KOMPILASI MAKALAH ILMIAH
SEMINAR ILMIAH UNIVERSITAS

18 Desember 2010
di GAP Lantai 8,
Universitas Kristen Maranatha

"PENGEMBANGAN POTENSI SUMBER DAYA ILMIAH DALAM MENINGKATKAN DAYA SAING BANGSA"

Diselenggarakan oleh:
Lembaga Penelitian dan Pengabdian kepada Masyarakat
Universitas Kristen Maranatha
Jl. Prof. drg. Suria Sumantri No. 65, Bandung

KATA SAMBUTAN

Tolak ukur keberhasilan perguruan tinggi di Indonesia antara lain ditentukan dari implementasi tridharma perguruan tinggi, yaitu: pendidikan, penelitian, dan pengabdian kepada masyarakat. Dua dharma dikelola oleh Lembaga Penelitian dan Pengabdian kepada Masyarakat (LPPM).

Selain itu, keberhasilan pelaksanaan kegiatan penelitian dan pengabdian kepada masyarakat sangat berpengaruh pada akreditasi status perguruan tinggi dan program studi, serta kenaikan jabatan akademik dosen, maupun untuk peroleh dana hibah dari Ditjen Dikti. Dengan demikian, Lembaga Penelitian dan Pengabdian kepada Masyarakat (LPPM) Universitas Kristen Maranatha harus mampu mengelola kegiatan penelitian dan pengabdian kepada masyarakat secara profesional, ilmiah dan terarah sesuai dengan kebijakan Dirjen Dikti yang sekarang berlaku, agar dapat menciptakan keberhasilan Universitas Kristen Maranatha melaksanakan misinya. Untuk itu, perlu dilakukan upaya untuk meningkatkan intensitas dan kualitas kegiatan penelitian dan pengabdian kepada masyarakat Universitas Kristen Maranatha.

Dalam rangka pemikiran tersebut diatas, maka dilakukan seminar ilmiah Universitas Kristen Maranatha, yang bertujuan:

a. Mensosialisasikan dan mendeseminasikan hasil-hasil penelitian yang selama ini telah dilaksanakan oleh fakultas/program studi, agar dapat mendorong dan memacu minat meneliti para dosen dan mahasiswa.

b. Membangkitkan minat dosen dan mahasiswa untuk melakukan kegiatan penelitian dan pengabdian kepada masyarakat, dengan memanfaatkan peluang dukungan dana dari DP2M Ditjen Dikti, Kemneg Ristek, Dewan Riset Nasional, serta peluang kerja sama antar perguruan tinggi dengan industri dan pemerintah.

c. Mengembangkan penelitian unggulan yang dapat menjadi *distinctive competence* fakultas/program studi, dan menghasilkan perolehan HAKI.

d. Mengembangkan budaya akademik untuk meningkatkan profesionalisme dan kompetensi dosen dan mahasiswa melalui kegiatan penelitian, perekayasaan, inovasi dan difusi teknologi, yang dpat menghasilkan nilai tambah bagi peningkatan:

   - Daya saing industri nasional dalam menghadapi era globalisasi.
   - Kemampuan KUMKM untuk kesejahteraan masyarakat, yang juga merupakan program pembangunan pemerintah.
   - Penelitian dan pengembangan ilmu pengetahuan teknologi dan seni (ipteks) lebih lanjut.

e. Menjalin kerja sama yang sinergi dibidang penelitian dan pengabdian kepada masyarakat dengan lembaga-lembaga ipteks, baik pemerintah, industri maupun antar perguruan tinggi, agar dapat menghasilkan kinerja dan manfaat yang lebih besar, serta menghindari terjadinya tumpang tindih/duplikasi yang memboroskan sumber daya.

f. Membentuk konsorsium penelitian dan pengembangan ipteks dengan masyarakat industri yang bertujuan meningkatkan daya saing industri.

Tujuan seminar ilmiah Universitas Kristen Maranatha ini akan berhasil dengan baik, bila segenap sivitas akademika, khususnya para dosen dan mahasiswa menyambut sesuai harapan Keberhasilan bukan oleh Lembaga Penelitian dan Pengabdian kepada Masyarakat (LPPM) sendiri, tetapi keberhasilan karena kebersamaan segenap peran serta sivitas akademika Universitas Kristen Maranatha. Yes we can.

Kiranya Tuhan memberkati. Amin.

Bandung, Desember 2010
Ketua LPPM UK Maranatha,

Ir. Yusak Gunadi Santoso, M.M.
NIP. 194905231982031001

KATA PENGANTAR

Puji syukur ke hadirat Tuhan yang Maha Esa kami panjatkan atas segala berkat dan pimpinanNya sehingga terselenggaranya Seminar Ilmiah Universitas pada tanggal 18 Desember 2010 di Gedung Adimistrasi Pusat Universitas Kristen Maranatha.

Keberhasilan akademik suatu perguruan tinggi dalam melaksanakan Tri Dharma Perguruan Tinggi diukur dari keberhasilan penelitian yang dilakukan. Universitas Kristen Maranatha berusaha secara konsisten malaksanakan kegiatan penelitian dan telah beberapa kali beberapa kali berhasil memperoleh dukungan dana hibah penelitian baik dari DP2M Ditjen Dikti, Depkes, maupun dari Kementrian Ristek. Penghargaan tersebut sangat memacu para dosen untuk berkarya di bidang penelitian.

Hasil penelitian yang telah dicapai selama ini wajib didesiminasikan kepada seluruh sivitas akademika agar dapat merangsang segenap dosen dan mahasiswa untuk melakukan kegiatan penelitian dengan melanjutkan kegiatan penelitian yang telah dilakukan. Dengan demikian diharapkan dapat menghasilkan karya penelitian yang bermanfaat bagi peningkatan kesejahteraan masyarakat dan dapat memperoleh HAKI. Selain itu, hasil penelitian juga dapat dimanfaatkan oleh masyarakat industri untuk meningkatkan daya saing industri nasional dalam persaingan global dan selanjutnya diharapkan dapat dibentuk *research consorsium* antara Universitas Kristen Maranatha dengan masyarakat industri agar dapat lebih efektif dalam melayani industri dalam meningkatkan produktivitas dan daya saing industri.

Harapan dan tujuan tersebut di atas inilah yang sesungguhnya melatarbelakangi diselenggarakannya Seminar Ilmiah Universitas Kristen Maranatha. Semoga gayung bersambut dan kegiatan penelitian Universitas Kristen Maranatha dapat berkembang sesuai arahan kebijakan dan harapan pemerintah.

Atas terselenggaranya Seminar ini, kami sebagai panitia menghaturkan banyak terimakasih kepada berbagai pihak yang telah banyak membantu terlaksananya acara ini, khususnya kepada Bapak Koordinator Kopertis IV: Prof. Dr. Ir. Abdul Hakim Halim, M.Sc. sebagai *Keynote Speaker* dan Bapak Kasubdit Sistem Informasi dan Publikasi DP2M DIKTI: Drs. Yudi Agustono, M.Si. sebagai *Plenary Lecturer*. Ucapan terima kasih juga kami ucapkan kepada Dekan-dekan Fakultas di Universitas Kristen Maranatha, dan para pemakalah atas partisipasinya dalam membantu mendiseminasikan hasil penelitiannya.

Kami mohon maaf apabila banyak kekurangan dalam pelaksaan Seminar ini dan masukan serta saran Bapak dan Ibu kami harapkan untuk penyempurnaan di masa yang akan datang.

Bandung, Desember 2010
Ketua Pelaksana Seminar Ilmiah Universitas

Dr. dr. Susy Tjahjani, M.Kes.
NIP. 195109051981032001

KOMPILASI MAKALAH ILMIAH
SEMINAR ILMIAH UNIVERSITAS
"Pengembangan Potensi Sumber Daya Ilmiah dalam Meningkatkan Daya Saing Bangsa"
di GAP Lantai 8, Universitas Kristen Maranatha

Bandung, 18 Desember 2010

DAFTAR ISI

ANTIOXIDANT ACTIVITIES, ANTI CHOLESTEROL ACTIVITY AND PLATELET AGGREGATION INHIBITOR OF VARIOUS TEA: A POTENTIAL THERAPEUTIC AGENTS IN CARDIOVASCULAR DISEASE

Hana Ratnawati[1], Wahyu Widowati[2], Tati Herlina[3] dan Tjandrawati[4]
[1,2]Fakultas Kedokteran, Universitas Kristen Maranatha
[3]Fakultas Kedokteran, Universitas Padjajaran
[4]Fakultas Kedokteran, LIPI Bandung

Abstrak

Penyakit kardiovaskular menempati urutan pertama penyebab kematian di Indonesia, dan sebagian besar disebabkan penyakit jantung koroner/miokard infark dan stroke. Tingginya kadar radikal bebas, hiperkolesterolemi dan agregasi trombosit berperan penting dalam patogenesis aterosklerosis yang selanjutnya dapat mengakibatkan penyakit jantung koroner dan stroke. Untuk mengatasinya, diperlukan obat-obatan antara lain antikolesterol dan anti agregasi trombosit yang harus dikonsumsi dalam jangka waktu lama, namun efek sampingnya seringkali menimbulkan penyakit baru bagi pasien. Itu sebabnya diperlukan pencarian bahan alam yang aman dan tersedia melimpah di Indonesia. Berdasarkan penelitian terdahulu menunjukkan bahwa teh mempunyai kadar flavonoid yang sangat tinggi dan flavonoid ini merupakan antioksidan yang diharapkan dapat berperan sebagai anti kolesterol dan anti agregasi trombosit. Berdasarkan prosesnya terdapat tiga macam teh, yaitu teh hitam (fermentasi penuh), teh oolong (semi-fermentasi) dan teh hijau (tanpa fermentasi). Dari ketiga jenis teh ini akan diuji aktivitas antioksidan, anti kolesterol dan anti agregasi trombosit secara *in vitro*. Desain penelitian adalah prospektif eksperimental dengan Rancangan Acak Lengkap dengan sampel ekstrak metanol teh hitam, teh hijau, teh oolong dengan parameter uji antioksidan (pemerangkapan radikal hidrogen peroksida, radikal hidroksil dan aktivitas superoksid dismutase/SOD) dengan pembanding asam askorbat; anti kolesterol dengan pembanding simvastatin dan anti agregasi trombosit (inducer ADP 5 uM dan Collagen 2.5 uM) dengan pembanding aspirin. Data dianalisis dengan ANOVA dan post hoc test Tukey HSD. Hasil penelitian menunjukkan bahwa untuk aktivitas antioksidan: ekstrak teh hitam (15.62 µg/mL) mempunyai aktivitas pemerangkapan radikal hidrogen peroksida paling tinggi (96.12%), diikuti oleh teh hijau (91%), teh oolong (83.41%) dan asam askorbat (82%). Untuk pemerangkapan radikal hidroksil, semua ekstrak teh pada konsentrasi 31.25 µg/mL mempunyai aktivitas yang lebih baik dibandingkan asam askorbat (24.69%). Aktivitas teh oolong (87.86%), teh hitam (77.98%) dan teh hijau (70.78%). Pada konsentrasi 62.5 µg/mL, aktivitas SOD teh oolong adalah terbaik (85.59%), the hitam (71.05%), the hijau (41.47%) dan asam askorbat (17.8%). Semua jenis teh mempunyai aktivitas antikolesterol , teh oolong (62.5 ug/mL) dan teh hitam (31.25 ug/mL) mempunyai aktivitas antikolesterol setara dengan simvastatin. Aktivitas anti agregasi trombosit: dengan *inducer* ADP, semua jenis teh tidak mempunyai aktivitas anti agregasi trombosit, dengan *inducer* collagen, teh oolong dapat menghambat agregasi trombosit lebih baik dibandingkan aspirin.

I. Introduction

Cardiovascular diseases (CVDs) are the number one cause of death globally, more people die annually from CVDs than from any other cause. An estimated 17.1 million people died from CVDs in 2004, representing 29%of all global deaths. Of these deaths, an estimated 7.2 million were due to coronary heart disease and 5.7 million were due to stroke. Eighty two percent of CVD deaths take place in low- and middle-income countries and occur almost equally in men and women. By 2030, almost 23.6 million people will die from CVDs, mainly from heart disease and stroke. The largest increase in number of deaths will occur in the South-East Asia Region, including Indonesia, due to people in low- and middle-income countries are more exposed to risk factors leading to CVDs and are less exposed to prevention efforts than people in high-income countries (WHO, 2009).

There are two categories risk factors for coronary heart disease and stroke, the modifiable risk factors such as hypertension, hypercholesterolemia and smoking, and non-modifiable risk factors, such as older age, gender and genetic. Various studies have established that the atherosclerosis is the underlying disease process in most of the coronary artery disease that causes myocardial infarction, cerebral infarction (stroke), aortic aneurysms, and peripheral vascular disease (gangrene of the legs). Identification and control of modifiable risk factors is the best strategy to reduce the incidence of CVDs. The influence

of hypolipidemic drugs on high levels of circulating lipids is known for the reduction of mortality and morbidity in atherosclerosis related diseases.

The key processes in atherosclerosis are intimal thickening and lipid accumulation. Elevated levels of serum cholesterol are sufficient to stimulate lesion development, even if other risk factors are absent. The major component of total serum cholesterol associated with increased risk is low-density lipoprotein (LDL) cholesterol. Chronic hyperlipidemia, particularly hypercholesterolemia, may directly impair endothelial cells function through increased production of free radicals which are produced by membrane lipid degradation and mitochondrial dysfunction. Once oxidized by free radicals, LDL makes multiple contributions to plaque development. Superoxide anions can be produced in the tissue as a result of incomplete and vicarious reduction of oxygen by damaged mitochondria or because of the action of oxidase derived from leucocytes, endothelial cells, or parenchymal cells. Cellular antioxidant defense mechanisms (superoxide dismutase, enzyme catalase and glutathione peroxidase which detoxifies H2O2) may also be compromised by ischemia, favoring the accumulation of radicals. The most important mechanism of membrane injury involves the formation of reactive free radicals and subsequent lipid peroxidation. Thus, the influence of oxygen-derived free radicals depends on the balance between the production and the inactivation of these metabolites by cells and tissues. Free radical scavengers may be of therapeutic benefit.

In its oxidized form, LDL has several effects, it damaging to the endothelium, which cause endothelial injury/dysfunction and increased endothelial permeability, enhance monocytes, other leucocytes and platelet adhesion. Monocytes differentiate into macrophages and phagocytes the oxidized LDL through scavenger receptor forming foam cells. Oxidized LDL also stimulates growth factor and cytokines production by activated platelets, macrophages, or vascular cells which promotes cell proliferation and connective tissue deposition. Smooth muscle cells migrate from the media to the intima (intimal thickening), where they proliferate and deposit extra cellular matrix components, converting a fatty streak into a mature fibrofatty atheroma, and contribute to the progressive growth of atherosclerotic lesions. Trials have demonstrated that antioxidant and anti cholesterol treatment protects against the development of atherosclerosis in hypercholesterolemic experimental animals.

Endothelial injury also will cause platelets encounter extracellular matrix constituents that are normally sequestered beneath an intact endothelium, this include collagen (most important), proteoglycans, fibronectin, and other adhesive glycoproteins. On contact with extracellular matrix, platelets undergo three general reactions: adhesion and shape change; secretion (release reaction) and aggregation.

An intact endothelium prevents platelets and plasma coagulation factors from meeting the highly thrombogenic subendothelial extracellular matrix. If platelets are activated after endothelial injury, they are inhibited from adhering to the surrounding uninjured endothelium by endothelial prostacyclin (PGI2) and nitric oxide (NO). Both mediators are potent vasodilators and inhibitors of platelet aggregation, their synthesis by endothelial cells is stimulated by a number of factors (e.g. thrombin and various cytokines) produced during coagulation, but oxygen free radicals could deactivate NO. Endothelial cells also express adenosine diphosphatase, which degrades ADP and thereby contributes to the inhibiton of platelet aggregation. Normal platelet functions are important for achieving primary hemostasis. Under most circumstances, endothelial cells maintain an environment conducive to liquid blood flow by mechanisms that block platelet adhesion and aggregation, interfere with the coagulation cascade, and actively lyse blood clots.

Many large clinical trials have demonstrated that most antiplatelet agents reduce the risk of all important vascular atherothrombotic events in patients at risk for these events. Platelet antiaggregation agents can prevent atherothrombotic events, by inhibiting the formation of intraarterial platelet aggregates. Numerous medications have been shown to affect platelet functions. Aspirin, clopidogrel, and the combination of aspirin plus extended-release dipyridamole are the antiplatelet agents most commonly used for this purpose. Aspirin is the only antiplatelet agent that has been proven effective for the acute treatment of ischemic stroke. However, it causes epigastric discomfort, gastric ulceration, and gastrointestinal hemorrhage, which may be life-threatening. Consequently, not every patient should be advise to take aspirin regularly because the risk of adverse side effects.

Taken together, the most important determinants of atherosclerosis are adverse effects of hypercholesterolemia and platelets aggregation which cause hemodynamic disturbance and thrombosis. Therefore, antioxidant, anti cholesterol and platelet aggregation inhibitor is important in preventing atherosclerosis as the risk factor of cardiovascular diseases.

Recently, there has been increasing interest in finding natural antioxidants from plants to protect the human body from attack of free radicals and retard the progress of many chronic diseases. Tea, obtained from the leaf of plant *Camellia sinensis*, is the most widely consumed beverage world-wide. About 78%

of the tea production worldwide is black tea, which is the main tea beverage consumed in the US and Europe. Green tea, about 20% of worldwide production, is the main tea beverage in Asian countries such as Japan and parts of China, while the remaining 2% of tea production is oolong tea which is consumed mainly in Southern China and Taiwan. These various teas herbal were differences in the processing, green tea are steamed to avoid enzymatic oxidation; oolong tea is semi-fermented tea to permit a moderate level of enzymatic oxidation; and black tea is enzymatically oxidized. These various teas seem to be an effective natural antioxidants because in our previous study, the phytochemical assay of various teas, showed that tea extracts contain high level polyphenol as a natural antioxidants which suggested to influence the pathogenesis of atherosclerosis through their role in protect the cells and tissues from oxidative damage by scavenging oxygen-free radicals, thus lowering the cholesterol concentration and inhibit platelet aggregation. Therefore, we investigated the role of black tea, green tea and oolong tea methanol extracts as natural antioxidants in scavenging the free radical (hydrogen peroxide and hydroxyl) and superoxide dismutase (SOD) activity by *in vitro* assay compare to ascorbic acid, as well as the effectiveness of decreasing cholesterol level compare to simvastatin and the effectiveness as platelet aggregation inhibitor compare to aspirin.

II. Materials and Methods

A. Materials

Methanol 96 %, DMSO, 2- deoxyribose in phosphate buffer, $FeCl_3$ in EDTA, PBS, ascorbic acid, H_2O_2 (0.1 M), TCA (2.8 %), TBA 1 % in NaOH 50 mM, DMSO 1%, ADP 5uM, Collagen 2.5 uM, hyperaggregation blood, sodium citrate 3.8%, aspirin, simvastatin, HCl 2N, Substrate cholesterol 95%, Kit SOD (Ransod), Platelet Aggregation Chromogenic kinetic System, Kit Cholesterol Randox, The dry leafs of black tea, green tea and oolong tea and were purchased from PT. Walini, Subang.

B. Methods

1. Extraction

The dry leaf of green tea/oolong tea were chopped into 60 mesh size and then soaked in methanol (MeOH) 96 % for 3 days and the substance were collected and evaporated.

2. Hydroxyl Radical Scavenging Activity

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 (62.5, 31.25 and 15.625 µg/mL) of the methanol extract 5 µL in 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, then added 1.0 mM ascorbic acid (40 µL), 0.1 mM H_2O_2 (4 µL) and incubated at 37° C for 30 minute, then the mixtures cooled at absorbance was measured at 532 nm (Gomes et al., 2001; Safitri et al, 2002). Reactions were carried out in triplicate. Hydroxyl radical scavenging activity in percent was calculated as follows:

*OH scavenger (%) = {1-(Sample-blank sample)/(Control-blank control)}x 100

3. Hydrogen Peroxide Scavenging Activity

One milliliter of sample was mixed with 0.6 ml H_2O_2 (2mM/L in PBS pH 7.4). After 10 minutes the absorbance values at 230 nm of the reaction mixtures were recorded against a blank solution containing phosphate buffer without H2O2 for each sample. For each concentration, a separate blank sample was used for background substraction. The percentage inhibition activity was calculated from:

$$1 - \frac{\text{absorbansi sampel}}{\text{absorbansi kontrol negatif}} \times 100$$

All test were done in triplicate and the result were presented as means ± SD.

4. Superoxide Dismutase Assay

The role of superoxide dismutase (SOD) is to accelerate the dismutation of the toxic superoxide radical ($O_2^{\bullet-}$) 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 (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 substrate 170 µL, xanthine oxidase 25 µL. Control contain ransod control 5 µL, mixed substrate 170 µL, xanthine oxidase 25 µL. Blank contain 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.

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 to form a red formazan dye. The superoxide dismutase activity is then measured by the degree of inhibition of this reaction (Randox Laboratories, 2004).

$$\text{Xanthine} \xrightarrow{\text{XOD}} \text{uric acid} + O_2^{\bullet-}$$

$$\text{I.N.T.} \xrightarrow{O_2^{\bullet-}} \text{formazan dye}$$

$$O_2^{\bullet-} + O_2^{\bullet-} + 2\,H^{+} \xrightarrow{\text{SOD}} O_2 + H_2O_2$$

5. Anti Cholesterol Assay

The research was done *in vitro* at various concentration (62.5, 31.25 and 15.625 µg/mL methanol extract of black tea, green tea and oolong tea) using kit cholesterol Randox, measured by Elisa reader and the anti cholesterol activity was calculated from:

$$\text{Aktivitas antikolesterol (\%)} = \frac{\text{Absorbansi}_{\text{sampel-kontrol}}}{\text{Absorbansi kontrol}} \times 100$$

6. Platelet Aggregation Assay

The methanol extracts of Green Tea, Oolong Tea and Aspirin as positive control were diluted with DMSO 1%. Platelet Rich Plasma and Platelet Poor Plasma were obtained from hyperaggregation individual. Aggregation was induced with adenosine diphosphate (ADP, final concentration of 5 uM/L) and Collagen (COLL, final concentration 2.5 uM/L). Platelet aggregation activity was measured by Platelet Aggregation Chromogenic Kinetic System (PACKS-4).

7. Statistical Analysis

Differences between the variants were analyzed by one way ANOVA (a = 0.05) and post hoc test *Tukey* HSD.

III. RESULTS

3.1 Antioxidant Activities

In this research the antioxidant activities of herbal tea were evaluated on hydroxyl radical (*OH), hydrogen peroxide (H2O2) scavenging and the SOD activity of green tea extract, oolong tea extract, black tea extract compare to ascorbic acid. The scavenging ability to H_2O_2 and *OH is given in table 1 and 2.

Table 1 Scavenging Activity of Samples on Hydrogen Peroxide (%)

Concentrations (µg/mL)	Treatments			
	Black tea MeOH extract	Green tea MeOH extract	Oolong tea MeOH extract	Ascorbic Acid
62.5	77.94 ± 0.33	68.00 ± 1.00	64.35 ± 0.53	38.45 ± 1.19
31.25	90.39 ± 1.22	82.00 ± 3.46	83.79 ± 0.18	24.69 ± 1.23
15.625	96.12 ± 0.93	91.00 ± 1.00	83.41 ± 0.18	82.63 ± 0.18

It is noticed that at all concentrations tea extracts had higher H_2O_2 scavenging activity compared to ascorbic acid. The methanol extract of black tea (15.62 ug/ml) gives the best result in scavenging hydrogen peroxide free radical (96.12%), followed by green tea (91 %) and oolong tea (83.41%). The statistical analysis showed that in concentration 15.625 ug/mL, black tea and green tea extract have the best scavenging activity on H_2O_2. showed a highly significance difference ($p = 0.000$) with ascorbic acid, whereas oolong tea extract showed the same activity with ascorbic acid, no significant difference ($p = 0.548$).

Table 2 Scavenging Activity of Samples on Hydroxyl Radical (%)

Concentrations (µg/mL)	Treatments			
	Black tea MeOH extract	Green tea MeOH extract	Oolong tea MeOH extract	Ascorbic Acid
62.5	80.66 ± 1.55	73.66 ± 1.88	87.24 ± 5.18	75.31 ± 0.83
31.25	77.98 ± 2.17	70.78 ± 6.80	87.86 ± 2.34	24.69 ± 1.23
15.625	73.87 ± 1.98	75.31 ± 0.62	84.98 ± 2.92	28.19 ± 2.17

At all concentrations, tea extracts showed higher *OH scavenging activity compared to ascorbic acid. Oolong tea extract at concentration 62.5 and 31.25 µg/mL had the highest $^{\bullet}OH$ scavenging activity compared to black, green tea extract and ascorbic acid. At the smallest concentration, the $^{\bullet}OH$ scavenging activity of oolong tea extract highly significant difference ($p = 0.000$) with ascorbic acid and significant difference compared to green tea extract and black tea ($p < 0.05$).
The role of superoxide dismutase (SOD) in various tea extracts showed in table 3.

Table 3 Superoxide Dismutase (SOD) of Various Tea Extract (%)

Concentrations (µg/mL)	Treatments			
	Black tea MeOH extract	Green tea MeOH extract	Oolong tea MeOH extract	Ascorbic Acid
62.5	71.05 ± 3.06	41.47 ± 2.42	85.59 ± 4.84	17.80 ± 0.29
31.25	57.62 ± 0.75	40.87 ± 2.34	76.16 ± 1.50	46.65 ± 2.77
15.625	33.05 ± 1.74	25.85 ± 0.98	70.45 ± 2.65	48.13 ± 1.42

The role of superoxide dismutase (SOD) is to accelerate the dismutation of the toxic superoxide radical ($O_2^{\bullet-}$), produced during oxidative energy process. Oolong tea extract showed better SOD activity in all concentration compared to ascorbic acid. In concentration 62.5 ug/mL, all the tea extracts showed better SOD activity and highly significant difference with ascorbic acid ($p = 0.000$). In the smallest

concentration, various teas extracts had significantly difference ($p < 0.05$) SOD activities, the best SOD activity was oolong tea extract, followed by black tea and green tea.

3.2 Anti Cholesterol Activity

In this research, we also examine the effectiveness of various tea extract in decreasing cholesterol level compare to simvastatin. The result showed in table 4.

Table 4. The Anti Cholesterol Activity of Various Tea Extracts (%)

Concentrations (µg/mL)	Treatments			
	Black tea MeOH extract	Green tea MeOH extract	Oolong tea MeOH extract	Simvastatin
62.5	93.97 ± 1.16	95.34 ± 0.31	97.81 ± 0.08	99.02 ± 0.77
31.25	96.47 ± 0.20	92.90 ± 0.37	94.44 ± 0.52	98.26 ± 0.75
15.625	93.37 ± 0.65	92.83 ± 0.29	96.05 ± 0.77	99.93 ± 0.11
Negative control	64.23 ± 1.71 %			

Compared to negative control (64.23 %), all the tea extracts had better anti cholesterol activity, although all tea extracts showed less anti cholesterol activity compared to simvastatin. Statistical analysis showed, oolong tea (62.5 ug/mL) and black tea (31.25 ug/mL) had the same anti cholesterol activity with simvastatin ($p > 0.05$). In all concentrations, oolong tea had anti cholesterol activity and significantly difference with negative control. In the smallest concentration (15.625 ug/mL), oolong tea had the best anti cholesterol activity compare to the other teas, whereas green tea had the same anti cholesterol activity with black tea ($p = 0.444$).

3.3 Platelet Aggregation Inhibitor Activity

The effectiveness of various tea extracts as platelet aggregation inhibitor compare to aspirin, showed in table 5. Two platelet aggregation inducer used in this assay, adenosine diphosphate (ADP) 5 uM and collagen (COLL) 2.5 uM. There is no replication in this assay, due to the limited sample (hyperaggregation blood) and limited reagent, therefore only descriptive analysis.

Table 4 The Platelet Aggregation Inhibitor of Various Tea Extracts

Inducer	Treatments				
	Black Tea MeOH extract	Green Tea MeOH extract	Oolong Tea MeOH extract	Neg Control	Aspirin
ADP 5 µM	96.4	77.7	73.2	77.3	45
COLL 2.5 µM	18.2	15.9	6.4	82.3	13.7

In the present study, with ADP inducer, black tea and green tea extract had no anti-platelet aggregation activity compare to negative control. Whereas with collagen inducer, all the tea extract showed inhibition activity towards platelet aggregation compare to negative control , even oolong tea extract gives better result compare to aspirin. Oolong tea potently inhibited platelet aggregation induced by collagen 2.5 µM, better than aspirin.

IV. Discussion

The antioxidative capacity of various tea extracts were examined by comparing to the activity of known antioxidant, ascorbic acid, by in vitro assays: hydrogen peroxide scavenging, hydroxyl radical scavenging and superoxide dismutase (SOD) activity. Tea extracts (green, black and oolong tea extract) had high antioxidant activity (> 70 %) in hydroxyl scavenging activity; oolong tea had > 80% in

hydrogen peroxide scavenging activity; oolong tea had > 70% in SOD activity, this data were similar with previous research. Tea is particularly rich in polyphenols, the most significant group of tea components, especially the catechin. The degree of oxidation affects the polyphenol profile of the tea. These polyphenols scavenge reactive oxygen species and free radicals through several mechanisms, including depolarization of electrons, formation of intramolecular hydrogen bonds and prevent oxidative reactions.

Black tea had antioxidant capacities because black tea is a major source of flavonoids, a group of compounds in plants with antioxidant effects. The flavons present in black tea possess at least the same antioxidant potency as catechins present in green tea. Both green tea and black tea also contain flavonol glycosides (quercetin and kaempferol). These compounds showed strong antioxidant properties. Green tea contains a class of polyphenols known as catechins, which consist mainly of epigallocatechin gallate, epicatechin gallate, gallocatechin, and epigallocatechin. Green tea and catechins have been reported to have a variety of nutritional and pharmacological properties, including antioxidant effects. Oolong tea is also known to have similar properties.

In general, the antioxidative effect of tea extract is better than ascorbic acid, due to the complex compound in tea extract work together and give a potent antioxidative activity.

The high level of cholesterol in blood vessel can cause partial or total occlusion as a risk factor of cardiovascular disease. Cholesterol can be oxidized by cholesterol oxidase enzyme which reduce the cholesterol level. In this study oolong tea extract had the same anti cholesterol activity with simvastatin. due to the high poliphenol and flavonoids compound in oolong tea extract.

Blood platelets can be activated by different compounds such as adenosine diphosphate and collagen. In this study we found that oolong tea methanol extract was a potent anti-platelet aggregation agent through its activity with collagen inducer even better than aspirin. All tea extracts selectively inhibited collagen-induced platelet aggregation, but it did not suppress the aggregation induced by ADP. Therefore tea extract was considered to block collagen signaling from integrin a2/ß1 to arachidonic acid release. Whereas aspirin is the best antiplatelet agent at all level of inducer and as an anti-thrombotic compounds through the inhibition of platelet cyclooxygenase-1 by irreversible acetylation of a specific serine moiety, thereby blocking the formation of thromboxane A2 for the lifetime of the platelets.

V. Conclusion

The studies presented here confirm the value of black tea, green tea and oolong tea methanol extracts as antioxidant, anti cholesterol, and platelet aggregation inhibitor therefore could be a potential therapeutic agents in cardiovascular disease.

- Antioxidant activity:
  - Black tea extract (15.62 ug/ml) had the highest activity in scavenging hydrogen peroxide radical (96.12%), followed by green tea (91 %), oolong tea (83.41%), and ascorbic acid (82%)
  - Oolong tea extract (31.25 µg/mL) had the highest hydroxyl scavenging activity compared to black, green tea extract and ascorbic acid.
  - Oolong tea extract (62.5 ug/mL) had the best SOD activity followed by black tea and green tea.
- Anti cholesterol activity:
  - All the tea extracts had anti cholesterol activity; Oolong tea (62.5 ug/mL) and black tea (31.25 ug/mL) had the same anti cholesterol activity with simvastatin.
- Platelet aggregation inhibitor activity:
  - All tea extracts had no platelet aggregation inhibitor activity towards ADP inducer
  - Oolong tea potently inhibited platelet aggregation induced by collagen 2.5 µM, better than aspirin.

5.1 Acknowledgement

We are grateful to Directorate General for Higher Education, Ministry of National Education of Republic Indonesia, for Research Grant of Hibah Kompetitif Sesuai Prioritas Nasional (2009) for financial support.

References

American Heart Association. 2008. LDL and HDL Cholesterol. http://www. americanheart.org/LDL-and-HDL-cholesterol.htm. November 5th , 2008.

Aprami, T.M. 1993. Peranan Antioksidan Dalam Pencegahan Aterosklerosis. Bagian/UPF Ilmu Penyakit Dalam Fak. Kedokteran UNPAD. Bandung.

Arisandi, Y, Y. Andriani. 2006. Khasiat Berbagai Tanaman Untuk Pengobatan. Eksa Media. Jakarta.

Arts, I.C.W., P.C.H. Hollman, E.J.M. Feskens, H.B.B deMesquita dan D. Kromhout. 2001. Catechin intake might explain the inverse relation between tea consumption and ischemic heart disease: the Zutphen Elderly Study. American Journal of Clinical Nutrition, Vol. 74, No. 2, 227-232, August 2001.

Bucky, R. 2002. Flavonoid Inhibition of Platelet Procoagulant Activity and Phosphoinositide Synthesis. Journal of Thrombosis and Haemostasis: 1(8). http://www.interscience.wiley.com/ journal/118901286/abstract. 5 Mei 2008

Chan, Y.P., K.K. Lau, K.H. Yiu, S.W. Li, H.T. Chan, S.Tam, X.O. Shu, C.P. Lau dan H.F. Tse. 2007. Isoflavone intake in persons at high risk of cardiovascular events: implications for vascular endothelial function and the carotid atherosclerotic burden. American Journal of Clinical Nutrition, Vol. 86, No. 4, 938-945, October 2007

Dalimarta, S. 2005a. Atals Tumbuhan Obat Indonesia. Jilid I. Trubus Agriwidya. Jakarta.

Ganapaty S., Chandrashekhar V.M., Chitme H.R., Narsu M.L. 2007. Free Radical Scavenging Activity of Gossypin and Nevadensin: An in-vitro Evaluation. Indian Journal of Pharmacology, 6(39): 281-3.

Gomes, A.J., C.N. Lunardi, S. Gonzales, A.C. Tedesco. 2001. The antioxidant action of *Polypodium leucotomos* extract and kojic acid: reactions with reactive oxygen species. Braz J Med Biol Res, November 2001, Volume 34(11) 1487-1494

Hannekens, C,H. 1999. Antioxidant Vitamins and Cardiovascular Disesae. Division of Preventive Medicine. Brigham and Women's Hospital, Boston, Massachusetts.USA.

Harbourne, J.B. 1973. Phytochemical Methods a Guide to Modern Techniques of Plants Analysis. Chapman and Hall. London

Hollman, P. C. H., M. G. L. Hertog, dan M. B. Katan. 1996. Analysis and Health Effects of Flavonoids. Food Chemistry 57(1):43-46

Imai, K. dan K. Nakachi. 1995. Cross sectional study of effects of drinking green tea on cardiovascular and liver diseases. *BMJ* 1995;310:693-696 (18 March).

Jusman, S.W.A. dan N. Mudjihartini. 2001. Uji Peroksidasi Lipid. Pelatihan 2001, Radikal Bebas dann Antioksidan Dalam Kesehatan, dasar, Aplikasi dan Pemanfaatan Bahan Alam. Bagian Biokimia, Fakultas Kedokteran, Universitas Indonesia, Jakarta.

Kumar V., Abbas A.K., Fausto N. 2005. Robbins and Cotran Pathologic basis of Disease. Elsevier Saunders.

Leung , L.K., Y., Su, R. Chen, Z. Zhang, Y. Huang and Z.Y. Chen. 2001. Theaflavins in Black Tea and Catechins in Green Tea Are Equally Effective Antioxidants. Journal of Nutrition. *2001;131:2248-2251*

Lin, J., K.M. Rexrode, F. Hu, C. M. Albert, C.U. Chae, E. B. Rimm, M.J. Stampfer dan J.A.E. Manson. 2007. Dietary Intakes of Flavonols and Flavones and Coronary Heart Disease in US Women. American Journal of Epidemiology 2007 165(11):1305-1313

Muhilal. 2001. Peranan suplementasi Antioksidan Terhadap Kesehatan. Seminar Nasional dan Lokakarya Pemahaman Konsep Radikal Bebas Dalam Meningkatkan Kesehatan Menuju Indonesia Sehat 2010. Lemlit Universitas Padjadjaran. Bandung.

Mukhtar, H. Dan N. Ahmad. 2000. Tea polyphenols: prevention of cancer and optimizing health. American Journal of Clinical Nutrition, Vol. 71, No. 6, 1698S-1702s. June 2000

Nowak T.J., Handford A.G. 2004. Pathophysiology 3rd ed. McGraw Hill Companies. Inc.

Papas, A.M. 1999. Antioxidant Status, Diet, Nutrition and Health. CRC Press, Washington, D.C.

Pearson, D, E.N. Frankle, R. Aeschbach, J.B. German. Inhibitation of Endothelial Cell-Mediated Oxidation of Low-Density Lipoprotein by Rosemary and Plant Phenolics. J. Agric. Food Chem, 1997, 45, 578-582.

Randox Laboratories Ltd. 2006. Cholesterol (CHOL). Ardmore, Diamond Road, Crumlin, Co. Antrim, United Kingdom, BT29 4QY.

Randox Laboratories Ltd. 2004c. Superoxide dismutase (RANSOD).Ardmore, Diamond Road, Crumlin, Co. Antrim, United Kingdom, BT29 4QY.

Song, W.O. dan O.K. Chun. 2008. Tea Is the Major Source of Flavan-3-ol and Flavonol in the U.S. Diet. J. Nutr. 138:1543S-1547S, August 2008.

Shahidi, F. 1999. Natural Antioxidants. Chemistry, Health Effects, and Applicatins. AOCS Press. Champaign. Illionis.

U.S. Department of Agriculture, Agricultural Research Service. 2003. USDA Database for the Flavonoid Content of Selected Foods. Unilever Bestfoods, North America, Englewood Cliffs, NJ

Widowati, W. dan Ch. Retnaningsih. 2007a. Antioxidant Activity of Ethanol Extract and Fraction of Velvet bean (*Mucuna pruriens* L.). Poster presentation at the Sixth Princess Chulabhorn International Science Congres (PC-VI). The Interface of Chemistry and Biology in the "OMICS" Era: Environment & Health and Drug Discovery" November 25-29, 2007, Bangkok Thailand.

Widowati, W. Mendi Lili P dan Anastasia Ida Ratnasari. 2007. Potensi Fraksi Aktif Antioksidan, Antikolesterol Kacang Koro (*Mucuna pruriens* L.) Dalam Pencegahan Aterosklerosis. Laporan Hasil Penelitian Hibah Bersaing Tahun Anggaran 2007/2008. Direktorat Jenderal Pendidikan Tinggi, Departemen Pendidikan Nasional.