6th CMAPSEEC

6th Conference on Aromatic and Medicinal Plants of Southeast European Countries

April 18-22, 2010
Kervansaray Lara Hotel
Convention Center

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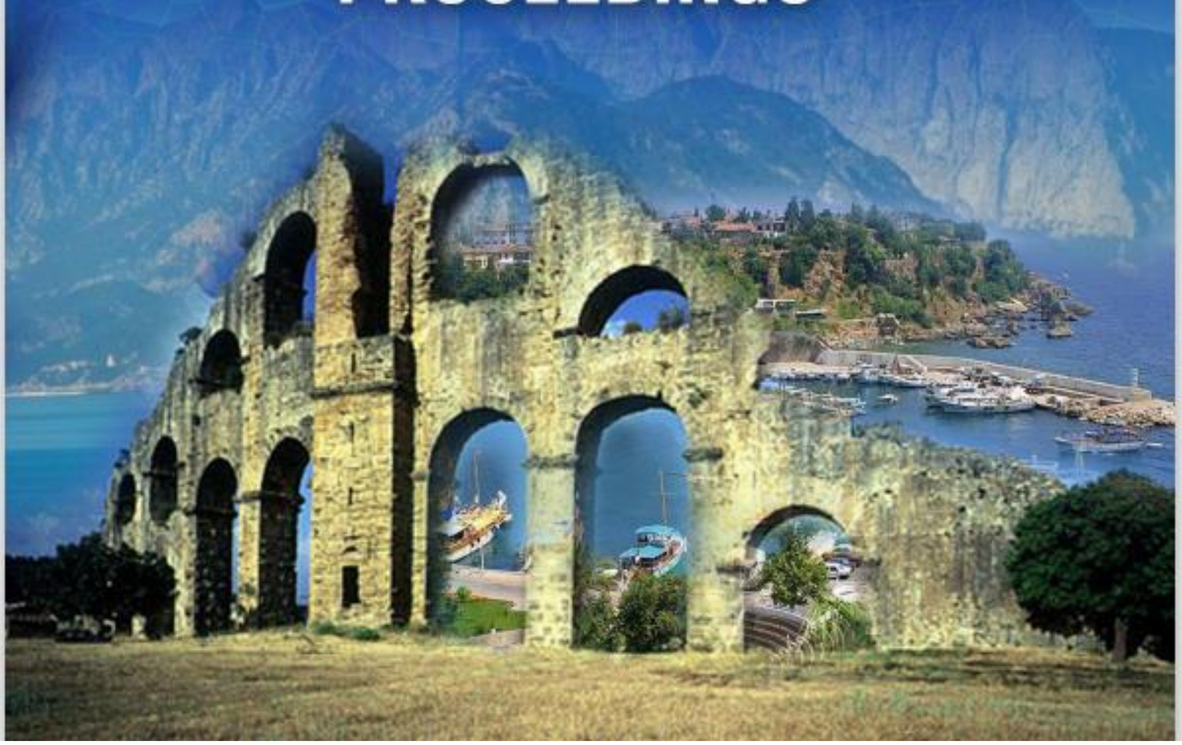

Published by: Damla Copy Center Matbaa
Kurbağalıdere Cad. No:37/1 Hasanpaşa Kadıköy/İstanbul
Tel: (0216) 550 31 90 Fax: (0216) 347 56 32
www.damlacenter.com

ISBN 978-605-61261-0-9

Published in Turkey

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Antioxidant and Anticholesterol *In vitro* Activity of Oolong Tea (*Camelia sinensis* L.) Extract

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Abstract

Epidemiologic studies have demonstrated an association between increased intake antioxidant and reduced cardiovascular disease. This association has been explained that atherogenesis is initiated by lipid peroxidation. The research was carried out to evaluate the free radical *1,1-diphenyl-2-picryl-hydrazyl* (DPPH) and anticholesterol activity of methanol extract of Oolong tea (*Camelia sinensis* L.). To know the antioxidant activity of oolong tea extract were compared with (-)-Epigallocatechine 3-gallate (EGCG) and to evaluate the anticholesterol of oolong tea extract were compared with simvastatin. The DPPH free radical scavenging activity and anticholesterol were carried out at 6 concentrations level (500 μg/mL; 250; 125; 62.5; 31.25 and 15.625 μg/mL). The results demonstrated that all concentrations of oolong tea extract had high antioxidant activity between 89.478 % and 92.923 % similar with EGCG, all concentrations of oolong tea extract had high anticholesterol activity between 91.813 % and 94.087 % were lower than simvastatin.

Keywords: *1,1-diphenyl-2-picryl-hydrazyl*, antioxidant, free radical, anticholesterol, oolong tea

Introduction

Epidemiological studies have shown an inverse correlation between diets rich in polyphenols and reduced risk of cardiovascular disease (CVD) (Mukamal et al., 2002). In a long-term study of a Dutch cohort the highest tea consumption was associated with a lower risk of death from coronary heart disease and lower incidence of stroke (Yang and Landau, 2000).

Tea is one of the most popular beverages in the world because of its attractive flavor and aroma. Among teas, green tea polyphenols have been extensively studied as cardiovascular disease (CVD). A number of biological mechanisms, including radical scavenging and antioxidant properties, have been proposed for the beneficial effects of green tea in different models of chronic disease (Frei and Hidgon, 2002; Kuriyama et al., 2006). Polyphenols are the most significant group of tea components, especially the catechin group of the flavonols. The major tea catechins are (-)-epigallocatechin-3-galllate (EGCG), (-)-epigallocatechin (EGC), (-)-epicatechin 3-gallate (ECG), (-)-epicathechin (EC), (+)-gallocatechin, and (+)-catechin. Many biological functions of tea polyphenols have been studied including anti-inflammatory, antioxidative (Lin et al., 1996), antihypercholesterolemic effects (Matsui et al., 2006). The possible protective effect of tea against cardiovascular diseases is that tea polyphenols inhibit the oxidation of LDL, which is known to be involved in the development of atherosclerosis (Yang and Landau, 2000).

Material and Methods

Plant and Chemical material

Dried Oolong tea leaves obtained from Tea Plantation in East Java, Indonesia. DPPH (1,1-diphenyl-2-picrylhydrazyl) (Sigma); HPLC grade methanol (Merck); EGCG (Sigma); dimethyl sulfoxide (Merck); Cholesterol KIT (Randox),Simvastatin (Kimia Farma); Cholesterol (Sigma).

Extraction and Sample preparation

The dried oolong tea leaves (*C. sinensis* L.) were milled and soaked in destillated methanol (MeOH) during 24 hours at maserator, separated filtrate and added MeOH at the maserator, then evaporated the filtrate at approximately 40°C. Two kg of dried oolong tea leaves produced 447,8 g methanol extract or 0.224 %.

Extract of oolong tea were prepared by dissolving 0.005 g of extract in 10 ml of HPLC methanol for antioxidant assay or DMSO 1% for anticholesterol assay as 500 µg/mL concentration level, therefore arranging series of concentration level (250; 125; 62.5 31.25; 15.625 smallest concentration was 15.625 µg/mL). To evalute the antioxidant activity by DPPH scavenging activity, were compared with EGCG and . anticholesterol activity, oolong tea extract were compared with simvastatin.

DPPH radical scavenging activity assay

The DPPH assay was carried out as described by Frum and Viljoen (2006). Pipetted 50 µL of sample (oolong tea extract, EGCG) of various concentrations of the samples, entered at the microtitre plate and then were added 200 µL of 0.077 mmol/L methanol solution of DPPH and the reaction mixture was shaken vigorously and kept in the dark for 30 min at room temperature, DPPH was determined by microplate reader at 517 nm. The radical scavenging activity of each sample was expressed by the ratio the of lowering of the absorption of DPPH (%), relative to the absorption (100%) of DPPH solution in the absence of test sample (negative control).

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

where A_s and A_c are absorbance at 517 nm of the reaction mixture with samples and without sample respectively

Anticholesterol activity assay

The anticholesterol assay was carried out as described by Iswantini et al (2005) and Cholesterol (Chol) Enzymatic Endpoint Method (Randox Laboratories Ltd, 2004). Cholesterol was disolved in chloroform until achieving 25 mg/10 mL. Pipetted 5 µL of sample (oolong tea extract, simvastatin disolved in DMSO 1%) of various concentrations of the samples, entered at the microtitre plate and then were added 1000 µL Randox reagent and 5 µL cholesterol as sample. Blank solution comprised 10 µL distilled water and 1000 µL Randox reagent; negative control comprised 10 µL cholesterol and 1000 µL Randox reagent; standard comprised 10 µL Randox standard and 1000 µL Randox reagent.

Mixed and incubated for 10 minutes at room temperature, measured the absorbance by microplate reader at 500 nm against reagent blank. The anticholesterol activity (%) :

$$\text{Anticholesterol (\%)} = 1 - \frac{Ac - As}{Ac} \times 100$$

where A_s and A_c are absorbance at 500 nm of the reaction mixture with samples and without sample (control) respectively

Statistical Analysis

To verify the statistical significance of the parameter, the data were calculated the values of means and standard deviation (M ± SD) and 95 % confidence interval (CI) of means. To compare several treatments, used analysis of variance (ANOVA) with complete randomized design. Furthermore to know the difference level among treatment and to know the best treatmet used Duncan's post-Hoc test 95 % confidence interval. Statistical analysis used SPSS 16.0 program

Results

The DPPH sacvenging activity

The DPPH free radical scavenging activity of oolong tea extract and EGCG is well known antioxidant as positive control of various concentration were measured to know the antioxidant activity. The DPPH free radical scavenging activity of oolong tea extract is shown in Table 1. The DPPH radical scavenging activity of oolong tea extract and EGCG showed high antioxidant activity. There were no different among concentrations of oolong tea extract, all cocentrations were high antioxidant activity. EGCG showed high antioxidant activity at concentrations 32.5 μg/mL – 500 μg/mL were same with oolong tea extract. EGCG antioxidant activity showed lower than oolong tea extract at level 15.625 μg/mL.

The anticholesterol activity

Oolong tea methanol extract and simvastatin as positive control of various concentration were measured to know the anticholesterol activity. The anticholesterol activity of oolong tea extract is shown in Table 2. Oolong tea extract had high anticholesterol, all concentrations > 94 %, the highest anticholesterol was oolong tea extract at level 62.5 μg/mL. Simvastatin had higher anticholesterol activity than oolong tea extraxt at all level concentrations. The highest anticholesterol activity was simvastatin at level 500 and 15.625 μg/mL.

Discussion

The scavenging of DPPH radicals is followed by monitoring the decrease in absorbance at 517 nm which occurs due to reduction by the antioxidant (AH) or reaction with radical species (R^*) :

$$DPPH^{*} + AH \longrightarrow DPP\text{-}H + A^{*}$$

$$DPPH^{*} + R^{*} \longrightarrow DPPH\text{-}R$$

The DPPH scavenging activity test if antioxidant or sample which contain antioxidant will be occurred hydrogen (H) capture by DPPH free radical or antioxidant donate hydrogen (H) was indicated purple color to become 1,1- diphenyl-2-picrylhydrazyn yellow color (Gordon, 2001). When $DPPH^{*}$ reacts with an antioxidant compound, which can donate hydrogen, it is reduced. The changes in colour (from deep—violet to light—yellow) were measured at 517nm (Miliauskas et al., 2003). The DPPH assay, showed that sample had highest antioxidant activity will occures the fastest colour changing compared to the others sample or progressive decrease in absorbance. The sample had lowest antioxidant activity may not be reached for several hours, even the sample is still purple (Gordon, 2001).

Oolong tea extract had high antioxidant activity because its contains flavonoids, there are flavan-3-ols namely EC 2.59 mg/100 g; ECG 6.73 mg/100 g; EGC 6.00 mg/100 g; EGCG 36.01 mg/100 g; (+) catechin 0.23 mg/100 g; flavonols namely kaempferol 0.90 mg/100g; myricetin 0.49; quercetin 1.30 mg/100 (USDA, 2003). Oolong tea contains catechins, polyphenols, gallic acid, caffeine (Rumpler et al; 2001). Many biological functions of tea polyphenols including antioxidative. Tea polyphenols act as antioxidants *in vitro* by scavenging reactive oxygen and nitrogen species and chelating redox-active transition metal ions (Frei and Higdon, 2003). Several epidemiological studies have shown correlations between a higher content of flavonoids in the diet and the decreasing coronary heart disease mortality. These associations were mainly ascribed to the antioxidant capacity of these compounds (Lolito and Fraga, 2000).

Tea is a major source of flavonoids, a group of compounds in plant foods with antioxidant effects that may help to retard atherosclerosis (Sesso et al., 1997). Catechins are a group of flavonoids that have attracted particular attention due to their relative high antioxidant capacity in biological systems (Lolito and Fraga, 2000).

The principle of anticholesterol assay is the cholesterol determined after enzymatic hydrolysis and oxidation. The indicator quinoneimine is formed from hydrogen peroxide and 4-aminoantipyrine in the presence of phenol and peroxidase (Randox Laboratories Ltd. 2004).

$$\text{Cholesterol ester} + H_2O \xrightarrow[\text{esterase}]{\text{Cholesterol}} \text{Cholesterol} + \text{fatty acids}$$

$$\text{Cholesterol} + O_2 \xrightarrow[\text{oxidase}]{\text{Cholesterol}} \text{Cholestene-3-one} + H_2O_2$$

$$2\,H_2O_2 + \text{phenol} + \text{4-Amnioantipyrine} \xrightarrow{\text{peroxidase}} \text{quinoneimine} + 4\,H_2O$$

Oolong tea extract contains polyphenol, flavonoids, catechins (USDA, 2003), by in vitro and in vivo studies that tea or catechins inhibit the intestinal absorption of dietary lipids. Studies *in vitro* indicate that catechins, particularly EGCG, interfere with the emulsification, digestion, and micellar solubilization of lipids, critical steps involved in the intestinal absorption of dietary fat, cholesterol, and other lipids. Tea or its catechins lower the absorption and tissue accumulation of other lipophilic organic compounds. The available information strongly suggests that tea or its catechins may be used as safe and effective lipid-lowering therapeutic agents (Koo and Noh, 2007).

The green tea extract standardized at 25% catechins (AR25) exhibiting marked inhibition of digestive lipases *in vitro* is likely to reduce fat digestion in humans (Juhel et al., 2000). Catechins have been shown to reduce plasma cholesterol levels and the rate of cholesterol absorption. Investigating the dose-response and the mechanism of action of EGCG on these parameters in rats which were fed a diet high in cholesterol and fat, after 4 weeks of treatment, total cholesterol plasma levels were significantly reduced in the group fed 1% EGCG when compared to the non-treatment group (Cabrera et al., 2006).

Base on this research (Table 1 and 2) showed that oolong tea extract had high antioxidant and anticholesterol activity, because oolong tea contained high polyphenols and flavonoids.

Acknowledgement

We are grateful to Directorate General for Higher Education, Ministry of National Education of Republic Indonesia, for Research Grant of Research Competitive According National Priority (2009) for financial support.

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