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IN VITRO ANTIPLATELET AND ANTICOAGULANT ACTIVITY OF INDIGENOUS VEGETABLES FROM SOUTHERN THAILAND

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ABSTRACT

Objective: Epidemiological studies have indicated that diets rich in fruits and vegetables help reduce the risk of cardiovascular diseases (CVDs). However, data about the antithrombotic activity of local vegetables is rare. The objective of this study was to evaluate antiplatelet and anticoagulant activity in indigenous vegetables with high phenolic compounds collected from Southern Thailand.

Methods: Five selected indigenous vegetables were crudely extracted by distilled water and 80% methanol. The extracts were screened for *in vitro* antiplatelet and anticoagulant activity at a concentration of 10 µg/µl. The antiplatelet activity was measured by inhibition of platelet adhesion to collagen and thrombin-induced platelet aggregation, while the anticoagulant activity was assessed by the prothrombin time (PT) and activated partial thromboplastin time (APTT) tests.

Results: Among the selected vegetables, the extracts of mon-pu (*Glochidion perakense* Hook.f.) and young cashew leaves (*Anacardium occidentale* L.) showed high antithrombotic properties. The highest antithrombotic activity was observed in the methanolic extract of mon-pu, which showed 92.79±0.78% of platelet adhesion inhibition, 102.9±1.53% of platelet aggregation inhibition, and a prolonged APTT assay (48.92±0.94 s). The prolonged APTT but normal PT results suggested that the extract could affect factors VIII, IX, XI, and XII of the intrinsic coagulation pathway.

Conclusion: Our findings demonstrated antiplatelet and anticoagulation properties of indigenous vegetables from Southern Thailand. The multi-potential effects of mon-pu extracts on antithrombosis evidently suggest that mon-pu can be considered as an excellent nutraceutical option in the prevention of thrombosis-related CVDs caused by different mechanisms.

Keywords: Indigenous vegetables, Antiplatelet activity, Anticoagulant activity, Southern Thailand.

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INTRODUCTION

Cardiovascular diseases (CVDs), a group of disorders of the heart and blood vessels, are the most common cause of death globally. Heart attacks and strokes are common events that are caused by sudden blockage of blood flowing to the heart or brain. Its pathogenesis involves fatty deposits inside the wall of the blood vessels and an increased risk of blood clots. It is very well known that thrombus formation after atheroma plaque rupture can be an important step in the pathophysiological mechanisms underlying CVDs, involving activated platelet adhesion, platelet aggregation, the coagulation system, and the fibrinolytic system. In individuals with risk factors such as an unhealthy diet, hyperlipidemia, hypertension, and diabetes, there is an increased risk of developing CVDs [1].

In recent years, the prevention of chronic diseases has mainly focused on fruit and vegetable consumption. Epidemiological data have indicated that a regular diet rich in fruits and vegetables can promote cardiovascular health as well as reduce the risk of CVDs [2]. The effect of fruit and vegetable consumption on health is related to phytochemical diversity; for example, dietary fiber, folate, vitamins, and phenolic compounds. Among these substances, phenolic compounds have been suspected to prevent vascular dysfunction and to have effects on platelets, endothelial functions, and the coagulation system [3].

In the southern region of Thailand, the climate is tropical and humid throughout the year. Thus, this area has indigenous vegetable diversity that is essential to local foods. In addition, these indigenous vegetables have been used for the supply of local medicine, for instance, in the treatment of diabetes, arthritis, hypertension, and gastrointestinal diseases [4,5]. Thai indigenous vegetables have been reported to be useful sources of antioxidants, and many also possess antidiabetic, antibacterial, anti-inflammatory, antimutagenic, and anti-carcinogenic properties [6,7]. On the other hand, information about the antithrombotic activity of southern Thai indigenous vegetables is limited. Some fruits and vegetables have demonstrated properties to inhibit platelet activation and coagulation activity. Antiplatelet activity has been described for garlic (*Allium sativum* L.) [8], onion (*Allium cepa* L.) [9], tomato (*Solanum lycopersicon* Mill.) [10], and green bean (*Phaseolus vulgaris* L.) [11]. Anticoagulant activity has been observed in pineapple (*Ananas comosus* (L.) Merr.) [12], grape (*Vitis vinifera* L.) [11], and raspberry (*Rubus idaeus* L.) [11]. Thus, if people have daily diets with the proper amounts of these fruits or vegetables, it would help to prevent thromboembolism.

In this study, we have selected five indigenous vegetables from Southern Thailand with high phenolic contents and antioxidant activity to investigate their antithrombotic activity [13]. We have evaluated these vegetables for *in vitro* antiplatelet adhesion, antiplatelet aggregation, and anticoagulant activity. It was anticipated that the results of antithrombotic activity in Southern Thai indigenous vegetables could promote Thai vegetable consumption.

METHODS

Sample collection

Indigenous vegetables used in this study were purchased from three regional supply markets in the southern Thai provinces of Nakhon Si

Thammarat and Surat Thani. The indigenous vegetables purchased were harvested in June-July 2018. Specimens were botanically identified by Chatchai Kanlayanapaphon, Ph.D. (Department of Biology, School of Sciences, Walailak University). The voucher specimens were deposited at the Walailak University Herbarium in Nakhon Si Thammarat and the Forest Herbarium in Bangkok, Thailand. Information on the individual vegetables is presented in Table 1.

Vegetable extracts

The vegetables were individually washed with water and rinsed with distilled water. For the extraction preparation, the vegetables were sliced into 50 g each, and then they were homogenized using a homogenizer at maximum speed for 1 min in 150 ml of either methanol (80%) or distilled water. The aqueous extraction of the vegetables was performed using previously described procedures with some modifications [14]. Briefly, the extraction was performed by heat-assisted extraction conditions, and then shaken in a water bath at 50°C for 15 h. The methanolic extraction (methanol:water, 80:20 v/v) was performed using previously described procedures with minor modifications [11]. Briefly, the suspensions were placed at 25 °C under stirring for 15 h. The two suspension types were sedimented by centrifugation at 700 g for 10 min, and then filtrated by using a filter paper (Whatman No. 1). The filtrated suspension of the aqueous extracts was lyophilized to obtain a dried form. The solvent of the methanolic extraction was removed from the extract using a rotary evaporator (Yamato, Rotary Evaporator, Model-RE 801, NY) at a temperature below 40°C. Both extracts were kept at −70°C. Stock solutions of the crude extracts were prepared by thoroughly mixing with 100% dimethyl sulfoxide (DMSO). After preparing the extraction suspension, the final concentration of 10 µg/µl was filtrated with a sterilized 0.22 µm syringe filter before using in the experiment.

Human blood samples

For the experiments, 10 ml of venous blood samples was collected from 30 healthy volunteers, both men and women between the age group of 18–40 years old, who have no medication history at least 10 days before the experiments and no history of bleeding or thrombosis before blood sampling collection. In addition, the volunteers were informed about the research and asked to sign an informed consent form. Protocol No. WUEC-18-024-01 was authorized by the Office of the Human Research Ethics Committee of Walailak University with the Declaration of Helsinki.

Platelet isolation and platelet-poor plasma (PPP) preparation

Venous blood was drawn by venipuncture and then transferred into a centrifuge tube containing 3.2% trisodium citrate solution at a ratio of 9:1 v/v. Platelet isolation was performed according to a previously described method with minor modifications [15]. Briefly, platelet-rich plasma (PRP) was isolated by centrifugation at 270 g for 10 min. PRP was then centrifuged further for 10 min at 2300 g to sediment platelet pellet. PPP was separated and stored at -70 °C for coagulation tests. The pellet was suspended in a Tyrode HEPES buffer (50 mM NaCl, 5 mM KCl, 10 mM glucose, and 10 mM HEPES, pH 7.3) and washed 2 times to remove other cellular debris. The platelets were suspended in a Tyrode HEPES buffer at a final concentration of 2×10^9/ml and immediately used for platelet adhesion and aggregation assays.

Platelet adhesion assay

Adhesion of platelets to collagen was evaluated according to a previously described method with minor modifications [16]. Platelets were pre-incubated with vegetable extracts at 37°C for 10 min, and 1% DMSO and 75 µg/µl of aspirin served as a vehicle control and a positive control, respectively. Using a collagen-coated 96-well plate (Millicoat Human Collagen Type IV Coated Strip, Merck Schuchardt OHG, Hohenbrunn, Germany), the wells were incubated with 200 µl of phosphate buffer saline (PBS) containing 1% BSA for 1 h and then washed 3 times with 200 µl of PBS. Next, 50 µl of platelet suspension was pipetted into each coated well, and 40 µl of the platelet activator, thrombin (0.25 U/ml), was added. The plate was incubated for 60 min at room temperature and then washed 3 times with 200 µl PBS to eliminate unattached platelets. After that, 140 µl of the substrate solution (0.1 M citrate buffer containing 5 mM p-nitrophenyl phosphate, and 0.1 % (w/v) Triton X-100, pH 5.4) was added to each well. The experiment reaction was stopped after 10 min of incubation at 25 °C by adding 100 µl of NaOH (2 N). The absorbance of the reaction was measured at 405 nm using a Multiskan™ GO UV/Vis microplate spectrophotometer (Multiskan GO, Thermo Fisher Scientific, Oy, Finland).

Platelet aggregation assay

Platelet aggregation was determined using a microtiter plate according to a previously described method with minor modifications [17]. Platelet suspension was pretreated with extracts at 37°C for 10 min, and 75 µl of suspension was then pipetted into 96-well plates. Next, 10 µl of thrombin (0.25 U/ml), agonist, was added to the wells. The optical density at 600 nm was read immediately and after incubation for 20 min using a Multiskan™ GO UV/Vis microplate spectrophotometer (Mutiskan GO, Thermo Fisher Scientific Oy, Finland). Platelet aggregation was calculated by subtracting the absorbance value at 20 min from the initial absorbance of the same well followed by normalization with the vehicle control (1% DMSO). Here, 75 µg/µl of aspirin was used as a positive control.

Prothrombin time (PT) activity assay

To evaluate anticoagulation activity of the vegetable extracts against the extrinsic pathways of coagulation, we measured the PT. The PT assay was carried out following the protocols of a previously described method with minor modifications [18]. The tests were conducted in a Coagulogram analyzer (Humaclot Duo Plus, Wiesbaden, Germany). Briefly, 90 µl of PPP was pre-incubated at 37°C for 5 min with 10 µl of a solution of 10 µg/µl of crude extract. Next, 100 µl of PT assay reagent (Thromborel® S, Siemens Healthcare Diagnostics Products GmbH, Germany) was added to initiate the reaction assay, and the clotting time was measured. Here, 1% DMSO was employed as a vehicle control and 3 IU/ml of heparin (Cristalia®, Itapira, SP, Brazil) was used as a positive control. The results of the PT were expressed as clotting time in seconds (s).

Activated partial thromboplastin time (APTT) activity assay

To assess the anticoagulation activity of the indigenous vegetable extracts toward the intrinsic pathway of coagulation, the APTT activity assay was performed following the protocols of a previously described method with minor modifications [18]. The sample of 90 µl of plasma collected from human volunteers was mixed with 10 µl of extract at 37 °C for 5 min. After 5 min, a pre-warmed APTT reagent (Actin® FS APTT, Siemens Healthcare Diagnostics Products GmbH, Germany) was added to the mixture. Clotting time was measured after the addition of 50 µl of 0.025 mol/l $CaCl_2$ solution (Siemens Healthcare Diagnostics Products GmbH, Germany) using a Coagulogram analyzer (Humaclot Duo Plus, HUMAN, Wiesbaden, Germany). Here, 1% DMSO was employed as a vehicle control and 3 IU/ml of heparin (Cristalia®, Itapira, SP, Brazil)

Table 1: The names of the selected vegetables used in the study and their percentage (w/w) of extraction yields

English name	Thai name	Scientific name	Aqueous (%)	Methanol (%)
No English name young leaves	Mon-pu	*Glochidion perakense* Hook.f.	1.61	2.93
Cashew young leaves	Yot mamuang himmaphan	*Anacardium occidentale* L.	2.67	7.00
Turmeric rhizomes	Kha-min-oan	*Curcuma longa* L.	2.53	4.60
Tangerine young leaves	Bai-som-paen	*Citrus reticulata* Blanco.	3.23	3.53
Joint-whip ginger young fruits	Kha-ling	*Alpinia conchigera* Griff.	1.64	3.13

was used as a positive control. The results of the APTT were expressed as clotting time in seconds (s).

Statistical analysis

The results were expressed as means ± standard error of the mean. Statistical comparisons of the data were achieved by a one-way analysis of variance (ANOVA) using GraphPad Prism Version 6.0 (San Diego, California, USA). $p<0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Extract yields

The list of the five selected indigenous vegetables from Southern Thailand and their percentage of extraction yields are shown in Table 1. The yields ranged from 1.61% to 7.00%. Comparatively, the methanol extracts had a higher yield than the aqueous extracts. The highest percent yield was from the young cashew leaves (*Anacardium occidentale* L.) at 7.00% by methanolic extraction. In contrast, the aqueous extract of the young mon-pu leaves (*Glochidion perakense* Hook.f.) showed the lowest percentage yield at 1.61%. It is generally known that phenolic compounds are widespread constituents of plant diets. In addition, they are more soluble in polar organic solvents due to the presence of a hydroxyl group [19]. Based on a previous study from Kongkachuichai *et al.*, these vegetables were shown to be rich in polyphenol compounds (mon-pu: 4,762.76, cashew: 4,075.79, turmeric: 1,037.31, tangerine: 609.22, and joint-whip ginger: 571.88 GAE/100 g) [13]. Hence, methanol could be more efficient in the extraction of major constituents from these vegetables than water.

Antiplatelet activity

Platelet adhesion and aggregation are essential for the formation of a platelet plug at damaged blood vessels. Interfering, the process of platelet activation is one of the therapeutic strategies for the treatment of platelet-related thrombosis [20]. We screened antiplatelet activity of the aqueous and methanolic extracts from the selected vegetables at a concentration of 10 µg/µl. According to the experiments with the five indigenous vegetables, both the aqueous and metabolic extracts of four of the vegetables including mon-pu, cashew, turmeric, and joint whip ginger revealed significant antiplatelet adhesion (17.82–92.79%) and antiplatelet aggregation (16.25–102.9%). In contrast, the methanolic extract of young tangerine leaves (*Citrus reticulata* Blanco) presented only antiplatelet adhesion by approximately 29%. The results of the platelet adhesion assay demonstrated that the methanolic extract of mon-pu and young cashew leaves showed high antiplatelet adhesion activity at 92.79% and 81.10%, respectively. Among the aqueous extracts, the highest inhibitory effect on antiplatelet adhesion was observed in mon-pu (90.08%), as shown in Table 2. In addition to antiplatelet adhesion activity, we found that the methanolic extracts of mon-pu and young cashew leaves presented high antiplatelet aggregation properties by approximately 100% (Table 2). Aspirin at 75 µg/µl concentration was used as a positive control, having percent inhibitions of 87.4±3.14 and 56.74±5.06 against platelet adhesion- and thrombin-induced platelet aggregation, respectively.

We had selected the extracts from mon-pu to study the inhibitory effect toward platelet adhesion and platelet aggregation at various concentrations (1, 2, 3, 4, 5, and 10 µg/µl). The analysis results demonstrated that both the aqueous and methanolic extracts of mon-pu showed dose-dependent platelet adhesion inhibition (Fig. 1) and platelet aggregation inhibition (Fig. 2). However, the methanolic extract had a higher effect toward platelet function than the aqueous extract. The methanolic extract of mon-pu at 1 µg/µl showed approximately 70% inhibition of platelet aggregation (Fig. 2). This dose amount of the extract showed a potentially similar inhibitory effect on platelet aggregation when compared to a previous study on crude rice (*Oryza*

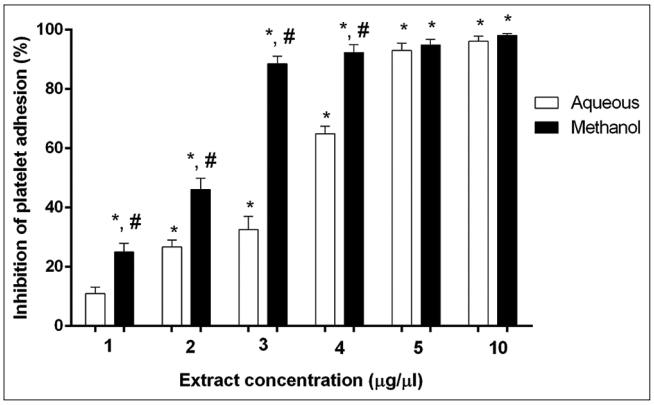

Fig. 1: The percentage of platelet adhesion inhibition of mon-pu extracts at various concentrations. *p<0.05, compared to the vehicle control (1% dimethyl sulfoxide); #p<0.05, compared to the aqueous extract from the same vegetable. Values are expressed in mean±standard error of the mean for each antiplatelet adhesion from duplicate analysis of three samples (n=3)

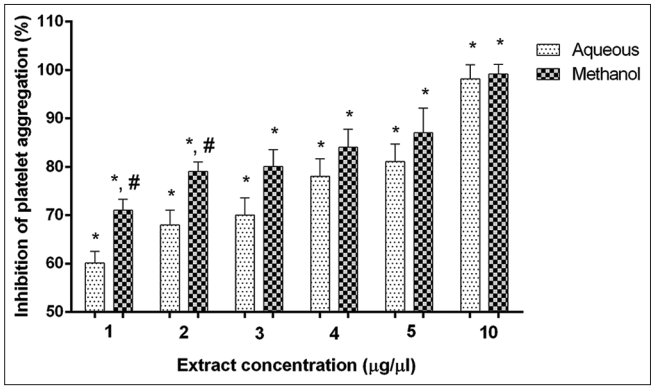

Fig. 2: The percentage of platelet adhesion inhibition of mon-pu extracts at various concentrations. *p<0.05, compared to the vehicle control (1% dimethyl sulfoxide); #p<0.05, compared to the aqueous extract from the same vegetable. Values are expressed in mean ± standard error of the mean for each antiplatelet aggregation from duplicate analysis of three samples (n=3)

Table 2: The percentage of the inhibition of platelet adhesion on collagen and thrombin-induced platelet aggregation of the extracts

Vegetables	Platelet adhesion inhibition (%)		Platelet aggregation inhibition (%)	
	Aqueous	Methanol	Aqueous	Methanol
Mon-pu	90.08±0.71^{*,a}	92.79±0.78^{*,a}	100.5±0.34^{*,a}	102.9±1.53^{*,a}
Cashew young leaves	71.85±3.36^{*,b}	81.10±4.30$^{*,\#,a}$	96.20±4.96^{*,a}	98.16±5.93^{*,a}
Turmeric	53.84±3.16^{*,c}	30.22±4.37$^{*,\#,b}$	16.25±4.01^{*,b}	50.12±4.12$^{*,\#,b}$
Tangerine young leaves	5.81±5.19^{d}	29.19±4.24$^{*,\#,b}$	2.09±1.62^{c}	15.48±3.31^{c}
Joint-whip ginger fruit	25.28±4.12^{*,d}	17.82±1.27^{*,b}	56.81±9.19^{*,d}	93.08±4.08$^{*,\#,a}$

Values are expressed in mean±standard error of the mean for each antiplatelet activity from duplicate analysis of ten samples (n=10); *p<0.05, compared to the vehicle control (1% dimethyl sulfoxide); #p<0.05, compared to the aqueous extract from the same vegetable. Mean values with different subscript letters within the same column of each antiplatelet activity are significantly different at p<0.05

Table 3: Effects of the vegetable extracts on anticoagulant activity based on the prothrombin time and activated partial thromboplastin time of normal human plasma

Vegetables	Prothrombin time (s)		Activated partial thromboplastin time (s)	
	Aqueous	Methanol	Aqueous	Methanol
Mon-pu	12.74±0.35	12.94±0.35	42.92±1.75*	48.92±0.94*
Cashew young leaves	12.99±0.18	12.85±0.17	27.27±0.64	28.37±0.89
Turmeric	14.30±0.17	14.46±0.15	27.32±0.54	27.86±0.61
Tangerine young leaves	13.80±0.15	14.20±0.22	26.64±0.67	28.31±0.77
Joint-whip ginger fruit	13.53±0.27	14.09±0.15	26.90±0.92	26.49±0.59

Values are expressed in mean ± standard error of the mean for each clotting time from triplicate analysis of ten samples (n=10). *p<0.05, compared to the vehicle control (1% dimethyl sulfoxide)

sativa) bran extract [21]. Although, the mon-pu extracts exhibited antiplatelet aggregation properties when thrombin was used as agonists, the potential of the extracts to reduce platelet aggregation stimulated by other agonists such as adenosine diphosphate, arachidonic acid, and collagen should be further investigated.

Several studies have reported that various extracts from vegetables and fruits such as green beans [10,11], tomatoes [11], and berries [22] have presented antiplatelet functions. In addition, the phenolic extracts from berries have been found to have effective antiplatelet functions in both *in vitro* and *in vivo* models [23]. A previous study regarding the total phenolic contents of mon-pu and young cashew leaves have indicated that mon-pu contained abundant amounts of gallic acid, epicatechin gallate, and apigenin [13]; whereas young cashew leaves contained abundant gallic acid, epigallocatechin-3-gallate, epicatechin, quercetin, and kaempferol [13]. Furthermore, the total polyphenol content of mon-pu and young cashew leaves was greater than that in blackberries by approximately 2 times [13]. Interestingly, gallic acid has been reported to inhibit platelet function through decreasing intracellular calcium mobilization and attenuated phosphorylation of PKC and Akt [24]. Moreover, flavonoid compounds, a class of phenolic compounds, isolated from plants have been reported to inhibit platelet adhesion and platelet aggregation as well [25]. The potential mechanisms by which flavonoids inhibit platelet functions include their ability to compete for binding to the thromboxane A(2) receptor and consequently decreasing in TxA(2) generation [26] or increasing platelet cyclic AMP levels [27]. In addition, evidence suggested that flavonoids such as quercetin and catechin significantly inhibited platelet functions by virtue antioxidant activity [25].

Anticoagulant activity

To investigate the effects of the aqueous and methanolic extracts of the selected vegetables on coagulation factors, we conducted the PT test for clotting time (coagulation factors VII, V, X, prothrombin, and fibrinogen) as well as the APTT test (coagulation factors XII, XI, IX, VIII, X, V, prothrombin, and fibrinogen). The study found that the aqueous and methanolic extracts of the selected vegetables had no significant effect on the extrinsic pathway by the PT assay when compared to the vehicle control (PT, 13.76±0.14 s) (Table 3). Regarding the intrinsic pathway, only the aqueous and methanolic extracts of mon-pu significantly increased the APTT (42.92±1.75 s and 48.92±0.94 s, respectively) when compared to the vehicle control (28.02±0.75 s) ($p<0.05$) (Table 3). A dose of 3 IU/ml of heparin was employed as a positive control with prolonged PT (17.23±0.31 s) and prolonged APTT (>200 s). Moreover, we tested the effect of lower concentrations (1, 2, 3, 4, 5, and 10 μg/μl) in the mon-pu extracts by the APTT assay. We found that the extracts showed an anticoagulant effect on the APTT in a dose-dependent manner (Fig. 3). Therefore, this result demonstrated that the extracts of mon-pu contained the bioactive compounds responsible for anticoagulant activity. In clinical assessment, a prolonged PT and/or APTT indicate an abnormality of specific coagulation factors in different coagulation pathways. For instance, abnormally long PT but normal APTT suggests an abnormality in activities of the coagulation factor VII of the extrinsic pathway; whereas if both the PT and APTT are affected, this effect is in the common pathway of coagulation cascades (factors V, X, prothrombin, and fibrinogen). Thus, only prolonged

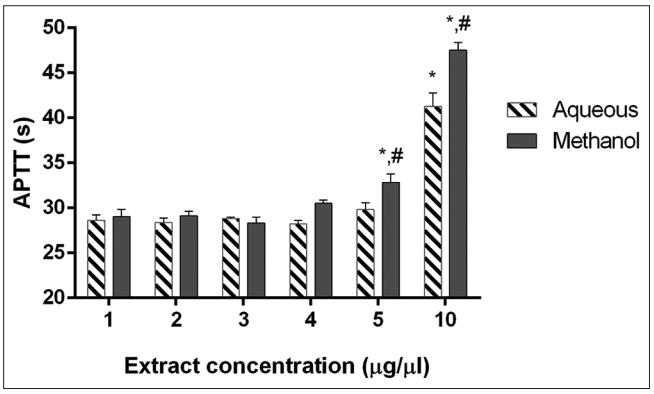

Fig. 3: Anticoagulant activity of the mon-pu extracts with various concentrations using activated partial thromboplastin time assay. *p<0.05, compared to the vehicle control (1% dimethyl sulfoxide); #p<0.05, compared to the aqueous extract from the same vegetable. Values are expressed in mean ± standard error of the mean for each clotting time from triplicate analysis of five samples (n=5)

APTT by the extracts from the mon-pu treatment suggested inhibition of coagulation factors VIII, IX, XI, or XII of the intrinsic pathway. Simple chelation of Ca^{2+} by the extracts, inhibiting coagulation factor activation, could be excluded because the PT was normal. Various plants showed anticoagulant activity against the coagulation factors of the intrinsic pathway, such as *Zingiber cassumunar* Roxb. [28], *Syzygium cumini* L. [29], and raspberry (*R. idaeus* L.) [11]. It was evident that the phytochemicals of *S. cumini* L. such as limonene, flavonoids, and phenolic could play an important role in interfering with the coagulation factors of the intrinsic pathway, resulting in prolonged APTT [29]. In a previous study regarding the effects of phenolic compounds on coagulation factors, Kuntic *et al*. demonstrated that quercetin-3-rutinoside could inhibit factors VIII and IX of the intrinsic coagulation pathway [30].

Collectively, our results suggested that young mon-pu leaves and young cashew leaves are good sources of bioactive compounds with antithrombotic properties, especially mon-pu which has both antiplatelet and anticoagulation activity. In addition, these two vegetables contain a high amount of phenolic contents and a great amount of antioxidant capacity among Southern Thai indigenous vegetables [13,14]. Therefore, the consumption of these vegetables can be beneficial in the prevention of CVDs related to thrombotic events.

CONCLUSION

These findings illustrated some antithrombotic activity in indigenous vegetables from Southern Thailand. Particularly, young mon-pu leaves can be used as a supplementary antithrombotic agent to improve and/or prevent CVDs and, therefore, have an important impact on human health. Further studies are essential to advance in the knowledge of the bioactive phytochemicals of these vegetables and their mechanisms of action.

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CONFLICTS OF INTEREST

All authors have no conflicts of interest to declare.

REFERENCES

1. Anderson KM, Odell PM, Wilson PW, Kannel WB. Cardiovascular disease risk profiles. Am Heart J 1991;121:293-8.
2. Hu FB, Willett WC. Optimal diets for prevention of coronary heart disease. JAMA 2002;288:2569-78.
3. Salvamani S, Gunasekaran B, Shaharuddin NA, Ahmad SA, Shukor MY. Antiartherosclerotic effects of plant flavonoids. Biomed Res Int 2014;2014:480258.
4. Neamsuvan O, Ruangrit T. A survey of herbal weeds that are used to treat gastrointestinal disorders from southern Thailand: Krabi and Songkhla provinces. J Ethnopharmacol 2017;196:84-93.
5. Andarwulan N, Kurniasih D, Apriady RA, Rahmat H, Roto AV, Bolling BW. Polyphenols, carotenoids, and ascorbic acid in underutilized medicinal vegetables. J Funct Foods 2012;4:339-47.
6. Nanasombat S, Yansodthee K, Jongjaited I. Evaluation of antidiabetic, antioxidant and other phytochemical properties of Thai fruits, vegetables and some local food plants. Walailak J Sci Technol 2018;16:856-66.
7. Povichit N, Phrutivorapongkul A, Suttajit M, Chaiyasut C, Leelapornpisid P. Phenolic content and *in vitro* inhibitory effects on oxidation and protein glycation of some Thai medicinal plants. Pak J Pharm Sci 2010;23:403-8.
8. 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;55:1280-8.
9. Ali M, Bordia T, Mustafa T. Effect of raw versus boiled aqueous extract of garlic and onion on platelet aggregation. Prostaglandins Leukot Essent Fatty Acids 1999;60:43-7.
10. Concha-Meyer A, Fuentes E, Palomo I. Antiplatelet protocol: Effects of ingesting a tomato pomace extract on human platelet aggregation. Methodsx 2019;6:1847-53.
11. Torres-Urrutia C, Guzman L, Schmeda-Hirschmann G, Moore-Carrasco R, Alarcon M, Astudillo L, *et al*. Antiplatelet, anticoagulant, and fibrinolytic activity *in vitro* of extracts from selected fruits and vegetables. Blood Coagul Fibrinolysis 2011;22:197-205.
12. Hawkey C, Howell M. Orally induced fibrinolysis. A critical assessment of pineapple protease. Thromb Diath Haemorrh 1964;12:382-90.
13. Kongkachuichai R, Charoensiri R, Yakoh K, Kringkasemsee A, Insung P. Nutrients value and antioxidant content of indigenous vegetables from Southern Thailand. Food Chem 2015;173:838-46.
14. Panpipat W, Suttirak W, Chaijan M. Free radical scavenging activity and reducing capacity of five Southern Thai indigenous vegetable extracts. Walailak J Sci Technol 2010;7:51-60.
15. Araki J, Jona M, Eto H, Aoi N, Kato H, Suga H, *et al*. Optimized preparation method of platelet-concentrated plasma and noncoagulating platelet-derived factor concentrates: Maximization of platelet concentration and removal of fibrinogen. Tissue Eng Part C Methods 2012;18:176-85.
16. Eriksson AC, Whiss PA. Measurement of adhesion of human platelets in plasma to protein surfaces in microplates. J Pharmacol Toxicol Methods 2005;52:356-65.
17. Krause S, Scholz T, Temmler U, Losche W. Monitoring the effects of platelet glycoprotein IIb/IIIa antagonists with a microtiter plate method for detection of platelet aggregation. Platelets 2001;12:423-30.
18. Mao WJ, Li HY, Li Y, Zhang HJ, Qi XH, Sun HH, *et al*. Chemical characteristic and anticoagulant activity of the sulfated polysaccharide isolated from *Monostroma latissimum* (*Chlorophyta*). Int J Biol Macromol 2009;44:70-4.
19. Xu BJ, Chang SK. A comparative study on phenolic profiles and antioxidant activities of legumes as affected by extraction solvents. J Food Sci 2007;72:S159-66.
20. Ruggeri ZM. Mechanisms initiating platelet thrombus formation. Thromb Haemost 1997;78:611-6.
21. Wong WT, Ismail M, Imam MU, Zhang YD. Modulation of platelet functions by crude rice (*Oryza sativa*) bran policosanol extract. BMC Complement Altern Med 2016;16:252.
22. Olas B. The multifunctionality of berries toward blood platelets and the role of berry phenolics in cardiovascular disorders. Platelets 2017;28:540-9.
23. Chong MF, Macdonald R, Lovegrove JA. Fruit polyphenols and CVD risk: A review of human intervention studies. Br J Nutr 2010;104:S28-S39.
24. Chang SS, Lee VS, Tseng YL, Chang KC, Chen KB, Chen YL, *et al*. Gallic acid attenuates platelet activation and platelet-leukocyte aggregation: Involving pathways of Akt and GSK3 beta. Evid Based Complement Altern 2012;2012:683872.
25. Pignatelli P, Pulcinelli FM, Celestini A, Lenti L, Ghiselli A, Gazzaniga PP, *et al*. The flavonoids quercetin and catechin synergistically inhibit platelet function by antagonizing the intracellular production of hydrogen peroxide. Am J Clin Nutr 2000;72:1150-5.
26. Guerrero JA, Lozano ML, Castillo J, Benavente-Garcia O, Vicente V, Rivera A. Flavonoids inhibit platelet function through binding to the thromboxane A(2) receptor. J Thromb Haemost 2005;3:369-76.
27. Iman RA, Priya BL, Chitra R, Shalini K, Vasanth J. *In vitro* antiplatelet activity guided fractionation of aerial parts of *Melothria maderaspatana*. Indian J Pharm Sci 2006;68:668-70.
28. Sukati S, Jarmkom K, Techaoei S, Wisidsri N, Khobjai W. *In vitro* anticoagulant and antioxidant activities of prasaplai recipe and *Zingiber cassumunar* Roxb. extracts. Int J Appl Pharm 2019;11:26-30.
29. Rehman AA, Riaz A, Asghar MA, Raza ML, Ahmed S, Khan K. *In vivo* assessment of anticoagulant and antiplatelet effects of *Syzygium cumini* leaves extract in rabbits. BMC Complement Altern Med 2019;19:236.
30. Kuntic V, Filipovic I, Vujic Z. Effects of rutin and hesperidin and their Al(III) and Cu(II) complexes on *in vitro* plasma coagulation assays. Molecules 2011;16:1378-88.