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Antiglucoxidase and Antioxidant Activities of Ginger, Cinnamon, Turmeric and Their Combination



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ABSTRACT

Background and Objective: Number of diabetic patients in Indonesia is expected to be 21.3% in 2030. Some traditional medicines have been using crude plant extracts for diabetes managements. Combination of extracts may have more complex effect on diabetes due to the interaction occurred among the individual components. The study aimed to evaluate the effect of combination of ginger, cinnamon and turmeric on anti-glucoxidase and antioxidant activities. Material and Methods: The α -glucoxidase inhibitory activity and antioxidant were measured by α -glucoxidase inhibitory and α , α -diphenyl- β -dipicryl-hydrazyl (DPPH) free radical scavenging assay, respectively. The total phenolic content of the extracts was determined by folin ciocaletus reagent method. The IC50 value of the most potential extract was calculated. Observation, findings, and Conclusion: Turmeric extract was the most potent to inhibit alpha-glucoxidase followed by extracts of ginger and cinnamon. Turmeric extract inhibited α -glucoxidase by 68.44 % followed by extracts of ginger and cinnamon, 22.46% and 19.613% respectively. The potentiality of the extracts to sequece free radical was not in concomitant with their anti-glucoxidase activities as cinnamon sequenced 82.6% free radicals, was higher than ginger and turmeric (79.75% and 79.53%, respectively). Combination of the extracts did not exert synergetic or antagonistic effect on anti-glucoxidase activity. Cinnamon extract has the highest ability to neutralize the free radical, followed by ginger and turmeric extracts and mixing of the extracts increase antioxidant activities. Extract containing higher total phenolic compound tend to have higher antioxidant activity but less effective to inhibit alpha glucoxidase enzyme activity.

INTRODUCTION

4 Diabetes mellitus (DM) is a metabolic disorder characterized primarily by abnormally high levels of blood glucose due to failure of glucose metabolism. Number of diabetic patients in Indonesia reached 7.0% in 2014 and has steadily increased since 1980 [1]. Around the world, prevalence of diabetes increases quadruplicate since 1980 in concomitant with the population growth and ageing [2].

4 Type 2 DM is characterized by two primary defects: insulin resistance (diminished peripheral tissue sensitivity to insulin) and relatively impaired beta-cell function (delayed or inadequate insulin release)[3]. In type 2 diabetic patient, blood glucose level should be controlled through increasing of insulin sensitivity or inducing of insulin release to decrease complications and provide better diabetes managements [4, 5]. Several types of glucose lowering drugs to sensitize tissue to insulin or induce insulin production have been developed such as metformin, sulphonylurea, alpha-glucosidase inhibitors, repaglinide, saxagliptin, empagliflozin, liraglutide, and pioglitazone [6, 7]. However, Most of the glucose-lowering drugs may have side effects, including severe hypoglycemia, lactic acidosis, idiosyncratic liver cell injury, permanent neurological deficit, digestive discomfort, headache, dizziness and even death [5-7]. Therefore, safe and effective pharmacological drugs should be developed especially from plant origin [8].

Some traditional medicines have been using crude plant extracts for diabetes managements. The plant extracts are generally considered safer than the synthetic one, less toxic, more accessible and cheap [8, 9]. Some compounds extracted from turmeric (*Curcuma longa*), for instance, has inhibition against alpha-glucosidase [10, 11], exhibited antioxidant defend in patients with T2DM [12] and been used for the treatment of diabetes in Ayurvedic and traditional Chinese medicine [13]. In combination with metformin, aqueous extract of *Curcuma longa* protects the islets β cells, decreases the insulin resistance and decreases the oxidative stress [14]. Ginger (*Zingiber officinale* (ROSC)) powder consumed daily, was used for patient with type 2 diabetes [13], and as a supplement, it effectively reduced fasting blood glucose concentrations and HbA1c levels[15]. Similarly, cinnamon (*Cinnamomum burmannii*) extract showed the strong anti-lipase and anti-amylase activities that may be important in the alleviation and prevention of the signs and symptoms of type 2 diabetes [16, 17]. These spices are not only used as medicine but also they have been used as condiments in Indonesia traditional foods and modern foods.

Plant extracts can be used as single herb or combination of herbs [16]. Combination of extracts may have more complex effect due to the interaction occurred among the individual components. Interaction is beneficial when the therapeutic effect of the combination is enhanced, however, combination does not always improve the efficacy of the extracts [16, 18]. Due to the fact that plant extract contains multiple compounds, effects of combinations are often unpredictable and complicated [18, 19]. One of mechanism how the plant extracts prevent increasing of blood glucose is by inhibition of starch metabolism through inactivating of starch hydrolysis enzymes such as alpha-glucosidase and alpha-amylase [8, 10, 15]. Some plant extracts also contain antioxidants that have been hypothesized to have a protective effect against the development of diabetes by inhibiting peroxidation chain reactions [12, 20, 21]. Therefore, we aimed to evaluate effect of combination of ginger, cinnamon and turmeric water extracts on anti-glucosidase and antioxidant activities, and elucidate the role of phenolic compound on the anti-glucosidase and antioxidant activities.

MATERIALS AND METHODS

Plant materials

Ginger, cinnamon and turmeric were collected from local market (Bandar Lampung). Ginger and turmeric, immediately, after collection were thoroughly washed with water, peeled, sliced and dried in an oven at 60°C. Cinnamon was purchased as dried bark. All dried materials were powdered using grinder to produce coarsely powder

Preparation of extracts

Dried plants powder (10 g) were boiled in 100 mL water for 20 minutes. The extract was filtered (extract 1), and the residue was reboiled in 100 mL for 20 minutes, then filtered to get extract 2. Extract 1 and extract 2 were then mixed and considered as 100% extract.

Chemicals, reagents and solvents

The materials needed for the analysis include sodium carbonate (Merck, Germany), Folin-Ciocalteu (Merck, Germany), pNPG (p-nitrophenyl- α -D-glucopyranoside) (Sigma Aldrich, Switzerland), crude enzyme α -glucosidase (Shandong Longda Bio-Products Co., Ltd.), DPPH (2,2-Diphenyl-1-Picrylhydrazyl) (Merck, Germany), ethanol (JT Baker), distilled water,

acarbose (Dexa Medica, Indonesia) and gallic acid hydrate (Tokyo Chemical Industry Co., Ltd).

Total phenolic analysis

Total phenolic content was determined using the Folin–Ciocalteu reagent [22] with slight modification. Briefly, 0.2 mL of plant extracts were thoroughly mixed with 0.2 mL of aquades and 0.2 mL of freshly diluted Folin–Ciocalteu reagent (1 N). Then 4 mL of sodium carbonate solution (2%) was added and the mixture was incubated for 30 min in the dark. The absorbance was measured at 760 nm using Spectrophotometer (Thermo Scientific Genesys 20, USA). Gallic acid (0.00–0.01 mg/mL) was used as a reference standard. The concentration of phenolic content was expressed as mg of gallic acid equivalents (GAE) per mL extract.

Antioxidant activity measurement

The antioxidant activity of the extracts was measured using DPPH radical scavenging activity methods [23]. Plant extracts (0.25 mL) was added with 2 mL DPPH solution (3.3 mg of DPPH in 100 mL methanol) and 8 mL ethanol, then vortexed gently. The mixture was incubated for 30 min in the dark and the absorbance (A1) was read at 517 nm (Thermo Scientific Genesys 20, USA). The absorbance (A0) of a reaction control (methanol instead of plant extract) was also recorded at the same wavelength. Scavenging ability (%) was calculated by using the formula = $[(A0 - A1)/A0] \times 100$, where A0 was the absorbance of reaction control and A1 was the absorbance of extracts.

Assay of α -glucosidase inhibitory activity

The effect of the plant extracts on α -glucosidase activity was determined according to the method described by Rao et al. [24] using α -glucosidase crude enzyme (Shandong Longda Bio-Products Co., Ltd.) with light modification. The substrate solution p-nitrophenylglucopyranoside (pNPG) was prepared in aquades (0.03012 g/100mL). 200 μ L of the plant extracts were preincubated with 2 mL of for 10min at 37°C. Then 1 mL of pNPG as a substrate added to start the reaction. The reaction mixture was incubated at 37°C for 30 min and stopped by adding 2 mL of 2% Na₂CO₃. The α -glucosidase activity was determined by measuring the yellow-colored paranitrophenol released from pNPG at 405 nm (Thermo Scientific Genesys 20, USA). Percentage inhibition is calculated as %Inhibition =

$[(\text{Abscontrol} - \text{Absextract})/\text{Abscontrol}] \times 100$. Concentrations of extracts resulting in 50% inhibition of enzyme activity (IC₅₀) were determined graphically.

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Statistical Analysis

Results are expressed as the mean of 3 replicates. Statistical analysis was carried out with a statistical program Minitab version 18. One way-ANOVA with Fisher test was used. Results were considered significant if $p < 0.05$.

RESULTS AND DISCUSSION

Alpha-glucosidase inhibitors were drugs for lowering blood glucose level that inhibits the digestion of carbohydrates in the gut and reduces postprandial hyperglycemia [25, 26]. Inhibition of the intestinal alpha-glucosidase by the drug cause prolongs starch digestion time, and thus reduces glucose absorption rate [24, 26]. Alpha-glucosidase inhibitors are an effective, safe and well tolerated treatment for diabetes [27] therefore, development of the drugs provides an interesting approach in the management of diabetes.

All of the extracts (single or combination) inhibit activity of alpha-glucosidase enzyme (Fig 1). Inhibitory activity of turmeric extract reached more than 50%, much higher than inhibitory effect of ginger or cinnamon extracts. Previously, turmeric ethyl acetate extract [28] and turmerin, as a turmeric water extract [20] were reported had higher alpha-glucosidase inhibitory effect than acarbose, a commercial glucose lowering drug. Bisdemethoxycurcumin, a curcuminoids compound isolated from turmeric, exhibited anti-glucosidase activity better than curcumin and has potentiality as new drug of diabetes [10]. Combination of ginger or cinnamon with turmeric increased their inhibitory activities but the percentages were lower than inhibitory effect of turmeric. Therefore, it is suggested that mixing of the extracts did not produce synergetic or antagonistic effect.

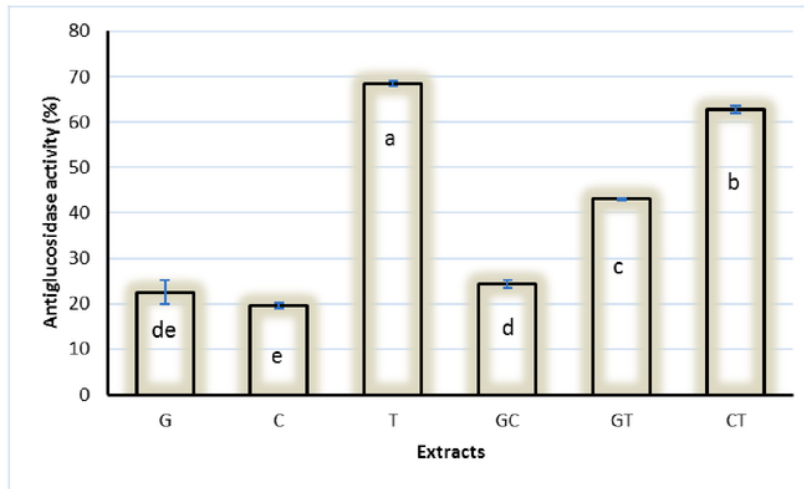


Figure 1. Effect of plant extracts on alpha-glucosidase inhibitory activities. Each value represents a mean \pm SEM (n = 3); G, C and T were ginger, cinnamon, and turmeric extracts, respectively. GC, GT and CT were combination G and C, G and T, C and T, respectively. The bars represent the mean of three replicates. Data points denoted by different superscripts differ significantly with $p < 0,05$.

Effect of turmeric on alpha-glucosidase activity was dose dependent (Fig 2). Inhibitory activity against alpha-glucosidase was upregulated when the turmeric extract level increased. The IC_{50} for turmeric extracts is 2,930 mg / dL. This value was lower than IC_{50} of turmeric ethyl acetate extract but higher than tumerin, a water soluble extract from turmeric, 1.71 mg/dL [28] and 8.1 mg/dL [20], respectively. IC_{50} is the concentration of the sample that is able to inhibit the activity of α -glucosidase as much as 50%.

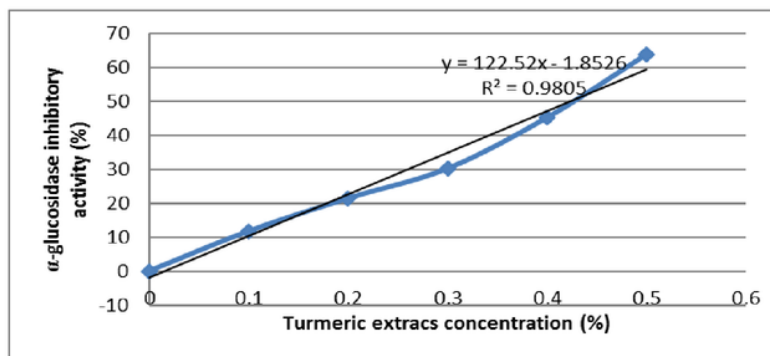


Figure 2. Effect of turmeric extracts concentration (%) on alpha-glucosidase inhibitory activities (%)

6 The aim of diabetes therapy is to reach normoglycemia to prevent complications. Alpha-glucosidase inhibitors decrease blood glucose level through delaying the absorption of ingested starch, therefore, reduces the postprandial glucose and insulin concentrations [25, 29]. Some plant extracts or their bioactive compounds have been shown effective as alpha-glucosidase inhibitors [10, 30, 31]. Kaempferol, a type of flavonoids found widely in the plant extracts, has high affinity against alpha-glucosidase and ultimately inhibiting the enzyme activity [32]. It is suggested that polyphenols in turmeric, cinnamon and ginger water extract also exhibit anti-alpha-glucosidase activity [33].

12 As the pathogenesis of DM involves oxidative stress, antioxidant therapies should have a potential value in its treatment [34, 35], therefore, the present work also involved the evaluation of the antioxidant activity of the single or combination of the extracts. Figure 3 shows a variation in free radical scavenging activities of the extracts which ranged from 79.53 to 82.86%. Among the single extract, cinnamon extract has the highest ability to neutralize the free radical (82.60%), followed by ginger and turmeric extracts (79.75% and 79.53% respectively). Mixing of the extracts increase antioxidant activities as seen in Fig 3, therefore, all combinations have antioxidant as high as cinnamon extract.

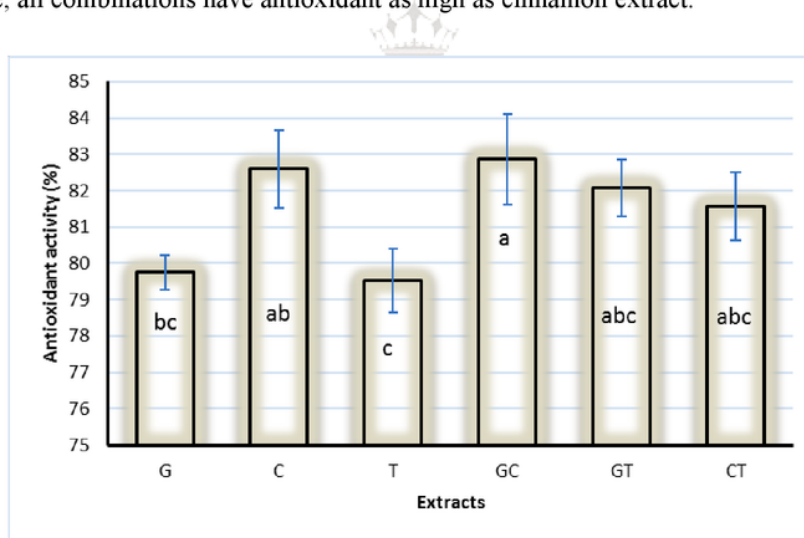


Figure 3. Effect of plant extracts on antioxidant activities. Each value represents a mean \pm SEM (n = 3); G, C and T were ginger, cinnamon, and turmeric extracts, respectively. GC, GT and CT were combination G and C, G and T, C and T, respectively. The bars represent the mean of three replicates. Data points denoted by different superscripts differ significantly with $p < 0.05$.

Extraction method and fraction of the extracts determine antioxidant activity of cinnamon extracts. Infusion of the cinnamon bark had the higher antioxidant activity than ethanol extract, and after fractionated, water fraction of the infusion had better antioxidant activity than ethyl acetate fraction [23]. Previous research showed ginger extract has antioxidant compounds such as gingerol related compounds, diarylheptanoids [36] diarylheptanoids and epoxidicdiarylheptanoids [32] those were suggested involved in initiation of antidiabetic effect of the extract [37]. Meanwhile, ethyl acetate extract of turmeric was very effective to scavenge free radicals and to reduce LDL oxidation and cellular oxidative stress [28].

15 Total phenolic content of the extracts was measured using the Folin-Ciocalteu method and the results are presented in Fig 4. There was a wide range of phenol concentration in the extract as the value varied from 1.44% (GAE) for turmeric to 5.80% (GAE) for cinnamon. 20 Free radical scavenging activity of the extracts may be attributed to their phenolic as indicated that their antioxidant activity levels were significantly positively correlated with their phenolic content ($p = 0.037$) (Table 1). Therefore, extract containing higher total phenolic compound, such as C and GC (Fig 4), tend to have higher antioxidant activity (Fig. 3). However, the phenolic compound of the extracts was significantly negatively correlated with anti-glucosidase activity ($p = 0.001$); extract containing low total phenolic compound was more effective in inhibiting of alpha glucosidase enzyme activity. Turmerin, that had the lowest phenolic content (Fig 4), exhibited the highest inhibitory action against alpha-glucosidase (Fig 1).

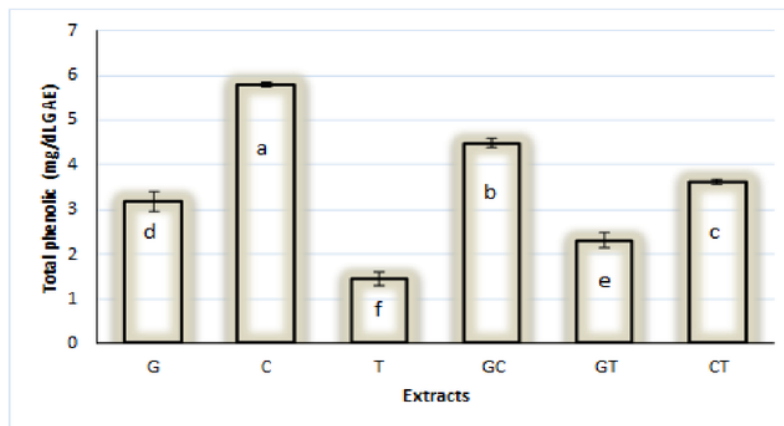


Figure 4. Total phenolic content of plant extracts, single or combination. Each value represents a mean \pm SEM ($n = 3$); G, C and T were ginger, cinnamon, and turmeric extracts,

respectively. GC, GT and CT were combination G and C, G and T, C and T, respectively. The bars represent the mean of three replicates. GAE is galic acid equivalent. Data points denoted by different superscripts differ significantly with $p < 0.05$.

Table 1. Correlation coefficients between total phenolic with antioxidant and anti-glucosidase activities of the extracts.

Activities	Total phenolic	
	Pearson correlation	p-value*
Antioxidant	0.495	0.037
Percent inhibition of glucosidase activity	-0.694	0.001

*Correlation is considered significant when $p < 0.05$

CONCLUSION

Turmeric extract was the most potent to inhibit alpha-glucosidase followed by extracts of ginger and cinnamon. Turmeric extract inhibited α -glucosidase by 68.44 % followed by extracts of ginger and cinnamon, 22.46% and 19.613% respectively. The potentiality of the extracts to sequence free radical was not concomitant with their anti-glucosidase activities as cinnamon sequenced 82.6% free radicals, was higher than ginger and turmeric (79.75% and 79.53%, respectively). Combination of the extracts did not exert synergetic or antagonistic effect on anti-glucosidase activity. Cinnamon extract has the highest ability to neutralize the free radical, followed by ginger and turmeric extracts and mixing of the extracts increase antioxidant activities. Extract containing higher total phenolic compound tend to have higher antioxidant activity but less effective to inhibit alpha glucosidase enzyme activity.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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