Antioxidative Properties of White Saffron Extract (Curcuma mangga Val) in The β-Carotene Bleaching and DPPH-Radical Scavenging Methods

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ABSTRACT

Study on antioxidative properties of white saffron extract in the emulsion system of b-carotene linoleic acid (β-carotene bleaching method) and DPPH-radical scavenging method was undertaken. The objective of this study was to examine the antioxidative activity of white saffron extract in the emulsion system of b-carotene linoleic acid and for radical scavenging activity by DPPH method. The extraction was carried out as follows: fresh white saffron was peeled and blanched in the 0.5% boiling citric acid solution for 5 minutes, the blanched white saffron was grated, and added distilled water. The ratio between grated white saffron and distilled water were 1 : 1 ; 1 : 2 ; 1 : 3, and 1 : 4. The mixture was then manually filtered through cloth to obtain white saffron extract. The antioxidative activity of white saffron extract was evaluated in the emulsion system of β-carotene linoleic acid and DPPH- radical scavenging method with reference standard of Butylated Hydroxy Anisole (BHA) and linoleic acid with no white saffron extract as a control. The results of this study showed that the oxidative inhibition of white saffron extract in the emulsion system of β-carotene linoleic acid was not significantly different from to BHA 200 ppm. The lower ratio of grated white saffron and distilled water, the lower percent free radical scavenging capacity. The higher white saffron extract concentration (white saffron : distilled water = 1 : 2) the higher percent free radical scavenging capacity.

Key words: white saffron, antioxidant activity, β-carotene bleaching method, DPPH-Radical scavenging method

INTRODUCTION

White saffron is spices commonly used as a raw material of traditional medicine. White saffron (temu mangga) is a bush perennial and has stalk tubers. When the tuber is cutted the yellow flesh will be seen on the outside layer and slightly light yellow in the center layer. The white saffron aroma and taste are similar to a ripe mangoes. The syrup of white saffron showed antioxidative activity (Dwiyati, 2003). This is hipotyzed due to curcuminoid contain.

Antioxidant can be obtained both from natural resources such as curcuminoid from turmeric (Curcuma domestica Val) (Kikuzaki and Nakatani, 1993) and from synthetic material such as Butylated Hydroxy Anisole (BHA), Butylated Hydroxy Toluene (BHT), Tert Butyl Hydroxy Quinone (TBHQ) and Propyl Gallate (PG) (Sherwin, 1990 in Wanasundara
The antioxidative activity of curcuminoid compounds (curcumin, demethoxy curcumin and bisdemethoxy curcumin) was 20, 9 and 8 times higher than α-tocopherol, when it was determined by modified active oxygen (Toda et al., 1985). Jitoe et al. (1992) examined the antioxidative activity of curcuminoid in the alcohol-water system and reported that each compound gave an antioxidative activity, and calculated 2.5 times higher than α-tocopherol. Kakaiau et al., (1974) and Revankar et al., (1975) in Majeed et al., (1995) reported that antioxidative activity of curcuminoid as a food additive had a potential to prevent the oxidation of oil and fat during storage and heat processing.

The objective of this study was to examine the antioxidative properties of white saffron extract in the emulsion system of β-carotene linoleic acid, and by DPPH radical scavenging method.

MATERIALS AND METHODS

The main substance of this study was white saffron tubers (Curcuma mangga Val) purchased from local market. Chemicals used were linoleic acid, Tween 40, ethanol, DPPH, BHA (pa) purchased from Sigma Chemical, and chloroform. The equipment used in this study were spectrophotometer (Shimadzu UV-1601), rotary evaporator centrifuge (BUCHI Rotavapor R-114), incubator, and aerator.

Preparation of white saffron extract

White saffron tubers were selected, washed, peeled, washed, and grated. The grated white saffron was added with distilled water in the ratio of 1:1, 1:2, 1:3, 1:4. and then, manually pressed through filter cloth to obtain white saffron extract. White saffron extract were determined their antioxidative activity in the emulsion system of β-carotene linoleic acid (β-carotene bleaching method) (Miller, 1971 in Wanasundara et al., 1994) and by DPPH radical scavenging method (Hatano et al., 1998 in Duh, 1998). The experimental diagram of this study is shown in Figure 1.
White saffron tubers

Peeling

Washing

Blanching

Extraction with distilled water ratio of
= 1:1, 1:2, 1:3, 1:4

White saffron extract

values of emulsion system in the incubation time 0, 30, 60, 90, 120, and 150 minutes were measured at \( \pi \) 470 nm on Spectrophotometer Shimadzu UV-1601. The antioxidant activity was defined as percent inhibition calculated according to the following equation:

\[
\% \text{ inhibition} = \frac{(A_0 \text{ sample} - A_{150} \text{ sample})}{(A_0 \text{ control} - A_{150} \text{ control})} \times 100\%
\]

Where \( A_0 \) = absorbance 470 nm at initial incubation (0 min)

\( A_{150} \) = absorbance 470 nm after 150 min incubation time

Antioxidant activity assay using \( \alpha, \alpha \) diphenyl picrylhydrazil (DPPH-Radical scavenging method)

White saffron extract sample was diluted with the absolute ethanol in order to obtain a given concentration. The mixture was then centrifuged at 300 rpm for 10 minutes. Supernatant obtained were determined using DPPH as follows: a 4 ml of 0.5 mM DPPH was added with 1 ml diluted extract of white saffron, and the absorbance was measured at 517 nm wave length. A 200 ppm of BHA in the ethanol was used as reference standard, control was ethanol without extract.

The capacity of free radical scavenging activity (Radical Scavenging Activity) was calculated by the following equation:

\[
\text{Radical Scavenging Activity (\%)} = \left[ \frac{1 - (\text{sample absorbance at 517 nm})}{(\text{control absorbance at 517 nm})} \right] \times 100\%
\]

Experimental design

Data was analyzed by using completed randomized design single factor. When there was significant difference it was followed by Duncan's Multiple Range Test (DMRT) at 95% confidence interval.
RESULTS AND DISCUSSION

Antioxidant activity in the β-carotene bleaching method

Antioxidant activity in the emulsion system was determined by using β-carotene bleaching method. The principle of this method was to evaluate white saffron extract protection as an antioxidant resource which determined the oxidation of β-carotene linoleic acid by O₂ in the water (O₂-saturated water) and heating. The results of those evaluation in the emulsion system of β-carotene linoleic acid is shown in Figure 2.

![Figure 2. The antioxidant activity of white saffron extract in the emulsion system of α-carotene linoleic acid at 50°C for 150 min.](image)

KP 1:1; KP 1:2; KP 1:3, and KP 1:4 (white saffron : distilled water ratio = 1:1; 1:2; 1:3, and 1:4 BHA : Butylated Hydroxy Anisole)

Kontrol : control, ethanal without white saffron extract.

The oxidation inhibition level of white saffron extract in the emulsion system of β-carotene linoleic acid is shown in Table 1.

Table 1. The inhibition of β-carotene bleaching by white saffron extract

<table>
<thead>
<tr>
<th>Sample</th>
<th>% inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>BHA</td>
<td>89.09b</td>
</tr>
<tr>
<td>Extract of KP: Dw=1:1</td>
<td>88.26b</td>
</tr>
<tr>
<td>Extract of KP: Dw = 1:2</td>
<td>87.69b</td>
</tr>
<tr>
<td>Extract of KP: Dw = 1:3</td>
<td>87.19b</td>
</tr>
<tr>
<td>Extract of KP: Dw = 1:4</td>
<td>85.90b</td>
</tr>
<tr>
<td>Control (ethanol without extract)</td>
<td>0.000a</td>
</tr>
</tbody>
</table>

KP: white saffron
Dw: Distilled water

White saffron extract had an antioxidant activity in the emulsion system as shown in the curve of decrease absorbance of β-carotene, which white saffron extract had a slope higher compared to the control, and it is not significantly differences compared with BHA (p<0.05). And the trend was similar to that of the moringa leaves extract (Siddharaju and Becker, 2003). White saffron extract had an antioxidant activity. The variation of ratio white saffron : distilled water did not significantly effect as shown in Figure 2 and Table 1. This might be due to the content of curcuminoid (Dwiyati and Sutardi, 2003).

According to Khurana and Ho (1980), turmeric tuber extract contained several compounds such as curcumin (54.5%), demethoxy curcumin (13%), and bisdemethoxy curcumin (13%), and other compounds (16.9%). Curcuminoid as individual had an antioxidant activity, but natural complex curcuminoid containing all of three types of curcumin had the highest antioxidant activity compared to both each curcumin and BHT, when it was determined by Rancimat method (Majeed et al., 1995).

Antioxidant activity using DPPH method

DPPH assay was conducted in order to evaluate the radical scavenging activity of white saffron extract. Analysis of antioxidant activity using DPPH method was conducted to white saffron extract with various ratio of white saffron : distilled water and various concentration of white saffron extract (white saffron : distilled water ratio 1 : 2).

Antioxidant activity using various extraction ratio of white saffron : distilled water

White saffron extract antioxidant activity is that was defined as the percent of free radical scavenging capacity (Radical Scavenging Activity) was described in Figure 3.
White saffron extract evaluated using DPPH method had an antioxidant activity as shown in Figure 3. The antioxidant activity of white saffron extract: destilled water ratio were in the order of 1:1 > 1:2 > 1:3 > 1:4, it may be due to the curcuminoid content. It is in accordance with previous report (Majeed et al., 1995), curcuminoid had an antioxidant activity as a free radical scavenger. According to Khurana and Ho (1980), the tumeric tuber extract contains curcumin (54.6%), demetoxy curcumin (13%), and bisdemetoxy curcumin (13%) and other compound (16.9%).

Antioxidant activity using various concentration

The sample white saffron: destilled water ratio 1:2 was used was for further experiment were shown in Figure 4.

As shown in Figure 4, the various concentration of white saffron extract (white saffron:destilled water = 1:2) had significant impact on radical scavenging activity. It was caused the higher concentration of white saffron extract, the higher antioxidant activity. It may be due to a higher curcuminoid content. Culivier et al (1992)reported that the efficiency of curcumin cold be explained by increased resonance stabilization of the free radical by delocalization of the unpaired electron. It is in accordance with the previous report by Suryanto et al (2003) that the andaliman extract, as the concentration ranging from 200 to 900 ppm showed increasing activity on scavenging DPPH free radical. It may be due to a higher antioxidant content.

CONCLUSION

In conclusion, white saffron extract exhibited antioxidant activity in the assay using the emulsion system of b-carotene linoleic acid. In the assay DPPH method white saffron extract also showed free radical scavenging activity. The higher concentration of white saffron extract, the higher antioxidant activity.

ACKNOWLEDGEMENT

Authors wish to thank to the Directorate General for Higher Education (DGHE) Development Research Project of Republic of Indonesia, for providing fund for this study through HIBAH PEKERJA II/I in 2004. Authors also wish to thank to Prof. Dr. Ir. Sri Raharjo, M.Sc. for his kind consultation, and to Pipiet Aribowo, S.TP, Dhani Rahaman, S.TP, and Elies Hastarisa S.TP for their technical assistance in the Laboratory.
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