



6th CMAPSEEC

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

April 18-22, 2010

Kervansaray Lara Hotel
Convention Center

ANTALYA

PROCEEDINGS



Copyright 2010 6th CMAPSEEC Organizing Committee

All rights reserved. No part of this publication may be reproduced, stored in a retrieval system or transmitted in any form or by any means, electronic, mechanical, photocopying, recording or otherwise, excluding short quotations for the use in preparation of reviews and technical and scientific papers, without prior written permission of Istanbul Technical University. Any recommendations, suggestions or conclusions expressed in the published papers are those of authors and in no way reflect the views of sponsors or the editions.

Although all care is taken to ensure the integrity and quality of this publication and the information herein, no responsibility is assumed by the publisher nor the author for any damage to property or persons as a result of operation or use of this publication and/or the information contained herein.

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

ORGANIZATION

Chairperson

Prof. Dr. Ilkay ERDOGAN-ORHAN (Gazi University, Ankara, Turkey)

Honorary chairperson

Prof. Dr. Zora Dajic-Stevanovic (President of AMAPSEEC, Serbia)

Organizing Committee

Prof. Dr. Murat KARTAL (Ankara University, Ankara, Turkey)
Assoc. Prof. Dr. Yüksel KAN (Selçuk University, Konya, Turkey)
Assoc. Prof. Dr. Nazım ŞEKEROĞLU (Kilis 7 Aralık University, Kilis, Turkey)
Assist. Prof. Dr. Ufuk KOCA (Gazi University, Ankara, Turkey)

Social Committee

Ipek PEŞİN (Gazi University, Ankara, Turkey)
Ali Rıfat GÜLPINAR (Ankara University, Ankara, Turkey)
F. Sezer ŞENOL (Gazi University, Ankara, Turkey)
Gülây ÇOKSARI (Selçuk University, Ankara, Turkey)
L. Tuba KADIOĞLU (Kilis 7 Aralık University, Kilis, Turkey)

Honorary Board

Prof. Dr. Rıza AYHAN (President – Gazi University)
Prof. Dr. Süleyman OKUDAN (President – Selçuk University)
Prof. Dr. Cemal TALUG (President – Ankara University)
Prof. Dr. İsmail GÜVENÇ (President – Kilis 7 Aralık University)
Prof. Dr. Mihailo RISTIC (Former President of AMAPSEEC)

International Scientific Board		
	Neşet ARSLAN	(Turkey)
	Yoshinori ASAKAWA	(Japan)
	Dea BARICEVIC	(Slovenia)
	Kemal Hüsnü Can BAŞER	(Turkey)
	Turhan BAYKAL	(Turkey)
	Emine BAYRAM	(Turkey)
	Strahil BERKOV	(Bulgaria)
	Ihsan ÇALIŞ	(Cyprus)
	Maksut COŞKUN	(Turkey)
	Zora DAJIC-STEFANIC	(Serbia)
	Raman DANG	(India)
	Ömür DEMİREZER	(Turkey)
	Hayri DUMAN	(Turkey)
	Bertalan GALAMBOSI	(Finland)
	Kazimierz GLOWNIAK	(Poland)
	Anilda GURI	(Albania)
	Monica HANCIANU	(Romania)
	Saliha KIRICI	(Turkey)
	Nada KOVACEVIC	(Serbia)
	Filiz MERİÇLİ	(Turkey)
	Menşure ÖZGÜVEN	(Turkey)
	Biljana PETROVSKA	(FYR of Macedonia)
	Dragoja RADANOVIC	(Serbia)
	Vassilios ROUSSIS	(Greece)
	Gabriela RUZICKOVA	(Czech Republic)
	Ivan SALAMON	(Slovak Republic)
	Gülçin SALTAN	(Turkey)
	Ekrem SEZİK	(Turkey)
	Dragana STOJANOVIC	(Serbia)
	Mircea TAMAŞ	(Romania)
	Deniz TAŞDEMİR	(UK)
	Milan ZEMLICKA	(Czech Republic)

ADDRESS FOR CORRESPONDENCE

Scientific Committee

Prof.Dr. Ilkay Erdogan-Orhan

Department of Pharmacognosy
Faculty of Pharmacy
Gazi University
06330 Ankara-Turkey
Tel: +90 312 202 31 86
Fax: +90 312 223 50 18
E-mail: iorhan@gazi.edu.tr

Congress Secretariat

Sandra Brinuma
TRIGA TOURISM CONGRESS &
ORGANIZATION
Libadiye Cd. Barajyolu Sk.
Aydoğan Apt. No:15/1 Istanbul-Turkey
Tel: +90 216 443 28 98
Fax: +90 216 443 25 27
E-mail: sandra@trigaturizm.com

Assist. Prof.Dr. Ufuk Koca (*Editing Person of Proceedings*)

ukoca@gazi.edu.tr
Tel: +90 312 202 31 87

Congress website

www.6thcmapseec.org

Congress Venue

Hotel Kervansaray Lara Convention Center (Antalya –Turkey)

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

Chemical composition of essential oil of <i>Allium ursinum</i> L. <i>S. Čavar, A. Čopra-Janičijević, S. Muradić, M. Maksimović and E. Sofić</i>	1057
Chemical composition and antioxidant activity of <i>Teucrium arduini</i> L. <i>S. Čavar, D. Vidic, A. Topčagić, M. E. Šolić and M. Maksimović</i>	1065
Breaking Seed Dormancy of <i>Centaurea behen</i> L. <i>S. Irani, E. Askari, K. Razmjoo, N. Khodaeian and A. Razzazi</i>	1077
Variability of traits in uncultivated marshmallow in Serbia (<i>Althaea officinalis</i> L.) <i>R Dang, K Das, T. N. Shivananda and L. Hegde</i>	1088
Biofungicide for powdery mildew control <i>S. Rajković, M. Tabaković-Tošić, M. Marković, Lj. Rakonjac, M. Ratknić and D. Mitić</i>	1096
Content and qualitative properties of essential oil of <i>Chamaemelum nobile</i> (L.) All. of Slovak provenience <i>Š. Váverková, M. Habán, M. Mikulášová, P. Farkaš and P. Otepka</i>	1107
Herbal products for lowering blood lipids <i>C. Svetlana and B. Biljana</i>	1114
Effects of planting date and irrigation date on morphologic characteristics of cumin (<i>Cuminum cyminum</i> L.) <i>Tahmineh Esfandiari, H. Shoorideh and A. Mollafilabi</i>	1122
Steroids from the <i>Erythrina variegata</i> plant of Indonesia and their anti-cancer properties against breast cancer cell t47d <i>Tati Herlina, Nurlelasari, Rani Maharani, Unang Supratman, Ukun MS Soedjanaatmadja, Zalinar Udin and Hideo Hayashi</i>	1132
Researches regarding the extractibility of some active principles from <i>Ajuga reptans</i> L. and <i>Ajuga genevensis</i> L. with solvents of different polarities <i>A. Hemicnschi, E. Gille, M. Hancianu, A. Trifan and U. Stanescu</i>	1141
Antioxidant and Anticholesterol <i>In vitro</i> Activity of Oolong Tea (<i>Camelia sinensis</i> L.) Extract <i>W. Widowati, T. Herlina and H. Ratnawati</i>	1150
Evaluation of Antifilarial Activity in Roots of <i>Plumeria alba</i> <i>W. Rizvi, A. Kumar, R. Kumar and N. Haider</i>	1160
The antihypertensive effect of aqueous extract of <i>O. africana</i> leaves <i>X. Wang, D. Dietrich and Q. Johnson</i>	1168
Effects of sowing date and nitrogen rate on yield and essential oil production of chamomile (<i>Matricaria chamomilla</i> L.) <i>Raei Yaegoub, Amir Aghaiy Mohammad Amin, Zehtab-Salmasi Saeide and Nasrollahzadeh Safar</i>	1178
Recent advances in phytochemistry of bryophytes: Chemical diversity and biological activity <i>Yoshinori Asakawa</i>	1187
Antimicrobial activity of four spontaneous species against a collection of plant pathogenic bacteria <i>Z. Krimi and H. Djellout</i>	1199

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

W. Widowati^{1*}, T. Herlina², H. Ratnawati¹

¹Medical Research Centre, Faculty of Medicine, Maranatha Christian University, Bandung, Indonesia

²Natural Sciences and Mathematic Faculty, Padjadjaran University, Sumedang, Indonesia

*Corresponding author, Tel : +62 22 201 7621; fax: +62 22 201 5154

E-mail address: wahyu_w60@yahoo.com

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-gallate (EGCG), (-)-epigallocatechin (EGC), (-)-epicatechin 3-gallate (ECG), (-)-epicatechin (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 distilled 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 evaluate 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 dissolved in chloroform until achieving 25 mg/10 mL. Pipetted 5 μ L of sample (oolong tea extract, simvastatin dissolved in DMSO 1%) of various concentrations of the samples, entered at the microtitre plate and then were added 1000 μ L Randox reagent and 5 μ L cholesterol as sample. Blank solution comprised 10 μ L distilled water and 1000 μ L Randox reagent; negative control comprised 10 μ L cholesterol and 1000 μ L Randox reagent; standard comprised 10 μ L Randox standard and 1000 μ L 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 (%):

$$\text{Anticholesterol (\%)} = 1 - \frac{A_c - A_s}{A_c} \times 100$$

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 ($M \pm SD$) 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 treatment used Duncan's post-Hoc test 95 % confidence interval. Statistical analysis used SPSS 16.0 program

Results

The DPPH scavenging 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 concentrations 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 extract 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^{\cdot}):



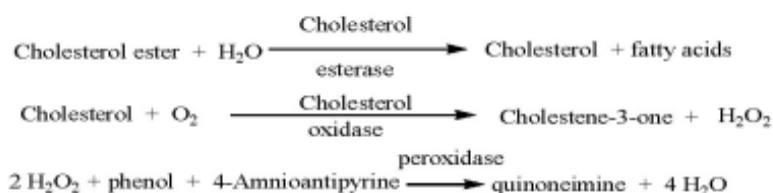
The DPPH scavenging activity test if antioxidant or sample which contain antioxidant will be occurred hydrogen (H) capture by DPPH free radical or antioxidant donate hydrogen (H) was indicated purple color to become 1,1- diphenyl-2-picrylhydrazyn yellow color (Gordon, 2001). When DPPH^{*} reacts with 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 occurs 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).



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.

References

- Cabrera, C., Artacho R., Giménez, R. 2006. Beneficial Effects of Green Tea—A Review. *Journal of the American College of Nutrition* 25, No. 2, 79–99.
- Frei, B., Higdon, J.V. 2003. Antioxidant Activity of Tea Polyphenols In Vivo : Evidence from Animal Studies. *J. Nutr.* 133,3275S-3284S.
- Frum, Y., Viljoen, A.M. 2006. In vitro 5-Lipoxygenase and Anti-Oxidant Activities of South African Medicinal Plants Commonly Used Topically for Skin Disease. *Skin Pharmacol Physiol.* 19, 329–335.
- Gordon, M.H. 2001. Measuring antioxidant activity. In Pokorny, J., N. Yanishlieva, M. Gordon, eds. *Antioxidant in food*. Woodhead Publishing Limited. Cambridge England.
- Iswantini, D., Nurenda, D., Sugita, P. 2005. Fraksinasi dan Karakterisasi Senyawa Aktif dari Bangle (*Zingiber cassumunar* Roxb) Sebagai Aktivator Enzim Kolesterol Oksidase. *Prosiding Simposium Nasional Kimia Bahan Alam XV*. 13-14 September 2005. Himpunan Kimia Bahan Alam. Bogor.
- Koo, S.I., Noh, S.K. 2007. Green Tea as Inhibitor of the Intestinal Absorption of Lipids: Potential Mechanism for its Lipid-Lowering Effect. *J Nutr Biochem.* 18 (3), 179–183.

- Kuriyama, S., Shimazu, T., Ohmori, K., Kikuchi, N., Nakaya, N., Nishino, Y., Tsubono, Y., Tsuji, I. 2006. Green tea consumption and mortality due to cardiovascular disease, cancer, and all causes in Japan : the Ohsaki study. *JAMA*. 296, 1255–1265.
- Lin, Y.L., Juan, I.M., Chen, Y.L., Liang, Y.C., Lin, J.K. 1996. Composition of polyphenols in fresh tea leaves and associations of their oxygen-radical-absorbing capacity with antiproliferative actions in fibroblast cells. *J. Agric. Food Chem.* 44, 1387–1394.
- Lotito, S.B., Fraga, C.G.. 2000. Catechins Delay Lipid Oxidation and α -Tocopherol and β -Carotene Depletion Following Ascorbate Depletion in Human Plasma. *Society for Experimental Biology and Medicine* 225, 32-38.
- Matsui, Y., Kumagai, Y., Masuda, H. 2006. Antihypercholesterolemic Activity of Catechin-free Saponin-rich Extract from Green Tea Leaves. *Food Sci. Technol. Res.* 12 (1), 50-54.
- Miliauskas, G. Venskutonis, P.R., van Beek, T.A. 2003. Screening of radical scavenging activity of some medicinal and aromatic plant extracts. *Food Chemistry*, Available online 24 October 2003.
- Mukamal, K.J., Maclure, M., Muller, J.E., Sherwood, J.B., Mittleman, M.A. 2002. Tea consumption and mortality after acute myocardial infarction. *Circulation*. 105:2476–81
- Randox Laboratories Ltd. 2004. Cholesterol (Chol) Enzymatic Endpoint Method Manual. Randox Laboratories Ltd, Ardmore, Diamond Road, Crumlin, Co. Antrim, United Kingdom. BT294 QY
- Rumpler, W., Seale, J., Clevidence, B., Judd, J., Wiley, E., Yamamoto, S., Komatsu, T., Sawaki, T., Ishikura, Y., Hosoda. 2001. Oolong Tea Increases Metabolic Rate and Fat Oxidation in Men. *J. Nutr.* 131, 2848–2852.
- Sesso, H.D., Gaziano, J.M., Buring, J.E., Hennekens, C.H. 1999. Coffee and Tea Intake and the Risk of Myocardial Infarction. *Am J Epidemiol.* 149, 162-167. *Agric. Food Chem.* 51, 63-67.