Medicinal Plants - International Journal of Phytomedicines and Related Industries
Year 2011, Volume-3, Issue-1 (March)
Print ISSN : 0975-4261
Online ISSN : 0975-6892
Issue DOI: 10.5958/j.0975-4261.3.1

Table of contents

Mini Review

Medicinal plant *Coleus forskohlii* Briq.: Disease and management
Rakshapal Singh, Surendera P. Gangwar, Deepmala Singh, Rachana Singh, Rakesh Pandey, Alok Kalra
DOI:10.5958/j.0975-4261.3.1.001

Research Articles

In vitro anticancer and antimalarial Erystagallin-A from *Erythrina variegata* (L.) stem bark
Tati Herlina, Nurlelasari, Dikdik Kurnia, Unang Supratman, Zalinar Udin
DOI:10.5958/j.0975-4261.3.1.015

In vitro culture and plant regeneration from nodal explants of *Verbesina encelioides* (Cav.) Benth & Hook
Monika Karnawat, Damini Singh, C.P. Malik
DOI:10.5958/j.0975-4261.3.1.002

Antioxidant and platelet aggregation inhibitor activities of black tea (*Camellia sinensis* L.) extract and fractions
Wahyu Widowati, Tati Herlina, Hana Ratnawati, Tjandrawati Mozef, Chandra Risdian
DOI:10.5958/j.0975-4261.3.1.003

Cross-cultural ethnobotany and conservation of medicinal and aromatic plants in the Nilgiris, Western Ghats: A case study
P. Navaneethan, Sunil Nautiyal, T. Kalaivani, C. Rajasekaran
DOI:10.5958/j.0975-4261.3.1.004

In vitro clonal propagation of *Centella asiatica* (L.) Urban using shoot tip explants pre-treated with thidiazuron
Tanavat Kaensaksiri, Puangpaka Soontornchainaksaeng, Noppamas Soonthornchareonnon, Sompop Prathanturarug
DOI:10.5958/j.0975-4261.3.1.005

Medicinal plants used by Nomadic tribals of Rajouri Pir Panjal Foothills of North-West Himalayas in Jammu and Kashmir, India
Mushtaq Ahmed, R.S. Upadhyay, M.A. Dar, H.B. Singh
DOI:10.5958/j.0975-4261.3.1.006

Augmented production of vincristine in induced tetraploids of *Agrobacterium* transformed shooty teratomas of C*atharanthus roseus*
Fathimunnisa Begum
DOI:10.5958/j.0975-4261.3.1.007

Insecticidal properties of *Gymnema sylvestre* R.Br. against the storage pest, *Tribolium castaneum* Herbst.
Ahalya Segarajah, Mikunthan Gunasingam
DOI:10.5958/j.0975-4261.3.1.008

Evaluation of antibacterial potential of *Embelia ribes*
A. Sabitha Rani, G. Sulakshana, V. Nagamani
DOI:10.5958/j.0975-4261.3.1.009

Short Communications

Triterpenoidal saponin and phenolic glycosides from *Rhododendron barbatum* flowers
U. Rawat, V. Joshi, D.K. Semwal, A. Bamola, S. Semwal, R. Badoni, O.P. Sati
DOI:10.5958/j.0975-4261.3.1.010

Preliminary antibacterial evaluation of *Lindsaea ensifolia* Sw. against bacteria involved in skin diseases
Toji Thomas
DOI:10.5958/j.0975-4261.3.1.011

Antidiarrhoeal effects of ethanolic leaf extract of *Erythrina indica* L. in experimental animals
J. Sonia, P.G. Latha, P. Gowsalya, G.I. Anuja, S.R. Suja, V.J. Shine, S. Shymal, P. Shikha, N.M. Krishnakumar, G. Sreejith, S. Sini, S. Raja Sekharan
DOI:10.5958/j.0975-4261.3.1.012

Emerging Medicinal Plant

Adhatoda zeylanica Medic.: A versatile plant with wide spectrum medicinal activities
S.K. Prabhuji, Deepanjali Srivastava, Richa Srivastava, Sarita Sheikh
DOI:10.5958/j.0975-4261.3.1.013

Conference Report

International Conference on Challenging and Emerging Dimensions in Medicinal/Herbal Plants and their Products: A Global perspective (November 26–28, 2010)DOI:10.5958/j.0975-4261.3.1.014

Top

Research Article

Antioxidant and platelet aggregation inhibitor activities of black tea (*Camellia sinensis* L.) extract and fractions

Wahyu Widowati[1], Tati Herlina[2], Hana Ratnawati[1], Tjandrawati Mozef[3] and Chandra Risdian[3]
[1]*Medical Research Center, Faculty of Medicine, Maranatha Christian University Bandung – Indonesia Jl. Prof. Drg. Suria Sumantri, MPH. No. 65 Bandung, West Java, Indonesia*
[2]*Faculty of Mathematics and Natural Sciences, University of Padjadjaran, Sumedang-Indonesia*
[3]*Research Centre for Chemistry, Indonesian Institute for Sciences, Bandung, Indonesia*

ABSTRACT

The dried leaves of *Camellia sinensis* L., is a popular beverage consumed worldwide and contains bioactive compounds. The research was carried out to evaluate antioxidant and antiaggregation activities of black tea extract and fractions namely hexane, ethyl acetate, butanol and water fraction. Antioxidant activity of extract and four fractions of black tea were evaluated by *1,1-diphenyl-2-picryl-hydrazyl* (DPPH) scavenging method and total phenolic compound were determined. Platelet aggregation was determined using whole blood of hyperaggregation individual and analyzed by aggregometer after induction by epinephrine (EPN). The results showed that black tea extract, exhibited highest DPPH free radical scavenging acitivity with IC_{50} = 5.405 µg/mL among black tea fractions, but still lower comparing to (-)-epigallocatechin-3-gallate (EGCG) with IC_{50} = 0.505 µg/mL. The highest total phenolic compounds was ethyl acetate fraction (499.07 EGCG µg/mg) and the lowest was water fraction (149.77 EGCG µg/mg). Extract and fractions of black tea were capable in lowering platelet aggregation both at high and low concentrations of EPN (300 and 75 µM) inducer compared to untreated blood. Ethyl acetate, water fractions and extract of black tea showed similar platelet antiaggregation compared with aspirin as positive control. **[Medicinal Plants 2011; 3(1) : 21-26]**.

Keywords : Antioxidant, aggregation platelet inhibitor, phenolic compound, *Camellia sinensis*

INTRODUCTION

Epidemiological studies suggest an inverse relationship between tea consumption and cardiovascular disease. There is also convincing evidence that dietary intake of antioxidant flavonoids from tea and other sources (eg, onions, apples, red wine, and broccoli) is associated with reduced cardiovascular risk. The benefit of high flavonoid intake may be greater for individuals with established coronary artery disease (CAD) (Duffy *et al*., 2001). Short- and long-term black tea consumption reverses endothelial vasomotor dysfunction in patients with coronary artery disease. This finding may partly explain the association between tea intake and decreased cardiovascular (Duffy *et al*., 2001). The Boston Area Health Study found that subjects who drank one (200–250 mL) or more cups of black tea per day had approximately half the risk of a heart attack compared with those who did not drink tea at all (Sesso *et al*., 1999; Yang and Landau, 2000). 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 (Wiseman *et al*., 1997; Yang and Landau, 2000). A number of biological mechanisms, including radical scavenging and antioxidant properties, have been proposed for the beneficial effects of 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), (-)-epicatechin3-gallate (ECG), (-)-epicatechin (EC), (+)-gallocatechin, and (+)-catechin. Many biological

Corresponding author : Wahyu Widowati
E-mail : wahyu_w60@yahoo.com

functions of tea polyphenols have been studied (Yang and Wang, 1993), including anti-inflammatory, antioxidative (Lin *et al*., 1996).

MATERIALS AND METHODS

Plant and Chemical material

Plant materials were dried black obtained from PT Walini Subang-West Java Indonesia. Chemical agent were 1,1-diphenyl-2-picrylhydrazyl (DPPH) (Sigma Chemical Co.), HPLC grade methanol (Merck), EGCG (Sigma Chemical Co.), dimethyl sulfoxide (DMSO) (Merck), Aspirin (PT Bayer Indonesia), platelet rich plasma (PRP), platelet poor plasma (PPP), sodium citrate, Epinephrine (Helene Laboratories), Folin-Ciocalteu's reagent (Sigma Chemical Co.), anhydrous sodium carbonate (Sigma Chemical Co.).

Preparation of extract and fraction

The dried leaves of black tea (7 kg) was chopped and immersed in 96% methanol. After 72 hours the filtrate was collected, and the residue was immersed again by 96% methanol for period of 72 hours. This treatment was repeated until the filtrate became colorless, and then evaporated with rotary evaporator at 40°C. The yield of methanolic extract was 546.1g. The methanolic extract was partitioned between hexane and water (7:3). The aqueous layer was fractioned respectively with ethyl acetate : water (1:1) and butanol : water (1:1). The hexane, ethyl acetate, butanol, water fraction were collected and concentrated with vacuum rotary evaporator at 40°C giving the yields 111.7 g; 59.7 g; 18.0 g; and 77.2 g respectively. The methanol extrac dan fractions of black tea were stored at 4 °C.

Sample Preparation

Evaluating antioxidant activity used HPLC methanol and antiaggregation activity used DMSO 1 % as solvent to obtain the series of concentration level. Extract and fractions of black were prepared by dissolving 0.005 g of extract in 10 ml of HPLC methanol or DMSO 1% as 500 µg/mL concentration level, therefore arranging series of concentration level (500; 250; 125; 62.5; 31.25; 15.625; 7.813 smallest concentration was 3.906 µg/mL). To evalute the antioxidant activity by DPPH scavenging activity, eight concentrations of black tea extract and fractions were compared with EGCG. Antiaggregation platelet activity of black tea extract and fractions (500 µg/mL) was compared with aspirin (500 µg/mL). Epinephrine (EPN) was diluted with distilled water at two concentrations of 300 µM and 75 µM, platelet rich plasma (PRP) obtained from hyperaggregation individual (Helena Laboratories,2008). To measure the total phenolic compound of black tea extract and fractions used EGCG as standard.

DPPH radical scavenging activity assay

The DPPH assay was carried out as described by Unlu *et al*. (2003), Han *et al*. (2004) and Frum and Viljoen (2006). Pipette 50 µL of sample (black tea extract and fractions, EGCG) of various concentrations of the samples (eight concentrations level) enter 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 minutes at room temperature. The DPPH radical scavenging activity 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

Antiaggregation activity assay

Nine mL blood was collected from hyperaggregation individual and added 1 mL 3.8% sodium citrate as anticoagulant and blood specimen was centrifuged at 100 × g for 10 minutes, the platelet rich plasma (PRP) was removed from the cells with a plastic pippete and place in a plastic tube, the PRP was maintained at room temperature for 30 minutes. Preparing platelet poor plasma (PPP) by recentrifuging the remaining blood samples at 1600 × g for 10 minutes, PPP was removed and placed in a plastic tube, the tube was maintained at room temperature (Chun-Han *et al*., 1993; Helena Laboratories, 2008).

Aggregation activity was measured by Platelet Aggregation Chromogenic Kinetic System (PACKS-4). Pipette 450 µL of PPP into a cuvette. Pipette 450 µL PRP and 40 µL the antiaggregation agents (aspirin, black tea extract, fractions at level 500 µg/mL) into cuvettes with stir bar, incubated the cuvettes at 37^0 C for 3-5 minutes. Insert the PPP cuvette into appropriate channel and set the instrument, insert the PRP cuvette into the appropriate channel. Add 50 µL of the aggregating reagent dilutions EPN at level 300 µM, 75 µM to the

PRP cuvette and record the percent aggregation (Helena Laboratories, 2008).

The total phenolic content

Phenolic compounds were assayed, according to the Folin–Ciocalteu method (Singleton and Rossi, 1965; Ivanova *et al*; 2005). Samples (15 µL) were introduced into microplate; 75 µL of Folin-Ciocalteu's reagent (2.0 M) and 60 µL of sodium carbonate (7.5%) were added. The samples were mixed and incubated at 45^0 C for 15 min. Absorption at 760 nm was measured. The total phenolic content was expressed as EGCG equivalent (EGCGE) was calculated by the following formula :

$$C = \frac{c \times V}{m}$$

C : total content of phenolic compounds, µg/mg plant extract, in EGCGE; c : the concentration of EGCG established from the calibration curve, µg/mL; V : the volume of extract (mL); m : the weight of pure plant extract (mg). Total phenol value was obtained from the regression equation : $y = 0.036 + 0.0048\ x$, with $R^2 = 0.965$.

Statistical Analysis

The amount of antioxidant actvity treatment were twenty forty eight treatment (eight level concentrations and six antioxidant), each treatment antioxidant activity was three replications. The amount of antiaggregation activity treatment were ten treatment (two level of EPN inducer and eight antiaggregation agents), each treatment antiaggregation activity was three replications. The amount of phenolic compound were five treatment (black tea extract and four fractions), each treatment phenolic compound was three replications.

The DPPH scavenging activity of black tea extract and fractions was expressed as IC_{50} To verify the statistical significance of antiaggregation and total phenolic content parameters, 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 completely randomized design. P-values of less than 0.05 were considered as statiscally significant. 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

DPPH scavenging activity

The DPPH free radical scavenging activity of black tea extract and fractions, EGCG antioxidant well known as positive control of various concentration were measured to know the antioxidant activity. The IC_{50} is the concentration of antioxidant activity to scavenge DPPH free radical 50 % (Fig. 1; Table 1) shows the DPPH scavenging activity, antioxidant activity among black tea extract, fractions and EGCG are same at high concentration, but reduced as compared with EGCG at low concentration.

The total phenolic content

The total phenolic compound of black tea extract, hexane, ethyl acetate, butanol and water fractions in EGCG equivalent is shown in Table 2. The phenolic content data of black extract and fractions were analyzed using one way ANOVA ($p<0.005$) continued by Duncan's post-Hoc test. The ethyl acetate fraction had highest

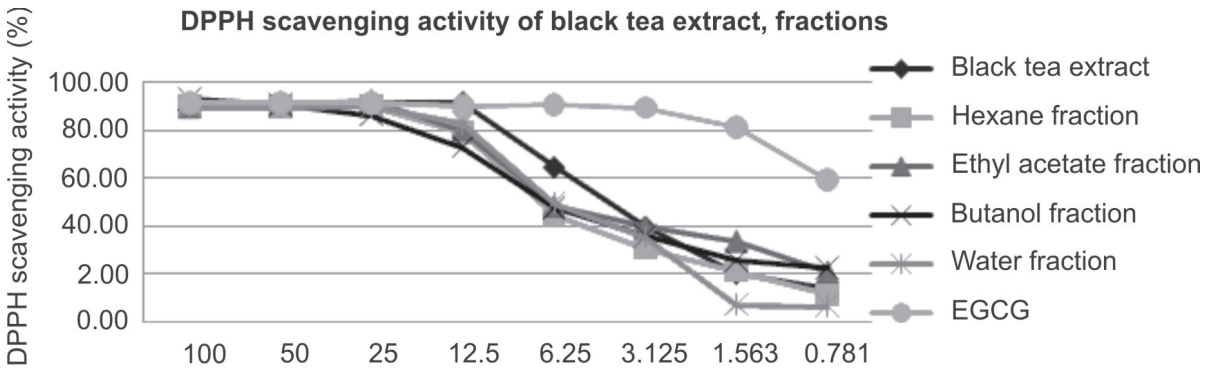

Fig. 1. The DPPH scavenging activity of black tea extract and fractions.

Table 1. The IC_{50} of DPPH scavenging activity of black tea extract and fractions

Sample	IC_{50} (µg/mL)
C. sinensis extract	5.405
Hexane fraction	7.124
Ethyl acetate fraction	6.785
Butanol fraction	8.581
Water fraction	6.895
EGCG	0.505

Table 2. Mean, standard deviation and Duncan's post hoc test of black tea extract and fractions on total phenolic content (µg EGCG/mg)

Sample	Phenolic content (µg/mg)
C. sinensis extract	338.53±28.45 c
Hexane fraction	244.77±6.32 b
Ethyl acetate fraction	499.07±39.05 d
Butanol fraction	351.48±34.66 c
Water fraction	149.77±14.80 a

The data showed mean and standard deviation. The same small letters show no significant at the 5% (Duncan's Post-hoc test)

Table 3. Mean, standard deviation and Duncan's post hoc test of black tea extract and fractions on antiaggregation platelet activity (%)

Sample	Aggregation platelet (%)	
	EPN 300 µM	EPN 75 µM
Hyperaggreagtion individu	45.83±4.31 e	88.27±3.31 h
C. sinensis extract	23.47±3.31 bc	34.40±3.05 d
Hexane fraction	76.50±3.15 g	79.97±6.35 g
Ethyl acetate fraction	28.33±3.70 bc	4.31±1.81 a
Butanol fraction	67.60±5.05 f	80.90±3.40 g
Water fraction	21.83±2.35 b	26.90±2.48 bc
Aspirin	22.97±3.01 bc	29.10±4.40 cd

The data showed mean and standard deviation. The same small letters show no significant at the 5 % (Duncan's Post-hoc test)

phenolic content and the lowest phenolic content was found in water fraction.

The antiaggregation activity

Black tea extract, fractions and aspirin as positive control (500 µg/mL or final concentration 37.03 µg/mL) and two EPN concentrations (300 µM; 75 µM) were measured to know the antiaggregation activity. Hyperaggregation individual showed very high platelet aggregation (88.27 % in 75 µg/mL EPN inducer and 45.83 % in 300 µM EPN inducer). All tea samples (black tea extract and fractions) were capable to reduce the platelet aggregation both at high and low concentration of EPN inducer (Table 3). Ethyl acetate fraction was highest antiaggregation activity both at high and low concentration similar with aspirin at high concentration. Water fraction was more active compared with aspirin both at high and low concentration of EPN inducer.

DISCUSSION

Black tea extract and fractions exhibited high antioxidant activity because its contains high polyphenol compounds (Table 1 and 2). Many biological functions of 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 show correlations between a higher content of flavonoids in the diet and a risk of coronary heart disease mortality (Lolito and Fraga, 2000). These associations are mainly ascribed to the antioxidant capacity of these compounds (Lolito and Fraga, 2000). Theaflavins (TF) present in black tea possess at least the same antioxidant potency as catechins present in green tea, and that the conversion of catechins to TF during fermentation in preparing black tea does not alter significantly their free radical–scavenging activity (Leung *et al*., 2001).

The major fractions of black tea polyphenols, accounting for >20% of the solids in brewed black tea, are known as thearubigens (Yang and Landau, 2000; Leung *et al*., 2001). Theaflavins and thearubigins are oligomeric polyphenolic compounds synthesized from monomeric tea flavanol units. Theaflavins (TF) are another group of polyphenol pigments found in black tea. TF are formed from polymerization of catechins at the fermentation stage during the manufacture of black tea (Leung *et al*., 2001). Ethyl acetate fraction of black tea extract contains high polyphenols (Table 2) and high flavonoids effected which resulted in high antioxidant activity (Table 1).

Aspirin was antiaggregation agent by inhibiting the production of thromboxane A2 (TXA2) and inhibiting the enzyme cyclooxygenase (Hyun-Jung *et al.*, 2006). Aspirin was an anti-thrombotic effects through the inhibition of platelet cyclooxygenase-1 (COX-1) by the irreversible acetylation of a specific serine moiety, thereby blocking the formation of TXA2 for the lifetime of the platelets (McKee *et al.*, 2002; Ohmori *et al.*, 2006). The black tea extract and fractions were able to decrease the platelet aggregation at all concentration level of EPN inducer (300 µM and 75 µM) compared with untreated on hyperaggregation individual (Table 3). Ethyl acetate fraction, water fraction and black tea extract showed high antiaggregation activity either in low and high concentration level of EPN inducer. Ethyl acetate was more active than aspirin in low concentration of EPN and was similar active with aspirin in high concentration of EPN (Table 3). Black tea extract and fractions contain polyphenol, flavonoids (USDA, 2003). Phenolic compound exhibits a wide range of biological effects, including antiplatelet, anti-inflammatory, anticancer, antimutagenic and antifungal properties. It is also a potent antioxidant, reactive oxygen species scavenger and metal chelators (Olas and Wachowicz, 2005). Earlier workers also discussed that eriodictyol and patuletin are flavonoids from *Leuzea carthamoides* inhibit platelet aggregation with collagen (COL) and arachidonic acid (AA) agonist (Koleckar *et al.*, 2008). Rutin is flavonoid, inhibit platelet aggregation in human platelets stimulated by collagen (COL) agonist and are concentration-dependent (250 and 290 µM) (Sheu *et al.*, 2004). Rapid phosphorylation of a platelet protein of M(r) 47000 (P47), a marker of protein kinase C activation, is reported to be triggered by collagen (Sheu *et al.*, 2004). The antiplatelet activity of rutin (flavonoid) may involve the following pathways : rutin inhibit the activation of phospholipase C, followed by inhibition of protein kinase C activity and TXA2 formation, thereby leading to inhibition of the phosphorylation of P47 and intracellular Ca^{2+} mobilization, finally resulting in inhibition of platelet aggregation (Sheu *et al.*, 2004). The combining *in vitro* 2 flavonoids, namely quercetin and catechin, demonstrate that they are synergistic in reducing platelet formation of H_2O_2 and inhibiting platelet function by interfering with the activation of phospholipase C pathway (Violi, 2002). Catechin and eugenol (flavonoids) also inhibit cyclooxygenase (COX) activities and platelet aggregation (Huss *et al.*, 2002). Flavonoids inhibit platelet aggregation because of their antioxidant activity, either by inhibiting the formation of endogenous mediators derived from phospholipid peroxidation, by blocking enzymatic free radical production, or by reducing platelet sensitivity to agonists by preventing lipid peroxidation (Murphy *et al.*, 2003). Various agonists may stimulate platelet Reactive Oxygen Species (ROS) production and aggregation, via regulating AA metabolism or via COX inhibition (Iuliano *et al.*,1997). In the presence of haemoglobin, ROS-induced platelet aggregation is enhanced (Iuliano *et al.*, 1992). The resting platelets also generated a low amount of ROS. AA stimulates platelet ROS production, which is inhibited by Hydroxychavicol flavonoid (HC), as a ROS scavenger (Chang *et al.*, 2002). Black tea extract, ethyl acetate and water fractions had higher antioxidant activity as compared with hexane and butanol fractions (Table 1). The other possible reason is that platelet ROS production can be mediated by COX as well as other enzymes such as platelet isoforms of NADPH oxidase, xanthine oxidase, mitochondrial respiration (Krotz *et al.*, 2004). Flavonoid quercetin (40–100 µmol/L) and catechin (100–420 µmol/L) inhibit platelet aggregation *in vitro* (Pignatelli *et al.*, 2001). Flavonoids inhibit platelet function by blunting hydrogen peroxide production and, in turn, phospholipase C activation (Pignatelli *et al.*, 2001). Flavonoids are phenolic compounds such as resveretrol, quercetin, and catechin act synergistically to inhibit platelet adhesion to collagen and collagen-induced platelet aggregation (Pignatelli *et al.*, 2001). Endothelial dysfunction in atherosclerosis is associated with increased oxidative stress and be reversed by antioxidant treatment (Duffy *et al.*, 2001). Black tea extract and fractions were capable to inhibit platelet aggregation due to high polyphenols (Table 2) and had high antioxidant activity (Table 1).

ACKNOWLEDGEMENT

We are grateful to Director General of Higher Education, Ministry of National Education of Republic Indonesia, for the Research Grant of Hibah Kompetitif Penelitian Sesuai Prioritas Nasional (2009-2010).

REFERENCES

Chang, MC, Uang BJ, Wu HL, Lee JJ, Hahn LJ and Jeng JH (2002). Inducing the cell cycle arrest and apoptosis of oral KB carcinoma cells by hydroxychavicol: roles of glutathione and reactive oxygen species. *Br. J. Pharmacol.,* 135: 619–630.

Duffy SJ, Keaney JF, Holbrook Jr H, Gokce N, Swerdloff PL, Frei B and Vita JA (2001). Short- and long-term black tea consumption reverses endothelial dysfunction in patients with coronary artery disease. *Circulation*., 104: 151-156.

Frei B and Higdon JV (2003). Antioxidant activity of tea polyphenols in vivo : evidence from animal studies. *J. Nutr.*, 133: 3275S-3284S.

Frum Y and Viljoen AM (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.

Han SS, Lo SC, Choi YW, Kim JH and Baek SH (2004). Antioxidant activity of crude extract and pure compounds of *Acer ginnala* Max. *Bull. Korean Chem. Soc.*, 25(3): 389-391.

Helena Laboratories (2008). Helena platelet aggregation system. Beaumont : Texas.

Huss U, Ringbom T, Perera P, Bohlin L and Vasange M (2002). Screening of ubiquitous plant constituents for COX-2 inhibition with a scintillation proximity based assay. *J Nat Prod.,* 65: 1517-1521.

Hyun-Jung L, Hak-Ju L, Kang-Hoon J, Jeing-Kon S, Seung-Jin L and Sun-Li B (2006). Compound isolated from *Ginkgo biloba* bark, isolation method thereof and antiplatelet composition containing the same. *United States Patent.,* 7341750.

Iuliano L, Violi F, Pedersen JZ, Pratico D, Rotilio G and Balsano F (1992). Free radical-mediated platelet activation by hemoglobin released from red blood cells. *Arch. Biochem. Biophys*, 299: 220-224.

Iuliano L, Colavita AR, Leo R, Pratico D and Violi F (1997). Oxygen free radicals and platelet activation. *Free Radical Biol. Med.,* 22: 999-1006.

Ivanova D, Gerova D, Chervenkov T and Yankova T (2005). Polyphenols and antioxidant capacity of Bulgarian medicinal plants. *J. Ethnopharmacol.*, 96: 145-150.

Kuriyama S, Shimazu T, Ohmori K, Kikuchi N, Nakaya N, Nishino Y, Tsubono Y and 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.

Leung LK, Su Y, Chen R, Zhang Z, Huang Y, Chen Z-Y (2001). Theaflavins in black tea and catechins in green tea are equally effective antioxidants. *J. Nutr.*, 131: 2248-2251.

Lotito SB and Fraga CG (2000). Catechins delay lipid oxidation and α-tocopherol and β-carotene depletion following ascorbate depletion in human plasma**.** *Soc. Exp. Biol. Med.,* 225: 32-38.

McKee SA, Sane DC and Deliargyris EN (2002). Aspirin resistance in cardiovascular disease: a review of prevalence, mechanisms, and clinical significance. *Thromb. Haemost.,* 88: 711-715.

Murphy KJ, Chronopoulos AK, Singh I, Francis MA, Moriarty H, Pike MJ, Turner AH, Mann NJ and Sinclair AJ (2003). Dietary flavanols and procyanidin oligomers from cocoa (*Theobroma cacao*) inhibit platelet function. *Am. J. Clin. Nutr.,* 77(6): 1466-1473.

Ohmori T, Yatomi Y, Nonaka T, Kobayashi Y, Madoiwa S, Mimuro J, Ozakis Y and Sakata Y (2006). Aspirin resistance detected with aggregometry cannot be explained by cyclooxygenase activity: involvement of other signaling pathway(s) in cardiovascular events of aspirin-treated patients. *J. Thromb. Haemostasis.,* 4: 1271-1278.

Olas B and Wachowicz B (2005). Resveratrol, a phenolic antioxidant with effects on blood platelet functions. *Platelets.*, 16: 251-260.

Pignatelli P, Pulcinelli FM, Celestini A, Lenti L, Ghiselli A, Gazzaniga PP and Violi F (2001). The flavonoids quercetin and catechin synergistically inhibit platelet function by antagonizing the intracellular production of hydrogen peroxide. *Am. J. Clin. Nutr.*, 73: 360.

Sesso HD, Gaziano JM, Buring JE and Hennekens CH (1999). Coffee and tea intake and the risk of myocardial infarction. *Am. J. Epidemiol*., 149: 162-167.

Sheu JR, Hsiao G, Chou PH and Shen MY (2004). Mechanisms involved in the antiplatelet activity of rutin, a glycoside of the flavonol quercetin, in human platelets. *Agric. Food Chem.*, 52(14): 4414-4418.

Singleton VL and Rossi JA (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am. J. Enol. Viticult.*, 16: 144-158.

Unlu, GV, Candan F, Sokmen FA, Dafefera D, Polissiou M, Sokmen E, Donmez and Tepe E (2003). Antimicrobial and antioxidant activity of the essential oil and methanol extracts of *Thymus pectinatus* Fisch. et Mey. Var. *pectinatus* (Lamiaceae). *J. Agric. Food Chem.*, 51: 63-67.

USDA (2003). USDA database for the flavonoid contents of selected foods. Beltsville: US Department of Agriculture.

Violi F (2002). Synergism among flavonoids in inhibiting platelet aggregation and H_2O_2 production. *Am. Heart Assoc. Circulation.*, 105: e53.

Wiseman SA, Balentine DA and Frei B (1997). Antioxidants in tea. *Crit. Rev. Food Sci. Nutr.*, 37: 705-718.

Yang CS and Landau JM (2000). Effect of Tea Consumptionon Nutrition and Health. *J. Nutr.*, 130: 2409-24112.

Yang CS and Wang ZY (1993). Tea and cancer. *J. Natl. Cancer Inst*., 85: 1038-1049.