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ScienceDirect
Food Chemistry 103 (2007) 528–535
Food Chemistry
www.elsevier.com/locate/foodchem

Preventive effects of guava (*Psidium guajava* L.) leaves and its active compounds against α-dicarbonyl compounds-induced blood coagulation

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Received 14 April 2006; received in revised form 31 July 2006; accepted 24 August 2006

Abstract

Diabetes is associated with a hypercoagulable state which may accelerate atherosclerosis, thrombosis and the diabetic microvascular complication. Endogenously produced α-dicarbonyl compounds are linked to the pathophysiology of diabetic complications. The effects of α-dicarbonyl compounds on coagulation parameters in vitro and the anticoagulant activities of aqueous extracts from guava leaves were examined. Incubation of plasma with glyoxal or methylglyoxal at 0.1 mM showed a significant decrease in thrombin clotting time (TT) ($P < 0.05$). However, they exhibited slight prolongation of the prothrombin time (PT) at 0.5 mM and no effect on the activated partial thromboplastin time (APTT). In order to define the action mechanism of the hypercoagulant activity, coagulation factors such as fibrinogen and antithrombin III activity were evaluated. The fibrinogen contents in plasma were decreased slightly with increasing concentrations of glyoxal and methylglyoxal. Moreover, methylglyoxal inhibited antithrombin III activity and over 80% of the activity was lost at 1.2 mM methylglyoxal. In contrast, guava leaf extracts exhibited significant inhibition of TT shortening induced by methylglyoxal. Guava leaf extracts and its active phenolic compounds including ferulic acid, gallic acid and quercetin also displayed a protective effect against methylglyoxal-induced loss of activity of antithrombin III. Thus, guava leaf extracts are a potent antiglycative agent and anticoagulant, which can be of great value in the preventive glycation-associated cardiovascular diseases in diabetes.

Keywords: α-Dicarbonyl compounds; Methylglyoxal; Blood coagulation; Guava leaf extracts

1. Introduction

Patients with diabetes mellitus are known to have an increased risk of cardiovascular disease, including atherosclerosis. Eighty percent of type II diabetic patients die of thrombotic complications; 75% of these deaths result from cardiovascular events and the remainder is due to cerebrovascular events and peripheral vascular complications (Carr, 2001; Marks & Raskin, 2000). The pathogenic factors contributing to cardiovascular disease associated with diabetes are not fully understood. Platelet hypersensitivity, endothelial cell dysfunction and alterations in coagulation mechanisms have been observed in diabetic patients and are implicated as possible factors contributing to vascular complications. Many investigators have shown that diabetic patients have enhanced platelet function and hypercoagulability (Bell, 1996). The hypercoagulation in diabetes is observed in increased plasma levels of the coagulative factors, including fibrinogen, factor VII, VIII, XI and XII, in decreased concentration of antithrombotic factors including antithrombin III and protein C (Carr, 2001; Schneider

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doi:10.1016/j.foodchem.2006.08.022

& Sobel, 2001). In the screening test for the coagulation pathway, the prothrombin time (PT) and activated partial thromboplastin time (APTT) were also shorter in diabetics (Acang & Jalil, 1993).

The hypercoagulable state in diabetes is possibly related to hyperglycemia (Ceriello, 1993). Aoki et al. (1996) indicated that good glycemic control might help to correct a hypercoagulable state in diabetic patients. Hyperglycemia is regarded as one key causal factor in the development of diabetic vascular complications. A large body of evidence converges to point to glycation as one key molecular basis of diabetic complications due to hyperglycemia (Carr, 2001; Gugliucci, 2000). In the glycation reaction, the chemical reaction between the aldehyde group of sugars and the amino group of proteins termed nonenzymatic glycation to form early glycation products (Amadori or fuctosamine). These early glycation products are considered to be intermediates in the reaction to form α-dicarbonyl compounds such as deoxyglucosone, methylglyoxal and glyoxal. They form afterwards irreversible advanced glycation end products (AGEs) which include heterogeneous structures of complex modifications (Gugliucci & Menini, 2002). Early products and AGEs accumulate in various tissues. They are capable of producing cross-linking of proteins and play an important role in diabetic pathology. Glycation of antithrombin III by glucose has been shown to decrease the binding to heparin and some in vivo data suggesting susceptibility for antithrombin III glycation in uncontrolled diabetes mellitus (Ceriello et al., 1990). Gugliucci and Menini (2002) also reported that a dose-dependent decrease in antithrombin III activity was shown when plasma was incubated with methylglyoxal. Moreover, AGEs exhibited an enhancing effect on platelet aggregability (Hasegawa, Suehiro, Higasa, Namba, & Kakishita, 2002). These data suggest that α-dicarbonyl compounds and AGEs may contribute to hypercoagulation and platelet hypersensitivity, resulting thrombus formation in the vasculature of diabetes patients.

Blood coagulation and platelet aggregation are crucial events in the pathogenesis of thrombotic disease. Therefore, the anticoagulant and antithrombotic agents also play major roles in preventing cardiovascular diseases. Several antithrombotic agents, such as heparin and aspirin, have been used for prevention and treatment of thromboembolic disorders. However, the use of heparin may be accompanied by side effects such as bleeding complications and a high incidence of aspirin resistance in diabetics has been reported (Watala et al., 2004). Therefore, there has been renewed interest in the use of natural compounds as anticoagulant and antithrombotic compounds for diabetes patients.

Based on the above mentioned observations, it appears that glycation, hypercoagulability and platelet dysfunctions contribute to the development of cardiovascular pathogenesis in diabetes. Therefore, the agents with antiglycation, anticoagulant and antiplatelet properties would have priority for search to alleviate diabetic complications. Guava (*Psidium guajava* L.) is widely cultivated and its fruit is popular in Asia. Guava was also used as a hypoglycemic in folk medicine. The leaves and skin of the fruit have greater effects. Guava tea, the infusion of dried guava fruit and leaves has recently become popular as a drink in Taiwan. Cheng and Yang (1983) reported that guava juice exhibit hypoglycemic effects in mice. The potent antioxidant activity in guava leaf extracts were previously reported and attributed to their phenolic compounds (Chen & Yen, 2007). Moreover, the guava leaf extracts also exhibited excellent antiglycation effect, being a rather powerful and effective inhibitor on low density lipoprotein glycation in both glucose- and glyoxal-induced models (Hsieh et al., 2005). In the present study, the inhibitory effects of guava leaf extracts against hypercoaulant effects induced by α-dicarbonyl compounds to evaluate the possible capacity of guava leaf extracts as anticoagulant in alleviating the development of cardiovascular complications in diabetes were examined.

2. Materials and methods

2.1. Material and chemicals

Psidium guajava L. budding leaves were provided by the local Sur-Tou Agricultural Community Association in Chang-Hua, Taiwan, their origins were identified and proved by the Institute of Medical Herb (Taichung, Taiwan). Glucose, glyoxal, methylglyoxal, aminoguandine, THROMBOMAX®HS WITHCALCIUM (T9561), ALEXIN™ (A1801/A1926), ACCUCLOT™ THROMBIN TIME (A8713/A4589) and FIBRINOGEN (886) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Protein assay kit purchased from BIO-RAD Co. (Hercules, CA). All other reagents were of analytical grade.

2.2. Sample preparation

Twenty grams of guava leaves were repeatedly extracted thrice with boiling water (200 ml) for 30 min. The extracts were filtered through a Whatman No. 2 paper and combined; the filtrate was lyophilized and pulverized. The percent yield (%, w/w on the starting dry base) of guava leaf extracts was 9.05.

2.3. Plasma preparation

Blood from healthy, euglycemic and normolipidemic volunteers was obtained by venipuncture and collected in evacuated tubes. Sodium citrate (0.38% final concentration) was used as an anticoagulant. Blood was centrifuged at 583*g*, at 4 °C for 10 min and separated plasma was immediately frozen at −80 °C until use.

2.4. Plasma glycation and coagulation parameters

Plasma (5.0 mg/ml final concentration) was incubated under sterile conditions in the absence or presence of

glucose (20–160 mM final concentration), glyoxal (0.1–2.5 mM final concentration) or methylglyoxal (0.1–2.5 mM final concentration) for a period of 2 h. Samples were incubated in the presence or absence of guava leaf extracts (0.001–0.05 mg/ml) or aminoguanidine (0.05 mM). Reactions were stopped by freezing at −80 °C and samples were processed on the same day. To rule out direct interference of guava leaf extracts on enzymatic activities, controls containing extracts with no addition of glucose, glyoxal or methylglyoxal were also run in parallel. The PT, APTT, thrombin clotting time (TT), and fibrinogen level were measured and recorded in a coagulometer (model KC4A, Amelung, German).

2.5. Plasma glycation and antithrombin III activity assay

Antithrombin III activity was measured with a chromogenic method as the reciprocal of the residual thrombin activity in a chromogenic assay using SAR-PRO-ARG-pNA as a specific thrombin substrate (Frantzen, Abildgaard, & Aasen, 1983). Briefly, plasma diluted 1:4 (v/v) in 10 mM PBS (pH 7.4) was incubated at 37 °C for 48 h with 1.2 mM methylglyoxal and 0–0.5 mg/ml guava leaf extracts. The reaction mixture (80 μL) was added to 90 μL thrombin solution (30 NIH U/ml) and 6 μL Heparin (1 U/μL), and incubated at 37 °C for exactly 20 min. Then, 20 μL of SAR-PRO-ARG-pNA (3.1 mM) was added and incubated at an ambient temperature for 2 min, and the absorbance was read at 385 nm.

2.6. HPLC analysis of phenolic compounds

Dried guava leaf extracts (20 mg) were dissolved in 10 ml 1 M HCl and then heated in a boil water bath for 1 h. After acid hydrolysis, the cooled solution was reextracted thrice by ethyl ether (20 ml). The combined ethyl ether layer was evaporated to dryness under nitrogen and the residue was redissolved in methanol (2.35 mg/ml final concentration) and was filtered through a 0.2 μm filter and analyzed by HPLC. HPLC performed with a Hitachi liquid chromatograph (Hitachi, Ltd., Tokyo, Japan) consisting of a model L-6200 pump, and a model L-4200 UV–Vis detector set at 280 nm. A reversed phase C_{18} Luna column (15 × 0.2 cm; particle size 5 μm, Phenomenex, Torrance, CA,) was used for HPLC analysis. The mobile phase consisted of two solvents; acetonitrile contained 17.5 mM glacial acetic acid (A) and 1% acetic acid (B). The solvent gradient in volumetric ratios was as follows: initial 10% A linear gradient to 25% A in 8 min; linear gradient to 80% A in 22 min and hold for 5 min; linear gradient to 10% A in 10 min. The flow rate was 0.2 ml/min. Phenolic compounds were identified by comparison of their retention time (R_t) values and UV spectra with those of known standards and determined by peak areas from the chromatograms.

2.7. Statistical analysis

All analyses were run in triplicate and mean values reported. Statistical analyses were performed according to the SAS Institute User's Guide. Analyses of variance were performed using the ANOVA procedure. Significant differences ($P < 0.05$) between the means were determined using Duncan's multiple range test.

3. Results

3.1. Effects of various AGEs precursors on PT, APTT, TT, and fibrinogen level

The effects of glucose, glyoxal and methylglyoxal on coagulation were evaluated by assays of APTT, PT, TT and fibrinogen content. As shown in Fig. 1, glucose did not change APTT, PT, TT and fibrinogen content. Glyoxal and methylglyoxal prolonged PT 44.95 ± 0.78 and 47.15 ± 0.21 s at concentration of 0.6 mM, respectively. However, TT was significantly decreased by incubation of plasma with glyoxal or methylglyoxal at 0.1 mM ($P < 0.05$). The fibrinogen contents in plasma were decreased slightly with increasing the concentrations of glyoxal.

3.2. Protective effect of guava leaf extracts on α-dicarbonyl compounds-induced hypercoagulation

The protective effect of guava leaf extracts on the α-dicarbonyl compounds-induced TT reduction is shown in Fig. 2. TT was reduced significantly by glyoxal and methylglyoxal at concentration of 0.1 mM. Guava leaf extracts (0.001 mg/ml) exhibited significantly inhibition on hypercoagulation induced by methylglyoxal, whereas no inhibition was observed in APTT, PT and fibrinogen assays at the same concentration (data not shown).

3.3. Determination of phenolic compounds in guava leaf extracts

Fig. 3 shows the chromatogram of mixed standards and guava leaf extracts. The results of HPLC analyses showed that three main peaks were found in guava leaf extracts at 280 nm. Gallic acid ($R_t = 4.96$ min), ferulic acid ($R_t = 35.87$ min) and quercetin ($R_t = 40.99$ min) were identified by comparison of their retention time values and UV spectra with those of known standards. The contents of three phenolic compounds in guava leaf extracts are shown in Table 1. The contents of gallic acid and quercetin were slightly higher than that of ferulic acid.

3.4. Effects of guava leaf extracts and its active compounds on methylglyoxal-induced loss of activity of antithrombin III

Table 2 shows the effects of guava leaf extract and its active compounds on methylglyoxal-induced loss of activ-

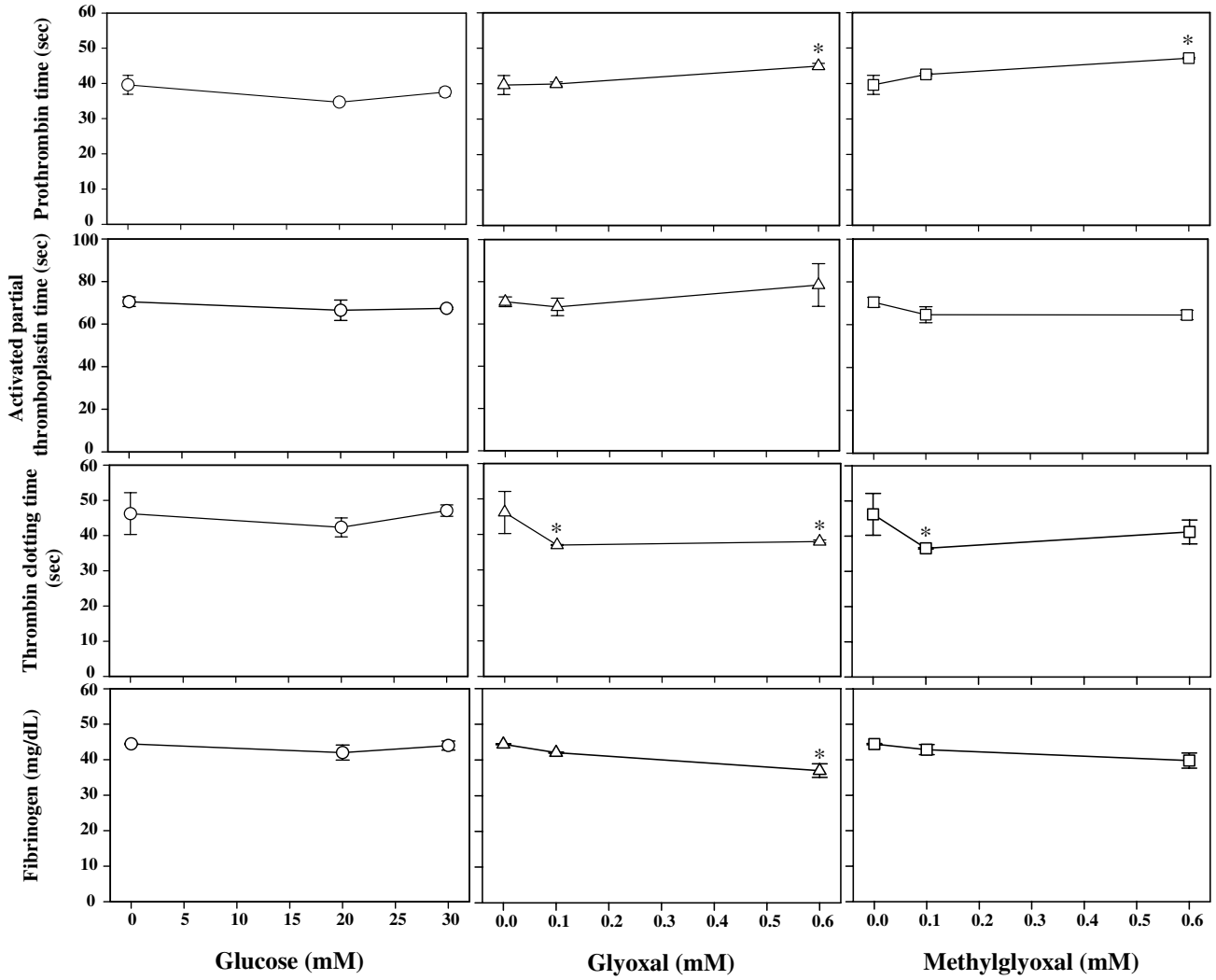

Fig. 1. Effects of various AGEs precursors on human plasma coagulation. Plasma was incubated with glucose (0–30 mM), glyoxal (0–0.6 mM) or methylglyoxal (0–0.6 mM) at 37 °C for 2 h. Data expressed in mean ± standard deviation from triplicate experiments. $^{*}P < 0.05$ versus controls.

ity of antithrombin III. Methylglyoxal inhibited significantly antithrombin III activity and over 80% of the activity was lost at 1.2 mM methylglyoxal. Guava leaf extracts displayed a protective effect against methylglyoxal-induced loss of activity of antithrombin III. Dose dependency of the effects is apparent. The content of ferulic acid, gallic acid and quercetin in 1 mg guava leaf extracts was 9.42, 12.18 and 12.26 μg, respectively (Table 1). Additions of these main phenolic compounds into methylglyoxal/plasma reaction system at the same levels present in guava leaf extracts also inhibited the activity loss of antithrombin III induced by methylglyoxal. There were no significant differences ($P > 0.05$) between the protective effects provided by individual phenolic compounds. However, combination of three main phenolic compounds exhibited more protective effect on methylglyoxal-induced loss of activity of antithrombin III. Table 2 also shows that aminoguanidine, an antiglycation agent, exhibited very well preventive effect against methylglyoxal-induced loss of antithrombin III activity at 4 mM.

4. Discussion

In humans with diabetes mellitus, many studies show disturbances of hemostatic and fibrinolytic mechanisms, namely, activation of blood coagulation (Ceriello et al., 1994), hypofibrinolysis (García-Frade et al., 1990), and platelet hyperaggregation (Winocour, 1994). It is well recognized that these abnormalities are associated with the development of diabetic complications and the increased incidence of cardiovascular events in diabetic subjects (Kwaan, 1992).

The hypercoagulable state in diabetes is possibly related to hyperglycemia. Endogenously produced α-dicarbonyl compounds, such as glyoxal and methylglyoxal are involved in hyperglycemia and AGEs formation. Therefore, our attention was focused on the effects of α-dicarbonyl compounds on coagulation. The concentrations of glyoxal and methylglyoxal in plasma of diabetes patients were 229 ± 127 nM (Agalou, Karachalias, Thornalley, Tucker, & Dawnay, 2002) and 742 ± 141 nM (Nemeta,

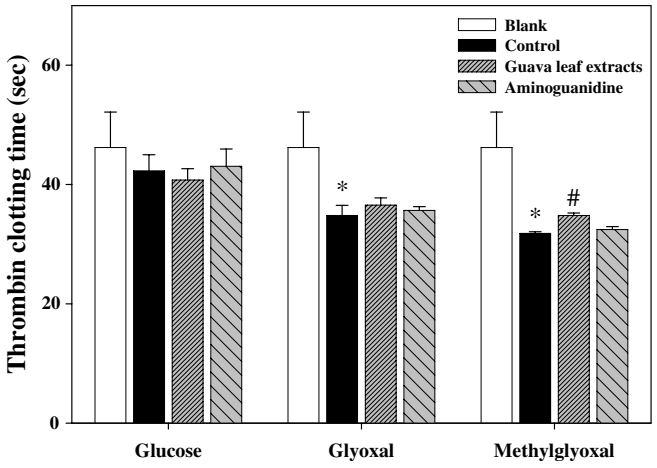

Fig. 2. Effects of guava leaf extracts on the AGEs precursors-induced hypercoagulation. Plasma was incubated with or without glucose (20 mM), glyoxal (0.1 mM) or methylglyoxal (0.1 mM) at 37 °C for 2 h in the absence or presence of guava leaf extracts (0.001 mg/ml) or aminoguanidine (0.05 mM). Data expressed in mean ± standard deviation from triplicate experiments. $^{*}P < 0.05$ versus blank, $\#P < 0.05$ versus control.

Turkb, Duvnjakc, Carc, & Varga-Defterdarovic, 2005), respectively. In the present study, the coagulant properties were assessed by APTT, PT and TT assays using human plasma incubated with α-dicarbonyl compounds at physiological concentrations. The results showed that glucose, glyoxal and methylglyoxal did not change APTT which was the screening test for the intrinsic coagulation pathway and common coagulation pathway. The PT, the screening test for the extrinsic coagulation pathway and common coagulation pathway, was slight prolonged by glyoxal and methylglyoxal at concentration of 0.6 mM. Prolongation of PT can be due to deficiency in any factors involved in the extrinsic and common pathway of blood coagulation or the presence of inhibitors (deGruchy, 1983). However, the possible mechanisms of PT prolongation induced by α-dicarbonyl compounds are remains as a matter to be studied further.

The TT is another important screening procedure for disorders of thrombosis and hemostasis as well as the presence of heparin. TT testing is a rapid assay procedure that measures the polymerization of fibrinogen to fibrin. Glyoxal and methylglyoxal could reduce TT at concentration of 0.1 mM (Fig. 1). Moreover, the decreasing of TT induced by methylglyoxal was inhibited significantly by adding 0.001 mg/ml guava leaf extracts (Fig. 2). Shortening of TT could be attributed to the variation in fibrinogen content or thrombin–antithrombin activities. The results shown in Fig. 1 indicated that the contents of fibrinogen changed unapparently by incubation of plasma with methylglyoxal. Antithrombin III is the most important physiological thrombin inhibitor and its reduction may be a contributing factor to hypercoagulability (Altes et al., 1995). To elucidate the hypercoagulant mechanism of methylglyoxal, its effect on activity of antithrombin III was measured using chromogenic substrates in the presence of heparin. As the data in Table 2 indicated the activity of antithrombin III was inhibited more than 80% after incubation of plasma with methylglyoxal at a concentration of 1.2 mM. A similar finding was reported by Gugliucci and Menini (2002) that over 70% of the antithrombin III activity was lost at 1 mM methylglyoxal. The well inhibition by addition of aminoguanidine into reaction system showed that the glycation played an important role in the inactivation of antithrombin III induced by methylglyoxal. Antithrombin III possesses a lysine-rich domain which is the glycation target. Glycation of antithrombin III may induce decreased heparin binding and result in loss of enzymatic activity (Ceriello et al., 1990; Gugliucci & Menini, 2002).

While search for synthetic new anticoagulants continues, much attention has been focused on potent anticoagulants in natural compounds, in particular herbal plant and fungus. Various anticoagulant-active fractions have been isolated and characterized from marine algae (Matsubara et al., 2001), fungus (Han, Yao, Yang, Liu, & Gao, 2005) and edible mushroom *Auricularia auricular* (Yoon et al., 2003). Mary, Babu, and Padikkala (2003) indicated that herbal formulation containing the extracts of nine plants exhibited anticoagulant, platelet antiaggregatory in rats. In the present study, guava leaf extracts also displayed well anticoagulant effects and protection on loss of activity of antithrombin III induced by methylglyoxal. The guava leaf extracts have shown excellent antioxidant and antiglycation properties (Chen & Yen, 2007; Hsieh et al., 2005). Therefore, guava leaf extracts can be of great value in the preventive glycation- and oxidation-associated cardiovascular disease.

Several kinds of compounds, such as chitosan, glucan, polysaccharides (Han et al., 2005; Yoon et al., 2003), protein (Yamaji et al., 2005), sulphated flavonoids (Guglielmone, Agnese, Núñez, Susana, & Cabrera, 2002), tannins (Dong, Chen, Kini, & Xu, 1998), tea catechins (Kang et al., 1999) and triterpenes (O'Neill et al., 1998) have shown potent anticoagulant and/or antithrombotic activities. In a previous report, the content of total phenolic compounds, as gallic acid equivalents, in guava leaf extracts was 165.61 mg/g (Hsieh et al., 2005). As shown in Fig. 3 and Table 1, the main phenolic compounds in guava leaf extracts were quercetin (12.26 mg/g), gallic acid (12.18 mg/g) and ferulic acid (9.42 mg/g). Moreover, additions of quercetin, gallic acid and ferulic acid individually into reaction system could effectively inhibit the activity loss of antithrombin III induced by methylglyoxal (Table 2). Ferulic acid showed the same inhibitory effects even though at the lowest dosages. The inhibition effects of combination of these three phenolic compounds at the same levels present in guava leaf extracts (1 mg) approximate to that of guava leaf extracts. These results suggested that these phenolic compounds were also the main anticoagulants in guava leaf extracts. Ferulic acid and its precursors, *p*-coumaric acid and caffeic acid are synthesized in plants.

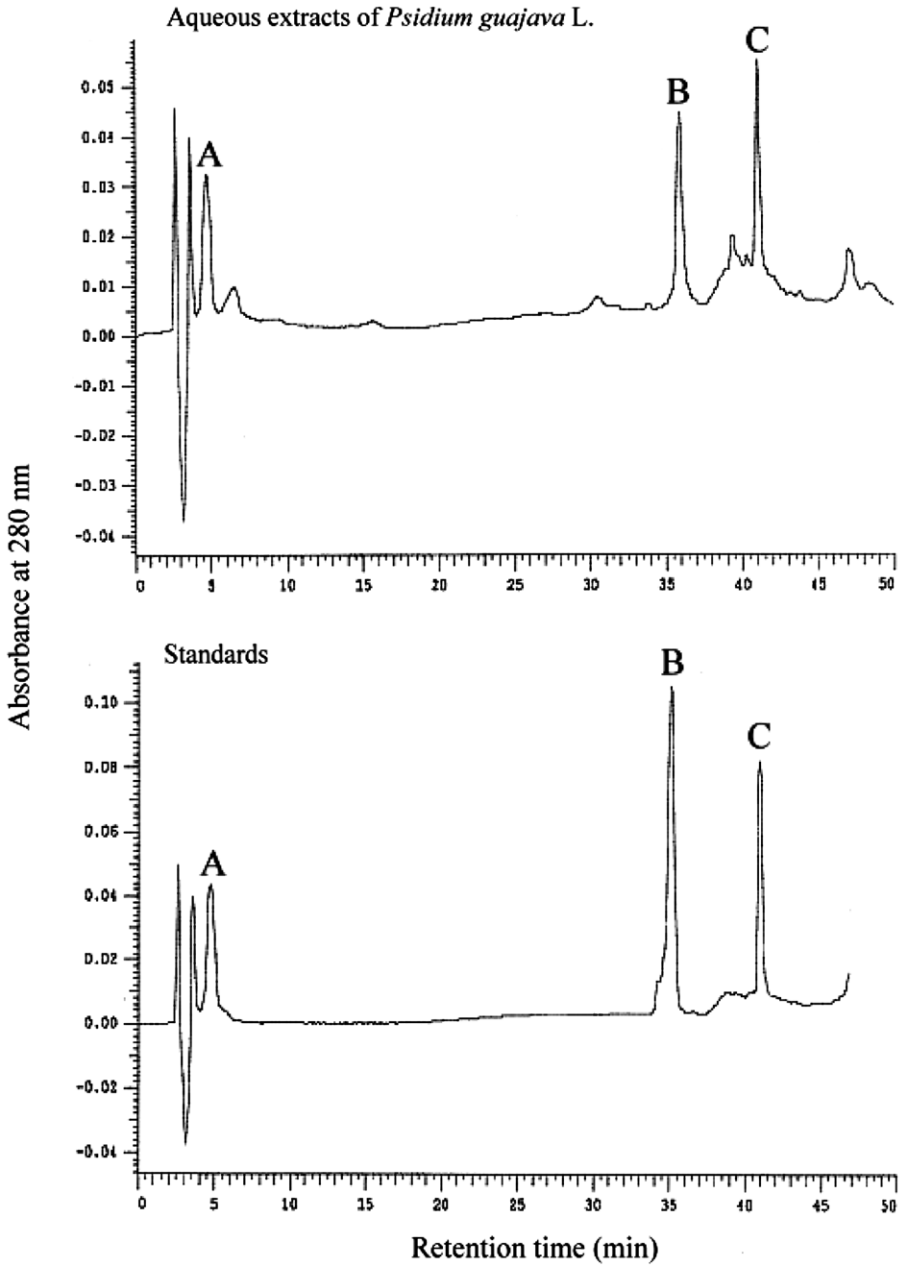

Fig. 3. HPLC chromatogram of guava leaf extracts and its active compounds. A, gallic acid; B, ferulic acid; C, quercetin.

Table 1
The contents of main phenolic compounds in guava leaf extracts

Phenolic compounds	Content (mg/g)[a]
Ferulic acid	9.42 ± 2.29
Gallic acid	12.18 ± 1.71
Quercetin	12.26 ± 0.69

[a] Data represent mean ± standard deviation from three experiments.

Ferulic acid occurs in cereals and vegetables such as rice, wheat, oat, tomatoes, asparagus, olives and many other plants. Recently, focus has been placed on physiological potential of ferulic acid (Anselmi, Centini, Andreassi, Buonocore, & Rosa, 2004). In vivo and in vitro studies have shown that ferulic acid and its sodium salt have antithrombotic and platelet aggregation inhibitory activities (Yin, 1980). Sodium ferulate is an effective component of Chinese herb and has been widely used in China to treat cardiovascular and cerebrovascular diseases and to prevent thrombosis for several decades (Wang & Ou-Yang, 2005). Besides, quercetin sulphate also showed anticoagulant activity. Guglielmone et al. (2002) demonstrated that quercetin sulphate obtained from *Flaveria bidentis* (Asteraceae) showed significant prolongation on the APTT and PT, enhancing thrombin inhibition by heparin cofactor II and markedly inhibited platelet aggregation. Therefore, phenolic compounds may play an important role in the anticoagulant ability of guava extracts.

Table 2
Protective effects of guava leaf extracts and its active compounds on methylglyoxal-induced antithrombin III inactivation

Samples[A]	Antithrombin III activity % of control[B]
Plasma control	100.00 ± 5.20^{a}
Methylglyoxal (1.2 mM)	17.77 ± 4.58^{f}
Methylglyoxal (1.2 mM) + aminoguanidine (4 mM)	78.90 ± 6.44^{b}
Methylglyoxal (1.2 mM) + guava leaf extracts (0.05 mg/ml)	30.67 ± 4.46^{e}
Methylglyoxal (1.2 mM) + guava leaf extracts (0.5 mg/ml)	51.37 ± 3.64^{cd}
Methylglyoxal (1.2 mM) + guava leaf extracts (1 mg/ml)	57.13 ± 2.34^{c}
Methylglyoxal (1.2 mM) + ferulic acid (9.42 μg/ml)	22.12 ± 2.54^{ef}
Methylglyoxal (1.2 mM) + gallic acid (12.18 μg/ml)	21.93 ± 2.04^{ef}
Methylglyoxal (1.2 mM) + quercetin (12.26 μg/ml)	23.66 ± 8.17^{ef}
Methylglyoxal (1.2 mM) + ferulic acid (9.42 μg/ml) + gallic acid (12.18 μg/ml) + quercetin (12.26 μg/ml)	43.83 ± 3.83^{d}

[A] Plasma was incubated with or without methylglyoxal (1.2 mM) at 37 °C CO_2 incubator in the absence or the presence of aminoguanidine, guava leaf extract, quercetin, ferulic acid and gallic acid.
[B] Data represent mean ± standard deviation from three experiments. Different letters between each treatments denote significantly different ($P < 0.05$).

In summary, the hypercoagulable state in diabetes may be related to α-dicarbonyl compounds formed in hyperglycemia and attributed to their inhibition on the activity of antithrombin III. The observation that guava leaf extracts are effective inhibitors on methylglyoxal-induced hypercoagulation, suggest that further investigations of the antithrombotic effects of guava leaf extracts in diabetes are required.

References

Acang, N., & Jalil, F. D. (1993). Hypercoagulation in diabetes mellitus. *The Southeast Asian Journal of Tropical Medicine and Public Health, 24*(Suppl. 1), 263–266.

Agalou, S., Karachalias, N., Thornalley, P. J., Tucker, B., & Dawnay, A. B. (2002). Estimation of α-oxoaldehydes formed from the degradation of glycolytic intermediates and glucose fragmentation in blood plasma of human subjects with uraemia. *International Congress Series, 1245*, 181–182.

Altes, A., Abellan, M. T., Mateo, J., Avila, A., Marti-Vilalta, J. L., & Fontcuberta, J. (1995). Hemostatic disturbances in acute ischemic stroke: a study of 86 patients. *Acta Haematologica, 94*(1), 10–15.

Anselmi, C., Centini, M., Andreassi, M., Buonocore, A., & Rosa, C. L. (2004). Conformational analysis: a tool for the elucidation of the antioxidant properties of ferulic acid derivatives in membrane models. *Journal of Pharmaceutical and Biomedical Analysis, 35*(5), 1241–1249.

Aoki, I., Shimoyama, K., Aoki, N., Homori, M., Yanagisawa, A., Nakahara, K., et al. (1996). Platelet-dependent thrombin generation in patients with diabetes mellitus: effects of glycemic control on coagulability in diabetes. *Journal of the American College of Cardiology, 27*(3), 560–566.

Bell, D. S. (1996). Diabetes mellitus and coronary artery disease. *Coronary Artery Disease, 7*(10), 715–722.

Carr, M. E. (2001). Diabetes mellitus. A hypercoagulable state. *Journal of Diabetes and its Complications, 15*(1), 44–54.

Ceriello, A. (1993). Coagulation activation in diabetes mellitus: the role of hyperglycaemia and therapeutic prospects. *Diabetologia, 36*(11), 1119–1125.

Ceriello, A., Marchi, E., Barbanti, M., Milani, M. R., Giugliano, D., Quatraro, A., et al. (1990). Non-enzymatic glycation reduces heparin cofactor II anti-thrombin activity. *Diabetologia, 33*(4), 205–207.

Ceriello, A., Taboga, C., Giacomello, R., Falleti, E., Stasio, G. D., Motz, E., et al. (1994). Fibrinogen plasma levels as a marker of thrombin activation in diabetes. *Diabetes, 43*(3), 430–432.

Cheng, J. T., & Yang, R. S. (1983). Hypoglycemic effect of guava juice in mice and human subjects. *The American Journal of Chinese Medicine, 11*(1–4), 74–76.

Chen, H. Y., & Yen, G. C. (2007). Antioxidant activity and free radical scavenging capacity of extracts from guava (*Psidium guajava* L.) leaves. *Food Chemistry, 101*(2), 686–694.

deGruchy, G. C. (1983). *Clinical haematology in medical practice* (p. 698). UK: Blackwell Scientific Publications.

Dong, H., Chen, S. X., Kini, R. M., & Xu, H. X. (1998). Effects of tannins from *Geum japonicum* on the catalytic activity of thrombin and factor xa of blood coagulation cascade. *Journal of Natural Products, 61*(11), 1356–1360.

Frantzen, H. G., Abildgaard, U., & Aasen, A. O. (1983). Simplified assay for antithrombin III activity using chromogenic peptide substrate. *Scandinavian Journal of Haematology, 31*(5), 427–436.

García-Frade, L. J., Calle, H., Torrado, M. C., Lara, J. I., Cuellar, L., & García Avello, A. (1990). Hypofibrinolysis associated with vasculopathy in noninsulin-dependent diabetes mellitus. *Thrombosis Research, 59*(1), 51–59.

Guglielmone, H. A., Agnese, A. M., Núñez, M., Susana, C., & Cabrera, J. L. (2002). Anticoagulant effect and action mechanism of sulphated flavonoids from *Flaveria bidentis*. *Thrombosis Research, 105*(2), 183–188.

Gugliucci, A. (2000). Glycation as the glucose link to diabetic complications. *The Journal of the American Osteopathic Association, 100*(10), 621–634.

Gugliucci, A., & Menini, T. (2002). The botanical extracts of *Achyrocline satureoides* and *Ilex paraguariensis* prevent methylglyoxal-induced inhibition of plasminogen and antithrombin III. *Life Sciences, 72*(3), 279–292.

Han, F., Yao, W. B., Yang, X. B., Liu, X. N., & Gao, X. D. (2005). Experimental study on anticoagulant and antiplatelet aggregation activity of a chemically sulfated marine polysaccharide YCP. *International Journal of Biological Macromolecules, 36*(4), 201–207.

Hasegawa, Y., Suehiro, A., Higasa, S., Namba, M., & Kakishita, E. (2002). Enhancing effect of advanced glycation end products on serotonin-induced platelet aggregation in patients with diabetes mellitus. *Thrombosis Research, 107*(6), 319–323.

Hsieh, C. L., Lin, Y. C., Ko, W. S., Peng, C. H., Huang, C. N., & Peng, R. Y. (2005). Inhibitory effect of some selected nutraceutic herbs on LDL glycation induced by glucose and glyoxal. *Journal of Ethnopharmacology, 102*(3), 357–363.

Kang, W. S., Lim, I. H., Yuk, D. Y., Chung, K. H., Park, J. B., Yoo, H. S., et al. (1999). Antithrombotic activities of green tea catechins and (−)-epigallocatechin gallate. *Thrombosis Research, 96*(3), 229–237.

Kwaan, H. C. (1992). Changes in blood coagulation, platelet function, and plasminogen-plasmin system in diabetes. *Diabetes, 41*(Suppl. 2), 32–35.

Marks, J. B., & Raskin, P. (2000). Cardiovascular risk in diabetes: a brief review. *Journal of Diabetes and its Complications, 14*(2), 108–115.

Mary, N. K., Babu, B. H., & Padikkala, J. (2003). Antiatherogenic effect of *Caps HT2*, a herbal Ayurvedic medicine formulation. *Phytomedicine, 10*(6–7), 474–482.

Matsubara, K., Matsuura, Y., Bacic, A., Liao, M. L., Hori, K., & Miyazawa, K. (2001). Anticoagulant properties of a sulfated galactan preparation from a marine green alga, *Codium cylindricum*. *International Journal of Biological Macromolecules, 28*(5), 395–399.

Nemeta, I., Turkb, Z., Duvnjakc, L., Carc, N., & Varga-Defterdarovic, L. (2005). Humoral methylglyoxal level reflects glycemic fluctuation. *Clinical Biochemistry, 38*(4), 379–383.

O'Neill, M. J., Lewis, J. A., Noble, H. M., Holland, S., Mansat, C., Farthing, J. E., et al. (1998). Isolation of translactone-containing triterpenes with thrombin inhibitory activities from the leaves of *Lantana camara*. *Journal of Natural Products, 61*(11), 1328–1331.

Schneider, D. J., & Sobel, B. E. (2001). Diabetes and thrombosis. In N. J. Totowa (Ed.), *Diabetes and cardiovascular disease* (pp. 68–149). Humana Press.

Wang, B. H., & Ou-Yang, J. P. (2005). Pharmacological actions of sodium ferulate in cardiovascular system. *Cardiovascular Drug Reviews, 23*(2), 161–172.

Watala, C., Golanski, J., Pluta, J., Boncler, M., Rozalski, M., Luzak, B., et al. (2004). Reduced sensitivity of platelets from type 2 diabetic patients to acetylsalicylic acid (aspirin)—its relation to metabolic control. *Thrombosis Research, 113*(2), 101–113.

Winocour, P. D. (1994). Platelets, vascular disease, and diabetes mellitus. *Canadian Journal of Physiology and Pharmacology, 72*(3), 295–303.

Yamaji, K., Wang, Y., Liu, Y., Abeyama, K., Hashiguchi, T., Uchimura, T., et al. (2005). Activated protein C, a natural anticoagulant protein, has antioxidant properties and inhibits lipid peroxidation and advanced glycation end products formation. *Thrombosis Research, 115*(4), 319–325.

Yin, Z. Z. (1980). The effect of dong quai (*Angelica sinsis*) and its ingredient ferulic acid on rat platelet aggregation and release of 5-HT. *Acta Pharmaceutica Sinica, 15*, 321–330.

Yoon, S. J., Yu, M. A., Pyun, Y. R., Hwang, J. K., Chu, D. C., Juneja, L. R., et al. (2003). The nontoxic mushroom *Auricularia auricula* contains a polysaccharide with anticoagulant activity mediated by antithrombin. *Thrombosis Research, 112*(3), 151–158.