

Antiglycation, α -glucosidase, and α -amylase Inhibitory Activity of Guava Leaf Hydrosol

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ABSTRACT

Introduction: Guava leaf oil (GLO) is one of the economic products obtained from the hydrodistillation process. It is widely used in the cosmetic, pharmaceutical, and nutraceutical industries. In this process, residual water (hydrosol) is generally drained out as effluent. There is no information available regarding this hydrosol's bioactivity for health.

Objective: This study aimed to evaluate the antiglycation and inhibitory activity of α -glucosidase and α -amylase enzymes which are related to diabetes mellitus.

Methods: Hydrosol used in this study was obtained from the essential oil production of guava leaves using the hydrodistillation process. This process was performed at guava leaves to water ratio of 1:10 (w/v) at 100 °C for 2 hours. The obtained hydrosol was then freeze-dried to obtain the hydrosol powder. Phenolic content (TPC) of hydrosol powder was determined using both Folin-Ciocalteu's reagent and HPLC methods. Inhibition of glycation, α -glucosidase, and α -amylase of hydrosol was also evaluated.

Results: The hydrosol yielded 5.4±0.8 %w/w of fresh leaves. TPC of the hydrosol was 134.3±3.6 mg of gallic acid/ g of hydrosol powder. The Antiglycation (IC₅₀) of the hydrosol was 9.6±1.3 µg/mL, which showed higher efficacy than aminoguanidine. Inhibition of the hydrosol (IC₅₀) against α -glucosidase, and α -amylase was 413.3±10.7 and 596.6 ±4.6 µg/mL, respectively.

Conclusion: Hydrosol byproduct obtained from essential oil distillation of guava leaves should be promising candidates for hypoglycemic functional product and antiaging ingredient.

Keywords: guava leaf; hydrosol; α -Glucosidase; phenolic compounds; glycation

Introduction

The nonenzymatic reaction between reducing sugar and the amino group of proteins spontaneously initiated the high reactive carbonyl radicals (the glycated products) in the body [1]. Accumulation of these glycated products induces irreversible skin collagen [2] and the pathologies such as Alzheimer's [3], chronic heart failure [4], and diabetes [5]. Carbohydrate hydrolytic enzyme such as α -glucosidase and α -amylase is one of the main causes to generate glycated products [6]. Several drugs have been widely used to inhibit two enzymes and glycated products. However, these drugs displayed side effects such as liver disorders [7,8].

Therefore, the natural product possesses both these enzymes' inhibitory and antioxidant activity and has been increasingly studied to use as an alternative source) [9]. Among medicinal plants, *Psidium guajava* is one of the interesting natural sources for solving these problems [10].

Psidium guajava L. (guava) is a plant of the Myrtaceae family. This is one of the most effective plants used in folk medicine for the treatment of various ailments such as gastroenteritis, and diarrhea rheumatism [11]. Guava leaf extract obtained from different solvent extraction such as chloroform, ethanol, and water also inhibited α -glucosidase and α -amylase

including displayed antioxidant activity [10]. Many phenolic compounds of this plant such as gallic acid, ellagic acid, and quercetin have been qualified and quantified [12,13]. These phenolics are related to the biological activities of guava leaves. In addition, several studies report on guava leaf oil used extensively, especially in the pharmaceutical, cosmetic, food, and perfume industries due to its chemical components attributed to a variety of effective biological activities, such as anticancer, antioxidant, anti-inflammatory, and antimicrobial [14,15]. However, residual water (hydrosol) is generally drained out as effluent in the hydro distillation of essential oil production. In recent years, many studies reported that polar volatile compounds and other secondary metabolites of the plant could interact with water via hydrogen bonds during the process of essential oils extracted from aromatic plants [16,17]. To water extract of guava leaves gave inhibitory activity against α -glucosidase and α -amylase [10] and information on the guava leaf hydrosol is no information. Therefore, the determination of the hydrosol activity against these enzymes is interesting. This study aimed to evaluate phenolic content and determine some phenolic compounds such as gallic acid, ellagic acid, and quercetin of this hydrosol. In addition, antiglycation and enzyme inhibition of α -glucosidase and α -amylase were also evaluated.

Methodology

Chemical reagents

The chemical reagents included p-nitrophenyl- α -glucopyranoside (4-pNPG), soluble starch from potatoes, α -glucosidase from *Saccharomyces cerevisiae*, α -amylase from *Aspergillus oryzae* were purchased from Sigma-Aldrich (St. Louis, MO, USA). Hydrosol is obtained from essential oil distillation of guava leaves at a weight ratio of guava leaves to water equal to 1:10 at 100 °C for 2 h. The hydrosol was further freeze-dried to obtain the powder and kept at 4 °C for further use.

Phenolic compound analysis

Phenolic compounds existing in the hydrosol that is obtained from hydro-distillation of guava leaves were identified using a reported method [12,13] with some modification. Briefly, HPLC analyses were performed using a UV detector set at 280 nm. Acetic acid (2% v/v) and acetonitrile (50%, v/v) were used as gradient solvents at a flow rate of 1 ml/min. Hydrosol was dissolved in absolute ethanol and filtrated through a 0.45 μ m Millipore filter prior to injecting into the HPLC system. The sample was analyzed in triplicate. Phenolic standards of interest such as gallic acid, ellagic acid, and quercetin were used for the identification of phenolic compounds that existed in the hydrosol. The identified phenolic compounds were quantified based on their peak area and compared with calibration curves obtained with the corresponding standards and then expressed as mg/g of extract.

Determination of total phenolic content

The total phenolic content of the hydrosol was determined by the Folin-Ciocalteu method according to described by the reported method [13] with some modifications. Briefly, the hydrosol was dissolved in absolute ethanol to a final concentration of 1 mg/mL prior to the test. An aliquot of 50 μ L of the hydrosol solution was mixed with 50 μ L of Folin-Ciocalteu reagent. After that, 100 μ L sodium carbonate (2% w/w) was added. The mixture was allowed to stand for 1 h at room temperature. The absorbance was measured spectrophotometrically at 750 nm. The experiment was done in triplicate. Gallic acid was used as the standard for the calibration curve. The total phenolic content of the sample was expressed as gallic acid equivalent (GAE) to 1.0 g of extract.

In vitro α -glucosidase inhibitory activity

Inhibition of α -glucosidase was determined as the reported method [9] with some modifications. Briefly, the hydrosol solution was mixed with 4-pNPG (0.25 mM) and α -glucosidase solution from *S. cerevisiae* (0.1 U/mL). Then, the reaction mixture was incubated at 37 °C for 20 min. After incubation, the enzyme activity in the reaction was stopped by adding 0.50 M Na₂CO₃. The release of the p-nitrophenolate group was measured at 405 nm using a UV/Vis spectrophotometer.

In vitro α -amylase inhibitory activity

Inhibitory activity of the guava hydrosol against α -amylase was determined according to the reported method [18] with some modifications. Aliquots of the hydrosol were mixed with soluble starch (1%, w/v) in 20 mM PBS (phosphate buffer saline, pH = 7.4). The mixture was incubated at 37 °C. After 10 min, α -amylase (1.5 U/mL) was added, and the mixture was incubated at 37 °C for a further 10 min. Then, 3, 5-dinitrosalicylic acid (DNS) solution was added and incubated at 100 °C for 5 min to completely inactivate the enzyme. The absorbance was measured at 540 nm using a spectrophotometer. Acarbose was used as a positive control.

In vitro antiglycation activity

The antiglycation activity of the hydrosol was determined according to the described assay [19] with some modifications. Briefly, Initially, the guava hydrosol, collagen (45 μ M), glucose (200 mM), and fructose (200 mM) were added in phosphate buffer (20 mM, pH 7.4) containing 100 mM sodium chloride. 0.31 mM sodium azide was added to achieve an aseptic condition. Aminoguanidine was used as a positive control. Reaction systems were incubated in the dark for 7 days. Antiglycation activity was quantified using Spectro fluorophotometer (λ_{ex} = 370 and λ_{em} = 440 nm). The experiment was conducted with three independent replicates. The IC₅₀ was calculated graphically, using a calibration curve, plotting the extract concentration versus the corresponding inhibition percentage.

Statistical analysis

To determine the statistical difference between means ($p < 0.05$), using SPSS statistical software package v. 22. Results were expressed as mean values $\pm$ SD.

Results

Hydrosol obtained from hydrodistillation of a ratio of guava leaves: water at 1:10 (w/v) at 100 °C for 2 h yielded 5.4 $\pm$ 0.8 %w/w of fresh leaves. Total phenolics of hydrosol powder was 134.3 $\pm$ 3.6 mg gallic acid/g of hydrosol powder.

Phenolics of interest that existed in hydrosol powder were characteristic and its HPLC profile is shown in Figure 1. Peak no. 1, 2, and 3 represented gallic acid, ellagic acid, and quercetin at the retention time of 6.6, 40.2, and 60.3 min, respectively. The identified phenolic compounds were quantified based on their peak area and compared with calibration curves of standard compounds. The calibration curves of the standards were linear with R^2 values of 0.99 for gallic acid, ellagic acid, and quercetin. The amount of gallic acid, ellagic acid, and quercetin that exist in the guava hydrosol is shown in Table 1.

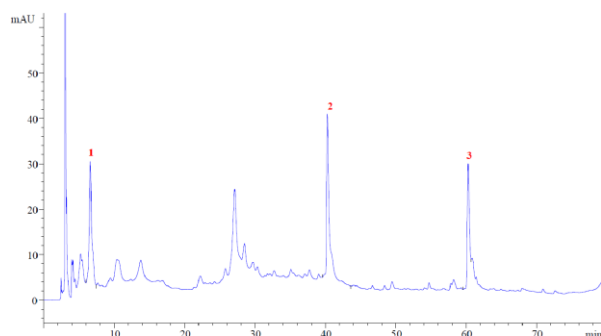


Figure 1 HPLC chromatogram of phenolic compounds existing in the guava hydrosol; (1) gallic acid; (2) ellagic acid; (3) quercetin.

Table 1 Phenolic content and phenolic compound existing in the guava hydrosol (GH).

Compound	Amount (mg/ g hydrosol)
Total phenolic content	134.3 $\pm$ 3.6
Gallic acid	6.9 $\pm$ 0.3
Ellagic acid	33.9 $\pm$ 1.7
Quercetin	24.7 $\pm$ 0.9

The guava hydrosol inhibited the mixture glycation of collagen, glucose, and fructose with an IC_{50} value of 9.6 $\pm$ 1.3 μ g/mL which was significantly higher efficacy than aminoguanidine. In addition, the guava hydrosol inhibited α -glucosidase and α -amylase at IC_{50} values of 413.3 $\pm$ 10.7 and 596.6 $\pm$ 4.1, respectively.

Table 2 displays that ellagic acid inhibited both α -glucosidase and α -amylase with IC_{50} of 37.4 $\pm$ 1.4 and 194.4 $\pm$ 2.8 μ g/mL which high efficacy than acarbose. Whereas gallic acid and quercetin inhibited only α -glucosidase with IC_{50} of 848.3 $\pm$ 4.3 and 82.0 $\pm$ 1.8 μ g/mL, respectively.

Table 2 IC_{50} values of guava hydrosol and the polyphenols exist in the guava hydrosol against α -amylase, α -glucosidase, and glycation of collagen-glucose-fructose.

Sample	Inhibitory activity (IC_{50} ; μ g/mL)		
	α -glucosidase	α -amylase	Glycation
GH	413.3 $\pm$ 10.7 ^d	596.6 $\pm$ 4.1 ^c	9.6 $\pm$ 1.3 ^a
Acarbos	361.4 $\pm$ 10.2 ^c	537.3 $\pm$ 4.4 ^b	-
e			
AM	-	-	76.19 $\pm$ 2.5 ^b
GA	848.3 $\pm$ 4.3 ^e	-	-
EA	37.4 $\pm$ 1.4 ^a	194.4 $\pm$ 2.8 ^a	-
QCT	82.0 $\pm$ 1.8 ^b	-	-

Note: GH= guava hydrosol; AM = aminoguanidine; GA= gallic acid; EA = ellagic acid; IC_{50} =concentration that inhibits 50% of the enzyme activity/glycation fluorescence. The different letters in the same column indicate a statistically significant difference ($P < 0.05$).

Discussion

The goal of this study was first to determine the phenolic content of the guava hydrosol due to previous studies reported that the amount of phenolic correlated with antiglycation activity and both enzyme inhibition [6,9,20]. Gallic acid, ellagic acid, and quercetin were chosen to examine their inhibitory effects on α -amylase and α -glucosidase since they are the most abundant polyphenol components in guava hydrosol. The pure polyphenol compound inhibited α -glucosidase more effectively than the hydrosol powder. This may be because the guava hydrosol had complicated phenolic compounds that prevented the action of individual polyphenol compounds attaching to the active sites of the enzymes or the enzyme-substrate complexes [10,21], so lowering their inhibitory impact. The inhibitory effect of polyphenol compounds on α -amylase may have been influenced not only by the overall polyphenols content but also by the identities and amounts of polyphenol compounds. Notably, unlike pure polyphenol compounds, quercetin and gallic acid exhibited no inhibitory impact on α -amylase. This may be owing to the different ring structures of polyphenol compounds. It has been shown that hydroxylation on the different positions of polyphenol rings can considerably alter their affinity for α -amylase, hence altering their inhibitory impact [22]. Regarding α -amylase inhibiting properties, the increased inhibitory impact of the guava hydrosol compared to the studied pure chemical components (gallic acid and quercetin) may be attributable to the effect of other unidentified components or the synergistic effect of some components that play a significant role in the enzyme's inhibition [23].

There is substantial evidence that the protein glycation process is one of the main factors contributing to the aging of the skin. AGEs build throughout the process of protein glycation and cause structural changes in proteins such as collagen. Natural and

manmade substances have been explored as AGE formation inhibitors. Compounds with antioxidant and photoprotective properties, such as gallic acid, ellagic acid, and quercetin, also prevent advanced glycation effectively [24]. In this work, the guava hydrosol inhibited the production of a glycation system made of collagen, glucose, and fructose very effectively, making it a great candidate for application as a natural treatment for degenerative disorders and aging. Prior to a prospective pharmaceutical application, however, additional research is required to determine the mechanism of action and the in vivo response in appropriate animals.

Conclusion

This work was first reported to evaluate the biological activity of hydrosol powder derived from essential oil distillation of guava leaves. The hydrosol powder expressed high activity against glycation, α -amylase, and α -glucosidase. The present activities can be attributed to bioactive phenols that exist in the hydrosol. The results suggest that guava hydrosol could be considered an alternative source of antiaging and antidiabetic ingredients.

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