for chlorinated solvents. Methanol, this solvent is used occasionally as a processing aid for purification. Ethanol, this solvent is used sparingly because curcumin is completely soluble in ethanol. Hexane (Stankovic, 2004).

Almost Curcuma longa L. (turmeric), and Curcuma xanthorrhiza Roxb. (is an original medicinal plant from In-
donesia, commonly known as temulawak) were used in Indonesian Jamu, which in many cities is commonly sold on the street by hawkers as a fresh handmade jamu or at jamu stalls that sold as dried jamu, and the other forms. Traditionally turmeric used for fever, diarrhea, hyperlipidemic, asthma, dyspepsia, rheumatic inflammation, jaundice, cholelithiasis (gallstones), hypertension, and Curcuma xanthorrhiza used for fever, chronic cholecystitis, hypercholesterolemia, anorexia, acne, cholelithiasis (gallstones), cholagogue (promotes bile secretion), healthy promoters as well as to increase the production of breast-milk (Hutapea et al., 2000/2001, Dalimartha, 2008). Nowadays, both of them were researched to use as modern medicine. Curcuma xanthorrhiza Roxb are used in the formal therapy, e.g. in vitamins as appetite stimulant which have been named as Apecur, Curfos, Curcuma Plus, Vitacur; for digestion upset have been named as Curliv; as aco-lereticum, colagogum and liver protector called as Curcil, Heparviton, Hepasil, Hepa Q, and other names. Combination of them as supplement is Gramuno (IPA et al., 2013, Medica Asia et al., 2010).

The research of both are the ethanol extract of Cur-\textit{cuma xanthorrhiza} and \textit{Curcuma domestica} powder (the size: 100 mesh) have been published, the main bioactive substances in the rhizomes of \textit{C. xanthorrhiza} and \textit{C. domestica} are curcuminoinds had efficacy as antioxidant and anti-inflammatory activities. Curcuminoid content in the rhizomes of \textit{C. xanthorrhiza} and \textit{C. domestica} were 31.27 and 66.32 mg/g, respectively. IC50 values for 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity was 81.99 and 73.31 μg/ mL, with \textit{C. domestica} having lowest value and most potent than \textit{C. xanthorrhiza}. Percent inhibition values for COX2 inhibitor activity were 74.84 and 67.96 %, with \textit{C. domestica} having the highest value (Nurcholis et al., 2012).

The ethanol extract of \textit{C. xanthorrhiza} powder (the size: 80 mesh) had cytotoxic activities, LC50 values for BSLT was 210.3 μg/ml (Nurcholis et al., 2012). The secondary metabolites in the \textit{C. xanthorrhiza} ethanol and aqueous extracts showed total phenol content (TPC) from the calibration curves of gallic acid, ethanol extract was 199.00 ± 1.31, and aqueous extract was 19.99 ± 0.16 mg GAE/g. Total flavonoid content (TFC) using the calibration curve generated from catechin from ethanol and aqueous extracts were 101.66 ± 0.8 and , 10.58 ± 0.83 respectively. The total saponin and total alkaloid contents present in the rhizomes of \textit{C. xanthorrhiza} were 80.90 mg/g and 14.06 mg/g, respectively (Halim et al., 2012).

The Minimum Inhibitory Concentration (MIC) of ethanol 70% extract toward \textit{S. aureus} and \textit{S. mutans} were 0.1% (w/v), while against \textit{B. cereus} it showed 2.0% (w/v). Phytochemistry analysis showed it consists of alkaloid, quinone, and terpenoids (Mangunwardoyo et al., 2012).

The antioxidant activity of the curcuminoinds of \textit{Curcuma domestica} L. and \textit{C. xanthorrhiza} Roxb. and eight compounds which are prevalent constituents of their rhizome oils was examined using thiobarbituric acid reactive substances (TBARSS) assay with human low-density lipoprotein LDL as the oxidation substrate. IC50 value (μg/mL) of the methanol extracts and curcuminoinds of \textit{Curcuma domestica} and \textit{C. xanthorrhiza} on human LDL peroxidation were 0.31 ± 0.01 and 0.78 ± 0.03 μg/mL respectively. IC50 value (μg/mL) of the essential oils of \textit{Curcuma domestica} and \textit{C. xanthorrhiza} and the essential oil standards on human LDL peroxidation were 7.8 ± 0.2 and 2.2 ± 0.1 μg/mL respectively (Jantan et al., 2012).

**MATERIALS AND METHOD**

**Material**

\textit{Curcuma longa} L. and \textit{Curcuma xanthorrhiza} Roxb., it was identified at School of Life Sciences and Technology of Institute Technology of Bandung, cultivated at Wonogiri (Central Java), was harvested in October, 2012. \textit{C. longa} has length 7.5 – 9.0 cm; diameter 3.5 – 4.5 cm, and \textit{C. xanthorrhiza} rhizome has length 13–17 cm; diameter 7–11 cm

Reagent : DPPH (Sigma Aldrich), methanol (E.Merck). Demineralization water

Equipment: Spectrophotometer Jenway 6305 UV-Vis single beam (United Kingdom).

**Experimental Details**

**Phytochemical screening of dried of \textit{Curcuma longa} L. and \textit{Curcuma xanthorrhiza} Roxb. rhizomes**

Both of the rhizomes analyzed for the presence of phytochemical constituents such as flavonoid, alkaloid, saponin, quinon, tannin, and steroid/terpenoid using standard procedure (Harbone, 1973).

**Preparation of \textit{C. longa} and \textit{C. xanthorrhiza} rhizome decocts, and isolation of curcumin** (Panigoro, 2013)

3.98 g of dried rhizome was boiled in 50 ml water at 90°C during 30 minutes, volume to be kept at constant. The decoction was filtered and supernatant was evaporated on the water bath. After dried, curcumin was isolated with methanol. Methanol was added until the total volume of solution was 50 ml. The solutions were used as samples.

**Preparation of DPPH stock solution**

1.0 x 10⁻³ M concentration of DPPH solution was prepared in methanol.

**Assay of antioxidant activity with 1, 1-diphenyl-2-picryl hydrazyl radical (DPPH)**

Each of the samples was mixed with 1 ml DPPH stock solution, kept in dark for 30 min and optical density was measured at 517 nm using Spectrophotometer Jenway 6305 UV-Vis single beam. The absorbance was recorded. The result is % effective inhibition, and was calculated by the equation below:
RESULT AND DISCUSSION

Table 1: Phytochemical constituents of Curcuma longa L. and Curcuma xanthorrhiza Roxb.

<table>
<thead>
<tr>
<th>Constituents</th>
<th>Curcuma longa L.</th>
<th>Curcuma xanthorrhiza Roxb.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloid</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoid</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Saponin</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Quinon</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tannin</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Steroid/Triterfenoid</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

+ = Present; – = Absent; Both of rhizomes contain alkaloid, saponin, quinon, and steroid/triterfenoid

Figure 1: Percent inhibition of decoction of dried Curcuma longa L. rhizome on DPPH

The linear regression is $y = 20.93x + 38.97$. Note: $y$ is % inhibition and $x$ is sample concentration. $EC_{50} = x$, $y = 25.00x + 26.98$, $50 = 25.00x + 26.98$, $EC_{50} = 0.9208$ mg/50 ml = 18.4 μg/ml. Antioxidant activity of decoctions of dried Curcuma longa L. rhizome on DPPH = 18.4 μg/ml.

Figure 2: Percent inhibition of decoction of dried Curcuma xanthorrhiza Roxb. rhizome on DPPH

The linear regression is $y = 20.93x + 38.97$. Note: $y$ is % inhibition and $x$ is sample concentration. $EC_{50} = x$, $y = 20.93x + 38.97$, $50 = 20.93x + 38.97$, $EC_{50} = 0.5269$ mg/50 ml = 10.5 μg/ml. Antioxidant activity of decoctions of dried Curcuma xanthorrhiza Roxb. rhizome on DPPH = 10.5 μg/ml

CONCLUSION

$EC_{50}$ of decoction of dried Curcuma longa is 18.4 μg/ml and Curcuma xanthorrhiza is 10.5 μg/ml. The antioxidant activity of dried decoction Curcuma xanthorrhiza is higher than Curcuma longa, and both of decoctions have very strong antioxidant activity.

Table 2: Percent inhibition of decoction of dried Curcuma longa L. rhizome on DPPH

<table>
<thead>
<tr>
<th>No. of samples</th>
<th>Concentration of samples (mg/50 ml)</th>
<th>Absorbance</th>
<th>% Inhibition</th>
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<td>2</td>
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<tr>
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<tr>
<td>1</td>
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<td>0.073</td>
<td>0.082</td>
</tr>
<tr>
<td>2</td>
<td>1.9900</td>
<td>0.122</td>
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<tr>
<td>3</td>
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<td>0.333</td>
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<tr>
<td>4</td>
<td>0.4975</td>
<td>0.641</td>
<td>0.614</td>
</tr>
<tr>
<td>5</td>
<td>0.2487</td>
<td>0.777</td>
<td>0.784</td>
</tr>
</tbody>
</table>

Table 3: Percent inhibition of decoction of dried Curcuma xanthorrhiza Roxb. rhizome on DPPH

<table>
<thead>
<tr>
<th>No. of samples</th>
<th>Concentration of samples (mg/50 ml)</th>
<th>Absorbance</th>
<th>% Inhibition</th>
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<td>0.689</td>
<td>0.685</td>
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ACKNOWLEDGEMENTS

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REFERENCES


