ANTIOXIDATIVE PROPERTIES OF WHITE SAFFRON EXTRACT (*Curcuma mangga* Val.) IN THE *IN VIVO* ASSAY

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ABSTRACT

A study on the antioxidative properties of white saffron extract (*in vivo*) has been conducted. The purpose of this study was to determine the antioxidative effects of white saffron extract in *in vivo* assay. Fresh white saffrons were peeled, washed, blanched at 100˚C in 0.5% citric acid solution for 5 minutes and grated. The ratio between grated white saffron and distilled water was 1:1; 1:2; 1:3 and 1:4. Then it was filtered in order to obtain white saffron extract. The extract was evaluated in terms of its antioxidant activity by using *in vivo*. Five-week old male Wistar rats were purchased from Experimental Animal Development Unit, Gadjah Mada University. After one week of adaptation, the rats were divided into six groups, feed and drinking water were provided *ad libitum*. White saffron extract was orally administrated using a syringe at 09.00 a.m and 14.00 p.m daily, for 14 days. The livers and serum were removed for analysis of thiobarbituric acid reactive subtances (TBARS), α-tocopherols and superoxide dismutase (SOD). The results of this study showed that white saffron extract has an antioxidative activity in the *in vivo* assay. The higher concentration of white saffron extract, the higher α-tocopherols and superoxide dismutase, but the TBARS value was lower.

Keywords: *Curcuma mangga* Val., rat, antioxidant activity, *in vivo*

INTRODUCTION

Antioxidant can be obtained both from natural resources such as curcuminoid from turmeric (*Curcuma domestica* Val) (Kikuzaki and Nakatani, 1993) and from synthetic materials such as butylated hydroxy anisole (BHA), butylated hydroxy toluene (BHT), tert butyl hydroxy quinone (TBHQ) and propyl gallate (PG) (Sherwin, 1990 in Wanasundara et al., 1994). In general, synthetic antioxidants are very effective, but its safety is still questionable. Therefore its use is tightly regulated in most countries. The current trend of increasing consumer's awareness and concern about the safety of synthetic additives in food products emphasizes the importance of continuing research in the application of natural antioxidants. Ginger is known for its ability to improve oxidative stability. The study on turmeric oleoresin added to peanut oil at a temperature of 60°C showed its capability to prevent oxidation. Kim et al. (1999) reported that chloroform fraction of *Rhus verniciflua* extract showed a higher antioxidative activity than commercial antioxidants, such as BHA and BHT. Therefore, the development of potential natural antioxidant, especially from tubers is needed.

White saffron aroma and taste are similar to ripe mangoes. It is the reason why people name it *temu mangga* (Fauziah, 1999). White saffron extracts contains phenolic compounds which is responsible for its antioxidative activity (Pujimulyani et al., 2010). Pujimulyani et al. (2004) reported that white saffron extract exhibited an antioxidant activity in an assay using the emulsion system of β-carotene linoleic acid. In the assay with 2,2-diphenyl-1-picrylhydrazyl (DPPH) method, and ferric-reducing antioxidant power (FRAP), white saffron extract also showed antioxidant activity (Pujimulyani et al., 2010).

Research on white saffron active substance has been done previously. Lestariana et al. (2000) and Atifah (1999) showed that the rhizome juice has cytotoxic activity against B-lymphoblastoid Cell-Lines (B-LCL), but not cytotoxic to B lymphocytes of healthy. White saffron contains ribosome-Inactivating Protein (RIP) that has cytotoxic activity. Juice of the rhizome is able to cut supercoiled DNA into nicked circular and linear, and has N-glycosidase activity that can bypass certain adenine bases in *Saccharomyces cereviceae* 26s rRNA (Lestariana et al., 2000).

Methanol extract of white saffron has cytotoxic activity against HeLa S3 cancer cells and Raji cells (Budiman, 2001). Major component of *Curcuma mangga* Val essential oils include β-felandren, β-mirsen, limonene, isopinokamfeol, and 2,6-nonadienal (Nurkhasanah, 2002). White saffron

essential oil showed cytotoxic properties against cancer cells Raji greater than HeLa S3 cancer cells. According Karioti et al. (2007), in general diterpenoid is potential immunomodulator. Shiyan (2008) showed that ethanol extract of white saffron can increase the antibody. According to Abas et al. (2004), white saffron extract showed antioxidant activity, that was tested by the FTC method and thiobarbituric acid (TBA).

Some studies showed that blanching increased antioxidant activity. Blanching of wheat with pressure at 100ºC after harvesting increased the total phenol of wheat powder (Cheng et al., 2006). Corn blanching with autoclave increased its total phenol (Randhir et al., 2008). The antioxidant activity of beans, corn, and tomato using DPPH method increased after blanching (Kwan et al., 2007). Brussel sprouts (*Brassica oleracea* L.), after water blanching at 100ºC for 2 and 3 minutes, have higher antioxidant activity compared to fresh brussel sprouts (Viña et al., 2007; Olivera et al., 2008). Bilberry extract was heated at 100ºC for 10 minutes had higher antioxidant activity compared to fresh extract, because glycoside was hydrolized into aglycon anthocyanidin and sugar (Yue and Xu, 2008). Zhang et al. (2004) and Sadilova et al. (2006) found that hydrolysis of glycoside anthocyanin into anthocyanidin occurred during heating in acidic condition.

The objective of this study was to examine the antioxidative properties of blanched white saffron extract in experimental rats (*in vivo*) and analysis was performed for TBARS, α-tocopherol and superoxidative activity of the livers and in serum.

MATERIALS AND METHODS

The main material in this study was white saffron rhizomes (*Curcuma mangga* Val.) purchased from local market, butylated hydroxyanisole (BHA), as a reference of antioxidant was purchased from Sigma Chemicals (St. Louis, MO, USA). The equipments used were HPLC, centrifuge, vortex, spectrophotometer (Shimadzu UV-Vis 1601), rotary evaporator (Buchi Rotavapor R-114), microsyringe, and magnetic stirrer.

Preparation of White Saffron Extract

White saffron rhizomes were selected, peeled, washed, blanched in the 0.5% boiling citric acid solution at 100°C for 5 minutes and grated. White saffron extract was prepared by extracting grated rhizomes with a 1:1, 1:2, 1:3 and 1:4 ratio of grated rhizomes to distilled water, and finally filtered with cheese cloth. White saffron extract was ready to use for *in vivo* assays.

The Assay of Antioxidant Activity *In Vivo*

The antioxidant activity of white saffron extract was *in vivo* method. Five-week old Wistar rats were purchased from UPHP, Gadjah Mada University. Forty two rats were divided into six groups (seven rats/group). The rats were kept individually in cages at a room with temperature of 27±1°C and humidity of 70 ± 5%. The feed and drinking water of rats were provided *ad libitum*. White saffron extract was orally administrated using a syringe at 09.00 a.m and 14.00 p.m daily for 14 days. After one week adaptation with standard diet of AIN-93G, the six groups were fed with rancid peanut oil, with the basic composition followed standard diet of AIN-93G (Table 1.).

Table 1. The composition of the normal diet and rancid peanut oil diet

Ingredient	AIN-93G# diet (mg/kgdiet)	peanut oil diet (mg/kgdiet)
Cornstarch	397.486	397.500
Casein (> 85% protein)	200.000	200.000
Dextrinized cornstarch (90-94% tetrasaccharides)	132.000	132.000
Sucrose	100.000	100.000
Soybean oil*	70.000	-
Rancid peanut oil**	-	70.000
Fiber	50.000	50.000
Mineral mix (AIN-95G-MX)	35.000	35.000
Vitamin mix (AIN-95G-VX)	10.000	10.000
L-Cystine	3.000	3.000
Choline bitartrate (41.1% choline)	2.500	2.500
Tert-butylhydroquinone	0.014	-

\# J. Nutr.123: 1939-1951 (1993)
* Wako Pure Chem. Ind. Ltd.
** Lab. of Mercubuana University (stored in the room temperature for 2 years)

The six group in the research: group I was fed with blanched white saffron extract (white saffron:distilled water = 1:1), group II was fed with blanched white saffron extract (white saffron : distilled water = 1:2), group III was fed with blanched white saffron extract (white saffron : distilled water = 1:3), group IV was fed with blanched white saffron extract (white saffron : distilled water = 1:4), group V was fed with non-blanched white saffron extract (white saffron : distilled water = 1:1), group VI was fed with distilled water (without white saffron extract)

At the end of the experimental period, the rats (seven in each group), were anaesthetized under Nembutal. After opening of the abdominal cavity the livers were perfused with 0,9% NaCl solution via the portal vein. The livers were removed for the analysis of α-tocopherol, thiobarbituric acid reactive substances (Ohkawa *et al*, 1996). The serum

was removed for TBARS analysis, α-tocopherols, and SOD (Oyanagui, 1984).

Statistical Analysis

Results from *in vivo* experiments were analyzed by Duncan's multiple range test to detect inter group differences where p-values ≤ 0.05 were considered statistically significant.

RESULTS AND DISCUSSION

TBARS Value of Serum and liver

The value of TBARS serum and livers, which were added with oxidized peanut oil diet and white saffron extract are shown in Table 2.

Table 2. TBARS Value of serum and liver

No	Treatment	TBARS serum (n mol MDA/ml)	TBARS liver (n mol MDA/g tissue)
1	Extract of KP : Dw = 1:1	2.85 ± 0.06 [a]	3.83 ± 0.12 [a]
2	Extract of KP : Dw = 1:2	3.09 ± 0.05 [b]	4.29 ± 0.16 [b]
3	Extract of KP : Dw = 1:3	4.83 ± 0.06 [c]	4.54 ± 0.06 [c]
4	Extract of KP : Dw = 1:4	5.50 ± 0.03 [d]	4.73 ± 0.07 [d]
5	Extract of KP : Dw = 1:1 (TB)	6.73 ± 0.07 [e]	4.97 ± 0.07 [e]
6	Control (Distilled water)	7.16 ± 0.04 [f]	5.17 ± 0.06 [f]

KP : white saffron
TB : Non Blanching
Dw : Distilled water
Means with the same superscript in the same columns are not significantly different

Table 2 shows that the diet of oxidized peanut oil causes the value of serum lipid peroxides to be higher than the rats which were given white saffron extract orally with 1 ml/rat twice a day for 14 days. It shows that oxidized peanut oil containing unsaturated fatty acid causes cellular membranes to have lipid oxidation (Saito and Nakatsugawa, 1994; Simopoukus, 1991; Hue et al, 1989). Giving white saffron extract at all variation shows that TBARS value is significantly lower compared to the given with of distilled water. Giving blanched white saffron extract at all variation shows that TBARS value is significantly lower compared to that of given with non-blanched white saffron. This might be due to the hydrolysis of quercetin-3-rutinoside of white saffron during blanching into aglicone quercetin. It was found that quercetin showed higher antioxidant activity than quersetin-3-rutinoside as measured by FRAP method (Pujimulyani, 2010). Li et al. (2009) reported that aglicone had higher antioxidant activity than glycoside. Other study also reported that anthocyanin of bilberry extract was hydrolyzed during heating (Yue and Xu, 2008). The higher concentration of white saffron extract, the lower TBARS serum value. It shows that white saffron extract can inhibit the oxidation of lipid rat livers that were given with diet of oxidized peanut oil, this white saffron extract shows an evident of containing the antioxidant.

TBARS value of rat liver given with oxidized peanut oil diet have the same trend with that of serum, whereas, the higher concentration of white saffron extract given, the lower the liver TBARS. This suggests that white saffron extract is able to inhibit the lipid oxidation in rat livers given with oxidized peanut oil diet. Therefore, this proves that white saffron extract contains the antioxidant substance.

α-Tocopherol Serum Content and Livers of Rats

Alpha-tocopherol in the serum and rat livers with the diet of oxidized peanut oil and were given white saffron extract are shown in Table 3.

Table 3. α- tocopherol in serum and liver of rats

Kel	Treatment	α-tocopherol serum (µg/dl)	α-tocopherol liver (µg/g tissue)
1	Extract of KP : Dw = 1:1	13.97 ± 0.99 [e]	0.130 ± 0.0007 [f]
2	Extract of KP : Dw = 1:2	11.47 ± 0.46 [d]	0.105 ± 0.0005[e]
3	Extract of KP : Dw = 1:3	9.93 ± 0.33 [c]	0.098 ± 0.0004[d]
4	Extract of KP : Dw = 1:4	7.77 ± 0.18 [b]	0.067 ± 0.0008[c]
5	Extract of KP : Dw = 1:1 (TB)	2.40 ± 0.05 [a]	0.016 ± 0.0001[b]
6	Control (Distilled water)	2.06 ± 0.04 [a]	0.015 ± 0.0001[a]

KP : white saffron
Dw : Distilled water
TB : Non blanching
Means with the same superscript in the same columns are not significanlly different.

Rat serum which was added oxidized peanut oil diet without white saffron extract (control) shows the lowest α-tocopherol content, while rats which were given the higher white saffron obviously showed higher α- tocopherol content. It shows that white saffron extract is able to inhibit the lack of antioxidants such as α- tocopherol in the treatment rat. White saffron extract with blanching treatment significantly shows the higher antioxidant activity compared to the one without blanching. It is possible for white saffron without blanching process that existing pigment to function as sensitizer in the fotooxidation with the light existed (Raharjo, 2004). Pujimulyani et al. (2010) showed that total phenol and total flavonoid in blanched white saffron are higher than non-blanched ones.

Rat livers given oxidized peanut oil diet without white saffron extract showed the lowest α-tocopherol content, while the rats with the higher concentration of white saffron extract showed higher α-tocopherol content. Like in the serum,

α-tocopherol livers of rat control were the lowest. It proves that white saffron extract can inhibit the lack of antioxidants such as α-tocopherol in the rat treatment livers.

Serum Superoxide Dismutase (SOD) of Rats

SOD content in the serum of rat treatment is shown in Table 4. The table shows that there is a significant increase of SOD content of rat serums which were given white saffron extract in the various solvents used. This is related to the factors that cause the TBARS value of rats given with dietary feed to be lower. White saffron extract contains curcuminoid (Pujimulyani and Sutardi, 2003), but the existence of antioxidant activity is not only caused by the curcumnoid, but also by the fact that there are catechin (C), epicatechin (EC), epigallocatechin (EGC), epigallocatechingallat (EGCG) and gallocatechingallat (GCG) and quercetin (Pujimulyani, 2010).

Table 4. Superoxide dismutase (SOD) value of rats serum

No	Treatment	SOD (NU/dl)
1	Extract of KP : Dw = 1:1	234.14 ± 4.91^{e}
2	Extract of KP : Dw = 1:2	221.71 ± 4.00^{d}
3	Extract of KP : Dw = 1:3	199.71 ± 4.31^{c}
4	Extract of KP : Dw = 1:4	181.86 ± 3.98^{b}
5	Extract of KP : Dw = 1:1 (TB)	159.86 ± 4.02^{a}
6	Control (Distilled water)	159.00 ± 3.06^{a}

KP : white saffron
Dw : Distilled water
TB : Non blanching
NU : Nitrit unit
Means with the same superscript in the same columns are not significanlly different.

CONCLUSIONS

The white saffron extract exhibits an antioxidant activity in the *in vivo* assay. The higher concentration of white saffron extract results in a higher antioxidant activity, α-tocopherol, and SOD, but the TBARS decreases.

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