PROCEEDING

The International Conference on Pharmacy and Advanced Pharmaceutical Sciences

Faculty of Pharmacy UGM
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Preface from the Editor

The proceeding was produced based on papers and posters presented at the international Conference on Pharmacy and Advanced Pharmaceutical Sciences, held in Yogyakarta, Indonesia, 5 – 6 October 2009.

The proceeding clearly reflects broad interest; from there are participants coming from all around the world. Many contributions on Pharmaceutical Sciences there are quite a substantial number of papers on Pharmacist role in general. The papers presented file into a broad spectrum in Pharmaceutical sciences including Pharmacology, Toxicology, Analytical Chemistry and Drug Design, Drugs Synthesis, Formulation of Drugs, Pharmacy Social, Pharmacoepidemy, Traditional Medicine Natural Product Chemistry and Phytochemistry, etc.

In addition there are substantial numbers of paper deal with professional aspect of Pharmacist in general health care.

In this an opportunity, I would like to express my appreciation to the editorial team of the proceeding who have been working hard to review manuscripts, and making the first edition of this proceeding be possible.

I would like also to thanks to all invited speakers and presenters who participated in the International Conference on Pharmacy and Advanced Pharmaceutical Sciences and your contribution to this proceeding.

Finally, I hope this proceeding will give contribution to the advanced scientific research in the field of pharmaceutical sciences

Yogyakarta, July 2010

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Welcome Message
From the committee

Welcome to Yogyakarta

On behalf of the Scientific and Organizing Committees, it is a great pleasure for me to welcome all participants to Yogyakarta, to the International Conference on Pharmacy and Advanced Pharmaceutical Science 2009.
The international conference is organized by the faculty of Pharmacy UGM to celebrate its 63th anniversary and the Lustrum XII of Gadjah Mada University, as a collaboration work between the Faculty of Pharmacy UGM with the Nara Institute of Science and Technology (Japan) and the Universiti Sains Malaysia (Malaysia). In this conference 15 lectures within the field of Pharmaceutical Care and Advanced Pharmaceutical Science will be given by invited speakers. Besides, 55 posters and 75 paper will be presented in the parallels presentation sessions. Herewith, we express our gratitude to all speakers and presenter, who would like to share their advance knowledge in this scientific event.

The Organizing Committee gratefully acknowledges the Nara Institute of Science and Technology and the Universiti Sains Malyasia, for the nice collaboration in bringing forth this conference. A special acknowledgment is addressed to the Rector of Gadjah Mada University and the sponsors, for all supports that make this symposium possible. Furthermore, personally, I want to express my deep appreciation to the members of the Organizing Committee, for the good teamwork and their great effort given in the preparation for this symposium.

Finally, I wish all participants a scientifically rewarding and an enjoyable meeting in Yogyakarta.

Chairman

Dr. Hilda Ismail, M.Si., Apt.

CONTENTS

The difference of antioxidant activity of various tea (*Camellia sinensis* L.) methanol extract

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Abstract

The dried leaves of the plant *Camellia sinensis* L., is a popular beverage consumed worldwide and contained bioactive compounds. The three main categories of tea are black, green and oolong. Black tea, green tea and oolong tea came from the same tea plant species. The difference is the processing method of tea. Black tea undergoes several hours of oxidation during preparation, oolong tea is partially fermented and green tea is steamed to stop oxidation. This causes different bioactive content. This research was carried out to determine the antioxidant activity of tea methanol extracts. To evaluate various methanol extracts hydroxyl radical scavenging activity (*OH), and superoxide dismutase level (SOD) were used. To know the antioxidant activity of the methanol extract of black tea, green tea and oolong tea were arranged 6 level concentrations respectively 500 µg/mL; 250; 125; 62.5, 31.25 and 15.625 µg/mL and compared to quercetine as positive control. The results showed that oolong tea extract exhibited the highest hydroxyl radical scavenging acitivity at all level concentrations compared to green, black tea extract and quercetine, the highest hydroxyl scavenging activity was methanol extract at level of 500 µg/mL (92.593%). The oolong tea extract had the highest superoxide dismutase level at all level of concentrations compared to green, black tea extract and quercetine, the highest superoxide dismutase level was methanol extract at level of 500 µg/mL (157.067 U/mL).

Keywords : antioxidant, hydroxyl radical, superoxide dismutase, black tea, green tea, oolong tea

Introduction

Tea is one of the most frequently consumed beverages in the world. Tea is the dried leaves of the plant *Camellia sinensis*, is a popular beverage consumed worldwide. About 78% of the tea production worldwide is black tea, which is the main tea beverage consumed in the US and Europe. Green tea, which is the main tea beverage in Japan and parts of China, about 20% of worldwide production, while the remaining 2% of tea production is Oolong tea which is consumed mainly in Southern China and Taiwan (Chen et al., 1997; Sun et al., 2006). About three billion kilograms of tea are produced and consumed yearly. The possible beneficial health effects of tea are being investigated and have received a great deal of attention. Green tea, black tea and oolong tea from the same tea plant species, the difference are the processing of tea. Green tea is steamed to stop oxidation during processing and is manufactured by drying fresh tea leaves. Black tea undergoes several hours of enzymatic oxidation during preparation (accelerated by heat and humidity). Oolong tea is partially fermented (Balentine, 1992; Lunder, 1992).

Green tea and black tea contain flavonol glycosides (quercetin and kaempferol) (Balentine, 1992; Hertog et al., 1992). Dried tea extract contains 25%- 40% polyphenols in green tea, these are flavonols (catechins), of which epigallocatechingallate is the most prevalent compound (Balentine, 1992; Lunder, 1992). Black tea contains mainly thearubigins and theaflavins, complex condensation products of tea catechins (Miura et al., 2001). Most of the catechins are oxidized to thearubigens and theaflavins, which give the extract its characteristic red-brown color (Miura et al., 2001). Plant polyphenols, a large group of natural antioxidants, are potential candidates and have protective effect, are mainly contained in beverages, such as tea and wine. The antioxidant may heal, prevent disease in humans (Halliwel and Gutteridge, 1999). The high level of flavonoids in tea can protect cells and tissues from oxidative damage by scavenging oxygen-free radicals. Chemically, the flavonoids found in tea are very effective radical scavengers (Rietveld and Wiseman, 2003).

Reactive oxygen species (ROS) (including superoxide anion $^{*}O_2^{-}$, hydroxyl radical $^{*}OH$, singlet oxygen $^{1}O_2$, and hydrogen peroxide H_2O_2) are important agents of various human diseases (Halliwel and Guuteridge, 1999; Pokorny et al, 1999) such as cancer, heart disease, multiple sclerosis, Parkinson's disease, autoimmune disease, stroke, and others. Among the various radicals, the hydroxyl radical ($^{*}OH$) is presumed to play a central role due to its strong activity (Wang et al, 1993; Halliwel and Gutteridge, 1999). Superoxide is ROS primarily formed by phagocytic cells. In inflammation processes superoxide may react with hydrogen peroxide leading to the most deleterious ROS, the hydroxyl radical. ROS, like singlet oxygen, hydroxyl and superoxide radicals, are often generated in biological systems during photosensitized oxidation processes (Chapple, 1997).

The kind of antioxidants are natural and synthetic, have been proposed for use in the treatment of many human diseases. Among naturally occurring antioxidants are superoxide dismutase, α-tocopherol, ascorbic acid, carotenoids, quercetine (Halliwel and Gutteridge, 1999, Papas, 1999).

In the present investigation we studied the antioxidant properties of three natural products, methanol extract of dried leaf of various tea (green, black and oolong teas) antioxidant agent against ROS (hydroxyl and anion superoxide).

Methodology

Materials

The dried leaf of various tea (green, black and oolong teas), methanol 96 %, DMSO, 2- deoxyribose (2.5 mM) in phosphate buffer (10 mM, PH 7.4), $FeCl_3$ (25 mM) in EDTA (100 mM), PBS (0.1 M, pH 7.4), ascorbic acid (1.0 mM), H_2O_2 (0.1 M), TCA (2.8 %), TBA 1 % in NaOH 50 mM. KIT SOD (RANSOD) comprises of Reagent 1 (mixed substrat), Reagent 2 (Ransod diluent), Reagent 3 (xanthine oxidase), Reagent 4 (standard S 6), Ransod Control.

Methods

Extraction

Three dried leaf tea (green, black tea and oolong teas) were chopped into powder (60 mesh size) and the powder dried leaf tea were soaked in MeOH 96 % during 3 days and the liquid were collected. The liquid were evaporated then produced methanol extract of various tea.

Hydroxyl radical scavenging assay

Hydroxyl radical scavenging activity was carried out by measuring the competition between deoxyribose and the extract for hydroxyl radicals generated from the Fe^{3+} /ascorbate/EDTA/H_2O_2 system. The attack of the hydroxyl radical to deoxyribose leads to thiobarbituric acid reactive substances (TBARS) formation. Various concentrations (500, 250, 125, 62.5, 31.25 and 15.625 µg/mL) of three methanol extract 5 µL at the eppendorf tube were added to the reaction mixture containing phosphate buffer 0.1 M pH 7.4 (40 µL), 2.5 mM deoxyribose in phosphate buffer 10 mM pH 7.4 (276 µL), 25 mM $FeCl_3$ in 100 mM EDTA (40 µL). The reaction mixture was incubated at 37 °C for 1 hour. The reaction mixture were added 1.0 mM ascorbic acid (40 µL), 0.1 mM H_2O_2 (4 µL) and incubated at 37 °C for 30 minute. 400 µL trichloroacetic acid (TCA, 2.8%) and 200 µL of thiobarbituric acid (TBA, 1%) were added to test tubes and incubated at 100 °C for 8 minute. After that the mixtures cooled at the ice, absorbance was measured at 532 nm. Blank (sample) contained phosphate buffer, deoxyribose, phosphate buffer Blank (control) contained phosphate buffer, DMSO, deoxyribose. Control contained phosphate buffer, deoxyribose, $FeCl_3$ (Gomes et al., 2001; Safitri et al, 2002). Reactions were carried out in triplicate. Hydroxyl radical scavenging activity in percent was calculated in the following formulation:

$$^{*}OH \text{ scavenger } (\%) = \{1-(\text{Sample-blank sample})/(\text{Control-blank control})\} \times 100$$

Superoxide dismutase assay

The role of superoxide dismutase (SOD) is to accelerate the dismutation of the toxic superoxide radical (O_2^{*-}) produced during oxidative energy processes to hydrogen peroxide and molecular oxygen. Prepare various tube respectively as diluted sample, sample diluent, standards, control and blank. Diluted sample contained 5 µL sample (500, 250, 125, 62.5, 31.25 and 15.625 µg/mL extract), mixed substrate 170 µL, xanthine oxidase 25 µL. Sample diluent contained Ransod sample diluent 5 µL, mixed substarte 170 µL, xanthine oxidase 25 µL. Standards (Standard 2,3,4,5,6) contained standard 5 µL, mixed substarte 170 µL, xanthine oxidase 25 µL. Control conatined Ransod control 5 µL, mixed substrate 170 µL, xanthine oxidase 25 µL. Blank contained ransod diluent 200 µL. Absorbance was measured at 505 nm against blank, initial absorbance (A1) after 30 seconds and final absorbance (A2) after 30 minutes. Level SOD were compared to Ransod control (Randox Laboratories, 2004).

Results and Discussion

The hydroxyl free radical (*OH) scavenging activity of three MeOH extract were measured to know the antioxidant activity especially to scavenge the *OH free radical (Gomes et al, 2001; Unlu et al, 2003). The antioxidative capacity of various MeOH extract were examined by comparing to the activity of known antioxidant quercetine by in vitro assays : hydroxyl scavenging and superoxide dismutase (SOD) activity. The highest *OH scavenging activity were oolong tea extract at level 500 µg/mL, the lowest activity was quercetine at level 15.625 µg/mL At all concentrations oolong tea extract had higher *OH scavenging activity compared to black, green tea extract and quercetine. The highest *OH scavenging activity of black tea extract was black tea extract 62.5 µg/mL, green tea extract had same *OH scavenging activity at all concentrations. The highest *OH scavenging activity of quercetine was quercetine 125 µg/mL.

Table 1. Hydroxyl radical scavenging activity of various tea extract

Concentrations	Type of antioxidant sources			
	black tea MeOH extract	green tea MeOH extract	oolong tea MeOH extract	quercetine
500 µg/mL	70.987±1.235 a A	74.074±1.852 b A	92.593±1.633 c B	74.074±0.617 b AB
250 µg/mL	72.634±0.943 a A	75.720±0.356 b A	89.300±1.886 c AB	73.869±1.285 ab AB
125 µg/mL	72.428±0.943 a A	74.074±1.069 a A	87.654±2.691 b AB	74.897±1.886 a B
62.5 µg/mL	80.658±1.554 bc B	73.663±1.886 ab A	87.243±5.177 c AB	72.016±6.484 a AB
31.25 µg/mL	77.984±2.168 a B	70.782±6.799 a A	87.860±2.337 b AB	72.634±0.356 a AB
15.62 µg/mL	73.868±1.984 b A	75.309±0.618 b A	84.980±2.917 c A	69.136±1.069 a A

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

Hydroxyl radical can be generated in biologically systems by multiple reactions : Fenton reaction, UV induced homolytic fission, hypochlorus acid reacting with O_2^{*-} (Halliwel and Gutteridge, 1999).

Fenton reaction is prime example of damaging free radical reactions catalysed by transition metals. A mixture of H_2O_2 with Fe^{2+} salt oxidizes many different organic molecules.

$$Fe^{2+} + H_2O_2 \longrightarrow \text{intermediate complex} \longrightarrow Fe(III) + {}^*OH + OH^-$$

Ultra violet induced homolytic fission of the O-O bond in H_2O_2 makes $^*OH$

$$H\text{-}O\text{-}O\text{-}H \xrightarrow{UV} 2\ ^*OH$$

Hypochlorus acid reacting with O_2^{*-}

$$HOCl + O_2^{*-} \longrightarrow O_2 + Cl^- + {}^*OH$$

To know the $^*OH$ radical scavenging activity by 2-deoxyribose oxidized method through Fenton reaction, 2-deoxyribose be degraded and produce Malonaldialdehyde (MDA), Fenton reaction decomposes H_2O_2 and need Fe^{2+} salt. MDA and thiobarbituric acid (TBA) undergoes heating at the low pH will produce the chromogen (pink colour) can be measured by spectrophotometer with absorbance 532 nm (Gomes et al, 2001; Safitri, 2002; Unlu et al, 2003). In the $^*OH$ scavenging activity assay, radicals were generated by incubating the reagents at 37ºC for 60 minute and are known as a Fenton system : the mixture of H_2O_2, and Fe^{3+} EDTA with adding ascorbic acid pH 7.4. The adding of ascorbic acid will increase the rate of $^*OH$ forming through Fe reduction and preserve the Fe^{2+} level (Halliwel and Gutteridge, 1999; Gomes et al., 2001, Safitri, 2002).

$$Fe^{3+}\text{-EDTA} + \text{ascorbic acid} \longrightarrow Fe^{2+}\text{-EDTA} + \text{radical ascorbic}$$

$$Fe^{2+}\text{-EDTA} + O_2 \longrightarrow Fe^{3+}\text{-EDTA} + O_2^-$$

$$2\ O_2^- + 2\ H^+ \longrightarrow H_2O_2 + O_2$$

$$Fe^{2+}\text{-EDTA} + H_2O_2 \longrightarrow Fe^{3+}\text{-EDTA} + {}^*OH + OH^-$$

$$^*OH + \text{deoxyribose} \longrightarrow \text{fragment} \longrightarrow \text{MDA}$$

$$2\ TBA + MDA \longrightarrow \text{chromogen}$$

This assay also could give the information of compound are capable to chelate Fe which could inhibit forming $^*OH$ and also reduce the degradation of deoxyribose. The antioxidant capacity to inhibit deoxyribose degradation on this reaction indicate the capability to disturb and destroy Fenton reaction.

Tea extracts including green, black and oolong tea extract had high antioxidant activity (> 70 %) in $^*OH$ scavenging activity, this data were similar with previous research. Black tea had antioxidant capacities because theaflavins present in black tea possess at least the same antioxidant potency as catechins present in green tea (Re et al., 1999). Both green tea and black tea also contain flavonol glycosides (quercetin and kaempferol). These compounds showed strong antioxidant properties (Hertog et al., 1992).

The role of superoxide dismutase (SOD) is to accelerate the dismutation of the toxic superoxide radical (O_2^{*-}), produced during oxidative energy processess to hydrogen peroxide and molecular oxygen (Halliwel and Gutteridge, 1999; Randox Laboratories, 2004). Superoxide compared to $^*OH$ is far less reactive with non-radical species in aquaeous solution. Superoxide reacts quickly with some other radicals such as NO^* (Halliwel and Gutteridge, 1999). Superoxide radical generated by the xanthine/xanthine oxidase (XOD) system was determined spectrophotometrically which react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride (INT) to form a red formazan dye. The superoxide dismutase activity is then measured by the degree of inhibition of this reaction (Randox Laboratories, 2004).

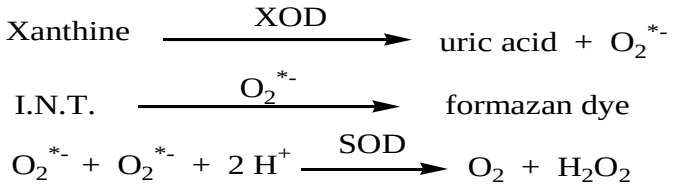

Table 2. Superoxide dismutase (SOD) of various tea extract

Concentration	Type of antioxidant sources			
	black tea MeOH extract	green tea MeOH extract	oolong tea MeOH extract	quercetine
500 µg/mL	114.653±7.345 c E	60.400±0.711 b D	157.067±14.121 d D	0.000±0.000 a A
250 µg/mL	105.480±11.309 c E	59.787±1.023 b D	149.720±12.166 d D	6.773±1.964 a BC
125 µg/mL	91.680±8.120 c D	56.507±0.740 b C	106.427±3.470 d C	11.240±4.561 a C
62.5 µg/mL	71.053±3.059 c C	41.467±2.420 b B	85.587±4.840 d B	20.493±4.020 a D
31.25 µg/mL	57.627±0.751 c B	40.867±2.343 b B	76.160±1.504 d AB	31.747±3.226 a E
15.625 µg/mL	33.053±1.740 c A	25.853±0.980 b A	70.453±2.646 d A	5.253±0.808 a B

The superoxide dismutase (SOD) level of various tea at various concentrations can be shown at Table 2.

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

Based on the research data showed, oolong tea extract at all concentrations had highest SOD level compared black, green tea extract and quercetine. The black, green and oolong tea extract showed that higher concentration would increase the SOD level. Its was different with quercetine which higher concentration would increase SOD level but at the certain concentration would decrease the SOD level. The highest SOD level of black, green and oolong tea extract was 500 µg/mL and quercetine was 31.25 µg/mL. The antioxidant acivity in SOD level was contradictory with previous report. The antioxidant capacity per serving of green tea (436 mg vitamin C equivalents) was much higher than that of black tea (239 mg). Green and black tea contained total phenols equal to 165 and 124 mg gallic acid, respectively (Sun et al., 2006). The amounts of polyphenols in green tea are 30–40% weight of the water-extractable materials, as compared with 3–10 % in black tea (Balentine et al., 1997).

The SOD level of black, green and oolong tea extract were higher than quercetin, perhaps extract contains many and complex compound, each compound synergetically increases SOD level.

Conclusions

Black, green and oolong tea extract had high *OH scavenging activity
Black, green and oolong tea extract had high SOD level compared to quercetin
Oolong tea extract had highest antioxidant both *OH sacvenging acitivity and SOD level

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