Austin Journal of Nutrition & Metabolism

Open Access

Austin Publishing Group

Research Article

Antioxidant and Anti-Thrombotic Properties of Selected Plant Extracts of Asia

Rao TP[1]*, Htay HH[1], Yasuda NK[1], Sugino H[1], Ohkubo[1], Hayashi T[2], Okamoto T[2] and Suzuki K[2]

[1]Nutrition Division, Central Research Laboratories, Japan

[2]Department of Molecular Pathobiology, Mie University, Japan

***Corresponding author:** Rao TP, International Division, Taiyo Kagaku Co, Ltd, 800 Yamada-cho, Yokkaichi, Mie 512-1111, Japan

Received: September 12, 2014; **Accepted:** October 16, 2014; **Published:** October 17, 2014

Abstract

Emblica officinalis Gaertn, *Hibiscus sabdariffa* L, *Acacia concinna* DC, *Xanthium strumarium* L, *Swertia pulchella* Buch Ham, *Vitis repens* Wight & Arn Prodr, *Hizikia fusiforme* and *Momordica charantia* L. are commonly used in food and traditional medicine of Asia. In order to establish the scientific basis of their medicinal properties and to use them as a functional food ingredient in the improvement of thrombosis, their aqueous extracts were subjected to the analysis of anti-oxidative and anti-thrombotic properties. Superoxide and DPPH radical scavenging activities, anti-thrombotic activities such as inhibition of thrombin, collagen, ADP and ristocetin induced platelet aggregation and Activated Partial Thromboplastin Time (APTT), Prothrombin Time (PT) were measured *in vitro*. The plant species were ranked based on each specific activity. Among the species, *E. officinalis* ranked high with strong antioxidant activity and high inhibition of platelet aggregation against three agonists. Whereas, *H fusiforme* and *H sabdariffa* showed strong anti-coagulant activity despite having low antioxidant and anti-platelet activities. In conclusion, the plant species were significantly different in their antioxidant, anticoagulant and anti-platelet activities. The results suggest that the antioxidant activities might not necessarily to be related to the anti-thrombosis effect of the species, which means the components responsible for anti-oxidant and anti-thrombosis might not necessary be the same. *E. officinalis* may compose the components responsible for both antioxidant and antithrombosis activities could be considered as a strong functional food ingredient for prevention and suppression of oxidative stress and thrombosis.

Keywords: Antioxidants; Anti-thrombosis; *E. officinalis; H. sabdariffa; A. concinna; V. repens*

Abbreviations

APTT: Activated Partial Thromboplastin Time; PT: Prothrombin Time; CVD: Cardiovascular Disease; ROS: Reactive Oxygen Species

Introduction

According to World Health Organization report [1], eighty percent of population in Asia and African countries relies on traditional medicine for their primary health care. Folk medication is mostly based on natural food sources like fruits, vegetables and herbs. The polyphenols in grapes and cocoa [2] and tea [3] were reported to have preventive effects on arterial thrombosis and Cardiovascular Disease (CVD), which is the major mortality risk disease in many countries. Indeed, the polyphenols found in ordinary foods act as antioxidants to scavenge Reactive Oxygen Species (ROS), which are usually produced during the physiological and biochemical processes within the body. Various cultures and traditions around the world suggest a regular intake of foods like tomatoes, garlic, ginger and other indigenous vegetables for healthy living and good blood circulation. These findings have been partially accepted in some scientific investigations [4,5].

Oxidative stress occurs when there is an imbalance between free radicals production and antioxidants availability in the body cells. The oxidative stress in the vascular endothelium cells is largely associated to atherosclerosis and thrombosis [6,7]. On the other hand, high blood lipid profiles and platelet aggregation in blood vessels leads to hypertension, a major high risk factor in Cardiovascular Disease (CVD) and heart attack [8]. Therefore, oxidative stress and thrombosis are the common factors linked to metabolic syndrome and CVD [9]. From this view point, plant polyphenols are subjected to increased investigation for their anti-oxidant and anti-thrombotic properties.

The aim of this study was to identity the potent anti-oxidant and anti-thrombotic food material, which are commonly used in food and traditional folk medicine in south and south-east Asia. For this purpose we have selected plant species namely Amla (*Emblica officinalis Gaertn.)*, Chin Boung (*Hibiscus sabdariffa*), Kin Pun Chin (*Acacia concinna)*, Nigauri (*Mormodica charantia*), Sei Kar (*Swerita pulchella)*, *Onamomi (Xanthium strumarium*), *Tapindine Mya Nan (Vitis repens)*, Hijiki (*Hizikia fusiforme)*, which are popularly used in the indigenous medicine of tropical countries like India, Myanmar, Bangladesh, Indonesia and the Malay Peninsula area. In this study, aqueous extracts of these plant species were prepared and examined their antioxidant and antithrombotic effects.

Materials and Methods

Ethno-pharmacological background of the plant materials

The list of plant species and parts used in this study was listed

Citation: Rao TP, Htay HH, Yasuda NK, Sugino H, Ohkubo, Hayashi T, et al. Antioxidant and Anti-Thrombotic Properties of Selected Plant Extracts of Asia. Austin J Nutr Metab. 2014;1(1): 6.

in Table 1. Traditional medicinal uses of these plants are known to prevent diseases or medical conditions such as diabetes, hepatitis, ulcers, hypertension, and digestive dysfunction. The fruits of *E. officinalis* (India; Sp. Pl. 982.1753) has been prevalent in use in various traditional medicine [10] like Ayurvedic medicine in India, herbal medicine in Tibet, China, and Myanmar. It is considered as an almighty fruit with strong antioxidant and several prophylactic properties in Ayurveda. The leaves and fruits *H. sabdariffa* (Myanmar; Sp. Pl. 695.1753) are extensively used as anti-hypertensive herb in food and beverages in more than thirteen countries in Asia, Southern Africa, South America and Australia [11,12]. The leaves of *A. concinna* (Myanmar; Sp. P. 4: 1090.1806) have been used to prevent diabetes and skin diseases in Myanmar, India and Thailand [13]. The fruits of *M. charantia* (Japan; Sp. Pl. 1009.1753) are widely consumed to promote blood health in Asian countries including Japan. The whole parts of bitter species *S. pulchella* (India; GBIF 108634372) are used for the prevention of digestive dysfunction in the folk medicine of Myanmar and China [14]. The fruits of *X. strumarium* (Myanmar; Sp. Pl. 987.1753) are used as an herbal medicine in China. *V. repens* (Myanmar; AD96233094) is used for ulcers, hepatitis and jaundice in the herbal medicines of Myanmar and India [15,16]. *H. fusiforme* (Japan; GBIF 3196919) is well known for its nutritional benefits in Japan and Korea.

Aqueous extraction of plant species

It is common to preserve the sun dried plant parts of various herbal medicinal plant species at home and use their decoction (aqueous extracts) when necessary for specific ailment. Hence, we collected the sun dried plant parts of above plant species from the local markets of India, Myanmar and Japan. The dried parts were grounded to fine powder and 100 g of powder was mixed well in one liter of distilled water and heated at 50^0 C for 3 h. The solutions were cooled to room temperature and subjected to centrifugal separation at 4^0 C, at 6500 rpm for 15 min. Then the supernatant was obtained by filtering with Whatman No.2 filter paper under reduced pressure. The filtrate was concentrated at 50^0 C by a rotary evaporator and then freeze-dried into powder for use in this study. The dry-weight (g) of freeze-dried powder was considered as the yield of extract and expressed in percentage of initial amount of dry powder of plant parts (100 g) used for extraction.

Chemical materials

For the measurement of polyphenol content and antioxidant activities, the chemicals and free radicals namely the Folin-ciocalteu reagent, 1-1 diphenyl-2-picrylhydrazyl (DPPH) and xanthine/xanthine oxidase were procured from Wako Pure Chemicals Industry Ltd (Osaka, Japan). The chemical agents for measurement of anticoagulant activity, such as PT (prothrombin time) reagents, Thromboplastin and $CaCl_2$ (SYSMEX Corporation, Kobe, Japan), APTT (DADE BEHRING, Marburg, Germany) were procured from the specified sources. The MPEC luminescence reagent (2-methyl-6-p-methoxyphenylethynyl-imidazopyrazinone) was bought from ATTO Corporation (Fukuoka, Japan). The platelet inducer, Thrombin was purchased from SIGMA-Aldrich (St. Louis, MO, USA). The other platelet induces, such as ADP, collagen and the ristocetin (von Willebrand factor activator) were sourced from CHRONO-LOG Corporation (Havertown, PA, USA).

Table 1: List of plant species and their parts used for aqueous extraction.

Botanical name	Vernacular name	Country	Part of plant used
E. officinalis Gaertn.	Amla	India	Fruits
V. repens Wight & Arn. Prodr.	Tapindine Mya Nan	Myanmar	Whole plant
X. strumarium L.	Onamomi	Japan	Fruits
S. pulchella Buch. Ham.	Sei Kar	India	Whole plant
A. concinna DC.	Kin Pun Chin	Myanmar	Leaves
M. charantia L.	Nigauri	Japan	Fruits
H. sabdariffa L.	Chin Boung Thee	Myanmar	Fruits
H. fusiforme Okam.	Hijiki	Japan	Sea algae

Determination of polyphenol content

The amount of total soluble polyphenols was determined according to the Folin-ciocalteu method [19]. Samples were dissolved in de-ionized water at concentrations of 1.0, 0.5, 0.25, 0.12, 0.06, 0.03 mg/ml. The reaction mixture (1.5 ml) contained 500 µλ of various concentrations of extract samples, 500 µλ of 20% Folin-ciocalteu reagent and 500 µλ of 10% (w/v) sodium carbonate solution. The reaction mixture was mixed for 5 min and incubated for 1 h in dark at room temperature. Color development was determined by spectrophotometer at 760 nm. Gallic acid (0.1, 0.05, 0.025, 0.012, 0.006 mg/ml) was used as a standard. The total polyphenol contents in the samples were expressed as gallic acid equivalent (GAE mg/g) of sample.

Measurement of DPPH radical scavenging activity

The DPPH free radical scavenging activity was determined an assay previously described [17]. Briefly, one milligram of each plant extracts was dissolved in 1 ml of de-ionized water. The solutions were further diluted with de-ionized water to make 0.5, 0.25, 0.12, 0.06, 0.03, 0.01 mg/ml concentrations for *E.officinalis, V.repens, X.strumarium, S.pulchella, A.concinna, H.sabdariffa* and 5.0, 2.5, 1.25, 0.62, 0.31, 0.15 mg/ml for *H. fusiforme* and *M. charantia*. The DPPH radical scavenging activity was performed with 1.5 ml reaction mixture containing 1 ml of 0.1 mM DPPH in ethanol solution, 0.45 ml of 50 mM Tris- HCl buffer (pH7.4) and 0.05 ml of de-ionized water (as control) or equal amount of different concentrations of the above aqueous extracts. Mixture was incubated for 1 hr in the dark at room temperature and radical scavenging activity was determined at the absorbance of 517 nm. Inhibition activity of DPPH was expressed in 50% inhibitory concentration (IC_{50}) of each plant species.

Measurement of superoxide (O_2^-) scavenging activity

One milligram of each plant extracts was dissolved in 1 ml of de-ionized water and then further diluted to make 0.3, 0.15, 0.075, 0.037, 0.018 mg/ml solutions. The superoxide anion was generated by xanthine/xanthine oxidase and detected using the MPEC luminescence reagent in a Veritas Microplate Luminometer (PROMEGA KK, Tokyo, Japan) according to Shimomura et al. [18]. The reaction in this study was performed in a 50 mM KH_2PO_4/NaOH buffer (pH 7.5) solution. The reaction mixture (300 µλ) contained 10 µλ of 300 µM MPEC, 60 µl of 0.1 U/ml Xanthine oxidase, 170 µλ of 0.1 M KH_2PO_4 buffer (pH 7.5), 50 µλ of hypoxanthine and 10 µl of different concentrations of aqueous extracts. The reaction was performed with or without (as control) aqueous extracts. In case of control, KH_2PO_4 buffer solution was used in place of aqueous extract.

Table 2: Polyphenol contents, antioxidant activities of aqueous plant extracts.

Botanical name	Extraction yield (%) of dry weight	Polyphenol contents		DPPH IC_{50} (mg/ml)*	O_2^- IC_{50} (mg/ml)§
		GAE (mg /g)†	GAE (%)‡		
E. officinalis Gaertn.	45.5[a]	346.8±7.6[a]	34.7[a]	0.02±0.001[a]	0.02±0.001[a]
V. repens Wight & Arn.Prodr.	12[c]	119.4±1.4[b]	11.9[b]	0.09±0.009[b]	0.04±0.002[a]
X. strumarium L.	7[c]	108.2±3.7[b]	10.8[b]	0.15±0.011[c]	0.09±0.007[b]
S. pulchella Buch. Ham.	20[b]	84.8±1.6[c]	8.5[c]	0.15±0.012[c]	0.09±0.007[b]
A. concinna DC.	23[b]	78.4±2.4[c]	7.8[c]	0.35±0.018[d]	0.30±0.013[c]
M. charantia L.	58[a]	24.2±0.5[d]	2.4[d]	2.50±0.146[e]	0.60±0.026[d]
H. sabdariffa L.	48[a]	22.9±0.7[d]	2.3[d]	0.48±0.019[d]	0.20±0.012[c]
H. fusiforme Okam.	24[b]	16.3±0.6[d]	1.6[d]	1.70±0.114[e]	0.27±0.012[c]

† Data expressed as mg gallic acid equivalents mg /1g of extract sample.
‡ Data expressed as % of dry weight of aqueous plant extracts.
* Data expressed as 50% inhibition (IC_{50}) of DPPH free radical activity.
§ Data expressed as 50% scavenging (IC_{50}) of superoxide free radical activity.
[a, b, c, d, e]in column 2, 3 and 4 represent significant ($p < 0.005$) difference among the mean ± SEM amounts of the species.
[a, b, c, d, e]in column 5 and 6 represents the order of high to low activity and the mean ± SEM differs significantly ($p < 0.005$).

The percentage of inhibition of superoxide anion was calculated and expressed in IC_{50} values.

Measurement of anti-coagulant activity

The measurements of anti-coagulant activities of plant species were performed with APTT and PT assays. The assays were performed with the final concentration of 5 mg/ml of each plant species. The reaction mixture contained 10 µλ of each sample, 50 µλ of pool plasma, 100 µλ of PT or 50 µλ of APTT and 50 µλ of $CaCl_2$ to measure coagulation time (sec) by a CA50 coagulometer (SYSMEX corporation, Kobe, Japan). The assays were performed according to manufacturer's instructions. Ten micro liters of heparin with concentrations ranging between 0-1.0 U/ml was used for standard curve. The ant-coagulation activity of the aqueous plant extracts was calculated from the heparin standard curve and expressed as the equivalents of heparin unit per 5 mg (U/mg) of extract sample.

Measurement of Anti-platelet activity

The thrombin-, collagen-, ADP- and ristocetin-induced platelet aggregation was determined by Screen Filtration Pressure (SFP) using an aggregometer according to the manual of manufacturer (WBA-Neo, ISK, Tokyo, Japan). Briefly, 200 µλ of blood collected from volunteers was mixed with 22 µλ of each of above agonist seperately at the concentrations of 5 U/ml thrombin, 1.25 µg/ml of collagen, 5 µM of ADP or 1 mg/ml of ristocetin, respectively. The assay was performed with or without of aqueous plant extracts (22 µλ) at 5mg/ml concentration. The platelet aggregation was measured and expressed as % of inhibition against each agonist.

Statistical analysis

Data of polyphenol content and antioxidant activities are presented as the mean±SEM of 5 replicates. The data of anti-coagulant and anti-platelet activities were measured using the blood samples of three individual subjects and the average values were expressed as mean±SEM. Statistical analysis was performed by one-way analysis of variance followed by the Student's t-test. The P value < 0.005 was considered statistically significant.

Results and Discussion

Extraction yield

The aqueous extracts are common in traditional use and food preparations, so the effectiveness of extraction and final dry yield of aqueous extract may have high relevance for their use as antioxidants and antithromobotic agents. The yield of an extract refers to the most usable portion. Among the plant species, the extraction yield (dry weight) was differed significantly, ranging from 7 to 58% (Table 2). The differences were largely attributed to the plant parts used for the extraction than the difference in plant species. High percentage of yield was observed from fruits than from leaves or whole herb.

Polyphenol content and antioxidant activities

The polyphenol content of the plant extracts were significantly varied and ranged between 16.3 mg to 347 mg of GAE. The antioxidant activities against DPPH and superoxide were corresponded to the polyphenol content of plant species (Table 2). Among the eight plant species tested, *E.officinalis, V.ripens* and *X.stumarium* showed high antioxidant activities. Particularly, *E.officinalis* showed the highest polyphenol content and as well strongest antioxidant activity. While comparing the three plant species, the proportion of polyphenols in the yield of extracts was lower in *E.officinalis*. Thus, the strong antioxidant activity of *E.officinalis* could be postulated either from its unique polyphenolic constituents or other water soluble components such as vitamin C. Scartezzinia *et al.* [20] reported that *E. officinalis* is a fruit of high in vitamin C content, which may also contributed for significant antioxidant activity. The antioxidants are vastly accepted of having many physiologically beneficial effects for the human body, such as anti-aging [21] and anti-obesity or alleviate metabolic syndrome [22]. Moreover, therapeutic potential against cardiovascular disease [23] and anti-tumor effects [24] were long been observed with polyphenols. The polyphenols known to be effective in reducing the oxidative stress induced by various pathological conditions [25]. Recent studies suggest that Amla extract reduced the oxidative stress in Streptozotacin induced diabetic rats [26], hypercholesterolemia and lipid peroxidation in cholesterol fed rats [27], dyslipidemia and oxidative stress in ageing process [28], age-related renal dysfunction by oxidative stress [29] and lypopolysaccharides induced pro-inflammatory factors in endothelial cells [30]. The plant species rich in polyphenols are thus expected to attenuate several dysfunctions including thrombosis, which are related to oxidative stress.

In comparison to a study in Korea [31] on *H. fusiforme*, our study showed lower polyphenol content and corresponding lower antioxidant properties. This could be probably the differences in the collecting area and processing methods. Siriwardhana's [31] group prepared freshly collected *H.fusiforme* samples while we prepared dry samples from markets in Japan. Market samples may have lost some polyphenol content during the preparation and storage.

Anti-coagulation activities

The anti-thrombosis properties are assayed with an anti-coagulation system and an anti-platelet system. Anti-coagulation agents are recognized to be effective for primary prevention of venous thrombotic factors such as deep vein thrombosis and pulmonary embolism [32]. The anti-platelet agents are crucial for primary prevention of atherothrombosis such as myocardial infarctions and stroke [33]. The blood coagulation system could be divided to two pathways, namely intrinsic coagulation (contact activation) and extrinsic coagulation (tissue factor activation) systems. The effect of endogenous and exogenous materials on coagulation activity could be accessed through APTT for the intrinsic coagulation system and PT for the extrinsic coagulation system. The coagulation time under APTT and PT systems were expressed as unit of Heparin. The coagulation time under APTT system for Heparin was 39.6, 50.9. 86.4 and 180.2 seconds for 0.05. 0.1, 0.2 and 0.4 Heparin U/ml, respectively. Similarly, the coagulation time under PT system for Heparin was 10.9, 11.8, 12.3, 13.2, and 18.1 seconds for 0, 0.125, 0.25, 0.5 and 1.0 Heparin U/ml, respectively.

In this study, aqueous extracts from *H. fusiforme*, *H. sabdariffa and A. concinna,* were having high APTT and PT values, resembling their efficacy against both intrinsic and extrinsic coagulation effects (Table 3). *V. repens* had high APTT values that limit its efficiency against intrinsic coagulation only. Among the plant species tested, H. fusiforme showed significantly high APTT (0.37 U/ml) and PT (1.88 U/ml) values. Earlier studies suggest potential anti-coagulant effect of several plant species such as Panax ginseng [34], Sparganii rhizome [35] etc. On the other hand, this plant showed low polyphenols content and low antioxidant activity, which suggests that the high anti-coagulant activity of this plant species might be related to some other water-soluble components other than polyphenols. A previous study by Chen et al [35] also found no relation between anti-coagulant activity and flavonoid contents. Another study suggested that the polysaccharides are likely responsible for the anti-coagulant effects in this plant species [36].

Anti-platelet activity

Concerning to the anti-platelet activity, the response of plant species against each platelet inducer was different (Table 3). Four out of eight extracts; *M. charantia, E. officinalis, A. concinna* and *H. sabdariffa* had high inhibitory activity against thrombin-induced platelet aggregation. More than 85% inhibition against collagen induced platelet aggregation was observed with five out of eight extracts, *X. strumarium, E. officinalis, A. concinna, S. pulchella. V. repens.* In case of ADP-induced platelet aggregation, four aqueous extracts namely *E. officinalis, A. concinna, V. repens, S. pulchella* showed strong inhibition rate. Regarding ristocetin-induced platelet aggregation, six plants extracts from *M. charantia, X. strumarium, S. pulchella, E. officinalis* and *H. fusiforme* have decreased the platelet aggregation by more than 60%. Though the plant species varied in their response against each platelet inducer, *E. officinalis*, ranked high followed by *S. pulchella* and *A. concinna* for all four platelet inducers.

Although, the anti-platelet activities of various substances are often related to their polyphenol and antioxidant activities [37], in the present study the differential response of *V. repens* and *X. strumarium* between thrombin induced platelet aggregation and the antioxidant activity suggesting that the components other than polyphenols such as polysaccharides could be responsible for the anti-platelet activities in this plant species. The oxidative stress plays an important role in thrombosis, but the clinical data on the relevance of anti-oxidants with platelet dependent thrombosis have not been established completely [38]. The plant species *H. fsuiforme* and *H. sabdariffa*, which have low polyphenol content and low antioxidant activity while exhibiting very high anti-coagulant and moderate anti-platelet activities. This result suggests that the anticoagulation or antiplatelet activities may not always necessarily be related to each other and with antioxidant activities. Previous studies explicit the activation of antiplatelet activities by the components other than antioxidants [39]. This results underlay that the components responsible for each activity might be different and they may have different mode of mechanism.

Overall performance

In order to rank the plant species used in this study for their

Table 3: Anti-coagulation and anti-platelet aggregation activities of aqueous plant extracts.

Name of aqueous plant extract	Anti-coagulant activities		Anti-platelet activities			
	APTT† (U Heparin/5mg)	PT† (U Heparin/5mg)	Inhibition of Platelet aggregation (%)‡			
			Thrombin	Collagen	ADP	Ristocetin
E. officinalis Gaertn.	0.04 ± 0.01^{d}	0.07 ± 0.03^{c}	93.4±0.6^{a}	92.7±0.8^{a}	96.1±0.6^{a}	63.0±1.2^{b}
V. repens Wight & Arn.Prodr.	0.17 ± 0.03^{b}	0.04 ± 0.02^{d}	0.0±0.0^{d}	87.0±0.8^{a}	95.8±0.2^{a}	40.0±1.2^{c}
X. strumarium L.	0.08 ± 0.03^{c}	0.05 ± 0.02^{d}	0.0±0.0^{d}	96.3±0.8^{a}	14.3±0.5^{c}	90.0±0.9^{a}
S. pulchella Buch. Ham.	0.04 ± 0.01^{d}	0.08 ± 0.02^{c}	25.6±1.3^{c}	90.0±1.7^{a}	60.7±1.0^{b}	86.0±0.7^{a}
A. concinna DC.	0.18 ± 0.02^{b}	0.12 ± 0.02^{b}	89.9±0.7^{a}	92.5±0.7^{a}	95.9±1.2^{a}	15.0±0.6^{d}
M. charantia L.	0.02 ± 0.01^{d}	0.07 ± 0.05^{c}	93.9±2.3^{a}	11.0±0.6^{c}	0.0±0.0^{d}	94.0±0.6^{a}
H. sabdariffa L.	0.23 ± 0.03^{b}	0.18 ± 0.04^{b}	68.0±1.0^{b}	34.0±1.2^{b}	15.4±0.9^{c}	0.0±0.0^{e}
H. fusiforme Okam.	0.37 ± 0.03^{a}	1.88 ± 0.04^{a}	0.0±0.0^{d}	0.0±0.0^{d}	29.9±0.8^{c}	62.0±1.1^{b}

†APTT and PT were expressed as heparin equivalent unit per 5 mg of aqueous plant extracts.

‡ Inhibition (%) of platelet aggregation expressed against the 100% aggregation of agonists namely Thrombin, Collagen, ADP and Ristocetin.

$^{a, b, c, d, e}$ the mean ± SEM represents the order of high to low activity and differs significantly ($p < 0.005$).

Table 4: The ranking system of overall performance of plant extracts.

Rank	Name of aqueous plant extract	Antioxidant activity		Anti-coagulant activity		Anti-platelet activity				Total score
		DPPH	O_2^-	APTT	PT	Thrombin	Collagen	ADP	Ristocetin	
1	*E. officinalis*.	5	5	2	3	5	5	5	4	34
4	*V. repens*	4	5	4	2	0	5	5	3	28
5	*X. strumarium*.	3	4	3	2	0	5	3	5	25
3	*S. pulchella*	3	4	2	3	3	5	4	5	29
2	*A. concinna*	2	3	4	4	5	5	5	2	30
7	*M. charantia*	1	2	2	3	5	3	0	5	21
6	*H. sabdariffa*	2	3	4	4	4	4	3	0	24
7	*H. fusiforme*	1	3	5	5	0	0	3	4	21

Ranking system: The overall performance of the plant extracts was assessed by assigning the values 5 to 1 for high to low activity graded as "a to e" in table 2 and 3 for antioxidant and antithrombotic activities. The value 0 was assigned for no activity.

overall performance in the antioxidant, anti-coagulant and anti-platelet activities, we adopted grading system and summarized the results in Table 4. The details of the grading system were explained under the table. According to this data, *E. officinalis* ranked top of all plant species used with a high score for polyphenols content, antioxidant and anti-platelet activities. These findings suggest that the extract of *E. officinalis* fruits and its components could be effective for the development of agents for anti-thrombosis. *A. concinna* and *S. pulchella* were ranked second and third, respectively.

Interestingly, the bitter taste plant species, such as, *S. pulchella, X. strumarium* and *M. charantia* have shown strong inhibition against ristocetin induced-platelet aggregation, which has high relevance to the anti-atherothrombosis. This result may leave a clue to study further the details on the relationship between bitter components particularly against the platelet aggregation responsible for atherothrombosis.

Conclusion

This study aimed for preliminary investigation of various food materials used in traditional medicine for their potent antioxidant and antithrombotic activities and the relationship between these two activities. Platelet aggregation and thrombus formation in vascular circulation may depend on the redox system within blood with the presence of endogenous or exogenous antioxidants. In the present study the antioxidant capacity of the aqueous plant extracts on the inhibition of various thrombotic factors could not be explained. Although the present study observed limited relationship between acute antioxidant activities and inhibition of thrombotic factors, the successive use of antioxidants may play significant role in the prevention of oxidative stress and subsequent control of the progression of thrombotic factors. The present data provides a preliminary evidence that the development of functional food ingredients for healthy blood circulation and prevention of thrombosis based on their polyphenol content and antioxidant properties alone would not be sufficient. Therefore, other water-soluble components present in these functional food materials should also be considered for anti-coagulant and anti-platelet activities for the management of thrombosis and heart heath. Further investigation is necessary to find the components responsible for these effects in these plant extracts. *E. officinalis*, which ranked top in overall performance may have all the components necessary to use it as an effective functional food ingredient for the inhibition of oxidative stress and promote healthy blood circulation to prevent vascular diseases like venous and arterial thrombosis.

Acknowledgement

We sincerely acknowledge Dr. D. C. Chu, Retired General Manager of Taiyo Kagaku, for coordinating with Mie University to conduct this joint research and to avail its facilities and assistance in this project. We gratefully acknowledge the Japan Sciences and Technology Agency, Ministry of Education, Culture, Sports, Science and Technology for providing a grant (no. 00432-017) through Tokai Plaza for conducting the research and development of food materials for the prevention of angiopathic lifestyle-related disease.

References

1. World health organization facts sheets.
2. Rein D, Paglieroni TG, Pearson DA, Wun T, Schmitz HH, Gosselin R, Keen CL. Cocoa and wine polyphenols modulate platelet activation and function. J Nutr. 2000; 130: 2120S-6S.
3. Stangl V, Dreger H, Stangl K, Lorenz M. Molecular targets of tea polyphenols in the cardiovascular system. Cardiovasc Res. 2007; 73: 348-358.
4. O'Kennedy N, Crosbie L, van Lieshout M, Broom JI, Webb DJ, Duttaroy AK. Effects of antiplatelet components of tomato extract on platelet function *in vitro* and *ex vivo*: a time-course cannulation study in healthy humans. Am J Clin Nutr. 2006; 84: 570-579.
5. Cavagnaro PF, Camargo A, Galmarini CR, Simon PW. Effect of cooking on garlic (Allium sativum L.) antiplatelet activity and thiosulfinates content. J Agric Food Chem. 2007; 55: 1280-1288.
6. Harrison D, Griendling KK, Landmesser U, Hornig B, Drexler H. Role of oxidative stress in atherosclerosis. Am J Cardiol. 2003; 91: 7A-11A.
7. Day SM, Duquaine D, Mundada LV, Menon RG, Khan BV, Rajagopalan S, et al. Chronic iron administration increases vascular oxidative stress and accelerates arterial thrombosis. Circulation. 2003; 107: 2601-2606.
8. Hansel H, Hansel B, Giral P, Nobecourt E, Chantepie A, Bruckert E, et al. Metabolic syndrome is associated with elevated oxidative stress and dysfunctional dense high-density lipoprotein particles displaying impaired antioxidative activity. The J Clin Endocrinol Metab. 2004; 89: 4963-4971.
9. Saito K, Kohno M, Yoshizaki F, Niwano Y. Extensive screening for edible herbal extracts with potent scavenging activity against superoxide anions. Plant Foods Hum Nutr. 2008; 63: 65-70.
10. World Health organization, regional office of South East Asia, New Delhi. The use of traditional medicine in primary health care; A manual for the health worker in South-East Asia. 1990; 40.
11. Herrera-Arellano A, Flores-Romero S, Chávez-Soto MA, Tortoriello J. Effectiveness and tolerability of a standardized extract from Hibiscus

sabdariffa in patients with mild to moderate hypertension: a controlled and randomized clinical trial. Phytomedicine. 2004; 11: 375-382.

12. Kao ES, Hsu JD, Wang CJ, Yang SH, Cheng SY, Lee HJ. Polyphenols extracted from Hibiscus sabdariffa L. inhibited lipopolysaccharide-induced inflammation by improving antioxidative conditions and regulating Cyclooxygenase-2 expression. Biosci Biotechnol Biochem. 2009; 73: 1-6.

13. Sekine T, Fukasawa N, Ikegami F, Saito K, Fuji Y, Murakoshi I. Structure and synthesis of a new monoterpenoidal carboxamide from the Seeds of the Thai medicinal plant Acacia concinna. Chem Pharm Bull. 1997; 45: 148-151.

14. Chen J, Komatsu K, Namba T, Yoshizawa T, Yamaji S. Pharmacognostical studies of Chinese folk medicines, Zhang ya cai (Shougasai) and Qing ye dan (Seihatan). Nat Med. 2001; 55: 165-173.

15. Han T, Li HL, Zhang QY, Han P, Zheng HC, Rahman K, et al. Bioactivity-guided fractionation for anti-inflammatory and analgesic properties and constituents of Xanthium strumarium L. Phytomedicine. 2007; 14: 825-829.

16. Ministry of Health. Commonly used herbal plants. 1997; 1: 55-58.

17. Blois MS. Antioxidant determination by the use of a stable free radical. Nature. 1958; 181: 1199-1200.

18. Shimomura O, Wu C, Murai A, Nakamura H. Evaluation of five imidazopyrazinone-type chemiluminescent superoxide probes and their application to the measurement of superoxide anion generated by Listeria monocytogenes. Anal Biochem. 1998; 258: 230-235.

19. Ragazzi E, Veronese G. Quantitative analysis of phenolic compounds after thin-layer chromatographic separation. J Chromatogr. 1973; 77: 369-375.

20. Scartezzini P, Antognoni F, Raggi MA, Poli F, Sabbioni C. Vitamin C content and antioxidant activity of the fruit and of the Ayurvedic preparation of Emblica officinalis Gaertn. J Ethnopharmacol. 2006; 104: 113-118.

21. Meng Q, Velalar CN, Ruan R. Effects of epigallocatechin-3-gallate on mitochondrial integrity and antioxidative enzyme activity in the aging process of human fibroblast. Free Radic Biol Med. 2008; 44: 1032-1041.

22. Simao ANC, Dichi JB, Barbosa DS, Cecchini R, Dichi I. Influence of uric acid and gamma-glutamyltransferase on total antioxidant capacity and oxidative stress in patients with the metabolic syndrome. Nutrition. 2008: 24: 675-681.

23. Curin Y, Andriantsitohaina R. Polyphenols as potential therapeutical agents against cardiovascular diseases. Pharmacol Rep. 2005; 57 Suppl: 97-107.

24. Valcic S, Timmermann BN, Alberts DS, Wächter GA, Krutzsch M, Wymer J, et al. Inhibitory effect of six green tea catechins and caffeine on the growth of four selected human tumor cell lines. Anticancer Drugs. 1996; 7: 461-468.

25. Ciocoiu M, Mirón A, Mares L, Tutunaru D, Pohaci C, Groza M, et al. The effects of Sambucus nigra polyphenols on oxidative stress and metabolic disorders in experimental diabetes mellitus. J Physiol Biochem. 2009; 65: 297-304.

26. Rao TP, Sakaguchi N, Juneja LR, Wada E, Yokozawa T. Amla (Emblica officinalis Gaertn.) extracts reduce oxidative stress in streptozotocin-induced diabetic rats. J Med Food. 2005; 8: 362-368.

27. Kim HJ, Yokozawa T, Kim HY, Tohda C, Rao TP, Juneja LR. Influence of amla (Emblica officinalis Gaertn.) on hypercholesterolemia and lipid peroxidation in cholesterol-fed rats. J Nutr Sci Vitaminol (Tokyo). 2005; 51: 413-418.

28. Yokozawa T, Kim HY, Kim HJ, Okubo T, Chu DC, Juneja LR. Amla (Emblica officinalis Gaertn.) prevents dyslipidaemia and oxidative stress in the ageing process. Br J Nutr. 2007; 97: 1187-1195.

29. Yokozawa T, Kim HY, Kim HJ, Tanaka T, Sugino H, Okubo T, et al. Amla (Emblica officinalis Gaertn.) attenuates age-related renal dysfunction by oxidative stress. J Agric Food Chem. 2007; 55: 7744-7752.

30. Rao TP, Okamoto T, Akita N, Hayasahi T, Yasuda NK, Suzuki K. Amla (Emblica officinalis Gaertn.) extract inhibit lipopolysaccharide-induced procoagulant and pro-inflammatory factors in cultured vascular endothelial cells. Br J Nutr. 2013; 110: 2201-2206.

31. Siriwardhana N, Lee KW, Kim SH, Ha JW, Jeon YJ. Antioxidant activity of Hizikia fusiformis on reactive oxygen species scavenging and lipid peroxidation inhibition. Food Scic Tech Int. 2003; 9: 339-436.

32. DeLoughery TG. Coagulation abnormalities and cardiovascular disease. Curr Opin Lipidol. 1999; 10: 443-448.

33. Maranhão RC, Leite AC. Development of Anti-Atherosclerosis Therapy Based on the Inflammatory and Proliferative Aspects of the Disease. Curr Pharm Des. 2014.

34. Li CT, Wang HB, Xu BJ. A comparative study on anticoagulant activities of three Chinese herbal medicines from the genus Panax and anticoagulant activities of ginsenosides Rg1 and Rg2. Pharm Biol. 2013; 51: 1077-1080.

35. Chen GY, Wu QN, Wang XS, Liang QL, He XX. [Study on quality evaluation of Sparganii rhizoma by biopotency determination method]. Zhongguo Zhong Yao Za Zhi. 2012; 37: 2913-2916.

36. Dobashi K, Nishino T, Fujihara M, Nagumo T. Isolation and preliminary characterization of fucose-containing sulfated polysaccharides with blood-anticoagulant activity from the brown seaweed Hizikia fusiforme. Carbohydrate Res. 1989; 194: 315-320.

37. Mattiello T, Trifirò E, Jotti GS, Pulcinelli FM. Effects of pomegranate juice and extract polyphenols on platelet function. J Med Food. 2009; 12: 334-339.

38. Freedman JE. Oxidative stress and platelets. Arterioscler Thromb Vasc Biol. 2008; 28: s11-16.

39. Vilahur G, Badimon L. Antiplatelet properties of natural products. Vascul Pharmacol. 2013; 59: 67-75.

Austin J Nutr Metab - Volume 1 Issue 1 - 2014
Submit your Manuscript | www.austinpublishinggroup.com

Citation: Rao TP, Htay HH, Yasuda NK, Sugino H, Ohkubo, Hayashi T, et al. Antioxidant and Anti-Thrombotic Properties of Selected Plant Extracts of Asia. Austin J Nutr Metab. 2014;1(1): 6.