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THE COMPARISON ANTIOXIDANT, ANTIAGGREGATION OF VARIOUS TEA EXTRACT (*CAMELLIA SINENSIS* L.)

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Abstract: The dried leaves of *Camellia sinensis* L., is a popular beverage consumed worldwide and contained bioactive compound. The three main categories of tea are black, green and oolong, the difference are the processing of tea, caused the three teas had different bioactive. The research was carried out to evaluate antioxidant and antiaggregation activity of black, green and oolong tea extract. To evaluate antioxidant of various methanol extract of teas used *1,1-diphenyl-2-picryl-hydrazyl* (DPPH) and total phenolic compound. To know the DPPH free radical scavenging activity, methanol extract of black tea, green tea and oolong tea were arranged at 6 level concentrations respectively 500 µg/mL; 250; 125; 62.5, 31.25 and 15.625 µg/mL and compared to quercetine as positive control. The antiaggregation activity were carried out 2 concentrations (10 µg/mL and 2.5 µg/mL) of Collagen inducer and 1 concentration (500 µg/mL) of antiaggregation agent. The results showed that black, green and oolong tea extract exhibited had high DPPH free radical scavenging acitivity, quercetine as positive control was higher antioxidant activity at level 500 and 250 µg/mL compared with black, green and oolong tea. Total phenolic compound of black, green and oolong tea were not significantly difference (751.967– 815.700 mg quercetine/g extract). Black, green and oolong tea extract are capable to inhibit platelet aggregation both in high and low concentration of Collagen inducer. The black, green and oolong tea as antiaggregation platelet more active in high concentration of Collagen compared with Aspirin, oolong tea extract more active than Aspirin in low concentration of Collagen.

Ke words : antioxidant, aggregation platelet, phenolic compound, 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]. Variety of factors contribute to the beneficial effects of plant foods, much attention has been addressed to plant polyphenols [Grassi et al., 2008]. Polyphenols constitute the most interesting group of tea leaves components, and in consequence, tea can be considered an important dietary source of polyphenols. Three billion kilograms of tea are produced each year worldwide. Because of the high rates of tea consumption in the global population, even small effects in humans could have large implications for public health [Kris-Etherton et al., 2002; Kuriyama et al., 2006]. Tea is generally consumed in the forms of green, oolong, and black tea, all of which originate from the leaves of the plant *Camellia sinensis* [Frei and Hidgon, 2002, Kuriyama et al., 2006]. Alterations in the *C. sinensis* manufacturing process result in black, green, and oolong tea, which account for approximately 75%, 23%, and 2% of the global production, respectively [Bliss, 2003; Carlson et al., 2007]. In the production of black tea, the plant leaves are picked and then allowed to wither

indoors, ferment, and oxidize. For green tea, the plant leaves are steamed and parched after picking to prevent oxidation of the catechins present in the leaf [Frei and Hidgon, 2003; Carlson et al., 2007]. Oolong tea is produced by "semifermenting" the green leaves, resulting in a tea that is chemically a mixture of green and black teas [Frei and Hidgon, 2003; Carlson et al., 2007]. The qualitative and quantitative chemical differences of various tea are resulted from the different processing techniques [Carlson et al., 2007].

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 [Geleijnse 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; Duffy et al., 2001]. Short- and long-term black tea consumption reverses endothelial vasomotor dysfunction in patients with coronary artery disease [Duffy et al., 2001]. Previous research with intragastric administration of black tea inhibited platelet aggregation and prevented experimental coronary thrombosis in dogs and that consumption of green tea polyphenols decreased ADP-induced platelet aggregation and possible mechanism for preventing cardiovascular diseases [Matsumoto, 1998; Yang and Landau, 2000].

MATERIALS AND METHODS

Plant and Chemical material

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

Extraction

The dried black, green and oolong tea leaves (*C. sinensis* L.) were milled and soaked in destilled methanol (MeOH) during 24 hours in macerator, separated filtrate and added MeOH in the macerator. The filtrate was collected until 3 times soaking, then evaporated the filtrate at approximately 40 °C. Two kg of dried oolong tea leaves yielded 447.8 g methanol extract or 22.39 %; seven kg of dried green tea leaves yielded 869.5 g methanol extract or 12.42 % and seven kg of dried black tea leaves yielded 546.1 g metanol extract or 7.80 %.

Sample Preparation

Evaluating antioxidant activity used HPLC methanol and antiaggregation activity used DMSO 1 % as solvent to obtain the series of concentration level. Extract of black, green and oolong 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 (250; 125; 62.5 31.25; 15.625 smallest concentration was 15.625 µg/mL). To evalute the antioxidant activity by DPPH scavenging activity, six concentrations of black, green and oolon tea extract were compared with quercetin. Antiaggregation platelet activity one concentration of black, green and oolong tea extract (500 µg/mL) was compared with aspirin (500 µg/mL). Collagen (COL) was diluted with distilled water at two concentrations of 10 µg/mL and 2.5 µg/mL, platelet rich plasma (PRP) obtained from hyperaggregation individual (Helena Laboratories,2008). To measure the total phenolic compound of variuos tea extract used quercetin 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 (oolong, green and black tea methanol extract,

quercetin) of various concentrations of the samples (six concentration 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

Whole blood 9 mL was collected from hyperaggregation individual and added 1 mL 3.8% sodium citrate as anticoagulant and blood specimen was centrifuged at 100xg 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 1600xg 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). Pippete 450 µL of PPP into a cuvette. Pippete 450 µL PRP and 40 µL the antiplatelet agents (aspirin, various tea extract 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 (Collagen) at level 10 µg/mL, 2.5 µg/mL 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, six replicates) 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 were mixed and incubated at 45^0 C for 15 min. Absorption at 760 nm was measured. The total phenolic content was expressed as quercetin equivalents (QE) was calculated by the following formula : $C=c \cdot V/m'$

C : total content of phenolic compounds, mg/g plant extract, in QE; c : the concentration of quercetin established from the calibration curve, mg/ml; V; the volume of extract (mL); m: the weight of pure plant extract (g). Total phenol value was obtained from the regression equation : $y = 1.325 + 0.0019\ x$, with $R^2 = 0.956$

Statistical Analysis

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

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 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

The DPPH sacvenging activity

The DPPH free radical scavenging activity of black, green and oolong tea methanol extract and quercetin antioxidant well known as positive control of various concentration were measured to know the antioxidant activity. The DPPH free radical scavenging activity of black, green and oolong tea extract is shown in Table 1. The DPPH radical scavenging activity of black, green, oolong tea extract and quercetin showed high antioxidant activity. The highest antioxidant activity of black tea extract were 91.513 % - 91.796 % (level 62.5 -250 µg/mL), green tea extract were 88.906%- 90.534% (level 62.5 - 500 µg/mL), oolong tea extract was 90.923 % (level 500 µg/mL), quercetin were 92.202% - 94.495% (level 125 – 500 µg/mL). Quercetin was the highest antioxidant activity in level 250 µg/mL and 500 µg/mL compared with various tea extract, at level 125 µg/mL all tea extract and quercetin had same antioxidant activity (89.720 % - 92.202%), the highest antioxidant activity at level 62.25 µg/mL was black tea extract (91.702%).

Table 1. Mean, standard deviation and Duncan's post hoc test of various methanol tea extract on DPPH scavenging activity (%)

Concentrations	Type of antioxidant sources			
	black tea MeOH extract	green tea MeOH extract	oolong tea MeOH extract	quercetin
500 µg/mL	90.005±0.432 a C	88.906±3.405 a C	90.923±0.278 a C	94.292±0.385 b C
250 µg/mL	91.513±0.748 b CD	89.924±0.916 a C	90.120±0.241 a B	94.495±0.404 c C
125 µg/mL	91.796±0.490 a D	89.720±3.473 a C	90.120±0.241 a B	92.202±2.807 a C
62.5 µg/mL	91.702±0.432 d D	90.534±0.808 c C	89.559±0.278 b AB	82.205±0.387 a B
31.25 µg/mL	64.828±1.598 a B	78.931±1.907 b B	89.478±0.139 b A	80.013±0.591 b AB
15.625 µg/mL	39.961±1.598 a A	54.300±12.344 b A	89.960±0.278 c AB	79.110±1.686 c A

The data showed mean and standard deviation. The same small letters at the same row (among tea extract and quercetin), and same capital letters at the same coloumn show no significant at the 5 % (Duncan's post hoc test)

The total phenolic content

The total phenolic compound of black, green and oolong tea in querctein equivalent is shown in Table 2. The phenolic content data of black, green and oolong tea were analyzed using one way ANOVA and there were not significantly difference. The black, green and oolong tea extract had same total phenolic compound (765.800 – 815,700 mg quercetin/g extract)

The antiaggregation activity

Black, green, oolong tea methanol extract and aspirin as positive control (500 µg/mL) and two collagen concentrations (10 µg/mL, 2.5 µg/mL) were measured to know the antiaggregation activity. The antiaggregation activity of various tea extract is shown in Table 3. Hyperaggregation individual showed very high platelet aggregation (82.267 % in 2.5 µg/mL collagen inducer and 97.900 % in 10 µg/mL Collagen inducer). All tea extracts (black, green, oolong extract) are capable to reduce the aggregation platelet both in high and low concentration of Collagen inducer. Oolong tea extract was more active as antiaggregation compared with aspirin on

low concentration of Collagen, black and green tea extract had same antiaggregation platelet with aspirin.

Table 2. Mean, standard deviation of various methanol tea extract on total phenolic content (mg quercetin/g extract)

Various tea extract		
black tea MeOH extract (mg/g extract)	green tea MeOH extract (mg/g extract)	oolong tea MeOH extract (mg/g extract)
815.700±82.236	765.800±24.970	751.967±23.174

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

Concentrations Collagen	Aggregation platelet (%)				
	Hyperaggregation individual	Aspirin	Black tea MeOH extract	Green tea MeOH extract	Oolong tea MeOH extract
10 µg/mL	97.900±1.947 g	70.500±4.812 e	17.533±2.558 b	26.233±3.431 c	55.300±3.959 d
2.5µg/mL	82.267±4.821 f	13.700±2.835 b	18.733±3.431 b	15.667±2.259 b	6.400±1.700 a

The data showed mean and standard deviation. The same small letters (among tea extract and concentrations of Collagen) show no significant at the 5 % (Duncan's Post Hoc test)

DISCCUSSION

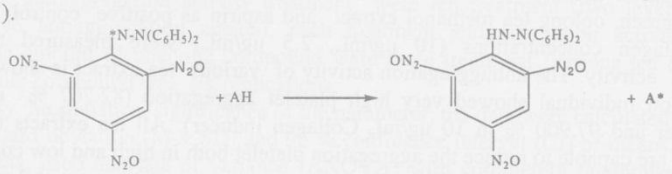
The scavenging of DPPH radicals is followed by monitoring the decrease in absorbance at 517 nm which occurs due to reduction by the antioxidant (AH) or reaction with radical species (R^*)

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

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

The DPPH scavenging activity test if antioxidant or sample which contain antioxidant will be occurred hydrogen (H) was captured by DPPH free radical or antioxidant donate hydrogen (H) was indicated purple color to become 1,1- diphenyl-2-picrylhydrazyn yellow color [Kikugawa et al., 1999; 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].

Fast reaction of DPPH radical occurs with some phenols e.g. α-tocopherol, flavonoid (Gordon, 2001).



1,1- diphenyl-2-picrylhydrazyl (purple color) **1,1- diphenyl-2-picrylhydrazyn (yellow color)**

Black, green and oolong tea had high antioxidant activity because its contain high polyphenol compound (Table 1 and 2). 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]. They may also function indirectly as antioxidants through : *1*). inhibition of the redox-sensitive transcription factors, nuclear factor-(kappa) B and activator protein-1; *2*). inhibition of "pro-oxidant" enzymes, such as inducible nitric oxide synthase, lipoxygenases, cyclooxygenases and xanthine oxidase; and *3*). induction of phase II and antioxidant enzymes, such as glutathione S-transferases and superoxide dismutases [Frei and Higdon, 2003]. Several epidemiological studies have shown correlations between a higher content of flavonoids in the diet and a risk of cancer and coronary heart disease mortality [Lolito and Fraga, 2000]. These associations were 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 making black tea does not alter significantly their free radical–scavenging activity [Leung et al., 2001].

Black, green and oolong tea had qualitative and quantitative chemical differences are resulted from the different processing techniques [Carlson et al., 2007]. In manufacturing black tea, the tea leaves are crushed to allow the polyphenol oxidase to catalyze the oxidation, leading to polymerization of catechins. The remaining catechins account for 3–10% of the solids in brewed black tea. Theaflavins, which include theaflavin (TF1), theaflavin-3-gallate (TF2A), theaflavin-3'-gallate (TF2B) and theaflavin-3,3'-digallate (TF3B), are key to the characteristic color and taste of black tea, and account for 2–6% of the solids in brewed black tea. The major fractions of black tea polyphenols, accounting for >20% of the solids in brewed black tea, are known as thearubigens. They have larger molecular weights and are poorly characterized chemically [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 both black and oolong teas. TF are formed from polymerization of catechins at the fermentation or semifermentation stage during the manufacture of black or oolong tea [Leung et al., 2001].

Green tea is a rich source of polyphenols, especially flavanols and flavonols, which represent approximately 30% dry weight of the fresh leaf [Balantine et al., 1997; Wolfram, 2007]. The main flavanols are catechins found in green tea, and to a lesser extent in black tea, are (-)-epigallocatechin-3-gallate (EGCG), (-)-epigallocatechin (EGC), (-)-epicatechin3-gallate (ECG), and (-)-epicatechin (EC). EGCG is the most abundant and widely studied tea polyphenol [Sano et al; 2001; Henning et al., 2004; Wolfram, 2007]. Green tea also contains gallic acid (GA) and other phenolic acids such as chlorogenic acid and caffeic acid, and flavonols such as kaempferol, myricetin and quercetin [Cabrera et al., 2006]. Oolong tea is produced by "semifermenting" the green leaves, resulting in a tea that is chemically a mixture of green and black teas [Frei and Hidgon, 2003; Carlson et al., 2007]. Oolong tea 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. Oolong tea 3 g were steeped in 300 mL hot water contained catechins EGCG 49.7 mg; EGCG 48.7 mg; EC 14.2; ECG 12.1 mg; catechin 3.93 mg; catechin gallate (CG) 2.28 mg; gallocatechin gallate (GCG) 1.56 mg; polyphenols including polymerized polyphenols 52.8 mg; gallic acid 5.25 mg; caffeine 53.9 mg [Rumpler et al; 2001]. Green tea contains flavonoids, there are flavan-3-ols namely EC 8.47 mg/100g; ECG 20.95 mg/100g; EGC 17.08 mg/100g; EGCG 82.89/100g; catechin 2.73 mg/100g; TF 0.05 mg/g; TF3B 0.01 mg/g; TF2B 0.01 mg/100g; TF2A 0.01 mg/100g; thearubigins 1.08 mg/100g; flavones namely apigenin 0.17 mg/100g; luteolin 0.17 mg/100g; flavonols namely kaempferol 1.42 mg/100g; myricetin 1.10 mg/100 g; quercetin 2.69 mg/100 g (USDA, 2003). Black tea contains flavonoids, there are flavan-3ols namely EC 2.33 mg/100g; ECG 7.24 mg/100g; EGC 10.43 mg/100g; EGCG 11.48 mg/100g; catechin 1.52 mg/100g; gallocatechin 1.26 mg/100g;

TF 1.58 mg/100g; TF3B 1.75 mg/100g; TF2B 1.51 mg/100g; TF2A 1.25 mg/100g; thearubigins 73.44 mg/100g; flavones namely apigenin 0.00 mg/100g; luteloin 0.00 mg/100g; flavonols namely kaempferol1.34 mg/100g; myricetin 0.45 mg/100g; quercetin 2.07 mg/100 [USDA, 2003]. Black, green and oolong tea contains high polyphenols were not significanly differences (Table 2) and high flavonoids effected and resulted high antioxidant activity (Table 1).

Activation of platelets by collagen is a multistep event. In fact, after an initial attachment to platelets through second messenger pathways, collagen stimulates release of thromboxane and ADP, which are important platelet agonists that induce aggregation [Pignatelli et al., 2001].

Aspirin was antiplatelet agent by inhibiting the production of thromboxane A2 (TXA2), 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, green and oolong tea extract decreased the platelet aggregation at all concentration level of collagen agonist (10 µg/mL and 2.5 µg/mL) compared with hyperaggregation individual. Tea extracts showed higher antiaggregation activity than aspirin either in low and high concentration level of collagen agonist. Black, green and oolong tea extract contains 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]. Eriodictyol and patuletin are flavonoids from *Leuzea carthamoides* inhibited platelet aggregation with agonist collagen (COL) and arachidonic acid (AA) [Koleckar et al., 2008]. Rutin is flavonoid, inhibited platelet aggregation in human platelets stimulated by COL agonist with concentration-dependently (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, was triggered by collagen [Sheu et al., 2004). The antiplatelet activity of rutin (flavonoid) may involve the following pathways : rutin inhibited 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, and demonstrated 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]. Flavonoid bioavailability and its relationship with antioxidant activity and platelet function [Violi, 2002]. Catechin and eugenol (flavonoids) also inhibit cyclooxygenase (COX) activities and platelet aggregation [Huss et al., 2002]. The isolation of flavonoids from *Cephalotaxus wilsoniana* (Cephalotaxaceae) had antiplatelet (Wu et al., 2007). The ginkgetin flavonoid was isolated from *Cephalotaxus wilsoniana* and *Justicia species* had inhibitory effect on cyclooxygenase-1 (COX-1). The flavonoids including ginkgetin, taiwanhomoflavone C, justicidin B and justicidin D were isolated from *Cephalotaxus wilsoniana* and *Justicia* showed inhibition of secondary aggregation induced by adrenaline [Wu et al., 2007]. Quercetin and catechin are flavonoids antioxidant, synergistically inhibited platelet function (*in vitro* assay) by blunting the release of hydrogen peroxide (H_2O_2) from platelets, subsequently reducing phospholipase C activation, calcium mobilization, and inositol phosphate synthesis. 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), supporting HC flavonoid as a ROS scavenger [Chang et al., 2002]. Base on the data (Table 3.) showed that black, green and oolong tea extract decreased platelet aggregation (as an antiaggregation activity) caused black, green and oolong tea extract had antioxidant activity (Table 1 and 2). Effects of mixed tocopherols as

antioxidant were associated with increased NO release, ecNOS activation, and SOD protein content in platelets, which may contribute to the effect on platelet aggregation [Liu et al., 2003]. AA-induced thromboxane production or induced by the other agonists is not solely mediated by ROS production. 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]. HC flavonoid inhibits the enzymes responsible for platelet ROS formation [Finazzi-Agro et al., 1982]. Previous studies showed that quercetin (40–100 μmol/L) and catechin (100–420 μmol/L) inhibited 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, green and oolong tea extract are capable to inhibit platelet aggregation (Table 3.) were due to black, green and oolong tea extract contains high polyphenols (Table 2) and had high antioxidant activity (Table 1.).

Base on this research (Table 1, 2 and 3) showed that black, green and oolong tea extract had high antioxidant, phenolic content and antiaggregation activity, becaused black, green and oolong tea contained high polyphenols and flavonoids.

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