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Guava Leaf Extract and Topical Haemostasis

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The effects of guava leaf extract on the bleeding time and the three main mechanisms of haemostasis: vasoconstriction, platelet aggregation and blood coagulation, were investigated. The water extract of guava leaves did not shorten bleeding times in rats. Guava leaf extract potentiated the vascular muscle contraction induced in rabbits by phenylephrine, and when given alone it stimulated human platelet aggregation *in vitro* in a dose-dependent manner. On the other hand, it significantly prolonged blood coagulation; activated partial thromboplastin time (APTT) test ($p < 0.05$). The higher the concentration of the extract, the longer APTT was observed. Thus, a water extract of guava leaves showed ambiguous effects on the haemostatic system. Guava leaf extract did not affect bleeding times, it stimulated vasoconstriction and platelet aggregation but it inhibited blood coagulation. Therefore, guava leaf extract is not recommended as a haemostatic agent.

Keywords: guava leaf; haemostasis; vasoconstriction; platelet aggregation; blood coagulation.

INTRODUCTION

Psidium guajava Linn is commonly known as guava, Psidium, Farang (Thai). This plant belongs to the Myrtaceae family and is native to Thailand (Pery, 1980) and Asia. *P. guajava* has been claimed to be useful in the treatment of diarrhoea and dysentery (Farnsworth and Bunyapraphatsara, 1990; Saralamp *et al.*, 1996). An alcohol extract of guava leaves exhibited spasmolytic effects on isolated rat and guinea-pig ileum (Lozoya *et al.*, 1990). When applied in the mouth, it was used to treat mouth sores and gum swelling, to aid wound healing and to prevent bad odour (Pongboonrod, 1979). Patients using a mouth rinse containing *P. guajava* leaf extract showed a significant reduction in gingivitis and had fewer sites with severe gingival disease than those using a placebo mouth rinse (Kraivaphan *et al.*, 1991). Antibacterial, antiviral and antihyperglycaemic activities in alloxan-diabetic rats have been demonstrated in biological testing of extracts of *P. guajava* (Collier and Van de Piji, 1949; Malcolm and Sofowara, 1969; Simons *et al.*, 1963; Maruyama *et al.*, 1985). An ethanol extract of *P. guajava* leaves was able to inhibit the growth of *Vibrio cholerae* and *Vibrio parahaemolyticus* which cause diarrhoea (Gritsanapan and Chulasiri, 1983). Leaves of *P. guajava* have been reported to contain several compounds, e.g. sesquiterpene (sesquiterguavaene: Bhati, 1967), triterpenoid (oleanolic acid: Arthur and Hui, 1954; Osman *et al.*, 1974), flavonoid (quercetin, guaijavarin, leucocyadin, amritoside: Seshadri and Vasishta, 1965; Seetharaman and Manjula, 1996), coumarin (ellagic acid: Seetharaman and Manjula, 1996), alkaloid and tannin (guavin A, B, C and D: Okuda *et al.*, 1984; Okuda *et al.*, 1987). Quercetin, the best known flavonoid from guava leaf, exerts spasmolytic action through a calcium-mediated mechanism (Capasso *et al.*, 1991; Morales *et al.*, 1994).

In China, guava leaves are used as antiinflammatory and haemostatic agents (Hon-Ning, 1988). They are also used as a topical haemostatic agent and externally used as an astringent for wounds in Thailand (The Office of the Primary Health Care; 1994; Aajsalee, 1981; Aajsalee, 1979). There is little scientific evidence for a haemostatic effect of guava leaves. In this study, we investigated effects of guava leaf extract on the bleeding time and the three mechanisms of haemostasis: vasoconstriction, platelet aggregation and blood coagulation.

MATERIALS AND METHODS

Plant material. Fresh leaves of guava were collected from Samut Songkram Province, Thailand. The plant was identified by an officer of the Royal Forestry Department, Ministry of Agriculture and Co-operatives. Voucher specimens were deposited at Bangkok Herbarium, Botany and Weed Science section, Department of Agriculture, Ministry of Agriculture, Bangkok, Thailand and filed under code BK 62543 and BK 62544.

Animals. Wistar rats (180–200 g) were obtained from the National Laboratory Animal Center, Mahidol University, Salaya. Rabbits (locally bred, 1.5–2 kg) were purchased from Bangkunthien market. The animals were housed in the animal room of the Faculty of Pharmacy, Mahidol University. Food and water were available *ad libitum*.

Blood sample. Human blood was obtained from 50

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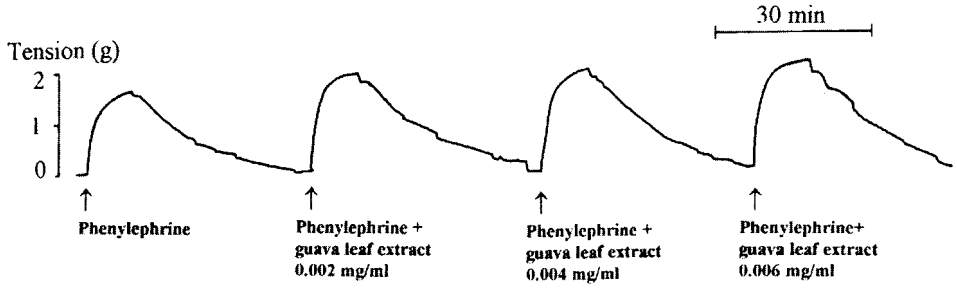

Figure 1. Tracing showing the effect of guava leaf extract on the contraction of rabbit aortic strips induced by phenylephrine. The spiral aortic strips (1 cm long) were placed in 25 mL water-jacketed organ baths containing Krebs–Henseleit solution maintained at 37 °C and aerated with a 95% oxygen and 5% carbon dioxide gas mixture. After 45 min of equilibration time, guava leaf extract at final concentrations of 0.002, 0.004 or 0.006 mg/mL was added and then the spiral aortic strips were induced to contract by a submaximal dose of phenylephrine (final concentration of phenylephrine: 4 μg/mL). Two minutes after the maximal tension was achieved, aortic strips were washed 2–3 times, refilled by Krebs–Henseleit solution then left to return to basal tension.

healthy donors aged between 25 and 45 years old who had not taken any medication known to affect blood coagulation and platelet function for 2 weeks. Venous blood was obtained and transferred to a plastic tube. Nine volumes of blood were decalcified with one volume of 3.8% sodium citrate solution. The blood was tested within 4 h of venepuncture.

Reagents. Activated thrombofax (Ortho Diagnostic system, USA), ADP (M.W. 427.2, Sigma Chemical Co., USA), adrenaline hydrogen tartrate (M.W. 333.3, BPH Chemical Limited, England), and phenylephrine (Thainakornpatana, Bangkok, Thailand) were used. Human brain thromboplastin, thrombin and collagen were prepared from Siriraj Hospital, Bangkok, Thailand.

Preparation of guava leaf extract. Guava leaves (4 kg) were washed, chopped into small pieces, and ground in blender with water (2560 mL). The leaf extract was filtered through muslin cloth and then through filter paper (Whatman paper no.1) with the aid of a suction pump. The filtrate was lyophilized to dryness (37.2 g). The dried extract was kept in an air tight bottle and freshly redissolved in normal saline solution before use.

Effect of guava leaf extract on bleeding time in rats. Thirty female Wistar rats were studied. An incision was made in a foot pad of each rat using a surgical blade no. 11 to make a wound 1 cm long and 0.1 cm deep. These rats were divided into three groups. The control group had normal saline solution 0.05 mL applied topically to the wound. The second and third groups were treated with guava leaf extract 0.05 mL, at concentrations of 1:10 and 1:5 w/v, respectively, topically applied to the wounds. Bleeding time (the duration of time after making the wound until bleeding stopped) was recorded. Cessation of bleeding was indicated by no blood flow to the collecting tube and was confirmed by no blood stain on a filter paper used to blot the wound.

Effects of guava leaf extract on the contraction of isolated vascular smooth muscle. Rabbits were killed and the descending aorta was removed, cleaned, and cut into a spiral shape. The spiral aortic strips (1 cm long) were placed in 25 mL water-jacketed organ baths containing Krebs–Henseleit solution maintained at 37 °C and aerated with a 95% oxygen and 5% carbon dioxide gas mixture. The spiral aortic strips were attached to a force-displacement transducer and Dynograph recorder under a resting tension of 1 g. After 45 min of equilibration time, guava leaf extract at final concentrations of 0.002, 0.004 or 0.006 mg/mL were added and then the spiral aortic strips were induced to contract by a submaximal dose of phenylephrine (final concentration of phenylephrine: 4 μg/mL). The amplitude of vasoconstriction in tension (g) was recorded.

Effect of guava leaf extract on platelet aggregation. Platelet aggregation was induced by ADP 20 μM. Various concentrations (at final concentrations 0.25, 0.5, 1.0 and 2.0 mg/mL) of guava leaf extract were added to platelet rich plasma and the pattern of aggregation was recorded using an aggregometer (Payton aggregation module, dual channel, USA) (Born and Cross, 1963).

Effect of guava leaf extract on blood coagulation. Blood coagulation was examined by determining prothrombin time (PT), activated partial thromboplastin time (APTT) and thrombin time (TT) of normal plasma by the methods of Denson, Quick and Langdell, respectively (Denson, 1976; Quick, 1935; Langdell *et al.*, 1953). Various concentration of guava leaf extract (at final concentrations 0.625, 1.25, 2.5 and 6 mg/mL) were used. In the control group, normal saline solution was used instead of guava leaf extract.

Statistical analysis. Statistical differences were tested using Student's *t*-test (paired group). A level of probability of 0.05 was accepted as significant.

RESULTS

Effect on bleeding time in rats

Topical application of guava leaf extract at concentrations of 1:5 and 1:10 had no significant effect on bleeding time (85.00 ± 6.30 and 82.99 ± 6.74 s, respectively vs control 81.20 ± 5.91 s, $n = 30$).

Table 1. Potentiation of rabbit aortic tension by guava leaf extract ($n = 6$)

	Aortic tension (g)
Phenylephrine	1.43 ± 0.16
Phenylephrine + guava 0.002 mg/mL	1.67 ± 0.22
Phenylephrine + guava 0.004 mg/mL	1.78 ± 0.23[a]
Phenylephrine + guava 0.006 mg/mL	1.74 ± 0.26[a]

$p < 0.05$ compared with phenylephrine-treated group.

Effect on vasoconstriction

Guava leaf extract significantly potentiated the contraction of blood vessels induced by phenylephrine (Fig. 1, Table 1) when added at concentrations of 0.004 mg/mL and 0.006 mg/mL ($p < 0.05$)

Effect on platelet aggregation

Guava leaf extract at a 0.25 mg/mL final concentration induced aggregation of human platelets in a dose-dependent manner. Furthermore, guava leaf extract could potentiate human platelet aggregation induced by ADP 20 μM in a dose-dependent manner (Table 2).

Effect on blood coagulation

Guava leaf extract significantly prolonged the coagulation time. The APTT test was most sensitive to the guava leaf extract. However, at a very high concentration of guava leaf extract (5 mg/mL, final concentration) the PT and TT were also prolonged (Table 3).

DISCUSSION

Guava leaf extract showed no effect on bleeding time when applied topically to the wound. Guava leaf has been widely used to stop bleeding (Hon-Ning, 1988). Thus, we further studied its effects on the main mechanisms in haemostasis. Haemostasis consists of five mechanisms: vasoconstriction, platelet aggregation, blood coagulation, clot retraction and clot dissolution. The first three mechanisms contribute to stop bleeding while the latter two mechanisms contribute to join the edges of broken vessels and allow blood flow to be reestablished (Porth, 1998). In this study we focused only on the three main mechanisms to stop bleeding. We found that, for the stimulation of haemostatic process, guava leaf extract exhibited both platelet-aggregating properties and a vasoconstrictive effect in a large artery, the aorta, in a dose-dependent manner. However, although guava extract potentiated the vasoconstrictive effect induced by phenylephrine, it did not induce blood vessel constriction by itself. In contrast, for the inhibition of haemostasis, guava leaf extract inhibited coagulation or the blood clotting process. The prolongation of coagulation, especially on APTT with little effect on PT and TT, was similar to the effect of heparin (serine protease inhibitor). Heparin exhibited the antithrombin III (AT-III) in order to inhibit blood clotting factors, factor Xa, II and XII. (Keijer *et al.*, 1991; Bombardini and Picano, 1997; Dace *et al.*, 1997). Guava leaf extract may have similar coagulation effects to heparin. However, AT-III was not measured in this study and the coagulation action requires further investigation.

Quercetin, the best known flavonoid from guava leaf, has various pharmacological properties, e.g. it causes vasodilatation and antiplatelet aggregation (Lin *et al.*, 1997; Janssen *et al.*, 1998). The guava extract must be hydrolysed in the gastrointestinal tract to produce quercetin (Lozoya *et al.*, 1990). Flavonoids were not detected in guava leaf extract using the cyanidin and the leucoanthocyanin test (personal communication with Dr W. Chuakul). Free quercetin was also not detected in guava leaf extract by thin layer chromatography (TLC) but trace amounts of free quercetin could be detected by TLC when the extract was hydrolysed with 20% sulphuric acid (unpublished data). In this study, the guava leaf extract was applied topically and thus was not hydrolysed to produce free quercetin. Quercetin produced the opposite haemostatic mechanisms from the guava leaf extract. Therefore, the effects of guava extract on the mechanisms of haemostasis in this study were not related to quercetin.

In conclusion, guava leaf extract did not shorten the bleeding time in rats. It promoted haemostasis by stimulation of platelet aggregation and vasoconstriction but inhibited blood coagulation. Thus, guava leaf extract has ambiguous effects on haemostasis. Therefore, guava leaf extract is not recommended as a haemostatic agent.

Table 2. Effects of guava leaf extract on human platelet aggregation ($n = 10$)

Concentration of guava leaf extract (mg/mL)	% Aggregation without ADP	% Aggregation with 20 μM ADP
0	0	51.0 ± 15.7
0.25	10.63 ± 6.5[a]	51.25 ± 8.7
0.5	46.75 ± 14.5[a]	69.25 ± 17.3[a]
1.0	69.25 ± 14.9[a]	75.9 ± 12.3[a]
2.0	75.5 ± 17.3[a]	76.9 ± 20.1[a]

$p < 0.05$ compared with control group.

Table 3. Effects of guava leaf extract on blood coagulation ($n = 10$)

Concentration of guava leaf extract (mg/mL)	APTT (s)	PT (s)	TT (s)
0	40.7 ± 3.3	13.7 ± 0.8	8.0 ± 0
0.625	47.4 ± 5.6	13.4 ± 1.4	8.3 ± 0.6
1.25	60.3 ± 14.3[a]	13.6 ± 0.7	8.0 ± 0
2.5	79.7 ± 19.4[a]	14.1 ± 0.8	8.8 ± 1.5
5.0	276.7 ± 26.2[a]	18.8 ± 3.0[a]	19.6 ± 3.2[a]

$p < 0.05$ compared with control group.

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