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Full Length Research Paper

Inhibitory effects of the extracts of *Garcinia* species on human low-density lipoprotein peroxidation and platelet aggregation in relation to their total phenolic contents

Ibrahim Jantan[1]*, Farra Aidah Jumuddin[1], Fadlina Chany Saputri[1] and Khalid Rahman[2]

[1]Drug and Herbal Research Center, Faculty of Pharmacy, Universiti Kebangsaan Malaysia, Jalan Raja Muda Abdul Aziz, Kuala Lumpur 50300, Malaysia.
[2]School of Biomolecular Sciences, Liverpool John Moores University, Bryom Street, Liverpool, L3 3AF, UK.

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Twenty-two methanol extracts from different parts of nine *Garcinia* species were investigated for their ability to inhibit platelet aggregation and low-density lipoprotein (LDL) peroxidation and their total phenolic contents (TPC). The antioxidant activity of the extracts was examined using thiobarbituric acid reactive substances (TBARS) assay with human LDL as the oxidation substrate and their antiplatelet activity in human whole blood was determined by using an electrical impedance method. The TPC of the extracts were measured by the Folin-Ciocalteau method. Among all samples studied, the leaf extract of *Garcinia eugenifolia* Wall showed the highest inhibitory activity on LDL peroxidation with IC_{50} value of 12.5 µg/ml. The twig extract of *Garcinia mangostana* Linn. was the most effective sample against platelet aggregation caused by arachidonic acid (AA) with IC_{50} value of 15.6 µg/ml. The TPC of the extracts varied from 4.4 to 62.8 mg of gallic acid equivalents per g (mg GAE/g). The Pearson correlation analysis revealed that TPC showed moderate positive correlations with antioxidant ($r = 0.30$, $p < 0.05$) and antiplatelet activities (AA-induced, $r = 0.62$, $p < 0.05$; ADP-induced, $r = 0.42$, $p < 0.05$; collagen-induced, $r = 0.54$, $p < 0.05$). Thus, it was concluded that the antioxidant and antiplatelet activities of the *Garcinia* extracts could partly be due to their total phenolic contents.

Key words: *Garcinia* species, low-density lipoprotein, LDL antioxidant activity, antiplatelet activity, total phenolic contents.

INTRODUCTION

Free radicals, generated in the human body as metabolic by-products or acquired from the environment, have been claimed to play a key role in affecting human health by causing oxidative damages associated with many degenerative diseases such as coronary heart diseases, atherosclerosis, aging, cancer and inflammatory conditions (Finkel and Holbrook, 2000). Free radicals react rapidly and destructively with various substrates in the body including all cellular components, proteins, polyunsaturated fatty acids, carbohydrates and nucleic acids leading to chronic disease development (Patthamakanokporn et al., 2008). Oxidation of low-density lipoproteins (LDL) has been considered as playing an important role in the initiation and progression of early stage of atherosclerosis and the development of cardiovascular diseases (Heinecke, 1998). Antioxidants are compounds that can delay or inhibit the oxidation of the biomolecules by inhibiting the oxidizing chain reactions by free radicals and they may reduce oxidative damage to the human body.

The antioxidant activity of phenolic compounds is mainly due to their redox properties, which play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen or decomposing peroxides (Rice-Evans et al., 1997). Many studies have

*Corresponding author. E-mail: ibj@pharmacy.ukm.my Tel: +603 92897315. Fax: +603 26983271.

indicated that there was a high correlation between the antioxidant activity of some plants and their phenolic contents (Velioglu et al., 1998; Odabasoglu et al., 2004; Scalzo et al., 2005). Some studies have indicated that phenolic substances such as flavonoids and phenolic acids are considerably more potent antioxidants than vitamins C and E. The bioactive components can effectively inhibit LDL oxidation and may prevent atherosclerosis by reducing and slowing down the progression to advance stage (Hodzic et al., 2009). Platelets play a basic role in hemostasis by sealing vessel damaged endothelium and initiating the repair process. 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 arachidonic acid (AA), adenosine diphosphate (ADP), platelet activating factor (PAF), thrombin and collagen (Gibbins, 2004).

In recent years, phenolic compounds (Nurtjahja-Tjendraputra et al., 2003), oxygenated xanthones (Chung et al., 2002), coumarins (Tsai et al., 1998), isothiocyanates (Morimitsu et al., 2000), a diterpene (Shen et al., 2000), quinines (Liao et al., 2000), prenylflavonoids (Lin et al., 1993) and alkaloids of diverse chemical structures (Jantan et al., 2006) which have been isolated from various plants, showed potent antiplatelet activity. Many epidemiological and biological studies showed that plant-derived polyphenolics are beneficial in the prevention of cardiovascular diseases (Hughes, 2005). The chronic antioxidant, hypolipidemic, antithrombotic and antiplatelet activities of these compounds have important roles in prevention of lipoprotein oxidation and atherosclerotic lesion development (Wollin and Jones, 2001). 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).

However, direct evidence of acute therapeutic benefits of phenolic compounds in cardiovascular disorders remains sparse. *Garcinia* is the biggest genus in the family Guttiferae with about 400 species widely distributed in tropical Asia, Australia, tropical and southern Africa and Polynesia. The fruits of many species are edible and some species are used in traditional medicine to treat various diseases. The most popular is the fruit hull of *Garcinia mangostana* Linn. which is used for the treatment of skin infections, wounds, diarrhea and antidiabetic (Perry, 1980). *Garcinia* species are known to be a rich source of oxygenated and prenylated phenol derivatives including xanthones, flavonoids, benzophenones, lactones and phenolic acids (Ji et al., 2007). Extracts and pure isolates of *Garcinia* species exhibited various biological activities such as antimicrobial (Naldoni et al., 2009), anticancer (Han et al., 2008), anti-inflammatory (Obolskiy et al., 2009), antiplatelet (Jantan et al., 2009) and antioxidant properties (Joseph et al., 2005; Okoko, 2009; Kolodziejczyk et al., 2009).

The present study was carried out to investigate the LDL antioxidant and antiplatelet activities of the methanol extracts of nine *Garcinia* species and to establish the relationships between these activities and their total phenolic contents.

EXPERIMENTAL

Chemicals and reagents

The chemicals used in this study were of analytical grade that include methanol, dimethyl sulfoxide (DMSO) and sodium citrate (Merck, Darmstadt, Germany). Optiprep^{TM}, Sudan black B, Follin-Ciocalteau reagent, gallic acid, acetyl salicylic acid (ASA), probucol, and protein kit were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Sub-cell electrophoresis and agarose gel were obtained from Bio-Rad, USA. TBARS kit was purchased from Zeptometrix Corporation, New York. Arachidonic acid (AA), ADP and collagen were purchased from Chrono-Log Corp. (USA).

Plant samples, extraction and sample preparation

Nine *Garcinia* species, namely *G. atroviridis* Griff. ex. T. Anderson (FF5), *G. cowa* Roxb. ex DC (FF4), *G. cantleyana* var. *cantleyana* Whitm. (SM785), *G. eugenifolia* Wall (SM762), *G. griffithii* T. Anders. (AZ60), *G. hombroniana* Pierre (FF3), *G. mangostana* Linn. (FF2), *G. nervosa* Miq. (AZ64), *G. prainiana* King (FF6) were collected from Cameron Highlands, Pahang, Malaysia in the month April and May of 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. The plants were separated to different parts and were allowed to dry under shade. Each dried plant material (100 g) was 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 thrice on the residue. The filtrates were combined and methanol was removed under reduced pressure. Serial dilutions of the plant extracts were prepared by dissolving the methanol extract in DMSO to obtain concentrations of 20, 10, 5, 2.5 µg/µl.

LDL Isolation

The use of human whole blood in this study was approved by the Ethics Committee of the Universiti Kebangsaan Malaysia (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 0.8 ml of Optiprep^{TM} (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^{TM} tube (Dillon et al., 2003). The tube was topped up with saline and ultracentrifuged at 402 000 g and

16°C for 3 h 10 min in a Ti.70.1 rotor. The subfractions of lipoprotein were labelled as very low-density lipoprotein (VLDL), LDL 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 using bovine serum albumin as standard (Bradford, 1976). LDL was diluted with phosphate buffer saline (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 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 (20, 10, 5, 2.5 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.

TBARS Assay

The inhibition of copper-catalysed LDL oxidation by the plant extracts was determined by using TBARS (thiobarbituric acid reactive substances) assay (Buege and Aust, 1978). The sample was dissolved in DMSO to obtain serial concentrations of 20, 10, 5 and 2.5 µg/µl. Fifty microlitres of the sample was added to a cuvette containing 900 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 1000, 500, 250, 125 and 62.5 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 lyse the membrane protein while TBA was used 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. 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:

$$\frac{(1-\text{oxidation of sample})}{\text{oxidation of control}} \times 100$$

Antiplatelet aggregation assay

Blood was collected from volunteers who were selected based on the criteria that they were healthy, non-smokers, had not taken any medications, including acetyl salicylic acid, within the last 2 weeks and had not taken any food within the last 8 h. The platelet count of the blood of volunteers must exceed more than 200 000 mg/dl. Whole blood (20 ml) was withdrawn from the right arm of a subject into a vacutainer containing 3.8% sodium citrate. 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 sample was dissolved in DMSO to obtain serial concentrations of 20, 10, 5 and 2.5 µg/µl. Five microlitres 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 and 12.5 µg/ml. The platelet aggregation was measured by a 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 identified by the variable in impedance over 6 min after the addition of the inducers, by comparison to that of a control group impedance. A mixture containing 0.5% DMSO in the diluted whole blood was used as control. Acetyl salicylic acid (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).

Total phenolic contents

The total phenolic contents (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, 0.1 ml aliquot of 50 mg/ml methanol solution of each plant extract was mixed with 7.9 ml of distilled water and 0.5 ml of the FC reagent. After 5 min, 1.5 ml of 20% sodium carbonate were added. The absorbance of the resulting colored solution was measured at 750 nm after incubation at 30°C for 2 h with intermittent shaking. Quantitative measurements were performed, based on a standard calibration curve of serial dilutions of gallic acid in 80% methanol. The total phenolic content was expressed as mg of gallic acid equivalents per g (mg GAE/g).

Statistical analysis

All data in the assays are shown as means ± SD from experiments ran in triplicate. A one-way analysis of variance (ANOVA) was used for multiple comparison, and if significant variation occurred between treatment groups, the mean values for inhibitors were compared with those for controls by Student's *t*-test; p values of 0.05 was considered to be statistically significant. The IC_{50} values, that is, the concentrations of the compounds required to inhibit oxidation or aggregation by 50%, were obtained from at least three determinations. The correlation between TPC in the Garcinia extracts and antioxidant and antiplatelet properties was described by the Pearson product-movement correlation coefficient (*r)* using SPSS V16.0. Differences were considered statistically significant if $p < 0.05$.

RESULTS AND DISCUSSION

Total phenolic contents

The results showed that generally, the methanol extracts of the different parts of selected *Garcinia* species

Table 1. Total phenolic contents (mg gallic acid equivalent/g) in the methanol extracts of *Garcinia* species.

Sample	Part used	Total phenolics* (mg GAE/g)
Garcinia atroviridis Griff. ex. T. Anderson	Leaf	29.1±4.3
	Twig	45.5±6.8
	Fruit	4.4±1.7
Garcinia cowa Roxb. ex DC	Leaf	19.2±2.5
	Twig	61.5±4.6
Garcinia eugenifolia Wall	Leaf	62.5±9.1
	Twig	60.9±2.3
Garcinia cantleyana var. *cantleyana* Whitm.	Leaf	52.4 ±3.3
	Twig	61.3±1.5
	Bark	62.3±9.6
Garcinia hombroniana Pierre	Leaf	50.3±4.7
	Twig	45.6±3.4
	Fruit	20.7±3.8
Garcinia prainiana King	Leaf	52.4±4.5
	Twig	39.0±1.0
	Fruit	33.6±6.0
Garcinia nervosa Miq.	Leaf	57.7±8.2
Garcinia griffithii T. Anders.	Leaf	51.9±1.3
	Twig	59.0±3.2
Garcinia mangostana Linn.	Leaf	62.8±6.2
	Twig	59.7±2.5
	Fruit	61.9±1.4

*Mean ± SD obtained from analysis of three independent samples, in triplicate.

contained high levels of total phenolic contents. The concentrations of phenolic contents in the extracts varied considerably, ranging from 4.4 to 62.8 mg of gallic acid equivalents per g (mg GAE/g) (Table 1). Among all the plant extracts, the highest total phenolic content was observed in the leaf of *G. mangostana* and the lowest was in the fruit of *G. atroviridis.*

All parts of *G. mangostana* studied (leaf, twig and fruit) contained high levels of phenolic contents ranging from 59.7 to 62.8 mg GAE/g. *Garcinia* species are known to be a rich source of oxygenated and prenylated phenol derivatives including xanthones, flavonoids, benzophenones, lactones and phenolic acids. The total content of phenolic acids in the fruit of *G. mangostana* has been reported and the major phenolic acids identified were protocatechuic acid, p-hydroxybenzoic acid, m-hydroxybenzoic acid and 3,4-dihydroxymandelic acid (Ji et al., 2007).

Inhibition of LDL peroxidation

The antioxidant activity of botanical materials has been measured by various methods such as DPPH radical scavenging activity assay, ABTS radical cation scavenging activity assay, superoxide anion radical scavenging activity assay, ferric reducing/antioxidant power (FRAP) assay and metal chelating activity assay (Zhao et al., 2008). The different methods used to determine the antioxidant activity often gave different results as the methods are based on different reaction mechanisms. In this study, a total of 22 methanol

Table 2. Percentage inhibition (µg/ml) and IC_{50} values (µg/ml) of the methanol extracts of *Garcinia* species on LDL peroxidation.

Sample	Part used	Concentration (µg/ml)	% Inhibition	IC_{50} value (µg/ml)
Garcinia atroviridis	Leaf	1000	46.0±0.7	
	Twig	1000	72.5±0.7*	38.0 ± 0.1
		500	71.5±2.1	
Garcinia atroviridis		250	64.5±1.4	
		125	63.0±7.1	
		62.5	51.0±5.7	
Garcinia atroviridis	Fruit	1000	53.0±0.5	
Garcinia cowa	Leaf	1000	23.0±0.2	
	Twig	1000	50.0±0.5	
Garcinia cantleyana	Leaf	1000	42.0±0.9	
	Twig	1000	74.5±3.5*	20.5 ± 0.1
		500	66.5±0.7	
		250	65.5±0.7	
		125	62.0±3.5	
		62.5	58.0±0.7	
	Bark	1000	83.5±5.0*	15.6 ± 3.3
		500	72.5±4.9	
		250	71.0±1.4	
		125	65.0±0.9	
		62.5	63.5±0.7	
Garcinia eugenifolia	Leaf	1000	75.0±0.8*	12.5 ± 3.3
		500	70.0±0.6	
		250	67.0±0.5	
		125	64.0±2.8	
		62.5	60.0±1.4	
	Twig	1000	79.0±1.4*	58.9 ± 3.4
		500	70.1±0.8	
		250	66.5±0.7	
		125	54.0±2.8	
		62.5	53.0±0.4	
Garcinia hombroniana	Leaf	1000	43.0±0.6	
	Twig	1000	71.0±7.1*	153.0 ± 9.2
		500	56.5±3.5	
		250	54.0±0.9	
		125	52.5±0.7	
		62.5	39.5±0.7	
	Fruit	1000	86.0±7.1*	50.0 ± 4.8
		500	85.0±5.7	
		250	70.5±7.8	
		125	64.0±4.2	
		62.5	53.0±1.4	
Garcinia prainiana	Leaf	1000	31.0±0.0	
	Twig	1000	27.0±0.5	

Table 2. Contd.

	Fruit	1000	69.5±0.7*	144 ± 4.8
		500	60.1±0.7	
		250	51.5±2.1	
		125	47.2±2.8	
		62.5	45.5±2.1	
Garcinia griffithii	Leaf	1000	36.0±0.9	
	Twig	1000	49.0±0.5	
Garcinia nervosa	Leaf	1000	45.0±0.8	
Garcinia mangostana	Leaf	1000	35.0±2.0	
	Twig	1000	51.0±0.4	
	Fruit	1000	34.0±0.5	
Probucol		20	85.4±2.9	0.3 ± 0.1
		10	79.7±1.5	(0.6 µM)
		2.5	74.2±1.2	
		0.3	48.9±2.3	

Probucol was used as a positive control. Concentration of probucol in reaction mixture: 20 µg/ml. Data represent mean ± SD of three independent experiments performed in triplicate. *$p < 0.05$ as compared with the respective control. The IC_{50} values in µM are presented in parenthesis.

extracts from different parts of nine *Garcinia* species were evaluated for their ability to retard LDL oxidation induced by copper ions. The LDL was incubated with copper ions in the presence or absence (negative control) of each of the extract and the level of LDL oxidation was measured quantitatively by the TBARS method. Probucol was used as the positive control.

Among the extracts tested, the extracts of the fruits of *G. hombroniana* and *G. prainiana,* the twigs of *G. eugenifolia, G. atroviridis, G. cantleyana* var*. cantleyana* and *G. hombroniana*, the bark of *G. cantleyana* var*. cantleyana* and the leaves of *G. eugenifolia* showed high inhibition of the LDL oxidation at 20 µg/µl, with all extracts exhibiting greater than 70% inhibition (Table 2). Table 2 shows the inhibitory effects of the extracts at various concentrations. The plant extracts retarded the LDL oxidation 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. The most active extract was from the leaves of *G. eugenifolia* with IC_{50} value of 12.5 µg/ml. The IC_{50} values of all the active extracts were higher than that of the positive control, 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 some of the *Garcinia* species contained compounds that are relatively strong inhibitors of LDL peroxidation.

Antiplatelet activity

In the antiplatelet aggregation study, the twig extracts of *G. cowa, G. cantleyana* var. *cantleyana* and *G. mangostana,* the bark extract of *G. cantleyana* var*. cantleyana* and the leaf extract of *G. mangostana* showed marked inhibitory effects on platelet aggregation caused by all three inducers at 100 µg/ml in human whole blood *in vitro,* with all extracts exhibiting about 75 to 100% inhibition (Table 3). Except for the fruit extracts of *G. hombriana* and *G. prainiana* and the leaf extracts of *G. cowa,* which showed weak activity on platelet aggregation at 100 µg/ml, all the other extracts showed strong inhibitory effects on platelet aggregation caused by one, two or all three inducers. Among the extracts tested, only the leaf extract of *G. griffithii* exhibited selective inhibitory activity on platelet aggregation induced by AA and the twig extract of *G atroviridis* selectively retarded collagen-induced platelet aggregation. The leaf extracts of *G. atroviridis, G. eugenifolia, G. prainiana* and *G. nervosa*, the twig extracts of *G. eugenifolia* and *G. prainiana* and the fruit extract of *G. atroviridis* showed selective inhibitory activity on platelet aggregation induced by ADP. Table 3 shows the inhibitory effects of the extracts at various concentrations. The extracts inhibited platelet aggregation in a dose-dependent manner, as the concentration of the extract increased the percentage inhibition increased.

Table 3. Percentage inhibition of methanol extracts of *Garcinia* species on platelet aggregation of human whole blood induced by arachidonic acid (AA) (0.5 mM), ADP (10 µM) and collagen (2 µg/ml).

Sample	Part used	(µg/ml)	AA	ADP	Collagen
Garcinia atroviridis	Leaf	100	23.0 ± 0.1	89.0 ± 0.2*	24 ± 0.1
		50		86.5 ± 3.5	
		25		77.5±0.7	
		12.5		41.5±2.1	
Garcinia atroviridis	Twig	100	39.0 ± 0.6	50.0 ± 0.1*	95.0 ± 0.3*
		50			92.5 ± 0.7
		25			27.6 ± 0.4
		12.5			24.0 ± 1.4
Garcinia atroviridis	Fruit	100	14.0 ± 0.2	72.0 ± 0.03*	24.0 ± 0.1
		50		66.5 ± 2.1	
		25		66.0 ± 1.4	
		12.5		25.0 ± 7.1	
Garcinia cowa	Leaf	100	46.0 ± 0.1	56.0 ± 0.3*	22.0 ± 0.1
Garcinia cowa	Twig	100	99.0 ± 0.4*	100.0 ± 0.3*	90.0 ± 0.2*
		50	98.0 ± 0.3	97.5 ± 0.7	74.0 ± 1.4
		25	49.5 ± 0.7	93.5 ± 0.7	64.5 ± 0.9
		12.5	31.0 ± 1.4	82.5 ± 3.5	29.0 ± 0.0
Garcinia cantleyana	Leaf	100	61.0 ± 0.6*	95.0 ± 0.7*	28.0 ± 0.1
		50	53.0 ± 2.8	94.0 ± 0.5	
		25	25.0 ± 7.1	90.0 ± 0.6	
		12.5	27.5 ± 3.5	41.5 ± 2.1	
Garcinia cantleyana	Twig	100	82.0 ± 0.3*	95.0 ± 0.3*	92.0 ± 0.9
		50	45.0 ± 1.4	89.5 ± 0.7	50.5 ± 0.7
		25	42.5 ± 0.7	82.5 ± 0.7	29.0 ± 0.1
		12.5	9.0 ± 1.4	56.5 ± 0.7	24.5 ± 0.7
Garcinia cantleyana	Bark	100	88.0 ± 0.2*	99.0 ± 0.0*	75.0 ± 0.3*
		50	40.5 ± 0.7	94.5 ± 0.7	58.5 ± 2.1
		25	25.0 ± 7.1	73.0 ± 0.0	18.0 ± 2.8
		12.5	21.5 ± 5.0	72.5 ± 2.1	11.0 ± 8.5
Garcinia eugenifolia	Leaf	100	38.0 ± 0.6	85.0 ± 0.6*	44.0 ± 0.1
		50		75.5 ± 0.7	
		25		54.0 ± 1.4	
		12.5		31.0 ± 1.4	
Garcinia eugenifolia	Twig	100	39.0 ± 0.6	85.0 ± 0.2*	44.0 ± 0.
		50		66.0 ± 0.7	
		25		44.0 ± 1.4	
		12.5		45.0 ± 0.8	
Garcinia hombroniana	Leaf	100	100.0 ±0.0*	96.0 ± 0.7*	49.0 ± 0.
		50	64.0 ± 1.4	76.0 ± 1.4	
		25	58.0 ± 0.0	59.5 ± 0.7	
		12.5	19.0 ± 1.4	42.0 ± 0.8	

Table 3. Contd.

Garcinia hombroniana	Twig	100	100.0 ±0.0*	96.0 ± 0.3*	49.0 ± 0.1*
		50	59.0 ± 2.8	95.5 ± 0.7	
		25	48.0 ± 0.0	81.0 ± 1.4	
		12.5	15.5 ± 6.4	75.5 ± 1.4	
Garcinia hombroniana	Fruit		50.0 ± 0.1*	50.0 ± 0.9*	41.0 ± 0.1
Garcinia prainiana	Leaf	100	31.0 ± 0.1	79.0 ± 0.4*	40.0 ± 0.1
		50		69.0 ± 1.4	
		25		56.5 ± 2.1	
		12.5		36.5 ± 3.5	
Garcinia prainiana	Twig	100	59.0 ± 0.0*	100.0 ± .0*	30.0 ± 0.1
		50		88.5 ± 0.7	
		25		66.5 ± 0.8	
		12.5		50.5 ± 1.4	
Garcinia prainiana	Fruit	100	36.0 ± 0.1	33.0 ± 0.1*	37.0 ± 0.1
Garcinia griffithii	Leaf	100	87.0 ± 0.4*	55.0 ± 0.1*	56.0 ± 0.3*
		50	42.5 ± 3.5		
		25	37.0 ± 4.3		
		12.5	9.9 ± 0.1		
Garcinia griffithii	Twig	100	91.0 ± 0.8*	94.0 ± 0.7*	58.0 ± 0.8*
		50	45.6 ± 2.0	89.5 ± 0.8	
		25	29.2 ± 1.1	54.5 ± 0.7	
		12.5	18.9 ± 1.6	33.5 ± 3.5	
Garcinia nervosa	Leaf	100	59.0 ± 0.9*	92.0 ± 0.7*	28.0 ± 0.1
		50		69.5 ± 0.8	
		25		35.0 ± 0.7	
		12.5		33.5 ± 3.0	
Garcinia mangostana	Leaf	100	92.0 ± 0.2*	98.0 ± 0.6*	93.0 ± 0.9*
		50	69.7 ± 0.5	89.5 ± 0.8	73.5 ± 2.1
		25	52.8 ± 0.3	77.5 ± 0.0	67.5 ± 0.9
		12.5	49.1 ± 1.3	38.0 ± 0.6	27.5 ± 3.6
Garcinia mangostana	Twig	100	99.0 ± 0.3*	98.0 ± 0.6*	98.0 ± 0.5*
		50	97.0 ± 0.1	89.5 ± 0.8	63.0 ± 2.8
		25	55.7 ± 0.4	82.5 ± 3.5	49.0 ± 1.4
		12.5	47.6 ± 3.4	62.5 ± 7.1	11.0 ± 1.4
Garcinia mangostana	Fruit	100	92.0 ± 0.5*	97.0 ± 0.2*	44.0 ± 0.1
		50	63.4 ± 2.3	83.5 ± 4.3	
		25	57.8 ± 3.1	62.5 ± 3.5	
		12.5	28.3 ± 2.4	47.5 ± 4.2	
Acetyl salicylic acid		25	98.5 ± 0.6*	58.0 ± 0.4*	38 ± 0.1
		12.5	92.0 ± 0.1		
		6.25	47.0 ± 0.1		
		3.13	38.5 ± 0.7		
	1.56	14.6 ± 0.6			

Acetyl salicylic acid was used as a positive control. Concentration of acetyl salicylic acid in reaction mixture: 25 µg/ml. Values are presented as mean ± SEM (n=3);* $p < 0.05$ as compared with the respective control.

Table 4. IC_{50} values (µg/ml) of the methanol extracts of *Garcinia* species on platelet aggregation induced by arachidonic acid (AA) (0.5 mM), ADP (10 µM) and collagen (2 µg/ml).

Sample	Part used	AA	ADP	Collagen
Garcinia atroviridis	Leaf	-	12.9 ± 1.0*	-
	Twig	-	-	26.2 ± 0.8
	Fruit	-	24.5 ± 1.5	-
Garcinia cowa	Leaf	-	-	-
	Twig	19.8 ± 0.2*	4.1 ± 0.1*	20.8 ± 0.2*
Garcinia cantleyana	Leaf	56.0 ± 6.5	12.1 ± 0.2*	-
	Twig	42.5 ± 3.9	8.8 ± 0.1*	35.9 ± 0.2
	Bark	43.3 ± 3.9	7.1 ± 0.1*	48.1 ± 0.2
Garcinia eugenifolia	Leaf	-	22.6 ± 0.4	-
	Twig	-	21.5 ± 0.1	-
Garcinia hombroniana	Leaf	25.6 ± 1.3	17.6 ± 0.1	-
	Twig	29.5 ± 2.7	4.2 ± 0.1*	-
	Fruit	-	-	-
Garcinia prainiana	Leaf	-	20.8 ± 0.2	-
	Twig	-	13.9 ± 0.2*	-
	Fruit	-	-	-
Garcinia griffithii	Leaf	42.6 ± 3.0	-	-
	Twig	39.5 ± 0.5	19.5 ± 1.3*	-
Garcinia nervosa	Leaf	-	26.7 ± 1.1	-
Garcinia mangostana	Leaf	16.8 ± 0.6*	15.1 ± 1.1*	20.6 ± 0.4*
	Twig	15.6 ± 0.9*	8.2 ± 0.4*	30.2 ± 1.0
	Fruit	24.1 ± 2.0	14.9 ± 0.0*	-
Acetyl salicylic acid		4.6 ± 0.2 (25.5 µM)		

Data represent mean ± SE of three independent experiments performed in triplicate. *$p < 0.05$ as compared with the control. The IC_{50} values in µM are presented in parenthesis.

The IC_{50} values of the active plant extracts with the mean values of three measurements are shown in Table 4. Among the extracts tested, the twig and leaf extracts of *G. mangostana* showed strong inhibition on platelet aggregation induced by AA, with IC_{50} values of 15.6 and 16.8 µg/ml, respectively. The antiplatelet mechanism is probably similar to that of ASA, that is due to the inhibition of thromboxane A_2 formation. However, the mechanism of action of the extracts requires further investigation, as the effect of other inducers has not been investigated. The IC_{50} values of the oils evaluated were higher than that of ASA (4.6 µg/ml or 25.5 µM), a potent cyclooxygenase inhibitor (Lloyd and Bochner, 1996). This is the first report on the antiplatelet activity of the extracts of *G. mongostana.* Previous studies have demonstrated that the extracts of *G. mangostana* have antioxidant, antitumoral, antiallergic, anti-inflammatory, antibacterial and antiviral activities (Pedraza-Chaverri et al., 2008). The twig extracts of *G. hombroniana, G. cowa, G. mangostana*, and *G. cantleyana* var*. cantleyana* and the bark extract of *G. cantleyana* showed marked inhibition on platelet aggregation induced by ADP, with IC_{50} values ranging from 4.1 to 8.8 µg/ml. Of all the samples studied, the leaf extract of *G. mangostana* and the twig extract of *G. cowa* showed relatively strong inhibition on collagen-induced aggregation with IC_{50}

values of 20.6 and 20.8 µg/ml, respectively.

The correlations between the antioxidant, antiplatelet activity and total phenolic contents of *Garcinia* species

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 *Garcinia* species 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 bivariate correlation between these two variables was positive and medium relationship strength, (r = 0.30). The correlation was significant with $p < 0.05$. The results indicated that a significant positive relationship exists between TPC and antioxidant activity.

Thus, the phenolic contents of *Garcinia* extracts contributed significantly to the inhibition of LDL peroxidation. A correlation was also observed between TPC of the Garcinia extracts and their antiplatelet activity. The results revealed that TPC showed moderate positive correlations with antiplatelet activity. TPC of *Garcinia* species significantly ($p < 0.05$) correlated (r = 0.62) with AA-induced, with ADP-induced (r =0.42; $p < 0.05$) and with collagen-induced (r = 0.53; $p < 0.05$). AA-induced (r = 0.62, $p < 0.05$), ADP-induced (r = 0.42, $p < 0.05$) and collagen-induced aggregations (r = 0.54, $p < 0.05$).

Conclusion

It was concluded that the antioxidant and antiplatelet activities of the *Garcinia* extracts could partly be due to their total phenolic contents. The leaf extract of G. *eugenifolia* and the twig extract of *G. mangostana* were potential sources of cardioprotective phenolic compounds. Further studies need to be carried out to isolate and identify the bioactive compounds and develop them into lead structures with maximum inhibitory activities on low-density lipoprotein peroxidation and platelet aggregation.

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