Free Radicals Scavenging Activities of Spices and Curcumin

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ABSTRACT

Antioxidants possess ability to protect body from damage caused by free radical-induced oxidative stress. In order to evaluate the antioxidant activity of spice ethanol extracts of cucurmin has been used as positive control. Antioxidant activities have been determined by measuring *1,1-diphenyl-2-picryl-hydrazyl* (DPPH) free radical scavenging activity and hydrogen peroxide (H_2O_2) scavenging activity of spice extracts including turmeric (*Curcuma longa* L.), white saffron (*Curcuma mangga* Val.), temulawak (*Curcuma xanthorrhiza* Roxb), ginger (*Zingiber officinale* Roscoe). Ten concentrations of extracts 100; 50; 25; 12.5; 6.25; 3.125; 1.563; 0.781; 0.391 and 0.195 µg/mL were performed to determine the DPPH and H_2O_2 scavenging activities. Results showed that Inhibitory Concentrations (IC)-50 of DPPH were as followed *C. longa* 8.33 µg/mL; *C. mangga* 277.79; *C. xanthorrhiza* 39.58 µg/mL µg/m; *Z. officinale* 10.51 µg/mL; and curcumin 7.85 µg/mL. Meanwhile, the highest H_2O_2 scavenging activity each extract were as followed *C. longa* 55.82%, *C. mangga* 44.52%, C. *xanthorrhiza* 49.04%, *Z. officinale* 46.21 and curcumin 52.77%. In our current study, *C. longa* extract showed the highest antioxidant activity among all tested extracts and the lowest antioxidant activity was *C. mangga. C. longa* could be a potential candidate to inhibit the oxidative stress.

Keywords: DPPH, antioxidant, free radical, hydrogen peroxide, *C. longa, C. mangga, C. xanthorrhiza, Z. officinale* , curcumin

INTRODUCTION

Nowadays, the fact of harmful effect of reactive oxygen species (ROS) on human health is well-known. The capability of natural defense systems of living organisms against excess production of these ROS will decrease when influenced with negative environmental factors and different cellular and extracellular components will be damaged, causing or enhancing a number of degenerative diseases (Zaporozhets *et al*, 2004). Therefore, antioxidants are important in preventing such "oxidative" pathologies. To defend against damage from free radicals attack, living organisms and humans need to develop powerful and complex antioxidant system. Antioxidant will remove free radicals and other reactive species, protect biomolecules against damage, scavenge free radicals (Halliwel and Gutteridge, 1999; Zaporozhets *et al*, 2004). Types of antioxidant are synthetic and natural antioxidants. Many synthetic antioxidant used to phenolic compounds such as butylated hydroxyanisol (BHA), 1,4 butylated hydroxytoluen (BHT), tertiary butylhydroquinone (TBHQ). Natural antioxidants such as α-tocopherol and L-ascorbic acid are widely used because are safer and causing fewer adverse reactions, but natural antioxidant activities are lower activities than those of synthetic antioxidants. Antioxidant compounds present in spices, herbs have recently been promoted with no toxic side effects (Maslarova, 2001).

Moreover, in recent years many different methods have been proposed for the evaluation of antioxidant activities. Most of them are based on the measurement of the relative abilities of antioxidants to scavenge free radicals in comparison with standard antioxidant compound. This research was to determine antioxidant activity markedly DPPH assay to scavenge 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical and hydrogen peroxide (H_2O_2) scavenging activity of four spice ethanol extracts including turmeric (*C. longa* L.), white saffron (*C. mangga* Val.), temulawak (*C. xanthorrhiza* Roxb), ginger (*Z. officinale* Roscoe).

MATERIALS AND METHODS

Plant material. Four spices including turmeric (*C. longa* L.), white saffron (*C. mangga* Val.), temulawak (*C. xanthorrhiza* Roxb), ginger (*Z. officinale* Roscoe) were collected from farmer plantation located in Bogor-west Java (May 2009). The plants were identified by staff of herbarium, department of biology, school of life sciences and technology, Bandung institute of technology, Bandung, west Java, Indonesia. Four rhizome were chopped and dried using drying device (40-45 ^{0}C) until achieve the stable water with level (±13%), the dried rhizomes were milled producing 60 mesh size of flour.

Chemical materials 1,1-diphenyl-2-picrylhydrazyl (Sigma Chemical Co.), ethanol (96 %), hydroperoxide (Merck), phosphate buffer saline, HPLC grade methanol (Merck), curcumin (Sigma Chemical Co.).

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Extraction procedure. One kilogram of dried materials were extracted with distilled ethanol by macerasi extraction, filtered and evaporated using rotatory evaporator. Our process resulted ethanol extract of turmeric extract 138.51 g (13.851 %), white saffron extract 67.43 g (6.743 %), ginger extract 110.54 g (11.054 %) & temulawak extract 119.25 g (11.925 %).

Sample Preparation. Each extract of turmeric, *white saffron, temulawak* and ginger were dissolved with HPLC methanol to reach series of concentrations as followed: 100; 50; 25; 12.5; 6.25; 3.125; 1.563; 0.781; 0.391 and 0.195 µg/mL. To evalute the DPPH scavenging activity and H_2O_2 scavenging activity, the spices extract were compared to curcumin.

DPPH Free Radical Scavenging Activity Assay. DPPH assay was carried out as described by Unlu *et al.* (2003), Han *et al*. (2004) and Frum and Viljoen (2006). A 96-well microtitre plate was used to generate a quantitative measure of extracts' radical-scavenging activities. Fifty µL of each extracts' various concentrations were introduced in microtitre plate, followed by addition of 200 µL of DPPH solution (0.077 mmol/L DPPH in methanol). Mixtures were then mixed gently and kept in the dark for 30 min at room temperature. Absorbance of DPPH was determined by microplate reader at 517 nm. DPPH free radical scavenging activity of each sample was measured according to the formula below:

$$\text{scavenging\%} = \frac{A_c - A_s}{A_c} \times 100$$

A_s: absorbance of samples, A_c: negative control absorbance (without sample, only DPPH).

Hydrogen peroxide scavenging assay. Hydrogen peroxide solution (2 mM/L) was prepared with standard phosphate buffer saline (pH 7.4). Four hundred µL different concentration of the extracts (sample) was added to 100 µL of hydrogen peroxide in phosphate buffer. Absorbance was determined at 230 nm after 10 min at room.

$$\text{scavenging\%} = \frac{A_c - A_s}{A_c} \times 100$$

A_s: absorbance of samples, A_c: negative control absorbance (without sample, only H_2O_2 in phosphat buffer).

Statistical Analysis. The treatment were run in triplicate. To know the DPPH free radicals scavenging activities using median inhibition concentration (IC_{50}). The IC_{50} is the inhibition extract concentration that reduces 50% DPPH free radicals activity. The IC_{50} value for DPPH were obtained from DPPH scavenging activities and calculated using linear regression analysis in Microsoft Excel software. Completely randomized design has been utilized to figure out the H_2O_2 scavenging activity and continued by Duncan's post hoc test to know the highest activity among concentrations and kind of spices (Steel and Torrie, 1993).

Results and Discussion

The DPPH Radical Scavenging Activity

The DPPH free radical scavenging activity of spices extract and curcumin which has been well known as positive control on various concentrations were measured for their antioxidant activity. The DPPH free radical scavenging activity of spices ethanol extract are shown in Figure 1. and Table 1.

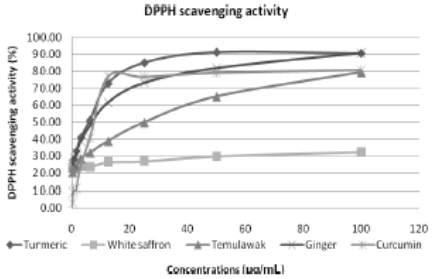

Figure 1. DPPH scavenging activity of spices extract and curcumin

Base on the data (figure 1), it showed that DPPH free radical scavenging activity of spices extract as well as curcumin showed that the highest concentration increased the antioxidant activity. Turmeric and ginger showed highest DPPH scavenging activity at level 100 µg/mL, 50 and 25 µg/mL. Base on the IC_{50} value (Table 1.). However, the hisghest DPPH scavenging activity was curcumin 7.85 µg/mL, followed by turmeric 8.33 µg/mL and ginger 10.51 µg/mL. The moderate antioxidant activity was showed by temulawak 39.58 µg/mL respectively. The lowest DPPH scaveving activity was showed by white saffron 277.79 µg/mL. Among spices extract, turmeric extract was strongest antioxidant and furthermore ginger extract, and temulawak. Turmeric as well as its active constituent *curcumin*, have been reported had high antioxidant activity to inhibits lipid peroxidation & cyclooxygenase activity (Hussain, 2002).

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Temulawak contains curcuminoid is one of the compounds in *Curcuma* exhibit antioxidant activity as free radical scavenger (Majeed *et al*., 1995; Pujimulyani *et al*., 2004; Achmad *et al*., 2007). The antioxidative activity of curcuminoid compounds (curcumin, demethoxy curcumin and bisdemethoxy curcumin) is 20, 9 and 8 times higher compared with α-tocopherol using modified active oxygen method (Pujimulyani *et al*., 2004; Widowati *et al*., 2011).

Table 1. The median inhibitory concentration (IC50) of DPPH scavenging activity of spices extract and curcumin

No	Sample	IC50
1	Turmeric	8.33
2	White saffron	277.79
3	Temulawak	39.58
4	Ginger	10.51
5	Curcumin	7.85

White saffron extract had no antioxidant activity; it was very contradictory with previous research by Ruangsang *et al*. (2009) which white saffron rhizomes have antioxidant. According to previous study water extract of white saffron exhibit antioxidant activity using β-carotene bleaching and DPPH scavenging method. Higher concentration of white saffron extract will increase the antioxidant activity, it may be due the curcuminoid content (Pujimulyani *et al*., 2004). This research, white saffron rhizomes were extraxcted by ethanolic and previous study using water as solvent to extract, the different solvent would result the different compound and bioactivity, validated by previous study that water extract of Forsythia koreana flowers exhibited a higher phenolic content than ethanol extract (Yang and Kang, 2011). Many biological functions of polyphenols including antioxidative (Frei and Higdon, 2003). Phenolic possess a wide spectrum of biochemical activities such as antioxidant, antimutagenic, anticarcinogenic (Marinova., 2005), phenolic in foods is important for their oxidative stability (Carbanaro *et al*., 2002).

The Hydrogen Peroxide Scavenging Activity

The hydrogen peroxide (H_2O_2) scavenging activity of spices extract and curcumin as positive control showed at Table 2.

The data showed mean and standard deviation. The small letters at the same column (among extract concentrations) and capital letters at the same row (among antioxidant agent) show no significant at the 5 % (Duncan's Post Hoc test).

The H_2O_2 scavenging activity the extracts were as follow: turmeric was 55.82% at level 0.195 µg/mL, white saffron was 44.52% at level 0.915 µg/mL, temulawak was 49.04% at level 0.781 µg/mL, ginger was 46.21 at level 0.195 µg/mL and curcumin was 52.77% at level 0.391 µg/mL. The highest H_2O_2 scavenging activity was showed by *turmeric*. The activity of H_2O_2 scavenging was contradicatory with DPPH scavenging activity according to the concentration level, by assuming H_2O_2 scavenging activity have specific and certain reaction whereas DPPH scavenging activity have general reaction which only based on the sample ability to donate hydrogen (H).

Table 2. H_2O_2 Scavenging Activity (%) on Spices Extract and Curcumin

Concentration µg/ml	Samples				
	Turmeric	White Seffron	Temulawak	Ginger	Curcumin
100	0.00±0.00 a A	0.00±0.00 a A	0.00±0.00 a A	0.00±0.00 a A	0.00±0.00 a A
50	0.00±0.00 a A	0.00±0.00 a A	0.00±0.00 a A	0.00±0.00 a A	0.00±0.00 a A
25	0.00±0.00 a A	0.00±0.00 a A	0.00±0.00 a A	0.00±0.00 a A	0.00±0.00 a A
12.5	0.00±0.00 a A	0.00±0.00 a A	0.00±0.00 a A	0.00±0.00 a A	0.00±0.00 a A
6.25	0.00±0.00 a A	0.00±0.00 a A	0.00±0.00 a A	0.00±0.00 a A	0.00±0.00 a A
3.125	15.82±2.07 b B	12.88±1.55 b B	38.98±1.89 cd D	22.26±2.41 a C	3.84±3.06 a A
1.563	32.54±0.89 c C	27.34±1.28 c AB	49.38±2.41 e D	24.29±1.60 a A	29.72±1.87 c C
0.781	48.81±2.38 d D	36.95±3.67 d B	49.04±2.76 e D	30.28±1.41 c A	42.26±1.99 d C
0.391	48.59±1.93 d B	38.08±4.47 d A	39.55±5.18 d A	38.98±1.55 d A	52.77±2.55 e B
0.195	55.82±1.03 e C	44.52±1.09 e BC	33.90±6.22 c B	46.21±6.95 e BC	12.66±11.43 b A

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Hydrogen peroxide is usually removed in areobes by two types of enzyme. The catalases directly catalyse decomposition of H_2O_2 to ground-state O_2 (Halliwel and Gutteridge, 1999):

$$2\,H_2O_2 \rightarrow 2\,H_2O_2 + O_2$$

Peroxidase enzymes remove H_2O_2 by using it to oxidize another substrate :

$$\text{Substrate-}H_2 + H_2O_2 \rightarrow \text{Substrate} + 2H_2O$$

Hydrogen peroxide at high concentration is deleterious to cells and its accumulation causes oxidation of cellular targets such as DNA, proteins, and lipids leading to mutagenesis and cell death. Removal of the H_2O_2 from the cell by catalase provides protection against oxidative damage to the cell (Halliwel and Gutteridge, 1999). Catalase activity varies greatly between tissues and Aspecies or organism Halliwel and Gutteridge, 1999).

CONCLUSION

Turmeric (*C. longa* L.) extract was showed the highest DPPH scavenging activity among spices extract, but it was lower than curcumin. The lowest activity was showed by white saffron (*C. mangga* Val). Turmeric (*C. longa* L.) extract had the highest H_2O_2 scavenging activity among the tested spices extract and curcumin, while and the lowest was showed by white saffron extract (*C. mangga* Val). Turmeric extract showed both the highest DPPH and H_2O_2 scavenging activity, while white saffron was lowest DPPH and H_2O_2 scavenging activity.

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