

***In vitro* anti-platelet aggregation effects of fourteen fruits and vegetables**

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Abstract: In the present study, the anti-platelet aggregation activity of 14 vegetables and fruits was tested *in vitro*. The aqueous, 90% ethanol and ethyl acetate extracts, as well as concentrated juices of 14 foods (fruits and vegetables) were prepared, and the anti-platelet aggregation activity of those extracts was analyzed on a platelet aggregation analyzer *in vitro* with adenosine 5'-diphosphate (ADP), bovine thrombin (THR) and arachidonic acid (AA) as aggregation inducers, respectively. Aspirin (ASP) was used as the positive control. A number of the tested foods had inhibitory effects in concentration-dependent manner on platelet aggregations induced by various agonists. Especially, some foods such as lemon, leek, garlic, scallion, ginger, tomato and grapefruit showed good anti-platelet aggregation effect similar or higher than that of positive control group i.e. aspirin (ASP). The results of present study provide scientific reference for reasonable selection of daily dietary with supplementary curative effects or prevention of cardiovascular diseases (CVD).

Keywords: Anti-platelet aggregation, adenosine 5'-diphosphate, bovine thrombin, arachidonic acid, food materials.

INTRODUCTION

Cardiovascular disease (CVD), which is a broad term used for all pathological disorders affecting the cardiovascular and circulatory system, gives rise to the most challenging healthy problem among millions of people globally in recent years (Leal *et al.*, 2006). CVD comprises a wide range of serious diseases, including hypertension, venous thromboembolism (VTE), myocardial infarction (MI), stroke, coronary heart disease, atherosclerosis and coagulopathy (Gaziano *et al.*, 2010). Each year, people of all races, ages and gender suffer from these fatal diseases and approximately 17 million people die of CVD, which represents thirty percent of all deaths (Zimmer *et al.*, 2015). It has been estimated by the World Health Organization (WHO) that CVD will not only be the top leading cause of morbidity and mortality worldwide by the year 2020, but also a major healthcare burden that is associated with high healthcare cost (Dai and Ge, 2012). The danger of CVD has led to the numerous pre-clinical and clinical trials investigating the pharmacological agents such as anti-hypertension (captopril, spironolactone), anti-coagulation (heparin, warfarin) and anti-platelet (aspirin, clopidogrel) for both prevention and treatment purpose (Cohen *et al.*, 2015). Although much progress has been made over the last 40 years on the discovery and development of more effective drugs for cardiovascular diseases, the mortality rates of patients with CVD is still very high (Collaboration, 2002). The drug resistance, limited efficacy in some patients, and side effects such as higher bleeding risk and

gastrointestinal dysfunctions are the main problems for the present CVD therapies (Barrett *et al.*, 2008).

On the other hand, food (including vegetables, fruits, beverages and spices) is not only regarded as a basic source of nutrition, but also promises to be a source of effective supplements or substitutes of chemical agents on the treatment of CVD (Vilahur and Badimon, 2013), as their less side effects and lower costs. Epidemiological and clinical studies indicate that fruits, vegetables, unrefined grains, fish, and low-fat dairy products contribute to reduce the risk of CVD and hypertension (Arnoldi *et al.*, 2015). Okuda *et al.* examined the associations of fruit and vegetable (FV) intake with mortality risk from CVD in a representative Japanese sample, a total of 9112 participants aged from 24-year follow-up. The results showed that higher total intake of FVs was significantly associated with reduced risk of CVD mortality in Japan (Okuda *et al.*, 2015). As the cereal grain commodity, a large body of clinical evidences suggested that the consumption of 3g or more per day of β -glucan from oats or barley, as part of a diet low in saturated fat and cholesterol, may reduce the risk of coronary heart disease (Clemens and van Klinken, 2014).

Platelet accumulation (activation, aggregation, secretion) at vascular injury sites is the primary event in circulatory system and its activation is a critical component of thrombosis, which is the main leading cause of CVD (Fuentes *et al.*, 2014). Anti-platelet aggregation tests were widely applied to evaluate and investigate cardiovascular disease both in experimental (Xia *et al.*, 2012; Schemmer *et al.*, 2013; Yi *et al.*, 2011) and clinical observations

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(Wurtz *et al.*, 2012a; Wurtz *et al.*, 2012b; Nagata *et al.*, 2013). Increasing evidence indicated that anti-platelet therapy is beneficial in treating thromboembolic diseases and preventing serious cardiovascular events. There are four main action pathways been reported for anti-platelet aggregation medicines including inhibiting the metabolism of platelet arachidonic acid (AA), increasing the level of platelet cyclic adenosine monophosphate (cAMP), inhibiting the activation of adenosine diphosphate (ADP), and blocking the platelet membrane glycoprotein IIb/IIIa. In addition, many studies have observed and described the anti-platelet aggregation property of dietary nutrients of vegetables and fruits (Duttaroy and Jorgensen, 2004; Liao *et al.*, 2012). Duttaroy reported that volunteers consuming two or three kiwi fruit per day for 28 days reduced platelet aggregation response to collagen and ADP by 18% compared with the controls (Duttaroy and Jorgensen, 2004). Liao *et al.* isolated, purified and identified twenty-nine compounds from ginger, among them, 6-shogaol exhibited potent anti-platelet aggregation bioactivity, which displayed significant inhibitory effects on the aggregation of washed rabbit platelets stimulated by the agonists, including AA, collagen, platelet activating factor (PAF), and thrombin (Liao *et al.*, 2012). However, to date, the systematic investigation and comparison on the anti-platelet aggregation activities of vegetables and fruits has not been reported.

The aim of this study was therefore to investigate the effect of various commonly used vegetables and fruits on rabbit platelet aggregation *in vitro*. The results may be beneficial for those patients with CVD to select their daily intake fruits and vegetables. In doing so, 14 kinds of food materials (fruits and vegetables) were chosen and extracted by 90% ethanol (further liquid-liquid extracted by ethyl acetate), water and parts of fruit juices to produce extracts with total constituents. Then the inhibitory effects of those extracts on rabbit platelet aggregation induced by ADP, thrombin (THR) and AA were systematically investigated and compared accordingly.

MATERIALS AND METHODS

Materials, chemicals and reagents

AA, THR and pentobarbital sodium were obtained from Sigma (St Louis, MO, USA). ADP was the product of Wuhu Huaren Technology Company (Wuhu, China). Aspirin (ASP) was purchased from Chengdu Kelong Chemical Reagent Factory (Chengdu, China) and sodium citrate was obtained from Chengdu Aikeda Chemical Reagent Company (Chengdu, China). All other chemicals and reagents were of analytical grade.

Fourteen food materials, including ten vegetables: leek (*Allium tuberosum* Rottler ex Sprengle), garlic (*Allium sativum* Linn.), scallion (*Allium fistulosum* Linn.), onion

(*Allium cepa* Linn.), ginger (*Zingiber officinale* Roscoe), agaric (*Auricularia auricula* (L.ex Hook.) Underwood), yam (*Dioscorea opposita* Thunb.), champignon (*Lentinus edodes* (Berk.) Sing), hawthorn (*Crataegus pinnatifida* Bge.), tamato (*Lycopersicon esculentum* Miller) and four fruits: lemon (*Citrus limon* (L.) Burm. F.), grape (*Vitis vinifera* Linn.), grapefruit (*Citrus paradise* Macf.), pipeapple (*Ananas comosus* (L.) Merr.) were randomly collected from local markets in Chongqing during autumn of 2013. The voucher specimens of those food materials were deposited at the School of Chemistry and Chemical Engineering, Chongqing University, Chongqing, China.

Preparation of sample extracts

All the ten vegetables as well as grapefruit peel, grape seed were dried in the oven at 50°C and stored at trockenschale before use. The dried materials of ten vegetables as well as grapefruit peel, grape seed were ground into fine powder in a pulverizer, respectively. 30 g of the powder was extracted with 120 mL 90% ethanol in an ultrasonic cleanser tank for 20 min, along with reflux extraction for 1 h at 80°C, filtered and another fresh 120 mL 90% ethanol was added into the residue, repeated the reflux extraction and filtered. The filtrate was combined and dried at 45°C using a rotary evaporator, and then the residue was re-suspended in 20 mL water and liquid-liquid extracted with 20 mL ethyl acetate for two times. The ethyl acetate extract and the rest solution were separately dried at 45°C using a rotary evaporator and further dried in an oven at 50°C. After 90% ethanol extraction, the material residue continued to be reflux extracted by 120 mL×2 distilled water for twice at 100°C, filtered and combined the filtrate. The filtrate solution was evaporated by a rotary evaporator at 50°C to produce aqueous extract. Besides, lemon, leek, garlic, grapefruit, scallion, onion, ginger, tomato, pineapple as well as grape were squeezed into juice and concentrated. Then 30 g of the residue of tomato, pineapple and grapefruit after squeezed were extracted according to above mentioned processes. Finally, the aqueous extract, the rest part of 90% ethanol extract after ethyl acetate extraction, the ethyl acetate extract of 90% ethanol extract and juice concentrates were labeled as A1, A2, A3 and A4, respectively. The extraction yields of each extract for 14 food materials are listed in table 1. A1 and A2 extracts were dissolved in phosphate buffer saline (pH 7.4) and A3 in diluted dimethyl sulfoxide (DMSO) (DMSO: ethanol absolute: water = 4: 3: 2) before use, respectively.

Preparation of blood plasma sample

Rabbits, males, weighing (2.2±0.4 kg) were purchased from Animal farm in Chongqing. All experimental procedures were approved by the Institutional Animal Ethical Committee of Chongqing University and were conducted according to the Guide for the Care and Use of Laboratory Animal of the National Institute of Health (Publication No. 80-23, revised 1996).

Table 1: The extraction yields (%) of extracts of 14 food materials

Fruit/vegetable materials	Family (F) and genus (G)	A1	A2	A3
		Extraction yield	Extraction yield	Extraction yield
Lemon	(F) Rutaceae, (G) Citrus	1.7	19.4	2.2
Leek	(F) Liliaceae, (G) Allium	11.3	13.1	3.2
Garlic	(F) Liliaceae, (G) Allium	42.1	7.8	0.3
Scallion	(F) Liliaceae, (G) Allium	17.1	27.1	1.0
Onion	(F) Liliaceae, (G) Allium	14.6	15.6	0.3
Ginger	(F) Zingiberaceae, (G) Zingiber	6.5	5.8	5.3
Agaric	(F) Auriculariaceae, (G) Auricularia	1.6	2.6	1.6
Yam	(F) Dioscoreaceae, (G) Dioscorea	4.3	3.2	0.5
Champignon	(F) Tricholomataceae, (G) Lentinus	8.2	12.7	1.4
Hawthorn	(F) Rosaceae, (G) Crataegus	4.7	28.2	2.0
Grape seed	(F) Vitaceae, (G) Vitis	2.8	6.0	4.9
Grapefruit peel	(F) Rutaceae, (G) Citrus	15.1	10.3	1.0
Grape	(F) Vitaceae, (G) Vitis	-	23.8	-
Grapefruit	(F) Rutaceae, (G) Citrus	-	15.2	-
Tomato	(F) Solanaceae, (G) Lycopersicon	-	15.0	-
Pineapple	(F) Bromeliaceae, (G) Ananas	-	50.6	-

A1, the aqueous extract; A2, the rest part of 90% ethanol extract after ethyl acetate extraction; A3, the ethyl acetate extract of 90% ethanol extract; A4, the juice concentrate.

Rabbit blood samples were collected in 3.8% sodium citrate with the ratio of 9:1 (blood: anticoagulant) from carotid artery after anesthetizing by 1% pentobarbital sodium. Platelet-rich plasma (PRP) was obtained by centrifugation at $93\times g$ for 15 min at room temperature, and platelet-poor plasma (PPP) was obtained by further centrifugation from the remaining blood at $2325\times g$ for 15 min. The concentration of PRP was adjusted to $3\times 10^{11}/L$ by PPP (Iwashita *et al.*, 2007).

***In vitro* platelet aggregation assay**

Platelet aggregation test was performed using the method described in previous report with some modifications (Kim *et al.*, 1998). In brief, the level of light transmission was calibrated as 0% by PPP (300 μL) using SC-2000 platelet aggregometer (Beijing Success Technology Development Co., Ltd.). PRP (300 μL) and different concentrations of extracts (10 μL) were incubated for 3 min at 37°C. After incubation, platelet aggregation was induced by adding 10 μL of ADP, THR and AA, respectively, and monitored for 5 min with stirring. PBS was used as blank control for A1, A2, A4 extracts and DMSO (final concentration: 1.7%) for A3 extract. Aspirin was used as the positive control.

The percentage of aggregation inhibition is calculated by the following formula:

$$I\% = \frac{A - B}{A} \times 100$$

where I % is the inhibitory percentage, A is maximal aggregation of the blank control test and B is maximal aggregation of drug-treated PRP. Data expressed as means $\pm$ SD.

RESULTS

Effects of A1 extracts of 12 food materials on platelet aggregation

The results (table 2) showed that A1 extracts of various food materials had rather different inhibitory effect on rabbit platelet aggregation *in vitro* with different platelet inducers (ADP, THR, AA). For THR as inducer, five food materials had the relatively strong effect (inhibition ratio higher than 40%) with a concentration-dependent manner. The highest inhibitory ratio of these five foods were in the order: lemon (99.1%) > scallion (95.8%) > leek (95.6%) > grapefruit peel (79.7%) > garlic (72.6%). For ADP as inducer, lemon also inhibited platelet aggregation by 64.1%, followed with champignon by 52.1% and scallion by 43.4%. Compared with THR and ADP, when using AA as inducer, only lemon A1 extract had a high inhibition ratio which is higher than 40%. Other food materials A1 extracts did not show significantly inhibitory effects on AA-induced platelet aggregation.

Effects of A2 extracts of 16 food materials on platelet aggregation

As shown in table 3, the rest part of 90% ethanol extract after liquid-liquid extracting by ethyl acetate (A2) had much stronger anti-platelet aggregation effects. In particular, when using THR as inducer, half of these tested food extracts showed high inhibitory effects on the aggregation with the order: lemon > garlic > grapefruit > tomato > yam > leek > champignon > grapefruit peel. Among them, the inhibition ratio of top five foods exceeded 90%, which had the similar effects as positive drug (ASP). For ADP as inducer, six foods inhibited aggregation with the order: yam > lemon > grapefruit >

Table 2. Inhibition effects on the platelet aggregation of aqueous extracts (A1) of 12 fruits/vegetables (& aspirin)

Fruits/vegetables	Final concentration (mg/ml)	Inhibition of platelet aggregation (means $\pm$ SD, n=3)			Foods	Final concentrations (mg/ml)	Inhibition of platelet aggregation (means $\pm$ SD, n=3)		
		ADP 10 μ mol/L	THR 0.25 u/mL	AA 1.205 mmol/L			ADP 10 μ mol/L	THR 0.25 u/mL	AA 1.205 mmol/L
Aspirin (control)	0.3125 0.7813 1.5625	7.5 $\pm$ 4.7 41.8 $\pm$ 13.3 78.4 $\pm$ 7.2	12.3 $\pm$ 1.3 62.8 $\pm$ 6.8 97.7 $\pm$ 2.3	42.3 $\pm$ 2.5 82.9 $\pm$ 3.9 96.9 $\pm$ 3.9	Ginger	0.7813 1.5625 3.1250	6.8 $\pm$ 0.7 10.8 $\pm$ 3.0 17.5 $\pm$ 2.1	7.9 $\pm$ 4.9 17.2 $\pm$ 11.1 16.7 $\pm$ 2.9	12.6 $\pm$ 13.5 9.9 $\pm$ 9.8 0.4 $\pm$ 3.9
Lemon	0.7813 1.5625 3.1250	34.8 $\pm$ 4.4 44.5 $\pm$ 5.3 64.1 $\pm$ 3.0	61.0 $\pm$ 15.0 90.8 $\pm$ 5.1 99.1 $\pm$ 1.6	7.8 $\pm$ 11.7 18.5 $\pm$ 5.4 54.8 $\pm$ 7.5	Agaric	0.7813 1.5625 3.1250	4.2 $\pm$ 3.0 * *	0.8 $\pm$ 3.0 * *	8.7 $\pm$ 3.4 * *
Leek	0.7813 1.5625 3.1250	9.4 $\pm$ 1.2 -1.4 $\pm$ 3.1 28.9 $\pm$ 4.1	90.7 $\pm$ 4.6 95.3 $\pm$ 4.1 95.6 $\pm$ 4.2	3.4 $\pm$ 5.9 0.8 $\pm$ 4.1 31.4 $\pm$ 9.7	Yam	0.7813 1.5625 3.1250	5.2 $\pm$ 1.8 9.4 $\pm$ 3.0 7.9 $\pm$ 2.4	7.4 $\pm$ 1.7 6.4 $\pm$ 2.3 6.2 $\pm$ 7.7	13.4 $\pm$ 4.2 9.9 $\pm$ 5.0 11.9 $\pm$ 4.4
Garlic	0.7813 1.5625 3.1250	-0.1 $\pm$ 9.1 0.4 $\pm$ 0.7 10.1 $\pm$ 1.6	15.4 $\pm$ 9.0 46.7 $\pm$ 6.4 72.6 $\pm$ 9.9	14.0 $\pm$ 2.2 9.9 $\pm$ 3.8 6.4 $\pm$ 1.3	Champignon	0.7813 1.5625 3.1250	30.4 $\pm$ 9.3 39.6 $\pm$ 0.9 52.1 $\pm$ 1.0	13.7 $\pm$ 8.9 14.9 $\pm$ 9.4 25.7 $\pm$ 9.9	7.6 $\pm$ 3.8 15.3 $\pm$ 0.4 17.8 $\pm$ 3.5
Grapefruit peel	0.7813 1.5625 3.1250	13.2 $\pm$ 3.1 14.8 $\pm$ 4.5 13.3 $\pm$ 7.4	31.5 $\pm$ 2.2 60.6 $\pm$ 2.5 79.7 $\pm$ 1.0	2.6 $\pm$ 2.0 7.6 $\pm$ 8.0 10.7 $\pm$ 11.8	Hawthorn	0.7813 1.5625 3.1250	17.7 $\pm$ 1.9 35.5 $\pm$ 5.6 *	6.9 $\pm$ 7.7 17.6 $\pm$ 2.5 *	6.9 $\pm$ 10.1 11.3 $\pm$ 3.4 *
Scallion	0.7813 1.5625 3.1250	24.1 $\pm$ 2.4 21.6 $\pm$ 6.4 43.4 $\pm$ 1.6	13.2 $\pm$ 5.7 36.1 $\pm$ 0.8 95.8 $\pm$ 2.5	9.5 $\pm$ 11.2 5.7 $\pm$ 2.7 6.3 $\pm$ 2.2	Grape seed	0.7813 1.5625 3.1250	13.9 $\pm$ 2.0 21.9 $\pm$ 4.9 18.5 $\pm$ 7.1	13.7 $\pm$ 4.9 12.1 $\pm$ 2.9 11.9 $\pm$ 1.9	11.9 $\pm$ 2.0 11.1 $\pm$ 5.5 17.0 $\pm$ 5.4
Onion	0.7813 1.5625 3.1250	13.3 $\pm$ 4.7 11.2 $\pm$ 9.8 9.2 $\pm$ 5.6	2.3 $\pm$ 9.4 8.1 $\pm$ 6.7 32.1 $\pm$ 1.9	5.2 $\pm$ 4.4 1.5 $\pm$ 1.7 3.2 $\pm$ 3.3					

“*” represents for the extract with poor solubility at corresponding concentration; A1, the aqueous extract; A2, the rest part of 90% ethanol extract after ethyl acetate extraction; A3, the ethyl acetate extract of 90% ethanol extract; A4, the juice concentrate.

Table 3. Inhibition effects on the platelet aggregation of 90% ethanol extracts (A2) of 16 fruits/vegetables (& aspirin)

Fruits/vegetables	Final concentration (mg/ml)	Inhibition of platelet aggregation (means $\pm$ SD, $n=3$)			Foods	Final concentrations (mg/ml)	Inhibition of platelet aggregation (means $\pm$ SD, $n=3$)		
		ADP 10 μ mol/L	THR 0.25 u/mL	AA 1.205 mmol/L			ADP 10 μ mol/L	THR 0.25 u/mL	AA 1.205 mmol/L
Aspirin (control)	0.3125 0.7813 1.5625	7.5 $\pm$ 4.7 41.8 $\pm$ 13.3 78.4 $\pm$ 7.2	12.3 $\pm$ 1.3 62.8 $\pm$ 6.8 97.7 $\pm$ 2.3	42.3 $\pm$ 2.5 82.9 $\pm$ 3.9 96.9 $\pm$ 3.9	Agaric	0.3125 0.7813 1.5625	-7.1 $\pm$ 5.2 5.5 $\pm$ 4.6 17.0 $\pm$ 7.9	-0.9 $\pm$ 6.6 -0.7 $\pm$ 6.7 6.3 $\pm$ 4.0	-2.5 $\pm$ 1.1 11.2 $\pm$ 6.2 -0.1 $\pm$ 2.8
Lemon	0.3125 0.7813 1.5625	11.2 $\pm$ 8.2 29.8 $\pm$ 2.1 70.2 $\pm$ 2.0	-4.9 $\pm$ 2.6 64.3 $\pm$ 6.0 94.5 $\pm$ 4.8	7.8 $\pm$ 7.6 15.7 $\pm$ 4.5 53.6 $\pm$ 6.4	Yam	0.3125 0.7813 1.5625	-4.3 $\pm$ 4.4 39.5 $\pm$ 3.5 79.8 $\pm$ 6.1	3.8 $\pm$ 6.3 86.1 $\pm$ 7.0 90.0 $\pm$ 5.8	3.4 $\pm$ 3.2 12.2 $\pm$ 8.7 11.7 $\pm$ 4.1
Leek	0.3125 0.7813 1.5625	7.8 $\pm$ 6.6 30.1 $\pm$ 5.4 49.8 $\pm$ 7.5	38.5 $\pm$ 2.9 92.8 $\pm$ 5.5 87.8 $\pm$ 6.6	9.0 $\pm$ 14.7 0.4 $\pm$ 2.3 22.7 $\pm$ 4.8	Tomato	0.3125 0.7813 1.5625	20.9 $\pm$ 9.5 32.1 $\pm$ 17.5 47.8 $\pm$ 16.4	88.9 $\pm$ 5.6 99.7 $\pm$ 0.5 91.7 $\pm$ 1.7	32.3 $\pm$ 1.6 24.8 $\pm$ 3.1 41.2 $\pm$ 8.2
Garlic	0.3125 0.7813 1.5625	24.3 $\pm$ 8.3 16.3 $\pm$ 10.6 24.1 $\pm$ 5.5	84.8 $\pm$ 6.5 85.1 $\pm$ 6.9 94.3 $\pm$ 6.7	2.3 $\pm$ 8.3 10.7 $\pm$ 2.5 33.5 $\pm$ 7.4	Champignon	0.3125 0.7813 1.5625	23.7 $\pm$ 1.6 13.9 $\pm$ 2.6 42.5 $\pm$ 20.6	89.5 $\pm$ 2.3 86.5 $\pm$ 8.6 87.6 $\pm$ 8.3	4.2 $\pm$ 3.5 24.9 $\pm$ 2.9 26.3 $\pm$ 2.4
Grapefruit	0.3125 0.7813 1.5625	4.5 $\pm$ 2.1 9.6 $\pm$ 1.4 57.2 $\pm$ 14.4	30.8 $\pm$ 1.8 57.4 $\pm$ 4.4 92.7 $\pm$ 1.4	10.1 $\pm$ 8.9 9.2 $\pm$ 8.3 14.2 $\pm$ 1.8	Hawthorn	0.3125 0.7813 1.5625	9.1 $\pm$ 5.0 -5.4 $\pm$ 7.2 5.1 $\pm$ 5.4	-6.1 $\pm$ 4.9 5.0 $\pm$ 9.7 21.2 $\pm$ 1.5	8.8 $\pm$ 6.5 18.9 $\pm$ 6.0 10.5 $\pm$ 5.7
Grapefruit peel	0.3125 0.7813 1.5625	-2.8 $\pm$ 1.9 7.5 $\pm$ 3.3 29.8 $\pm$ 4.0	25.7 $\pm$ 10.5 32.4 $\pm$ 6.6 77.5 $\pm$ 6.3	19.5 $\pm$ 9.8 15.9 $\pm$ 8.7 78.7 $\pm$ 10.7	Pineapple	0.3125 0.7813 1.5625	8.3 $\pm$ 1.5 9.6 $\pm$ 13.1 11.1 $\pm$ 19.5	1.0 $\pm$ 0.5 2.8 $\pm$ 3.3 -1.8 $\pm$ 3.9	17.0 $\pm$ 2.1 7.9 $\pm$ 3.4 2.8 $\pm$ 8.1
Scallion	0.3125 0.7813 1.5625	6.4 $\pm$ 5.1 22.5 $\pm$ 6.4 11.5 $\pm$ 1.8	6.1 $\pm$ 2.2 6.1 $\pm$ 6.0 14.0 $\pm$ 1.8	3.6 $\pm$ 7.3 2.3 $\pm$ 1.4 8.2 $\pm$ 2.5	Grape	0.3125 0.7813 1.5625	18.1 $\pm$ 13.7 12.5 $\pm$ 4.7 3.1 $\pm$ 7.5	5.3 $\pm$ 2.3 14.5 $\pm$ 7.8 5.8 $\pm$ 2.5	6.6 $\pm$ 5.0 1.9 $\pm$ 1.3 19.7 $\pm$ 3.1
Onion	0.3125 0.7813 1.5625	-19.3 $\pm$ 11.2 -6.7 $\pm$ 19.5 7.5 $\pm$ 9.9	1.1 $\pm$ 4.7 9.6 $\pm$ 2.3 9.5 $\pm$ 3.0	10.1 $\pm$ 2.7 7.9 $\pm$ 4.4 12.2 $\pm$ 2.3	Grape seed	0.3125 0.7813 1.5625	5.4 $\pm$ 6.0 1.4 $\pm$ 1.2 *	6.1 $\pm$ 12.0 16.9 $\pm$ 5.1 *	10.9 $\pm$ 2.3 6.8 $\pm$ 2.0 *
Ginger	0.3125 0.7813 1.5625	2.0 $\pm$ 2.9 11.6 $\pm$ 5.3 5.8 $\pm$ 5.3	6.6 $\pm$ 8.6 23.6 $\pm$ 6.3 28.1 $\pm$ 10.4	7.1 $\pm$ 8.1 10.3 $\pm$ 7.8 12.2 $\pm$ 4.1					

“*” represents for the extract with poor solubility at corresponding concentration; A1, the aqueous extract; A2, the rest part of 90% ethanol extract after ethyl acetate extraction; A3, the ethyl acetate extract of 90% ethanol extract; A4, the juice concentrate.

Table 4: Inhibition effects on the platelet aggregation of acetate ethyl extracts (A3) of 16 fruits/vegetables (& aspirin)

Fruits/ vegetables	Final concentration (mg/ml)	Inhibition of platelet aggregation (means $\pm$ SD, $n=3$)			Foods	Final concentration (mg/ml)	Inhibition of platelet aggregation (means $\pm$ SD, $n=3$)		
		ADP 10 μ mol/L	THR 0.25 u/mL	AA 1.205 mmol/L			ADP 10 μ mol/L	THR 0.25 u/mL	AA 1.205 mmol/L
Aspirin (control)	0.3125 0.7813 1.5625	7.5 $\pm$ 4.7 41.8 $\pm$ 13.3 78.4 $\pm$ 7.2	12.3 $\pm$ 1.3 62.8 $\pm$ 6.8 97.7 $\pm$ 2.3	42.3 $\pm$ 2.5 82.9 $\pm$ 3.9 96.9 $\pm$ 3.9	Ginger	0.0313 0.1563 0.3125	5.7 $\pm$ 18.2 2.7 $\pm$ 2.9 5.4 $\pm$ 2.8	4.4 $\pm$ 1.4 14.1 $\pm$ 1.8 23.7 $\pm$ 4.4	13.0 $\pm$ 1.5 13.9 $\pm$ 13.1 12.6 $\pm$ 8.8
Lemon	0.0313 0.1563 0.3125	3.2 $\pm$ 0.9 0.8 $\pm$ 2.7 -4.4 $\pm$ 5.2	4.2 $\pm$ 3.4 4.4 $\pm$ 3.5 49.7 $\pm$ 3.2	10.0 $\pm$ 14.3 1.9 $\pm$ 8.8 39.4 $\pm$ 4.4	Agaric	0.0313 0.1563 0.3125	-10.0 $\pm$ 5.3 -5.6 $\pm$ 4.6 2.2 $\pm$ 13.6	-0.4 $\pm$ 2.8 7.6 $\pm$ 7.5 19.5 $\pm$ 15.3	-6.8 $\pm$ 2.6 8.7 $\pm$ 3.5 13.1 $\pm$ 10.4
Leek	0.0313 0.1563 0.3125	4.4 $\pm$ 4.5 5.9 $\pm$ 15.7 26.9 $\pm$ 12.3	5.8 $\pm$ 7.7 24.4 $\pm$ 1.8 69.3 $\pm$ 13.9	10.6 $\pm$ 9.6 59.1 $\pm$ 8.7 81.9 $\pm$ 12.3	Yam	0.0313 0.1563 0.3125	-2.9 $\pm$ 9.5 19.5 $\pm$ 19.8 8.9 $\pm$ 1.7	8.6 $\pm$ 4.2 22.7 $\pm$ 21.4 25.9 $\pm$ 2.6	38.6 $\pm$ 7.1 69.2 $\pm$ 9.9 91.9 $\pm$ 3.2
Garlic	0.0313 0.1563 0.3125	11.8 $\pm$ 11.1 10.0 $\pm$ 1.5 17.2 $\pm$ 27.2	1.6 $\pm$ 3.0 27.8 $\pm$ 5.6 77.9 $\pm$ 6.0	43.1 $\pm$ 8.5 72.0 $\pm$ 8.3 84.7 $\pm$ 1.0	Champignon	0.0313 0.1563 0.3125	-0.7 $\pm$ 3.6 9.5 $\pm$ 4.0 25.3 $\pm$ 4.9	6.1 $\pm$ 1.1 27.1 $\pm$ 8.4 9.8 $\pm$ 7.7	18.7 $\pm$ 5.5 14.6 $\pm$ 7.7 16.4 $\pm$ 5.8
Grapefruit peel	0.0313 0.1563 0.3125	11.1 $\pm$ 20.6 -1.1 $\pm$ 5.0 3.8 $\pm$ 6.4	1.2 $\pm$ 1.0 34.3 $\pm$ 3.8 18.4 $\pm$ 11.1	14.4 $\pm$ 5.8 5.4 $\pm$ 7.4 24.2 $\pm$ 1.6	Hawthorn	0.0313 0.1563 0.3125	-5.5 $\pm$ 4.1 5.1 $\pm$ 7.5 0.2 $\pm$ 8.1	-3.5 $\pm$ 1.7 9.8 $\pm$ 8.8 24.3 $\pm$ 13.7	11.9 $\pm$ 7.4 9.3 $\pm$ 2.8 37.6 $\pm$ 1.5
Scallion	0.0313 0.1563 0.3125	-0.4 $\pm$ 2.3 0.9 $\pm$ 6.8 2.8 $\pm$ 17.6	1.4 $\pm$ 0.8 39.0 $\pm$ 7.2 48.4 $\pm$ 1.5	12.3 $\pm$ 1.6 17.9 $\pm$ 5.0 14.0 $\pm$ 11.7	Grape seed	0.0313 0.1563 0.3125	6.4 $\pm$ 7.6 13.0 $\pm$ 10.4 21.1 $\pm$ 18.4	7.9 $\pm$ 0.8 12.5 $\pm$ 8.4 21.1 $\pm$ 7.8	12.1 $\pm$ 5.5 18.8 $\pm$ 7.8 25.6 $\pm$ 3.3
Onion	0.0313 0.1563 0.3125	5.0 $\pm$ 20.5 -3.9 $\pm$ 4.7 0.7 $\pm$ 25.9	4.5 $\pm$ 12.3 15.8 $\pm$ 7.1 15.4 $\pm$ 9.6	-8.2 $\pm$ 7.1 6.7 $\pm$ 10.6 56.1 $\pm$ 5.1					

A1, the aqueous extract; A2, the rest part of 90% ethanol extract after ethyl acetate extraction; A3, the ethyl acetate extract of 90% ethanol extract; A4, the juice concentrate.

Table 5: Inhibition effects on the platelet aggregation of fruit juice (A4) of 9 fruits/vegetables (& aspirin)

Fruits/ vegetables	Final concentration (mg/ml)	Inhibition of platelet aggregation (means $\pm$ SD, $n=3$)			Foods	Final concentration (mg/ml)	Inhibition of platelet aggregation (means $\pm$ SD, $n=3$)		
		ADP 10 μ mol/L	THR 0.25 μ /mL	AA 1.205 mmol/L			ADP 10 μ mol/L	THR 0.25 μ /mL	AA 1.205 mmol/L
Aspirin (control)	0.3125 0.7813 1.5625	7.5 $\pm$ 4.7 41.8 $\pm$ 13.3 78.4 $\pm$ 7.2	12.3 $\pm$ 1.3 62.8 $\pm$ 6.8 97.7 $\pm$ 2.3	42.3 $\pm$ 2.5 82.9 $\pm$ 3.9 96.9 $\pm$ 3.9	Onion	0.7813 1.5625 3.1250	3.0 $\pm$ 9.1 13.1 $\pm$ 9.4 22.0 $\pm$ 19.7	19.2 $\pm$ 3.7 65.9 $\pm$ 1.5 85.2 $\pm$ 4.3	-1.6 $\pm$ 4.4 -1.4 $\pm$ 1.4 15.1 $\pm$ 1.4
Leek	0.7813 1.5625 3.1250	23.6 $\pm$ 7.6 29.9 $\pm$ 0.9 53.0 $\pm$ 17.6	89.2 $\pm$ 10.2 94.0 $\pm$ 5.3 97.4 $\pm$ 4.4	2.0 $\pm$ 7.1 24.7 $\pm$ 4.4 28.5 $\pm$ 4.4	Ginger	0.7813 1.5625 3.1250	15.8 $\pm$ 5.1 15.3 $\pm$ 10.5 10.6 $\pm$ 4.6	39.4 $\pm$ 1.7 66.3 $\pm$ 4.9 83.9 $\pm$ 6.4	16.1 $\pm$ 2.0 38.9 $\pm$ 1.9 94.7 $\pm$ 2.3
Garlic	0.7813 1.5625 3.1250	21.9 $\pm$ 4.1 30.3 $\pm$ 3.2 50.3 $\pm$ 17.8	29.5 $\pm$ 2.0 70.7 $\pm$ 5.1 92.0 $\pm$ 6.4	3.4 $\pm$ 0.6 23.4 $\pm$ 2.0 20.2 $\pm$ 0.4	Tomato	0.7813 1.5625 3.1250	33.9 $\pm$ 4.1 50.2 $\pm$ 9.0 64.6 $\pm$ 6.2	94.9 $\pm$ 4.5 92.8 $\pm$ 3.0 98.2 $\pm$ 1.5	76.2 $\pm$ 4.2 90.0 $\pm$ 13.5 95.5 $\pm$ 7.2
Grapefruit	0.7813 1.5625 3.1250	8.9 $\pm$ 17.3 30.2 $\pm$ 5.0 45.2 $\pm$ 3.3	17.1 $\pm$ 4.7 62.9 $\pm$ 4.4 76.1 $\pm$ 8.0	-5.3 $\pm$ 4.9 16.4 $\pm$ 0.5 72.9 $\pm$ 4.1	Pineapple	0.7813 1.5625 3.1250	17.2 $\pm$ 5.3 3.3 $\pm$ 1.2 19.7 $\pm$ 8.6	4.4 $\pm$ 8.1 9.3 $\pm$ 4.1 12.1 $\pm$ 7.2	5.9 $\pm$ 6.2 5.1 $\pm$ 14.3 14.8 $\pm$ 8.4
Scallion	0.7813 1.5625 3.1250	26.3 $\pm$ 4.4 25.9 $\pm$ 14.1 30.2 $\pm$ 8.9	90.0 $\pm$ 12.3 89.2 $\pm$ 11.3 91.4 $\pm$ 7.7	21.5 $\pm$ 3.8 20.5 $\pm$ 2.2 45.8 $\pm$ 9.3	Grape	0.7813 1.5625 3.1250	21.7 $\pm$ 7.1 1.3 $\pm$ 4.0 17.6 $\pm$ 7.0	-9.7 $\pm$ 8.7 8.9 $\pm$ 3.7 -3.6 $\pm$ 7.6	-4.7 $\pm$ 3.5 8.1 $\pm$ 0.8 10.2 $\pm$ 2.4

A1, the aqueous extract; A2, the rest part of 90% ethanol extract after ethyl acetate extraction; A3, the ethyl acetate extract of 90% ethanol extract; A4, the juice concentrate.

leek > tomato > champignon. When using AA as inducer, only three extracts including grapefruit peel, lemon, tomato exhibited inhibition ratio over 40%. Grapefruit had the highest inhibitory effect, at 78.7%, compared with 53.6% for lemon and 41.2% for tomato. In a word, strong anti-platelet aggregation effects were observed for all three inducers in lemon and tomato A2 extracts. There were four foods A2 extracts (leek, grapefruit, yam and champignon) inhibiting the aggregation induced by ADP as well as THR strongly, while grapefruit peel A2 extract had effect on THR and AA as inducer.

Effects of A3 extracts of 12 food materials on platelet aggregation

As shown in table 4, the ethyl acetate extracts from 90% ethanol extracts (A3) showed relatively weaker inhibitory effect on platelet aggregation. For THR as inducer, garlic, leek, lemon and scallion ranked top four of tested foods on inhibitory effects, whose inhibition ratio was 77.9%, 69.3%, 49.7% and 48.4%, respectively. For AA as inducer, different from A1 and A2 extracts, several foods A3 extracts showed strong inhibitory effects, in which yam, garlic, leek exceeded 80% and followed by the figures for onion and lemon, at 56.1% and 39.6%, respectively. Among them, lemon, leek and garlic A3 extracts showed high inhibition ratio on aggregation induced by THR and AA. In contrast, none of these foods A3 extracts inhibited platelet aggregation induced by ADP.

Effects of A4 extracts of 9 food materials on platelet aggregation

As shown in table 5, many vegetables and fruits had much stronger inhibitory effects on platelet aggregation after squeezing into juice, especially that grapefruit and tomato strongly inhibited platelet aggregation when using all the three inducers. For THR as inducer, in particular, seven foods inhibited the aggregation significantly with the order: tomato > leek > garlic > scallion > onion > ginger > grapefruit. When using ADP as inducer, four foods inhibited aggregation over 40%. Among them, tomato had the highest inhibition ratio, at 64.6%, while the figure for leek and garlic were lower, at 53.0% and 50.3%, respectively and that for grapefruit was lowest, at 45.2%. Also, when induced by AA, four foods had strong anti-platelet aggregation effects with the order: tomato > ginger > grapefruit > scallion, in which ginger and tomato were remarkable, at 95.5% and 94.7%, respectively.

DISCUSSION

The present study was carried out to evaluate a wide range of daily food materials of their anti-platelet aggregation effects. ADP, THR and AA are used as platelet inducers because they are involved in three main platelet aggregation pathways. Of the extracts investigated, leek, garlic, scallion, ginger, lemon, tomato and grapefruit were proved to be the most potent inhibitors of platelet aggregation *in vitro* since they have

strong inhibitory activities on the aggregation induced by all three inducers (ADP, THR, AA). Among these foods, leek, garlic, scallion as well as onion are in the category of Liliaceae family, Allium genus. In our daily dietary, these foods are usually used as condiments and welcomed by many people as they possessed some medicinal purpose, such as antimicrobial (Casella *et al.*, 2013; Mnayer *et al.*, 2014) and anticancer (Iciek *et al.*, 2009; He *et al.*, 2014). What's more, there were previous reports indicating the efficacy of these foods in prevention and treatment of cardiovascular disease (Furusawa *et al.*, 2003; Thomson *et al.*, 2002; Koo *et al.*, 2001). The anti-platelet aggregation activity of extracts from Allium plants mainly contributes to the organosulfur compounds (Briggs *et al.*, 2000; Cavagnaro and Galmarini, 2012). Garlic and its chemical constituents have been tested for its possible beneficial effects on cardiovascular disease such as hyperlipidemia, hypertension, platelet aggregation, and blood fibrinolysis activity (Santhosha *et al.*, 2013). Lawson *et al.* demonstrated that some thiosulfonates, dialk(en)yl sulfides, disulfides, trisulfides, vinyl dithiols and ajoenes, which were found in garlic preparations have anti-platelet aggregation activity *in vitro* (Lawson *et al.*, 1992). Ginger is another food which exhibited strong anti-platelet aggregation activity in our study. Van *et al.* screened for approximately 20 phenolic compounds in ginger to inhibit arachidonic acid (AA) induced platelet aggregation in human whole blood. 8-paradol and its analogue 1,7-bis(4-hydroxy-3-methoxyphenyl) heptan-3-one were found to be the most active substances among the series tests and the inhibitory mechanism of their anti-platelet aggregation effect may be related to attenuation of COX-1/Tx synthase enzymatic activity (Nurtjahja-Tjendraputra *et al.*, 2003). Another two fruits (lemon and grapefruit), which belong to the same genus Citrus, have been identified their high anti-platelet aggregation activity in the present study. Citrus fruit species is one of the most popularly consumed fruits in the world today (Obot *et al.*, 2014) and a rich source of nutrient, including flavonoids, citric acid, vitamin C and minerals, which provide numerous health promoting properties (Del Rio *et al.*, 2003). But there were few previous works associated with the anti-platelet aggregation effect of lemon, so our result could supply consumers with experimental basis for using it as thrombotic prevention fruit. Grapefruit is native to Latin America which is similar to the original Citrus macima (Burm.) Merr. in China. Because of its low sugar degree, this beneficial fruit makes a big contribute to lose weight, lower cholesterol as well as inhibiting key enzymes linked with type 2 diabetes. Apart from the effects for weight loss, promoting cardiovascular health effects of grapefruit likely attribute to the conversion from flavonones, naringin as well as hesperidin to the aglycones naringenin and hesperitin in the gut (Erlund *et al.*, 2002; Dow *et al.*, 2012). In addition, Chang *et al.* found that yam have anti-oxidative effect on hyperhomocysteinemia rats and the mechanism mainly

included significantly inhibiting the platelet aggregation induced by thrombin as well as reducing lipid peroxidation, and oxidative stress (Chang *et al.*, 2004). In another study carried out by Wu *et al.*, they found that agaric (*A. auricula*) polysaccharides including glucose (72%), mannose (8%), xylose (10%) and fucose (10%) could improve heart function through its strong antioxidant activity on aged rats (Wu *et al.*, 2010). It has been identified that human treatment with grape seed extract (GSE) capsule which is a nutraceutical containing vasodilator phenolic compounds lowered blood pressure in subjects with pre-hypertension. And this finding suggested that GSE could be used as a nutraceutical in a lifestyle modification program for patients with pre-hypertension (Robinson *et al.*, 2012).

Most of the vegetables are treated by different methods, such as boiling in water, frying in oil or even squeezing into juice before consumption. According to some findings, cooking practice may cause a number of changes in physical and chemical property of vegetables (Turkmen *et al.*, 2005). In our study, when comparing the inhibitory effects of A1, A2, A3 and A4 extracts, the inhibitory degree of these four extracts varied a lot from one to another. Juice concentrates had much stronger anti-platelet aggregation effect than other three extracts in general. Some vegetable juices including leek, garlic, scallion, onion, ginger as well as tomato, plus one fruit juice – grapefruit all inhibited rabbit platelet aggregation strongly *in vitro*. Especially for grapefruit, tomato and scallion, they significantly inhibited both ADP-, THR- and AA-induced aggregation. Many studies were operated on the juice extracts of food since they may exert much more potential pharmaceutical activities. Inmaculada Navarro-Gonzalez *et al.* found that the bioactive compounds of tomato juice including lycopene, chlorogenic acid, rutin and naringenin have a significant reduction in 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR) activity for interfering the cholesterol biosynthetic pathway to treatment of CVD (Navarro-Gonzalez *et al.*, 2014). In addition, the study found that grapefruit juice inhibited angiotensin-1-converting enzyme (ACE) activity in a dose-dependent manner and administration of the juice to rats which fed a high-cholesterol diet caused a significant reduction in plasma total cholesterol, triglyceride, and low-density lipoprotein cholesterol levels as well as an increase in high-density lipoprotein cholesterol levels (Obloh *et al.*, 2014). Therefore, it is an effective and important way to intake more vegetable and fruit juices in daily dietary as they may contain numerous nutrition substances which could have prevention and treatment effects on diseases.

CONCLUSION

This study clearly identified that leek, garlic, scallion, ginger, lemon, tomato and grapefruit possess the

appreciable anti-platelet aggregation activity. Low polar substances in these foods may account for their anti-platelet aggregation effect and squeezed juice is a better way to intake more active substances. This study also provides a theoretical foundation for clinical prevention of platelet aggregation by food materials and for the treatment and prevention of cardiovascular disease. Besides, further investigation on the effects of individual food on platelet function and elucidation of each bioactive constituent is required.

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REFERENCES

- Arnoldi A, Zanoni C, Lammi C and Boschini G (2015). The role of Grain Legumes in the prevention of hypercholesterolemia and hypertension. *Crit. Rev. Plant Sci.*, **34**: 144-168.
- Barrett NE, Holbrook L, Jones S, Kaiser WJ, Moraes LA and Rana R (2008). Future innovations in anti-platelet therapies. *Br. J. Pharmacol.*, **154**: 918-939.
- Briggs WH, Xiao H, Parkin KL, Shen C and Goldman IL (2000). Differential inhibition of human platelet aggregation by selected *Allium* thiosulfonates. *J. Agr. Food Chem.*, **48**: 5731-5735.
- Casella S, Leonardi M, Melai B, Fratini F and Pistelli L (2013). The role of diallyl sulfides and dipropyl sulfides in the *in vitro* antimicrobial activity of the essential oil of garlic, *Allium sativum* L., and leek, *Allium porrum* L. *Phytother. Res.*, **27**: 380-383.
- Cavagnaro PF and Galmarini CR (2012). Effect of processing and cooking conditions on onion (*Allium cepa* L.) induced antiplatelet activity and thiosulfonate content. *J. Agr. Food Chem.*, **60**: 8731-8737.
- Chang SJ, Lee YC, Liu SY and Chang TW (2004). Chinese yam (*Dioscorea alata* cv. Tainung No. 2) feeding exhibited antioxidative effects in hyperhomocysteinemia rats. *J. Agr. Food Chem.*, **52**: 1720-1725.
- Clemens R and van Klinken BJW (2014). The future of oats in the food and health continuum. *Br. J. Nutr.*, **112**: S75-S79.
- Cohen AT, Imfeld S, Markham J and Granziera S (2015). The use of aspirin for primary and secondary prevention in venous thromboembolism and other cardiovascular disorders. *Thromb. Res.*, **135**: 217-225.
- Collaboration AT (2002). Collaborative metaanalysis of randomised trials of antiplatelet therapy for prevention of death, myocardial infarction, and stroke in high risk patients. *Brit. Med. J.*, **324**: 71-86.
- Dai Y and Ge J (2012). Clinical use of aspirin in treatment

- and prevention of cardiovascular disease. *Thrombosis*, **2012**.
- Del Rio JA, Fuster MD, Gomez P, Porras I, Garcia-Lidon A and Ortuno A (2003). Citrus limon: A source of flavonoids of pharmaceutical interest. *Food Chem.*, **84**: 457-461.
- Dow CA, Going SB, Chow HHS, Patil BS and Thomson CA (2012). The effects of daily consumption of grapefruit on body weight, lipids, and blood pressure in healthy, overweight adults. *Metab. Clin. Exp.*, **61**: 1026-1035.
- Duttaroy AK and Jorgensen A (2004). Effects of kiwi fruit consumption on platelet aggregation and plasma lipids in healthy human volunteers. *Platelets*, **15**: 287-292.
- Erlund I, Silaste ML, Alfthan G, Rantala M, Kesaniemi YA and Aro A (2002). Plasma concentrations of the flavonoids hesperetin, naringenin and quercetin in human subjects following their habitual diets, and diets high or low in fruit and vegetables. *Eur. J. Clin. Nutr.*, **56**: 891-898.
- Fuentes E, Pereira J, Mezzano D, Alarcon M, Caballero J and Palomo I (2014). Inhibition of platelet activation and thrombus formation by adenosine and inosine: Studies on their relative contribution and molecular modeling. *PLoS One*, **9**.
- Furusawa M, Tsuchiya H, Nagayama M, Tanaka T, Nakaya K and Iinuma M (2003). Anti-platelet and membrane-rigidifying flavonoids in brownish scale of onion. *J. Health Sci.*, **49**: 475-480.
- Gaziano TA, Bitton A, Anand S, Abrahams-Gessel S and Murphy A (2010). Growing epidemic of coronary heart disease in low- and middle-income countries. *Curr. Probl. Cardiol.*, **35**: 72-115.
- He Y, Jin H, Gong W, Zhang C and Zhou A (2014). Effect of onion flavonoids on colorectal cancer with hyperlipidemia: An *in vivo* study. *Onco. Targets Ther.*, **7**: 101-110.
- Iciek M, Kwiecien I and Wlodek L (2009). Biological properties of garlic and garlic-derived organosulfur compounds. *Environ. Mol. Mutagen.*, **50**: 247-265.
- Iwashita M, Oka N, Ohkubo S, Saito M and Nakahata N (2007). Piperlongumine: A constituent of *Piper longum* L., inhibits rabbit platelet aggregation as a thromboxane A₂ receptor antagonist. *Eur. J. Pharmacol.*, **570**: 38-42.
- Kim YS, Pyo MK, Park KM, Park PH, Hahn BS, Wu SJ and Yun-Choi HS (1998). Antiplatelet and antithrombotic effects of a combination of ticlopidine and Ginkgo biloba Ext (EGb 761). *Thromb. Res.*, **91**: 33-38.
- Koo KKK, Ammit AJ, Tran VH, Duke CC and Roufogalis BD (2001). Gingerols and related analogues inhibit arachidonic acid-induced human platelet serotonin release and aggregation. *Thromb. Res.*, **103**: 387-397.
- Lawson LD, Ransom DK and Hughes BK (1992). Inhibition of whole blood platelet aggregation by compounds in garlic clove extracts and commercial garlic preparations. *Thromb. Res.*, **65**: 141-156.
- Leal J, Luengo-Fernandez R, Gray A, Petersen S and Rayner M (2006). Economic burden of cardiovascular diseases in the enlarged European Union. *Eur. Heart J.*, **27**: 1610-1619.
- Liao YR, Leu YL, Chan YY, Kuo PC and Wu TS (2012). Anti-platelet aggregation and vasorelaxing effects of the constituents of the rhizomes of *Zingiber officinale*. *Molecules*, **17**: 8928-8937.
- Mnayer D, Fabiano-Tixier AS, Petitcolas E, Chemat F, Hamieh T, Nehme N, Ferrant C and Fernandez X (2014). Chemical composition, antibacterial and antioxidant activities of six essential oils from the Alliaceae family. *Molecules*, **19**: 20034-20053.
- Nagata Y, Inomata Ji, Kinoshita M, Kurokawa K, Aburadani I, Maruyama M and Usuda K (2013). Impact of proton pump inhibitors or famotidine on the antiplatelet actions during dual-antiplatelet therapy in Japanese patients. *Cardiovasc. Intervention Ther.*, **28**: 22-29.
- Navarro-Gonzalez I, Perez-Sanchez H, Martin-Pozuelo G, Garcia-Alonso J and Periago MJ (2014). The inhibitory effects of bioactive compounds of tomato juice binding to hepatic HMGCR: *In vivo* study and molecular modelling. *PLoS One*, **9**.
- Nurtjahja-Tjendraputra E, Ammit AJ, Roufogalis BD, Tran VH and Duke CC (2003). Effective anti-platelet and COX-1 enzyme inhibitors from pungent constituents of ginger. *Thromb. Res.*, **111**: 259-265.
- Oboh G, Bello FO and Ademosun AO (2014). Hypocholesterolemic properties of grapefruit (*Citrus paradisi*) and shaddock (*Citrus maxima*) juices and inhibition of angiotensin-1-converting enzyme activity. *J. Food Drug Anal.*, **22**: 477-484.
- Okuda N, Miura K, Okayama A, Okamura T, Abbott RD and Nishi N (2015). Fruit and vegetable intake and mortality from cardiovascular disease in Japan: A 24-year follow-up of the NIPPON DATA80 Study. *Eur. J. Clin. Nutr.*, **69**: 482-488.
- Robinson M, Lu B, Edirisinghe I and Kappagoda CT (2012). Effect of grape seed extract on blood pressure in subjects with pre-hypertension. *J. Pharm. Nutr. Sci.*, **2**: 155-159.
- Santhosha SG, Jamuna P and Prabhavathi SN (2013). Bioactive components of garlic and their physiological role in health maintenance: A review. *Food Biosci.*, **3**: 59-74.
- Schemmer P, Zhong Z, Galli U, Wheeler MD, Li X, Bradford BU, Conzelmann LO, Forman D, Boyer J and Thurman RG (2013). Glycine reduces platelet aggregation. *Amino Acids*, **44**: 925-931.
- Thomson M, Al-Qattan KK, Al-Sawan SM, Alnaqeeb MA, Khan I and Ali M (2002). The use of ginger (*Zingiber officinale* Rosc.) as a potential anti-inflammatory and antithrombotic agent. *Prosta. Leukotr. Ess.*, **67**: 475-478.
- Turkmen N, Sari F and Velioglu YS (2005). The effect of

- cooking methods on total phenolics and antioxidant activity of selected green vegetables. *Food Chem.*, **93**: 713-718.
- Vilahur G and Badimon L (2013). Antiplatelet properties of natural products. *Vasc. Pharmacol.*, **59**: 67-75.
- Wu Q, Tan Z, Liu H, Gao L, Wu S, Luo J, Zhang W, Zhao T, Yu J and Xu X (2010). Chemical characterization of *Auricularia auricula* polysaccharides and its pharmacological effect on heart antioxidant enzyme activities and left ventricular function in aged mice. *Int J. Biol. Macromol.*, **46**: 284-288.
- Wurtz M, Hvas AM, Kristensen SD and Grove EL (2012a). Platelet aggregation is dependent on platelet count in patients with coronary artery disease. *Thromb. Res.*, **129**: 56-61.
- Wurtz M, Hvas AM, Wulff LN, Kristensen SD and Grove EL (2012b). Shear-induced platelet aggregation in aspirin-treated patients: Initial experience with the novel PlaCor PRT device. *Thromb. Res.*, **130**: 753-758.
- Xia Q, Wang X, Xu DJ, Chen X and Chen FH (2012). Inhibition of platelet aggregation by curdione from *Curcuma wenyujin* essential Oil. *Thromb. Res.*, **130**: 409-414.
- Yi T, Chen HB, Zhao ZZ, Yu ZL and Jiang ZH (2011). Comparison of the chemical profiles and anti-platelet aggregation effects of two "Dragon's Blood" drugs used in traditional Chinese medicine. *J. Ethnopharmacol.*, **133**: 796-802.
- Zimmer S, Grebe A and Latz E (2015). Danger signaling in atherosclerosis. *Circ. Res.*, **116**: 323-340.