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Short communication

Optimization of ultrasound-assisted extraction conditions for total phenols with anti-hyperglycemic activity from *Psidium guajava* leaves

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ABSTRACT

The high concentration of total phenolic compounds (TPC) in *Psidium guajava* leaf extracts (GvEx) is correlated to its anti-hyperglycemic activity. In this study, we established the optimum ultrasound extraction conditions for maximizing TPC yield. The response surface methodology (RSM) was employed for empirical model building. The maximum value of TPC (26.12%) was obtained at solvent to solid ratio (v/w) of 12.1, extraction temperature of 59.8 °C, and extraction time of 5.1 min. The anti-hyperglycemic activity of GvEx was compared to the commonly used diabetic drug acarbose. The IC_{50} of GvEx for α-amylase and α-glucosidase inhibition was 50.5 μg/mL and 34.6 μg/mL, respectively. However, the IC_{50} of acarbose for α-amylase and α-glucosidase inhibition was 95.3 μg/mL and 1075.2 μg/mL, respectively. In conclusion, GvEx obtained under optimum extraction conditions had higher anti-hyperglycemic activity than acarbose. In addition, the recommended extraction procedures for GvEx save time and are environmentally friendly.

1. Introduction

Psidium guajava, a member of the Myrtaceae family, grows wild in the Torrid Zone and subtropics. The anti-hyperglycemic activity of the guava leaves extract has been reported *in vitro*, in animal models, and in human clinical studies [1–3]. Total phenols, which have beneficial antioxidant and anti-hyperglycemic activity [4–6], are one of the major biological active constituents of guava leaves.

Several environmentally friendly extraction technologies have been developed for extraction of bioactive components of plants [7–9]. Ultrasound-assisted extraction is one of the more popular methods because it is an inexpensive, simple, and efficient extraction technique. It offers high reproducibility, saves time, has low solvent consumption, and requires low energy input [10,11].

The rise in blood glucose levels is due to the hydrolysis of complex carbohydrates into absorbable monosaccharides by pancreatic α-amylase and intestinal α-glucosidase. Inhibiting the carbohydrate hydrolyzing enzymes is an effective therapeutic approach to moderate the postprandial increase of blood glucose level [12]. Therefore, inhibition of α-amylase and α-glucosidase can be a key strategy in the control of diabetes mellitus [13].

The purpose of this study was to develop an efficient and environmentally friendly ultrasound-assisted extraction procedure for total phenols in guava leaves. Total phenol yield, phenolic composition, antioxidant and anti-hyperglycemic activities of the extracts were also evaluated.

2. Materials and methods

2.1. Materials

Psidium guajava leaves of Jen Ju Pa (Myrtaceae family) were harvested in Jing-cin Farm (Tianzhong Township, Changhua County, Taiwan) during the stages of appearance of flower buds to flower buds visible. The resource of plant materials were taxonomically identified and deposited in Fengshan Tropical Horticultural Experiment Branch, Taiwan Agricultural Research Institute Council of Agriculture, Executive Yuan (FTHA000282).

2.2. Guava leaves extraction

The extraction was assisted with ultrasound probe (BRANSON, Danbury, CT, USA) and ultrasonic power was fixed at 1100 W. Distilled water was used as the only extraction solvent in this study. The aqueous extract was filtered through a filter paper and concentrated, freeze-dried and then stored at −80 °C pending further

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analysis. For all experiments performed in this study, individual extraction was carried out with an 800 mL solvent.

2.3. Single factor and optimization experimental design

The effects of v/w, temperature, and time on TPC values were studied by changing the level of each factor one at a time while keeping the other two constant. The keeping condition were 10 of v/w, 60 °C of temperature and 5 min of time. A 3-factor-5-level central composite rotatable design (CCRD) was used in optimization experimental design [14]. The ranges of three variables including v/w (X_1), extraction temperature (X_2), and extraction time (X_3) were determined by the single factor test.

2.4. TPC measurement

The concentration of TPC was determined according to the Folin-Ciocalteu method [15]. Gallic acid solution was used as a standard and TPC concentration in extract was then expressed as gallic acid equivalent (%).

2.5. HPLC analysis

Separation of phenolic compounds were performed on an Agilent C18 column (Santa Clara, CA, USA) with 1.0 mL/min flow rate as described by Yue et al. [16]. The phenolic compounds were calibrated to their respective standard curve and then expressed as a proportion (%) of total extract.

2.6. Antioxidant activity

2.6.1. ABTS assay

The ABTS assay was determined according to previous methods [17,18]. The appropriately diluted samples (1000, 500, 250, 125, and 62.5 μg/mL) and trolox (50, 40, 30, 20, 10, 5, 2.5 μg/mL) were used in the assay. The activities were expressed as μg of trolox equivalent.

2.6.2. Reducing power

This was determined according to a method described by Oyaizu [19]. The appropriately diluted samples (1000, 500, 250, 125, and 62.5 μg/mL) or ascorbic acid (70, 60, 50, 40, 30, 20, 10 μg/mL) were used in the assay. The results were expressed as μg of ascorbic acid equivalent.

2.7. In vitro anti-hyperglycemic activity

2.7.1. Inhibition of α-amylase

This assay was performed as described previously [20]. The sample solution at various concentrations (150, 100, 50, 25, 12.5 μg/mL) was used for analysis of enzyme inhibition. Absorbance was measured at 540 nm using a spectrophotometer (Agilent Varian Cary 50, Santa Clara, CA, USA). Acarbose was used as a positive control for this assay. In control group which had 100 μl of buffer solution in place of the sample solution. Percent inhibitory activity was calculated as follows: % inhibition = $[(A^{Control} - A^{Sample})/(A^{Control})] \times 100$.

2.7.2. Inhibition of α-glucosidase

The α-glucosidase from *Saccharomyces cerevisiae* was used for inhibitory assay described by Shim et al. [21]. The sample solution at various concentrations (75, 50, 25, 12.5 and 7.5 μg/mL) was used for analysis of enzyme inhibition and absorbance was recorded at 400 nm. Percent inhibitory activity was calculated as follows: % inhibition = $[(A^{Control} - A^{Sample})/(A^{Control})] \times 100$.

2.8. Statistical analysis

The response surface regression procedure of the SAS statistical software package [22] was used to analyze the experimental data.

3. Results and discussion

3.1. Single factor optimization for extraction

Our results revealed that v/w of 12 resulted in the highest TPC value (26.01%) (Fig. 1A). However, v/w above 12 resulted in a decrease could be due to dissolved polysaccharides and/or other compounds that interfered with phenol extraction. Raising extraction temperature from 25 to 60 °C increased TPC values, reaching a maximum of 23.38% at 60 °C (Fig. 1B). Beyond 60 °C, TPC values started to decline because excessively high temperatures may have degraded the phenolic compounds [16,23,24]. Fig. 1C shows that TPC values increased as extraction time increased from 0.5 to 5 min. No further increases were recorded beyond the 5 min extraction time.

3.2. Optimization of parameters by RSM

According to the results of single factor tests, the parameters for v/w (12), extraction temperature (60 °C) and extraction time (5 min) were selected for the subsequent RSM study to optimize extraction conditions. Multiple regression analysis was applied to the experimental data (Supplementary data, Table S1) producing the following second-order polynomial equation: $Y = 26.21 + 0.132X_1 - 0.097X_2 + 0.416X_3 - 0.218X_1X_2 + 0.118X_1X_3 + 0.018X_2X_3 - 3.718X_1^2 - 3.657X_2^2 - 2.738X_3^2$. The regression equation has a coefficient of determination (R^2) of 0.915, indicating a close agreement between the observed and predicted values. The model was significant ($p < 0.01$), and there was a moderate lack of fit ($p > 0.05$), indicating that the second order model was appropriate for describing the response surface (Supplementary data, Table S2). Fig. 2A shows that the optimum v/w was in the range of 11 to 13 while optimal temperature was around 60 °C. Fig. 2B shows the effect of v/w and time on the TPC of GvEx. The highest TPC value was obtained with a 4–6 min extraction time and v/w range of 11–13. The effect of extraction temperature and time on TPC content of GvEx is shown in Fig. 2C. The optimal conditions for maximum TPC extraction were 50–60 °C of extraction temperature and between 5 and 6 min of extraction time.

Analysis of the surface response revealed that the stationary point for the extraction of TPC yield from guava leaves was a true maximum. The maximum predicated value of TPC yield was 26.23% under the following extraction conditions: a solvent to solid ratio of 12.1, an extraction temperature of 59.8 °C, and an extraction time of 5.1 min. In addition, the verification studies also proved that the predicted value of TPC for the model could be realistically achieved within a 95% confidence interval of experimental values (Supplementary data, Table S3).

3.3. HPLC analysis

Phenolic compounds, such as gallic acid, catechins, chlorogenic acid, caffeic acid, and quercetin, are important plant components because of their antioxidant and anti-hyperglycemic activities [25–29]. Table 1 shows that catechin (2.25%) and epicatechin (1.45%) were the predominant polyphenols. Gallic acid (0.87%), quercetin (0.83%), chlorogenic acid (0.62%), epigallocatechin gallate (0.47%) and caffeic acid (0.11%) were present in lower concentrations in GvEx. The composition of phenolic compounds indicates

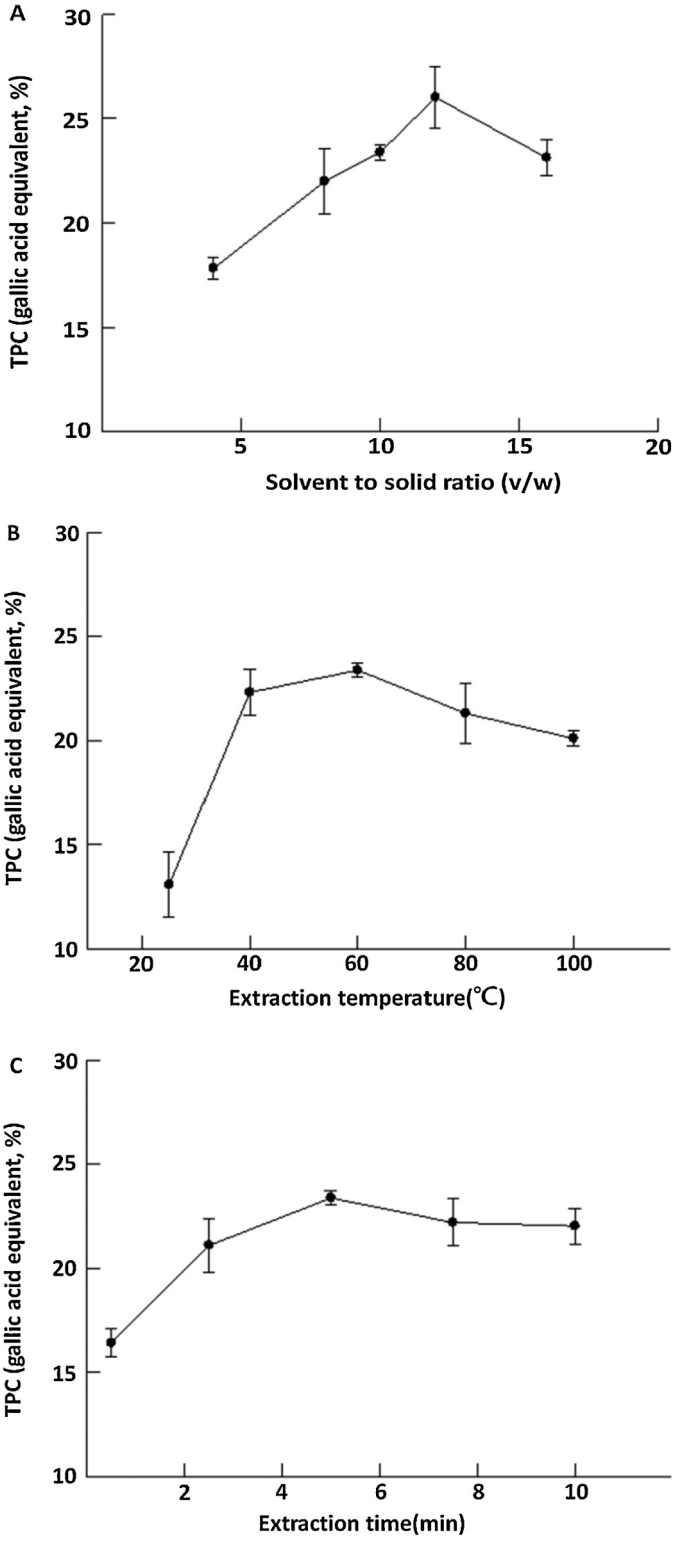

Fig. 1. Effects of (A) solvent to solid ratio, (B) extraction temperature, and (C) extraction time on total phenols content of extraction. The maximum TPC values were observed separately at a solvent to solid ratio of 12 (26.0 ± 1.46%), extraction temperature of 60 °C (23.4 ± 0.35%), and an extraction time of 5 min (23.4 ± 0.35%). Data are expressed as mean ± SD (n = 3).

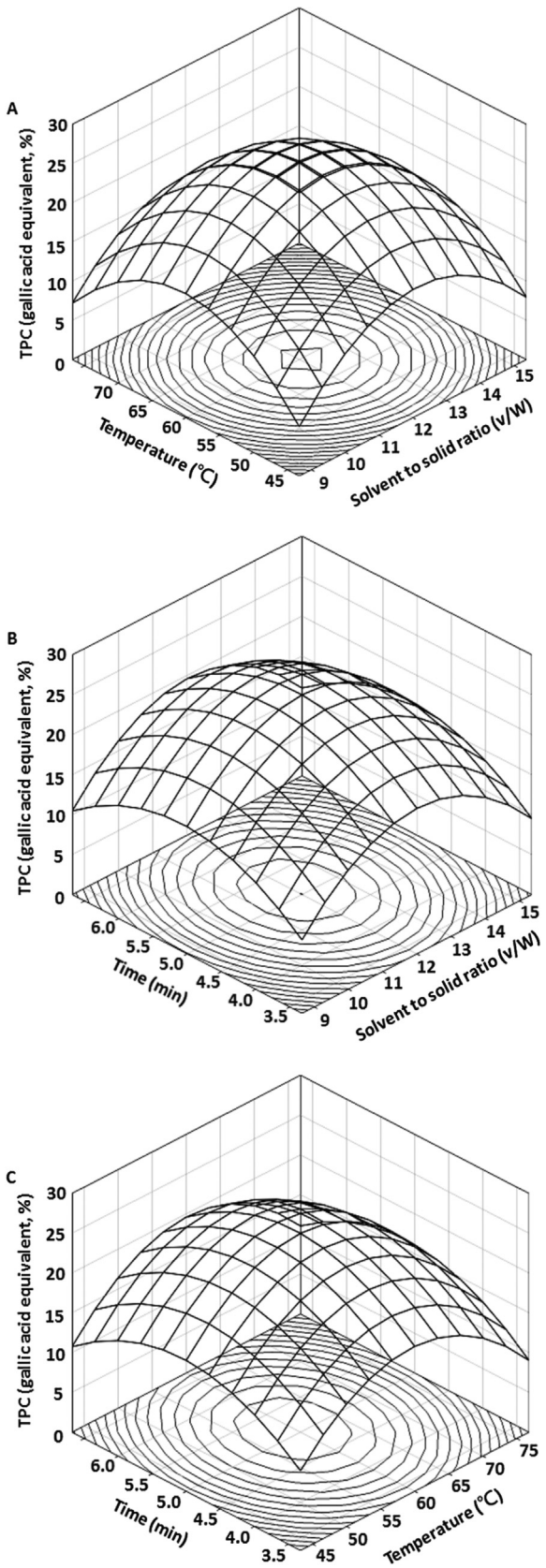

Fig. 2. Response surface plots showing the effects of variables one the TPC of extract. (A) Interaction of the solvent to solid ratio and extraction temperature. The optimum v/w was in the range of 11–13 while optimal temperature was around 60 °C. (B) Interaction of the solvent to solid ratio and extract time. The maximum TPC value was obtained with a 4–6 min time and a v/w range of 11–13. (C) Interaction of the extraction temperature and time. The optimal conditions for maximum TPC extraction were; 50–60 °C of temperature and between 5 and 6 min of time.

Table 1
Phenolic compounds of guava leaf extract[a]. The major components of extract were catechin (2.25 ± 0.29%) and epicatechin (1.45 ± 0.13%).

Compounds	Content (%)
Gallic acid	0.87 ± 0.02
Chlorogenic acid	0.62 ± 0.05
Caffeic acid	0.11 ± 0.01
Catechin	2.25 ± 0.29
Epicatechin	1.45 ± 0.13
Epigallocatechin gallate	0.47 ± 0.06
Quercetin	0.83 ± 0.06

[a] Data are expressed as mean ± SD (n = 5).

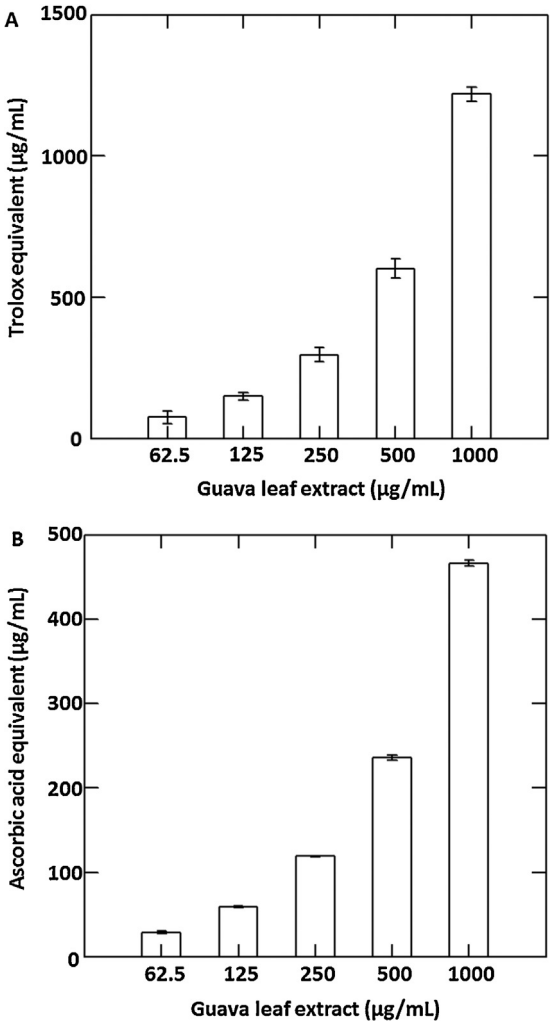

Fig. 3. (A) The trolox equivalent ranged from 75.3 ± 22.2 to 1217.5 ± 32.1 μg/mL when the concentration of GvEx was between 62.5 to 1000 μg/mL. (B) The reducing power of ascorbic acid equivalent ranged from 29.0 ± 1.82 to 466.5 ± 4.78 μg/mL when the concentration of GvEx was between 62.5 and 1000 μg/mL. Data are expressed as mean ± SD (n = 5).

that GvEx has potential antioxidant and anti-hyperglycemic activities.

3.4. Antioxidant activity of GvEx

The trolox equivalent ranged from 75.3 to 1217.5 μg/mL when the concentration of GvEx was between 62.5 to 1000 μg/mL (Fig. 3A). The ABTS radical scavenging activity of 1 g GvEx was equal to that of 1.22 g trolox, suggesting that GvEx has better antioxidant activity than trolox. The reducing power of ascorbic acid equivalent ranged from 29.03 to 466.49 μg/mL when the concentration of GvEx was between 62.5 to 1000 μg/mL (Fig. 3B). The reducing power of 1 g GvEx was equal to 0.47 g ascorbic acid. High correlation was found between ABTS radical scavenging activity and reducing power (R^2 = 0.996, p < 0.001), indicating the dual antioxidant mechanisms of GvEx.

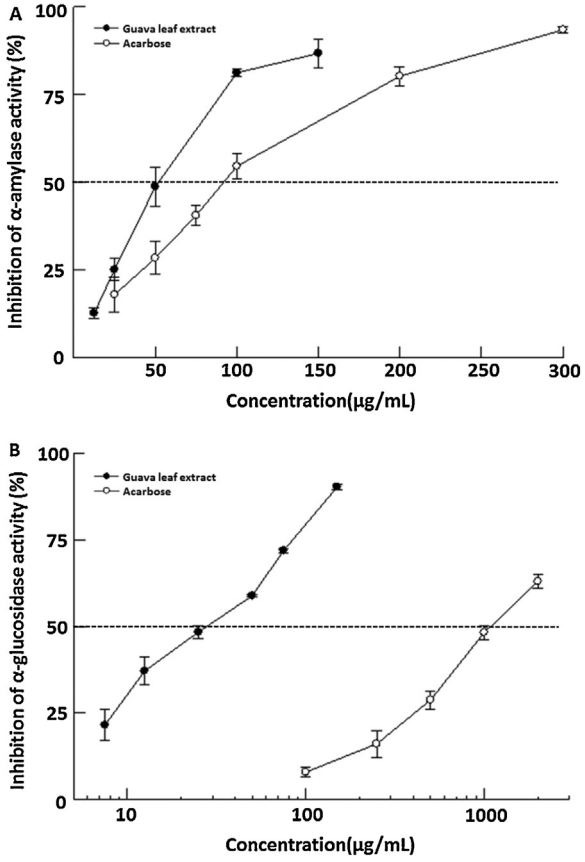

Fig. 4. (A) α-Amylase and (B) α-glucosidase inhibition by guava leaf extract and acarbose. The IC_{50} of guava leaf extract and acarbose on α-amylase are 50.5 μg/mL and 95.3 μg/mL respectively. The IC_{50} of guava leaf extract and acarbose on α-glucosidase are 34.6 μg/mL and 1075.2 μg/mL respectively. Data are expressed as mean ± SD (n = 3).

3.5. Anti-hyperglycemic activity of GvEx in vitro

Several studies have reported the inhibitory effects of total phenols on α-amylase and α-glucosidase activities [27–30]. The potency of GvEx as an inhibitor of pancreatic α-amylase activity was shown in Fig. 4A. The inhibition was dose-dependent with an IC_{50} value of 50.5 μg/mL. Acarbose, a known pancreatic α-amylase inhibitor, was showed an IC_{50} of 95.3 μg/mL. Effects of GvEx on α-glucosidase inhibition occurred in a concentration-dependent manner with an IC_{50} value of 34.6 μg/mL (Fig. 4B). It should be noted that acarbose showed the least α-glucosidase inhibitory activity with an IC_{50} value of 1075.2 μg/mL.

Deguchi et al. [2] indicated that hot water extracts of dried guava leaves inhibited pancreatic α-amylase, intestinal maltase and sucrase with IC_{50} values of 600, 2100, and 3600 μg/mL, respectively. Wang et al. [3] reported that 1500 μg/mL extracts of dried leaves, extracted with water, inhibited 29.3% of pancreatic α-amylase activity, 34.5% of sucrase activity and 27.3% of maltase activity, respectively. The differences in leaf extract inhibitory activity between our study and other studies could have been caused by differences in freshness of leaves, plant variety

and growing environment. In addition, our condition (ultrasound assisted extraction at moderate temperature for a short time) may extract and/or preserve more bioactive compounds which with anti-hyperglycemic activities.

4. Conclusions

In conclusion, the distinguishing features of the proposed ultrasound-assisted extraction of total phenols from guava leaves are the time-saving and environmentally friendly procedures. In addition, the optimization of extraction parameters for total phenols from guava leaves, presented in this study, gives a foundation for the development and utilization of guava leaf resources.

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References

[1] Cheng JT, Yang RS. Hypoglycemic effect of guava juice in mice and human subjects. Am J Chin Med 1983;11:74–6.
[2] Deguchi Y, Osada K, Uchida K, Kimura H, Yoshikawa M, Kudo T, et al. Effects of extract of guava leaves on the development of diabetes in the db/db mouse and on the postprandial blood glucose of human subjects. Nip Nog K J 1998;72:923–32.
[3] Wang H, Dub YJ, Song HC. α-Glucosidase and α-amylase inhibitory activities of guava leaves. Food Chem 2010;123:6–13.
[4] Gutiérrez RM, Mitchell S, Solis RV. Psidium guajava: a review of its traditional uses, phytochemistry and pharmacology. J Ethnopharmacol 2008;117:1–27.
[5] Deguchi Y. Effect of guava tea on postprandial blood glucose and diabetes. Assoc J Jpn Soc Med Use Funct Foods 2006;3:439–45.
[6] Vadivel V, Biesalski HK. Contribution of phenolic compounds to the antioxidant potential and type II diabetes related enzyme inhibition properties of *Pongamia pinnata* L. Pierre seeds. Process Biochem 2011;46:1973–80.
[7] Han HW, Cao WL, Zhang JC. Preparation of biodiesel from soybean oil using supercritical methanol and CO_2 as co-solvent. Process Biochem 2005;40:3148–51.
[8] Jacquemin L, Zeitoun R, Sablayrolles C, Pontalier PV, Rigal L. Evaluation of the technical and environmental performances of extraction and purification processes of arabinoxylans from wheat straw and bran. Process Biochem 2012;47:373–80.
[9] Chen RZ, Li SZ, Liu CM, Yang SM, Li XL. Ultrasound complex enzymes assisted extraction and biochemical activities of polysaccharides from *Epimedium* leaves. Process Biochem 2012;47:2040–50.
[10] Jiao Y, Zuo Y. Ultrasonic extraction and HPLC determination of anthraquinones, aloe-emodine, emodine, rheine, chrysophanol, and physcione, in Radix Polygoni multiflori. Phytochem Anal 2009;20:272–8.
[11] Wang C, Zuo Y. Ultrasound-assisted hydrolysis and gas chromatography–mass spectrometric determination of phenolic compounds in cranberry products. Food Chem 2011;128:562–8.
[12] Deshpande MC, Venkateswarlu V, Babu RK, Trivedi RK. Design and evaluation of oral bioadhesive controlled release formulations of miglitol, intended for prolonged inhibition of intestinal alpha-glucosidase and enhancement of plasma glucagon like peptide-1 levels. Int J Pharm 2009;380:16–24.
[13] Hirsh AJ, Yao SY, Young JD, Cheeseman CI. Inhibition of glucose absorption in the rat jejunum: a novel action of alpha-D-glucosidase inhibitors. Gastroenterology 1997;113:205–11.
[14] Myers RH, Montgomery DC. Response Surface Methodology. 2nd ed. New York: John Wiley and Sons; 2002. p. 85–143.
[15] Singleton VL, Orthofer R, Lamuela-Raventos RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Method Enzymol 1999;299:152–78.
[16] Yue T, Shao D, Yuan Y, Wang Z, Qiang C. Ultrasound-assisted extraction, HPLC analysis, and antioxidant activity of polyphenols from unripe apple. J Sep Sci 2012;35:2138–45.
[17] Miller LN, Rice-Evans CA, Davies MJ, Gopinathan V, Milner A. A novel method for measuring antioxidant status in premature neonates. Clin Sci 1993;84:407–12.
[18] Arnao MB, Cano A, Hernadez-Ruiz J, Carcia-Canovas F, Acosta M. Inhibition by L-ascorbic acid and other antioxidants of 2,2′-azino-bis (3-ethylbiazoline-6-sulfonic acid) oxidation catalyzed by peroxidase: a new approach for determining total antioxidant status of food. Anal Biochem 1996;236:255–61.
[19] Oyaizu M. Studies on products of browning reaction antioxidative activities of products of browning reaction prepared from glucosamine. Jpn J Nutr 1986;44:307–15.
[20] Ali H, Houghton PJ, Soumyanath A. α-Amylase inhibitory activity of some Malaysian plants used to treat diabetes; with particular reference to *Phyllanthusamarus*. J Ethnopharmacol 2006;107:449–55.
[21] Shim YJ, Doo HK, Ahn SY, Seong JK, Park IS, Min BH. Inhibitory effect of aqueous extract from the gall of *Rhus chinensis* on alpha-glucosidase activity and postprandial blood glucose. J Ethnopharmacol 2003;85:283–7.
[22] SAS User's Guide. Version V8. NC: SAS Institute Inc.; 2002.
[23] Alonso-Salces RM, Korta E, Barranco A, Berrueta LA, Gallo B, Vicente F. Pressurized liquid extraction for the determination of polyphenols in apple. J Chromatogr A 2001;933:37–43.
[24] Escribano-Bailon M, Santos-Buelga C. Polyphenol extraction from foods methods in polyphenol analysis. Lausanne: Gary Williamson Nestle Research Center; 2003.
[25] Paganga G, Miller N, Rice-Evans CA. The polyphenolic content of fruit and vegetables and their antioxidant activities. What does a serving constitute? Free Radic Res 1999;30:153–62.
[26] Rohn S, Rawel HM, Kroll J. Inhibitory effects of plant phenols on the activity of selected enzymes. J Agric Food Chem 2002;50:3566–71.
[27] Ma CM, Hattori M, Daneshtalab M, Wang L. Chlorogenic acid derivatives with alkyl chains of different lengths and orientations: potent alpha-glucosidase inhibitors. J Med Chem 2008;51:6188–94.
[28] Adisakwattana S, Chantarasinlapin P, Thammarat H, Yibchok-Anun S. A series of cinnamic acid derivatives and their inhibitory activity on intestinal alpha-glucosidase. J Enzyme Inhib Med Chem 2009;24:1194–200.
[29] Yilmazer-Musa M, Griffith AM, Michels AJ, Schneider E, Frei B. Grape seed and tea extracts and catechin 3-gallates are potent inhibitors of α-amylase and α-glucosidase activity. J Agric Food Chem 2012;60:8924–9.
[30] McDougall GJ, Shpiro F, Dobson P, Smith P, Blake A, Stewart D. Different polyphenolic components of soft fruits inhibit α-amylase and α-glucosidase. J Agric Food Chem 2005;53:2760–6.