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Full Length Research Paper

Effects of selected medicinal plants on human low-density lipoprotein oxidation, 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radicals and human platelet aggregation

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The effects of the methanol extracts of 20 selected medicinal plants on free radical scavenging capacity, human low-density lipoprotein (LDL) peroxidation and platelet aggregation were investigated. The antioxidant activity was evaluated using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging capacity and thiobarbituric acid reactive substances (TBARS) assay with LDL as the oxidation substrate. The antiplatelet activity in human whole blood was investigated using an electrical impedance method. The total phenolic contents (TPC) of the extracts were determined by the Folin-Ciocalteau method. Among the extracts, *Phyllanthus amarus* and *Labisia pumila* var*. alata* possessed potent radical scavenging activity with IC_{50} values of 3.4 and 5.7 µg/ml, respectively. The extracts of *Zingiber officinale, Curcuma xanthorrhiza* and *Curcuma domestica* showed strong inhibition of LDL peroxidation with IC_{50} values ranging from 0.1 to 0.9 µg/ml. The extract of *Z. officinale* was the most effective sample against platelet aggregation caused by arachidonic acid (AA) and adenosine diphosphate (ADP) with IC_{50} values of 10.9 and 7.7 µg/ml, respectively. The Pearson correlation analysis indicated that the TPC of the extracts showed significant positive correlations with DPPH scavenging activity (*r* = 0.846) and LDL antioxidant activity (*r* = 0.639) and moderate positive correlations with antiplatelet activities (AA-induced, r = 0.51; ADP-induced, r = 0.40; collagen-induced, r = 0.44). The antioxidant and antiplatelet activities of the plant extracts could partly be due to their TPC.

Key words: Medicinal plants, LDL antioxidant activity, antiplatelet activity, DPPH scavenging capacity, total phenolic contents.

INTRODUCTION

Oxidation of LDL by chain-propagating free radicals has been considered as playing an important role in the initiation and progression of early stage of atherosclerosis and the development of cardiovascular diseases. It is likely that oxidative modifications of LDL involve lipid peroxidation and the modification of apolipoprotein B-100, followed by macrophage uptake and cell accumulation of cholesterol to generate foam cells, causing early atherosclerotic lesions (Heinecke, 2006). Reduction of LDL oxidation may be one of the most important therapeutic approaches to prevent the development of atherosclerosis. Many antioxidants have been developed to delay or inhibit the oxidation of the biomolecules by terminating the initiation or propagation of the oxidizing chain reactions by free radicals and inhibiting the foam cell formation (Faure et al., 2004). Platelets have also been implicated in the pathogenesis of artherothrombotic conditions and play a key role in acute arterial thrombosis. Platelet aggregation is induced by the action of endogenous agonists such as AA, ADP, platelet activating factor (PAF), thrombin and collagen (Gibbins, 2004). Many epidemiological and biological studies showed that plant-derived polyphenolics are

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beneficial in the prevention of cardiovascular diseases (Hughes, 2005). The chronic antioxidant, hypolipidemic, antithrombotic and antiplatelet activities of these compounds has important roles in prevention of lipoprotein oxidation and atherosclerotic lesion development (Wollin and Jones, 2001). Phenolic compounds such as flavonoids and phenolic acids have been known to have a strong antioxidant activity and some studies have indicated that they are considerably more potent antioxidants than vitamin C and E (Pereira et al., 2009).

The protective effect of phenolics in biological system is principally due to their capacity to transfer electrons, redox properties and chelating abilities (Reiko et al., 2001; Hsu et al., 2007). Many studies have indicated that there was a high correlation between antioxidant activity and phenolic contents (Yu et al., 2005; Park et al., 2006). Phenolic compounds have significant anti-inflammatory effect, inhibition of platelet function, augmentation of endothelial nitric oxide release and other effects on proinflammatory factors (Jiang and Dusting, 2003). Although the antioxidant activity of many plants have been demonstrated, direct evidence of acute therapeutic benefits of plant extracts and their phenolic compounds in cardiovascular disorders remains sparse and data on LDL oxidation have been few (Bukhari et al., 2009; Okoko, 2009; Kolodziejczyk et al., 2009).

In search for sources of natural cardiovascular protective agents for pharmaceutical, food and nutraceutical applications, we investigated the antiplatelet and antioxidant effects of 20 methanol extracts of selected medicinal plants. These plants were selected for this study as they are widely used as spices to flavour dishes and in traditional medicine to treat various ailments including inflammatory conditions and as antioxidants (Table 1) (Burkill, 1966). The antiplatelet activity of the extracts was carried out *in vitro* in human whole blood and the antioxidant activity was determined against isolated human LDL oxidation and free radical scavenging activity against DPPH. The relationships between these activities and their total phenolic contents were also established.

MATERIALS AND METHODS

Chemicals and reagents

Methanol, dimethyl sulfoxide (DMSO) and sodium citrate used in this study were analytical grade and purchased from Merck Company (Merck, Darmstadt, Germany). Optiprep, Sudan black B, Follin-Ciocalteau's reagent, gallic acid, DPPH, phosphate buffer saline tablet (PBS), acetyl salicylic acid (ASA), ascorbic acid, probucol and protein kit were purchased from Sigma Chemical Company (St. Louis, MO, USA). Sub-cell electrophoresis and agarose gel were obtained from Bio-Rad, USA. TBARS kit was purchased from Zeptometrix Company (New York and USA) and Sigma Chemical Company (St. Louis, MO and USA). AA, ADP and collagen were purchased from Chrono-Log Company. (Havertown, USA).

Plant materials, extraction and sample preparation

Twenty plants were freshly collected from different locations in peninsular Malaysia in the month of March and July 2008. The voucher specimens were identified by Dr. Abdul Latiff Mohamad of Universiti Kebangsaan Malaysia (UKM) and deposited at the Herbarium of UKM, Bangi, Malaysia (Table 1). The plant materials were allowed to dry under shade. 100 g of dried material of each plant sample were ground and macerated in methanol at the ratio of 1:10 (w/v). The extract was filtered through Whatman filter paper No. 1 and the entire extraction process was repeated twice on the residue. The filtrates were combined and the methanol extracts were obtained by removing the solvent under reduced pressure to give various yields of crude extracts, calculated based on dry weight (Table 2).

Determination of total phenolic contents

The TPC of the methanol extracts of the plants were determined by the Folin-Ciocalteau (FC) method as described by Singleton et al. (1999). Gallic acid was used as a standard phenolic compound. Briefly, 20 µl aliquot of 20 mg/ml methanol extract solution was mixed with 1.58 ml of distilled water and 100 µl of the FC reagent. After 8 min, 300 µl of 20% sodium carbonate solution were added. The absorbance of the resulting colored solution was measured at 765 nm after incubation at 20°C for 2 h with intermittent shaking. Quantitative measurements were performed, based on a standard calibration curve of serial dilutions of gallic acid. The total phenolic content was reported as gallic acid equivalents per gram (mg GAE/g).

Determination of DPPH radical scavenging activity

DPPH scavenging activity of the extracts was carried out according to the method described by Kordali et al. (2005) with a slight modification. Briefly, 2 ml of each extract at various concentrations (100, 80, 60, 40 and 20 µg/µl) were, respectively added to 2 ml of freshly made DPPH methanol solution (4 mg/100 ml). The mixtures were vortexed vigorously and allowed to stand in the dark for 30 min at room temperature for the radical-antioxidant reaction to occur. Finally, the absorbance of these mixtures was measured by using a Shimadzu UV-1800 spectrophotometer (Shimadzu, Kyoto, Japan) at 517 nm. The difference in absorbance between the sample and the control (2 ml of DPPH solution in 2 ml of methanol) was calculated as percentage (%) inhibition of DPPH activity. The IC_{50} values, that is, concentration of sample providing 50% of radical scavenging activity was obtained through interpolation of linear regression analysis. Ascorbic acid was used as a positive control and the assay was conducted in triplicate for each sample concentration.

Human LDL isolation

The use of human whole blood in this study was approved by the Ethics Committee of the UKM (approval no. FF-120-2007). All subjects were healthy volunteers aged 24 to 70 years, normolipidemic, non-smoker had not taken any medications including vitamin supplements within the last 2 weeks and fasting within the last 8 h. Venous blood was drawn from the volunteers and 9 volumes of blood were added into 1 volume of 3.8% (w/v) sodium citrate solution as an anticoagulant. Plasma was obtained by centrifugation at 2000 g for 20 min. LDL was isolated by density gradient ultracentrifugation using a method developed by Graham et al. (1996) with slight modification using Optiprep^{TM} as the density gradient medium. Briefly, 3.2 ml of plasma obtained was mixed with

Table 1. Samples used in this study and their traditional uses.

Sample no	Scientific names	Family	Traditional uses	Part used	Voucher no
1	*Curcuma domestica* L.	Zingiberaceae	Spice, stomachache, carminative, diarrhea, itches and skin diseases	Rhizome	B-29789
2	*Curcuma xanthorrhiza* Roxb.	Zingiberaceae	Spice and pimples	Rhizome	B-29783
3	*Curcuma mangga* (Valton and Vazsjip).	Zingiberaceae	Spice, analgesic and anti-inflammatory	Rhizome	B-29796
4	*Curcuma aeruginosa* Roxb.	Zingiberaceae	Spice and uterine relaxant	Rhizome	B-29787
5	*Zingiber officinale* Rosc.	Zingiberaceae	Spice, anti-inflammatory, antioxidant, anti-asthmatic and antihypertension	Rhizome	B-29785
6	*Boesenbergia pandurata* (Roxb.) Schlecht	Zingiberaceae	Treatment for colic disorder	Rhizome	B-29786
7	*Kaempferia galanga* L.	Zingiberaceae	Spice, Urticaria, anti-allergy, asthma and rheumatism	Rhizome	B-29788
8	*Alpinia galanga* (L.) Willd	Zingiberaceae	Spice, analgesic, anti-emetic, hypertension, fever, anti-inflammatory and antiflatulent	Rhizome	B-29784
9	*Labisia pumila* var. *alata*	Myrcinaceae	Antioxidant and postnatal care	Whole plant	B-29773
10	*Orthosiphon aristatus* (Blume) Miq	Lamiaceae	Antioxidant, Renal inflammation, kidney stones and dysuria	Whole plant	B-29770
11	*Tinospora crispa* L.	Menispermaceae	Antihyperglycaemic and antimalaria	Stem	B-29793
12	*Phyllanthus amarus* Schum and Thonn	Euphorbiaceae	Liver diseases and tonic	Whole plant	B-29769
13	*Annona muricata* L.	Annonaceae	Chronic ulcer and skin eruption	Fruit peel	B-29783
14	*Piper nigrum* L.	Piperaceae	Spice, antioxidant, antibacterial and antipyretic	Seed	B-29788
15	*Cymbopogon citratus* (DC.) Stapf	Poaceae	Spice, antibacterial, antimalaria and stomach ache	Whole plant	F-001
16	*Polygonum minus* Huds	Polygonaceae	Spice, treatment for ailments and antioxidant	Leave	F-002
17	*Citrus hystrix* DC.	Rutaceae	Spice and insect repellent	Leave	B-29794
18	*Citrus aurantifolia* (Christm.) Swingle	Rutaceae	Spice and antifungal	Fruit	B-29790
19	*Andrographis paniculata* (Burn.f) Ness.	Acanthaceae	Antioxidant, hepatoprotective, imunostimulant and antihyperglycaemic	Whole plant	B-29795
20	*Averrhoa bilimbii* Linn.	Oxalidaceae	Spice, antidiabetic, cough, anti-inflammatory and rheumatism	Fruit	B-29782
21	Ascorbic Acid	-	-	-	-
22	Probucol	-	-	-	-

0.8 ml of Optiprep™ (60% iodixanol) to give a final iodixanol concentration of 12% (v/v); 4 ml of this was layered under 4 ml of 6% iodixanol in saline in an 8.9 ml Optiseal™ tube (Dillon et al., 2003). The tube was topped up with saline and ultracentrifuged at 402 000 g at 16°C for 3 h 10 min in a Ti. 70.1 rotor. The sub fractions of lipoprotein were labelled as Very low-density lipoprotein (VLDL), LDL, GAP and High density lipoprotein (HDL). The brightly coloured LDL band was located approximately one third of the way down the tube and was isolated using a pasteur pipette. LDL was characterized by measuring the amount of protein by the Bradford protein assay using bovine serum albumin as standard (Bradford, 1976). LDL was diluted with PBS (pH 7.4) to a final concentration of 200 µg protein/mL, prior to oxidation analysis. The purity of LDL was evaluated by using ultraviolet (UV) spectrophotometer as described by Galle and Wanner (1998) and agarose gel electrophoresis as described by Noble (1968). The electrophoretic mobility of LDL was measured using agarose gels. Samples were electrophoresed at a constant 45 mA/gel for 45 min, then oven dried at 85°C and stained with Sudan Black for 20 min.

Oxidation of LDL

LDL (200 µg protein/ml) was oxidised by exposing it to 10 µM$CuSO_4$ at 37°C for 5 h. (Dillon et al., 2003). This incubation was also carried out in the presence of serial dilutions (5, 2.5, 1.25, 0.63, 0.31 and 0.16 µg/µl) of each plant extract. The oxidation of LDL was terminated by rapid freezing. Samples intended for TBARS analysis were kept at -20°C for a maximum of 48 h. The plant extracts and probucol (as a positive control) were added to LDL directly before incubation.

TBARS assay

The inhibition of copper-catalysed LDL oxidation by the plant extracts was determined by using TBARS assay (Buege and Aust, 1978). The sample was dissolved in DMSO to obtain serial concentrations of 5, 2.5, 1.25, 0.63, 0.31 and 0.16 µg/µl. 5 µl of the sample was added

Table 2. IC_{50} values (µg/ml) of the methanol extracts of selected medicinal plants on DPPH radical scavenging activity, LDL peroxidation and their total phenolic content (mg GAE/g).

Sample no	IC_{50}		Total phenolic	Yield (%)
	DPPH radical	LDL peroxidation		
1	12.2 ± 0.2	0.9 ± 0.1	139.2 ± 0.6	35.2
2	34.4 ± 1.4	0.7 ± 0.1	73.5 ± 2.4	17.0
3	-	9.1 ± 0.1	14.6 ± 0.3	15.0
4	-	9.1 ± 0.1	30.7 ± 0.1	15.2
5	23.6 ± 1.5	0.1 ± 0.1	103.1 ± 0.1	10.5
6	-	7.6 ± 0.2	62.6 ± 4.5	10.1
7	-	5.5 ± 0.4	16.3 ± 0.1	12.5
8	48.2 ± 0.4	1.3 ± 0.2	38.1 ± 1.5	15.6
9	5.7 ± 0.8	1.0 ± 0.1	193.5 ± 0.1	15.3
10	13.4 ± 0.4	2.2 ± 0.1	80.0 ± 0.2	10.2
11	-	3.6 ± 0.2	39.1 ± 1.0	30.8
12	3.4 ± 0.1	1.9 ± 0.1	185.1 ± 0.1	10.4
13	40.3 ± 0.8	4.1 ± 0.2	38.4 ± 1.2	22.6
14	-	2.9 ± 0.2	41.6 ± 0.9	25.9
15	-	3.5 ± 0.4	27.0 ± 0.1	17.9
16	14.6 ± 3.3	1.2 ± 0.1	122.1 ± 1.6	22.3
17	-	12.0 ± 0.1	19.8 ± 0.9	24.8
18	-	-	20.2 ± 0.2	16.3
19	-	-	9.2 ± 0.5	19.7
20	-	-	9.0 ± 0.2	19.1
21	1.6 ± 0.1	-	-	-
22	-	0.3 ± 0.0	-	-

to a cuvette containing 945 µL of LDL, 50 µl of $CuSO_4$ and incubated at 37°C for 5 h. A mixture containing LDL and $CuSO_4$ was used as control and blank experiment consisted of LDL and 0.5% DMSO. The total volume of the mixture was 1 ml. The final concentrations of the sample in the mixture were 25.0, 12.5, 6.25, 1.13, 1.56 and 0.78 µg/ml. Probucol was used as the positive control in the assay. The final concentration of DMSO in the reaction mixtures was less than 0.5% to eliminate the effect of the solvent on the reaction as evidenced by control experiments. After the incubation, sodium dodecyl sulphate (SDS) and thiobarbituric acid (TBA) were added to the mixture followed by incubation at 95°C for 1 h to increase the peroxidation. SDS was used to lysis the membrane protein while TBA for the detection of lipid peroxides. The mixture was bathed with ice for 10 min to cool down and stop the peroxidation process. The precipitate formed was removed by centrifugation at 3000 rpm, 15 min. Malondialdehyde (MDA) in the supernatant was determined at 532 nm. TBARS are expressed in terms of MDA equivalents and the results are expressed as nmoles of MDA/mg LDL protein. MDA standard was used to construct a standard curve against which the samples can be plotted (Dillon et al., 2003). The percentage inhibition of LDL oxidation was calculated as follows:

$$\% \text{ Inhibition} = \left(1 - \frac{\text{Oxidation of sample}}{\text{Oxidation of control}}\right) \times 100$$

Antiplatelet assay

The use of human whole blood in this study was approved by the Ethics Committee of the UKM (approval no. FF-120-2007). Healthy volunteers were recruited based on the criteria that they were non-smokers and had not taken any medications within the last 2 weeks, including aspirin and had not taken any food within the last 8 h. Whole blood (20 ml) of a subject was collected in a vacutainer containing 3.8% sodium citrate (9:1 v/v). The blood and the anticoagulant were thoroughly mixed by inverting the vacutainers several times. The blood sample was diluted with normal saline in the ratio of 1:1. The plant sample was dissolved in DMSO to obtain concentrations of 20, 10, 5, 2.5 and 1.25 µg/µl. Five µl of the sample was added to a cuvette containing the diluted whole blood and the mixture was allowed to incubate at 37°C for 4 min prior to the addition of AA (0.5 mM), ADP (10 µM) or collagen (2 µg/ml). The total volume of the mixture was 1 ml. The final concentrations of the sample in the mixture were 100, 50, 25, 12.5 and 6.25 µg/ml.

The platelet aggregation was measured by whole blood Lumi-Aggregometer (Chrono-Log Corp., Havertown, PA) using an electrical impedance method (Ingerman-Wojenski and Silver, 1984). The mean platelet aggregation in whole blood was measured as a change in impedance over 6 min after the addition of the inducers by comparison to that of a control group impedance (Challen et al., 1982). A mixture containing 0.5% DMSO in the diluted whole blood was used as control. ASA was used as the positive control. The final concentration of DMSO in the whole blood was 0.5% to eliminate the effect of the solvent on the aggregation (Dong and Chen, 1998). The percentage inhibition of platelet aggregation was calculated as follows:

$$\% \text{ Inhibition} = \left(1 - \frac{\text{Aggregation of sample}}{\text{Aggregation of control}}\right) \times 100$$

Statistical analysis

All the data are presented as standard error of the mean ± (SEM) from triplicate experiments and were analysed using Statistically Package for Social Sciences (SPSS) software version 17.0. A one way analysis of variance (ANOVA) was used for multiple comparison.

The concentration of the compounds required to inhibit 50% oxidation (IC_{50}) for active extract was determined using probit programme. The correlation between TPC in the extracts and antioxidant properties was described by the Pearson product-movement correlation coefficient (*r*). *P<0.05* was considered to be statistically significant.

RESULTS

Extraction yields and total phenolic contents

Table 2 presents the yields and total phenolic contents of the methanol extracts of the selected 20 medicinal plants. The yields of the extracts varied from 10.2 to 35.2%. Among the tested extracts, the highest and the lowest yields were, respectively obtained from *C. domestica* and *Boesenbergia pandurata*. The phenolic contents in the plant extracts were determined through a linear gallic acid standard curve.

DPPH radical scavenging capacity

Twenty methanol extracts of selected medicinal plants which are commonly used in local dishes as spices and flavours were investigated for their radical scavenging capacity by using DPPH. The extracts which showed high inhibition of radical scavenging activity at 50 µg/ml, exhibiting greater than 52% inhibition were further investigated for their antioxidant effect at various concentrations (Figure 1).

The results demonstrated that the extracts inhibit the antioxidant activity in a dose-dependent manner, as the concentration of the extract increased the percentage inhibition of DPPH activity increased. The IC_{50} values of the extracts with radical scavenging activity are shown in Table 2.

Inhibition of LDL oxidation

The 20 methanol extracts were also investigated for their ability to inhibit copper-mediated oxidation on isolated human LDL. The human LDL was isolated by ultracentrifugation method and its purity was evaluated by using UV spectrophotometer and agarose gel electrophoresis. The LDL was incubated with copper ions which catalyzed a lipid peroxidation process, in the presence or absence (negative control) of each of the extract. The level of *in vitro* oxidative modification of LDL oxidation was measured quantitatively by the TBARS method based on MDA production. Probucol was used as the positive control. Most of the extracts showed significant antioxidant activity on LDL oxidation. The methanol extracts of *L. pumila, Curcuma domestica, C. xanthorrhiza, Z. officinale, Polygonum minus, Orthosiphon aristatus, P. amarus* and *Alpinia galanga* showed high inhibition of the LDL oxidation at 25.0 µg/ml with all extracts exhibiting greater than 90% inhibition (Figure 2). The extracts which showed more than 50% inhibition were further investigated for their antioxidant effect at various concentrations. The results demonstrated that the extracts inhibited the copper-mediated oxidation of LDL in a dose-dependent manner, as the concentration of the extract increased the percentage inhibition increased. The IC_{50} values of the extracts with inhibition of LDL oxidation are shown in Table 2.

Inhibition of platelet aggregation

In the antiplatelet aggregation study, the extracts of *C. domestica, C. xanthorrhiza, Z. officinale, B. pandurata, A. galanga, L. pumila* and *Polygonum minus* showed marked inhibitory effects on platelet aggregation caused by one, two or three inducers at 100 µg/ml in human whole blood *in vitro,* with all extracts exhibiting about 60 to 100% inhibition (Table 3).

Among the extracts tested, only the extract of *L. pumila* exhibited strong inhibitory activity against platelet aggregation induced by all the three inducers. The extracts of *C. mangga* and *P. minus* showed selective inhibitory activity on platelet aggregation induced by collagen and the extract of *B. pandurata* selectively retarded the ADP-induced platelet aggregation. The extracts inhibited platelet aggregation in a dose-dependent manner, as the concentration of the extract increased the percentage inhibition increased. The IC_{50} values of the active plant extracts with the mean values of three measurements are shown in Table 3.

DISCUSSION

The results showed that the methanol extracts of these plants contained high levels of phenolic contents. The TPC of the extracts varied considerably, ranging from 9.0 to 193.5 mg GAE/g (Table 2). Among all the plant extracts, the highest TPC was observed in the extract of *L. pumila* while the lowest was in the extract of *Averrhoa bilimbi.* The antioxidant activity of botanical materials has been measured by various methods such as DPPH radical scavenging activity assay, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS) radical cation scavenging activity assay, superoxide anion radical scavenging activity assay, oxygen radical absorbance capacity (ORAC) assay, ferric reducing/antioxidant power (FRAP) assay and metal

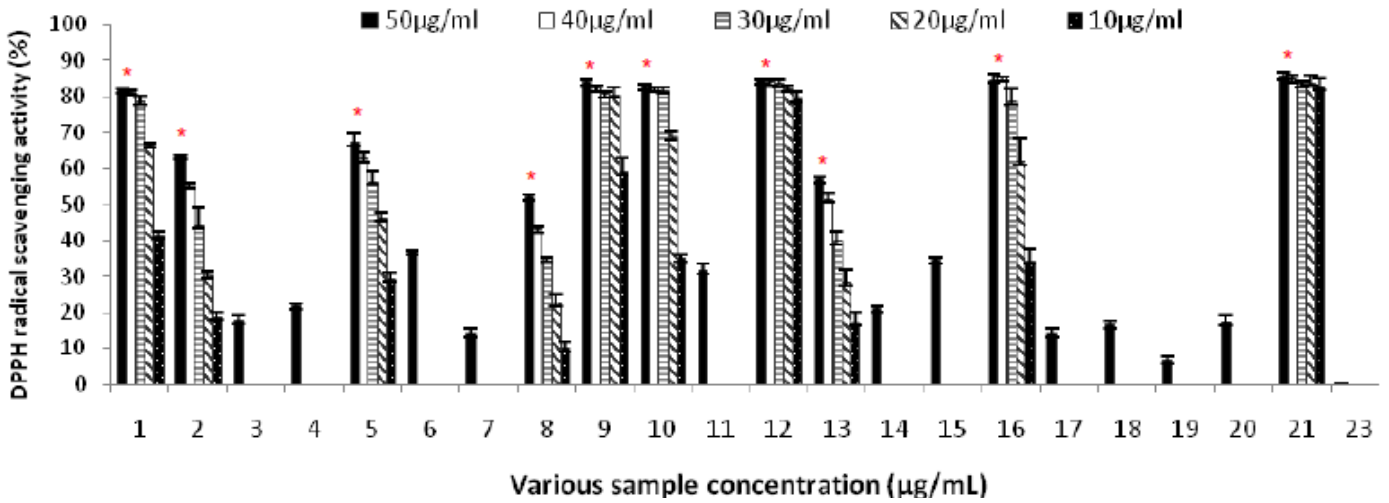

Figure 1. DPPH radical scavenging activity of the methanol extracts of selected medicinal plants. The bar graphs represent percentage inhibition to oxidation at various concentrations of plant extracts. Each bar represents a mean triplicate reading ± SEM. * Significant differences (*p<0.05*) compared with the respective control. Note: For all botanical names refer to the key in Table 1. Samples 21 and 23 represent Ascorbic Acid Acid and negative control, respectively.

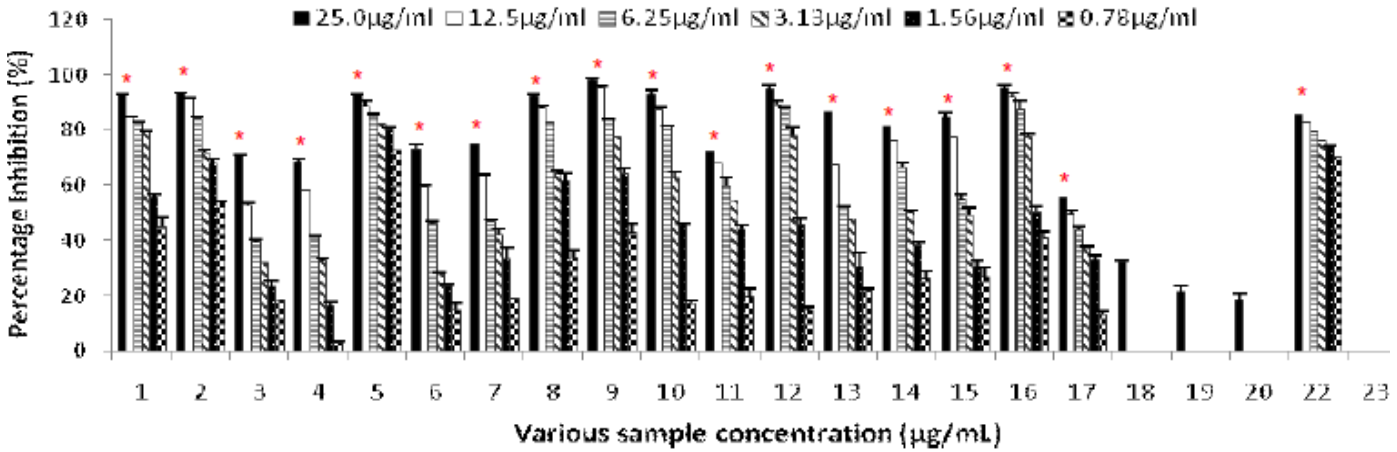

Figure 2. Effect of the methanol extracts of selected medicinal plants on human LDL oxidation. The bar graphs represent percentage inhibition in copper-mediated LDL oxidation to various concentrations of plant extracts after 5 h incubation at 37°C with native LDL. Each bar represents a mean triplicate reading ± SEM. * Significant differences (*p<0.05*) compared with the respective control. Note: For all botanical names refer to the key in Table 1. Samples 22 and 23 represent probucol and negative control, respectively.

Table 3. Percentage inhibition and IC_{50} values of the methanol extracts of plants on platelet aggregation of human whole blood induced by AA (0.5 µM), ADP (10 µM) and collagen (2 µg/ml).

Sample	Concentration (µg/ml)	AA	ADP	Collagen
Curcuma domestica	100	92.5 ± 2.9*	100.0 ± 0.0*	24.8 ± 3.4
	50	40.0 ± 3.9	78.3 ± 3.3	
	25	32.8 ± 6.9	45.8 ± 1.1	
	12.5	22.4 ± 5.0	32.1 ± 4.8	
	6.25	15.8 ± 6.0	14.6 ± 1.9	
		(39.7 ± 8.9)	(21.0 ± 0.7)	
Curcuma xanthorrhiza	100	100.0 ± 0.0*	100.0 ± 0.0*	33.6 ± 2.2
	50	48.0 ± 4.4	78.6 ± 3.7	
	25	30.5 ± 7.9	62.5 ± 2.7	
	12.5	23.1 ± 2.5	32.8 ± 1.4	
	6.25	13.4 ± 0.4	25.2 ± 1.6	
		(33.1 ± 3.8)	(16.8 ± 0.8)	
Curcuma mangga	100	31.1 ± 3.7	15.7 ± 4.9	57.1 ± 0.1
	50			45.7 ± 0.0
	25			45.0 ± 1.1
	12.5			34.3 ± 6.4
	6.25			28.2 ± 0.4
				(55.4 ± 1.9)
Curcuma aeruginosa	100	7.8 ± 0.1	45.6 ± 3.3	19.5 ± 0.4
Zingiber officinale	100	98.3 ± 0.1*	98.3 ± 0.1*	41.6 ± 2.1
	50	85.2 ± 0.3	85.7 ± 6.8	
	25	83.2 ± 0.6	70.5 ± 6.7	
	12.5	41.7 ± 1.1	67.9 ± 1.6	
	6.25	39.2 ±2.4	46.0 ± 1.4	
		(10.9 ± 0.6)	(7.7 ± 0.9)	
Boesenbergia pandurata	100	34.0 ± 2.3	100.0 ± 0.0*	31.6 ± 0.6
	50		66.8 ± 4.5	
	25		55.2 ± 3.0	
	12.5		48.8 ± 1.1	
	6.25		44.1 ± 1.6	
			(12.5 ± 1.2)	
Kaempferia galanga	100	5.7 ± 0.3	14.1 ± 1.1	46.6 ± 7.2
Alpinia galanga	100	65.1 ± 0.7*	98.9 ± 1.6*	35.0 ± 3.5
	50	36.9 ± 1.1	45.8 ± 1.1	
	25	24.5 ± 8.6	40.5 ± 1.3	
	12.5	17.1 ± 1.6	38.9 ± 1.3	
	6.25	10.9 ± 3.0	32.4 ± 1.9	
		(67.9 ± 6.7)	(22.9 ± 0.8)	
Labisia pumila	100	93.2 ± 1.3*	71.9 ± 2.5*	100.0 ± 0.0*
	50	41.3 ± 2.9	37.4 ± 1.3	67.1 ± 0.6
	25	33.6 ± 1.1	25.2 ± 2.9	50.3 ± 0.0
	12.5	26.3 ± 0.1	19.6 ± 3.2	45.4 ± 7.0
	6.25	15.0 ± 1.9	7.7 ± 2.7	39.0 ± 2.1
		(34.8 ± 1.8)	(57.6 ± 1.6)	(14.8 ± 1.6)
Orthosiphon aristatus	100	20.0 ± 1.9	14.9 ± 5.1	21.8 ± 1.8
Tinospora crispa	100	39.6 ± 1.9	16.7 ± 2.5	27.5 ± 3.3
Phyllanthus amarus	100	14.6 ± 3.9	41.5 ± 11.0	38.4 ± 1.4
Annona muricata	100	13.3 ± 3.4	23.9 ± 2.1	48.6 ± 0.6
Piper nigrum	100	30.7 ± 8.0	21.0 ± 2.2	37.6 ± 1.7
Cymbopogon citratus	100	13.5 ± 2.8	13.9 ± 0.9	38.3 ± 3.0

Table 3. Contd.

Polygonum minus	100	25.6 ± 0.8	24.0 ± 2.1	63.1 ± 1.1*
	50			55.0 ± 0.8
	25			38.6 ± 6.7
	12.5			31.7 ± 5.1
	6.25			24.9 ± 0.5
				(42.2 ± 9.6)
Citrus hystrix	100	16.4 ± 0.2	47.1 ± 1.2	28.2 ± 0.4
Citrus aurantifolia	100	34.1 ± 3.2	12.5 ± 2.6	34.9 ± 2.2
Andrographis paniculata	100	6.6 ± 1.1	28.3 ± 3.8	41.0 ± 0.8
Averrhoa bilimbii	100	23.2 ± 2.3	27.9 ± 7.6	32.3 ± 0.4
Aspirin	25	100.0 ± 0.0	46.4 ± 0.6	34.1 ± 2.2
	12.5	99.1 ± 0.4		
	6.25	48.3 ± 2.2		
	3.13	36.9 ± 6.1		
	1.56	24.5 ± 5.6		
		(3.97 ± 9.4)		

Acetyl salicylic acid was used as a positive control: Values are presented as mean ± SEM (n=3). IC_{50} values in µg/ml are in parentheses: *$p < 0.05$ as compared with the respective control.

chelating activity assay (Zhao et al., 2008). The need to use different methods of antioxidant capacity measurement is due to these various mechanisms of antioxidant action.

Determination of the antioxidant activity of plant extracts and compounds often gave different results as the methods used are based on different reaction mechanisms (Wong et al., 2006). Among the extracts tested, *P. amarus* was the most potent inhibitor of free radicals *in vitro* with IC_{50} value of 3.4 µg/ml, which was comparable to that of ascorbic acid (1.6 µg/ml), a potent antioxidant (Wang et al., 2003). Other extracts with significant radical scavenging activity were *L. pumila, C. domestica, O. aristatus, P. minus, Z. officinale, C. xanthorrhiza, Anona muricata* and *A. galanga,* arranged in order of increasing IC_{50} values (5.7 – 40.3 µg/ml) (Table 2). The results indicate that the extracts of these plants contained compounds that were relatively strong scavengers of free radicals.

The antioxidant effect is due to the ability of the compounds in the plants extracts to transfer electron or hydrogen atom to neutralize radicals of DPPH to form neutral DPPH molecules (Yu et al., 2005). Interestingly, most of the plants which showed strong antioxidant activity are being used in the local traditional medicine as antioxidants and to treat inflammatory conditions. Most of the extracts showed high inhibitory activity on LDL oxidation with IC_{50} values ranging from 0.1 to 12.0 µg/ml (Table 2). Among the extracts tested, *Z. officinale,* was the most potent inhibitor of human LDL peroxidation *in vitro,* followed by *C. xanthorrhiza, C. domestica* and *L. pumila,* with IC_{50} values of 0.1, 0.7, 0.9 and 1.0 µg/ml, respectively.

Their IC_{50} values were comparable to that of probucol (0.3 µg/ml or 0.6 µM), a potent inhibitor of copper-catalysed LDL peroxidation (Parthasarathy et al., 1986). The results indicate that the extracts of these plants contained compounds that were relatively strong inhibitors of LDL peroxidation. *Z. officinale* is a well known spice that has been reported to contain strong antioxidant substances identified as zingerone and dehydrozingerone (Stoilova et al., 2006; Kuo et al., 2005). The antioxidant effect is due to the ability of the compounds in the plants extracts to chelate Cu^{2+} ion and thus may inhibit the initiation of LDL oxidation and free radical formation at the lipoprotein.

The extracts may also form chelating complexes with transition metals to reduce their availability as catalysts for free radical generation (Yu et al., 2005). Among the extracts tested, the extract of *Z. officinale* showed the strongest inhibition on platelet aggregation induced by AA and ADP, with IC_{50} values of 10.9 and 7.7 µg/ml, respectively (Table 3). The IC_{50} value of the extract against platelet aggregation induced by AA was higher than that of ASA (4.6 µg/ml or 25.5 µM), a potent cyclooxygenase inhibitor (Lloyd and Bochner, 1996). The extract of *L. pumila* was the most effective extract against collagen-induced platelet aggregation with IC_{50} value of 14.8 µg/ml. Phenolic compounds have been reported to be the major contributor to the antioxidant activities of grain, vegetables and other botanical materials (Zhao et al., 2008). Statistical correlations have been studied between TPC of the extracts of 20 medicinal plants and their LDL antioxidant activity determined by the TBARS assay. To access the degree and the direction of the linear relationship between TPC and antioxidant activity,

a bivariate Pearson's product-movement correlation coefficient (r) was calculated. The Pearson correlation analysis indicates that the TPC of the extracts showed significant positive correlations with DPPH scavenging activity (r = 0.846, $p < 0.01$) and LDL antioxidant activity (r = 0.639, $p < 0.01$) and moderate positive correlations with antiplatelet activities (AA-induced, r = 0.51, $p < 0.05$; ADP-induced, r = 0.40, $p < 0.05$; collagen-induced, r = 0.44, $p < 0.05$). The high correlation between the antioxidant activity and their TPC indicated that phenolic compounds might be a major contributor of antioxidant and antiplatelet activities of these plants. These results were consistent with the findings by other workers who showed that there was positive correlation between antioxidant activity and TPC (Cai et al., 2004; Tawaha et al., 2007).

Conclusion

This study indicates that the order of antioxidant activity of the extracts using two types of antioxidant capacity measurement was broadly similar. Of the all the samples studied, the extract of *P. amarus* was the strongest scavenger of free radicals by using DPPH and the extract of *Z. officinale* was the most effective inhibitor of LDL peroxidation. Some of the plants particularly *P. amarus*, *L. pumila* and *C. domestica* were found to have strong DPPH radical scavenging activity and could also strongly inhibit LDL peroxidation, implying that antioxidants in these plants were capable of scavenging free radicals and reducing oxidants.

The extract of *Z. offinale* also showed the strongest inhibition on platelet aggregation induced by AA and ADP, while the extract of *L. pumila* was the most effective extract against collagen-induced platelet aggregation. The high correlation between the antioxidant and antiplatelet activities and their TPC indicated that phenolic compounds are the major contributor of antioxidant capacities and antiplatelet activity of these plants. The plants are valuable sources of natural antioxidants and antiplatelet compounds for further development into leads with maximum inhibitory activities on LDL peroxidation and platelet aggregation.

Abbreviations: LDL, Low-density lipoprotein; **TBARS,** thiobarbituric acid reactive substances; **TPC,** total phenolic contents; **AA,** arachidonic acid; **ADP,** adenosine diphosphate; **PAF,** platelet activating factor; **DMSO,** dimethyl sulfoxide; **PBS,** phosphate buffer saline; **ASA,** acetyl salicylic acid; **UKM,** universiti Kebangsaan Malaysia; **FC,** folin-ciocalteau; **GAE/g,** gallic acid equivalents per gram; **VLDL,** very low-density lipoprotein; **SDS,** sodium dodecyl sulphate; **TBA,** thiobarbituric acid; **MDA,** malondialdehyde; **HDL,** high density lipoprotein; **UV,** ultraviolet; **SEM,** standard error of the mean; **SPSS,** statistically package for social sciences; **ORAC,** oxygen radical absorbance capacity; **FRAP,** ferric reducing/antioxidant power; **ABTS,** 2,2'-azino-bis 3-ethylbenzothiazoline-6-sulphonic acid.

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REFERENCES

Bradford MM (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein uitlizing the principle of protein dye-binding. Anal. Biochem., 72: 248-254.

Buege JA, Aust SD (1978). Microsomal lipid peroxidation. Meth. Enzymol., 52: 302-310.

Bukhari SB, Bhanger MI, Iqbal S (2009). Antioxidant potential of commercially available cumin (Cuminum cyminuml inn) in Pakistan. Int. J. Food Sci. Nutr., 60(3): 240-247.

Burkill IH (1966). A Dictionary of Economic Products of the Malay Peninsula, Vol. 1 & II, Ministry of Agriculture and Co-operative, Kuala Lumpur, Malaysia.

Cai Y, Luo Q, Sun M, Corke H (2004). Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. Life Sci., 74: 2157-2184.

Challen A, Branch WJ, Cummings JH (1982). Quantitation of platelet mass during aggregation in the electronic (Wellcome) whole blood aggregometer. J. Pharmacol. Meth., 8: 115-122.

Dillon SA, Burmi RS, Lowe GM, Billington D, Rahman K (2003). Antioxidant properties of aged garlic extract: an *in vitro* study incorporating human low density lipoprotein. Life Sci., 72: 1583-1594.

Dong H, Chen SX (1998). A new antiplatelet diarylheptanoid from *Alpinia blepharocalyx*. J. Nat. Prod., 61: 142-144.

Faure P, Oziol L, Le Bihan ML, Chomard P (2004). Cell culture media are potent antioxidants that interfere during LDL oxidation experiments. Biochimie, 86: 373-378.

Galle J, Wanner C (1998). Oxidized LDL and Lp(a) preparation, modification and analysis. Meth. Mol. Biol., 108: 119-130.

Gibbins JM (2004): Platelet adhesion signaling and the regulation of thrombus formation. J. Cell Sci., 117: 3415-3425.

Graham JM, Higgins JH, Gillott T, Taylor T, Wilkinson J, Ford T (1996). A novel method for the rapid separation of plasma lipoproteins using self-generating gradients of iodixanol. Atherosclerosis, 124: 125-135.

Heinecke JW (2006). Lipoprotein oxidation in cardiovascular disease: chief culprit or innocent bystander. J. Exp. Med., 203: 813-816.

Hughes DA (2005). Plant polyphenols: Modifiers of immune function and risk of cardiovascular disease. Nutr., 21: 422-423.

Hsu CY, Chan YP, Chang J (2007). Antioxidant activity of extract from *Polygonum cuspidatum*. Biol. Res., 40: 13-21.

Ingerman-Wojenski CM, Silver MJ (1984). A quick method for screening platelet dysfunctions using the whole blood lumiaggregometer. *Thromb. Haemost*., 2: 154-156.

Jiang F, Dusting GJ (2003). Natural phenolic compounds as cardiovascular therapeutics: Potential role of their antiinflammatory effects. Curr. Vasc. Pharmacol., 1: 135-156.

Kolodziejczyk J, Masullo M, Olas B, Piacente S, Wachowicz B (2009). Effects ofgarcinol and guttiferone K isolated from *Garcinia cambogia* on oxidative/nitrative modifications in blood platelets and plasma. Platelets, 20: 487-492.

Kordali S, Cakir A, Mavi A, Kilic H, Yildirim A (2005). Screening of chemical composition and antifungal and antioxidant activities of the essential oils from three Turkish Artemisia species. J. Agric. Food Chem., 53: 1408-1416.

Kuo PC, Damu AG, Cherng CY, Jeng JF, Teng CM, Lee EJ (2005). Isolation of a natural antioxidant, dehydrozingerone from *Zingiber officinale* and synthesis of its analogues for recognition of effective

antioxidant and antityrosinase agents. Arch. Pharm. Res., 28: 518-528.
Lloyd J, Bochner F (1996). Aspirin: how low is the dose? Australian Prescr., 19: 79-81.
Noble RP (1968). Electrophoretic separation of plasma lipoproteins in agarose gel. J. Lipid Res., 9: 693-700.
Okoko T (2009). *In vitro* antioxidant and free radical scavenging activities of *Garcinia kola* seeds. Food Chem. Toxicol., 47: 2620-2623.
Park KH, Park YD, Han JM, Im KR, Lee BW, Jeong IY (2006). Anti-atherosclerotic and anti-inflammatory activities of catecholic xanthones and flavonoids isolated from *Cudrania tricuspidata*. Bioorg. Med. Chem. Lett., 16: 5580-5583.
Parthasarathy S, Young SG, Witztum JL, Pittman RC, Steinberg D (1986). Probucol inhibits oxidative modification of low density lipoprotein. J. Clin. Invest., 77: 641-644.
Pereira RP, Fachinetto R, Prestes AdS, Puntel RL, Santos da Silva GN, Heinzmann BM (2009). Antioxidant effects of different extracts from *Melissa officinalis*, *Matricaria recutita* and *Cymbopogon citrus*. Neurochem. Res., 34: 973-983.
Reiko H, Waka S, Akiyo M, Hiroshige I, Osamu I, Kazuo K (2001). Antioxidant ability of various flavonoids against DPPH radicals and LDL oxidation. J. Nutri. Sci. Vitaminol., 47: 357-362.
Singleton VL, Orthofer R, Raventos RML (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu Reagent. Meth. Enzymol., 299: 152-178.
Stoilova I, Stoyanova A, Denev P, Gargova S (2006). Antioxidant activity of a ginger extract (Zingiber officinale). Food Chem., 102: 764-770.
Tawaha K, Alali FQ, Gharaibeh M, Mohammad M, El-Elimat T (2007). Antioxidant activity and total phenolic content of selected Jordanian plant species. Food Chem., 104: 1372-1378.
Wang SY, Chang HN, Lin KT, Lo CP, Yang NS, Shyur LF (2003). Antioxidant properties and phytochemical characteristic of extracts from Lactuca indica. J. Agric. Food Chem., 51: 1506-1512.
Wollin SD, Jones PJH (2001). Alcohol, red wine and cardiovascular disease, J. Nutr., 131: 1401-1404.
Wong SP, Leong LP, Koh JHW (2006). Antioxidant activities of aqueous extracts of selected plants. Food Chem., 99: 775-783.
Yu L, Zhou K, Parry JW (2005). Inhibitory effects of wheat bran extracts on human LDL oxidation and free radicals. LWT Food Sci. Technol., 38: 463-470.
Zhao GR, Zhang HM, Ye TX, Xiang ZJ, Yuan YJ, Guo ZX, Zhao LB (2008). Characterization of the radical scavenging and antioxidant activities of danshensu and salvianolic acid B. Food Chem. Toxicol., 46: 73-81.