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Phytochemical Content, Antioxidant and α-Glucosidase Inhibitory Activities of *Gynostemma pentaphyllum* and *Gymnema inodorum* Extracts

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Abstract

The prevention of hyperglycemia and diabetic complications is essential for diabetes management. Chronic hyperglycemia accelerates the glycation process, increasing advanced glycation end products (AGEs), triggering oxidative stress, and inflammation, thereby causing adverse diabetic complications. Anti-hyperglycemic and antioxidant properties are described for *Gynostemma pentaphyllum* (GP) and *Gymnema inodorum* (GI). With the hypothesis that combined therapy may exert better benefits compared to monotherapy, this study aimed to investigate the anti-diabetic and antioxidant activities of combined GP and GI formulas. Plants were extracted with hot water extraction and subjected to testing of anti-α-glucosidase and antioxidant activities, as well as total phenolic content. The extract formula of GP:GI (1:1, w/w) exhibited the highest total phenolic content, and ABTS radical scavenging activity, with values of 4.16 ± 0.21 mg GAE/g extract and 5.06 ± 0.46 mg TE/g extract, respectively. In addition, the combination of GP and GI extracts at ratios of (1:1, w/w) and (2:1, w/w) demonstrated inhibitory activity against α-glucosidase, reducing its activity by 30.40% and 34.04%, respectively. This property was found to be higher, compared to the activity of a single GP or GI treatment at the same concentration. Thus, results indicated that combining aqueous extracts from *Gynostemma pentaphyllum* and *Gymnema inodorum* has better antioxidant and anti-diabetic properties. This study supports the use of a combined GP:GI formula as a therapeutic remedy for controlling complications in individuals with hyperglycemia.

Keywords: *Gynostemma pentaphyllum; Gymnema inodorum; hyperglycemia; anti-α-glucosidase; anti-diabetic; antioxidant*

1. Introduction

Diabetes mellitus is a chronic metabolic disorder caused by a lack of insulin or insensitivity to insulin, leading to high blood sugar levels and affecting the metabolism of carbohydrates, proteins, and fats (Mohammad et al., 2014). Hyperglycemia escalates the propensity for chronic complications such as microvascular and macrovascular diseases, in addition to acute coronary syndrome, among affected patients (Angeli et al., 2015). Furthermore, chronic high blood sugar leads to increased oxidative stress, which raises levels of pro-inflammatory proteins. This stimulates macrophages to produce inflammatory cytokines, causing inflammation in specific areas of the body (Wellen, & Hotamisligil, 2005). Recent studies on alternative medicines have revealed that natural pharmaceuticals can effectively manage hyperglycemia without inducing adverse side effects

(Hanhineva et al., 2010). *In vitro* and *in vivo* studies indicate that phytochemical-rich foods, particularly those abundant in phenolic compounds, hold promise in preventing chronic degenerative diseases and associated risk factors (GutiErrez-Grijalva et al., 2016). Phytochemical substances in functional foods exert health-promoting effects by scavenging free radicals, reducing oxidative stress, and preventing oxidation of biomolecules. These qualities hold promise for utilization in the management of diabetes (Shahidi, & Ambigaipalan, 2015). Furthermore, a practical therapeutic approach for managing the disease entails attenuating postprandial hyperglycemia by inhibiting α-glucosidase, an enzyme accountable for carbohydrate hydrolysis. This action consequently delays the overall absorption of glucose (Hossain et al., 2020). Consequently, many new studies have been conducted to find potent α-glucosidase inhibitors (AGI) from natural sources (Adisakwattana et al., 2012).

Gynostemma pentaphyllum (Thunb.) Makino, a plant belonging to the Cucurbitaceae family, is commonly known as Jiaogulan in China. This botanical species is renowned for its dual attributes as an edible and medicinal plant (Ahmed et al., 2023). In Thailand, *Gynostemma pentaphyllum* (GP) is predominantly cultivated in the northern area. GP contains various phytochemicals including saponins, flavonoids, polysaccharides and gypenosides (Choi et al., 2013). Gypenosides and dammarane triterpenoid structures found in *Gynostemma* species may play a role in developing natural drugs for metabolic diseases like diabetes mellitus (Ha et al., 2019). GP has previously been reported to exhibit antioxidant, antiproliferative, anti-inflammatory and hypoglycemic properties (Xie et al., 2010; Yang et al., 2013). According to the anti-diabetic effect, previous research indicated that GP exerts comparatively higher ability to inhibit α-glucosidase activity than acarbose. As shown by their result, GP exerted an inhibitory effect on α-glucosidase activity in mice's small intestine and improved glucose tolerance in comparable to the control group (Megalli et al., 2006). In addition, a previous study has reported the antioxidant, anti-inflammatory and anti-diabetic effects of GP extract in mice model, the study suggested such activities as fundamental mechanisms responsible for the potential of GP in prevention and treatment of diabetes (Wang et al., 2020).

Gymnema inodorum (Lour.) Decne, called Chiang Da in Thai, is a climbing vegetable cultivated throughout Southeast Asia (Norkum ai et al., 2023). It is utilized in traditional cuisine and herbal medicine practices in northern Thailand. The leaves of *Gymnema inodorum* (GI) contain several phytochemical components, mainly triterpenoids, gymnemic acids and saponins (Jeytawan et al., 2022). Gymnemic acid is capable of inhibit glucose absorption by binding to the mouth's sweet taste buds and intestinal epithelium receptors (Sanematsu et al., 2014). High concentrations of phenolics, flavonoids, quercetin and kaempferol in GI extracts demonstrated cellular solid antioxidant activity, restoring cell viability under oxidative stress and preventing peroxynitrite-induced cell death (Nuchuchua et al., 2024). Additionally, GI extract enhances glucose tolerance by inhibiting α-glucosidase activity, consequently reducing intestinal glucose absorption and slowing carbohydrate digestion. This mechanism effectively controls postprandial hyperglycemia (Srinuanchai et al., 2021).

While GP and GI exhibit comparable anti-diabetic properties and are widely regarded as safe and cost-effective herbal remedies, their individual therapeutic applications have garnered widespread acceptance. However, the potential synergistic effects arising from the combined administration of GP and GI extracts remain unexplored. Consequently, this study aims to investigate the anti-diabetic and antioxidant capabilities of formulations comprising both GP and GI extracts.

2. Objectives

To investigate the anti-diabetic and antioxidant activities of combined GP and GI formulas.

3. Materials and methods

3.1 Plant materials

This study used *Gynostemma pentaphyllum* cv. Chiang Rai 01, a cultivar developed by the Chiang Rai Horticultural Research Institute, Chiang Rai Province, Thailand. *G. pentaphyllum* plants were cultivated for a period of 3-4 months, with aerial parts harvested in August 2023. Concurrently, a *Gymnema inodorum* leaves sample was obtained from the Community Enterprise Sanmahaphon Organic Herbs, Chiang Mai Province, Thailand. Following collection, fresh samples of GP and GI were dried using a hot air oven for 6 hours, with the temperature set at 60°C.

3.2 Preparation of plant extracts

The extracts were prepared with minimal adjustments using the hot water extraction method

previously utilized in prior research (Ounjaijean et al., 2021). Briefly, dried GP and GI leaves were ground into powder. The powdered samples were then extracted in a 1:10 aqueous solution at 80°C for 10 min and filtered using filter paper (Whatman No.1). The aqueous extracts were dried using a Freeze Dryer (Alpha 1-4 LSCplus, Christ, Germany), operated at -50°C and a pressure of 0.2 mbar for 48 hours. The resulting GP extract (GPE) and GI extract (GIE) were stored at -20°C for further analysis.

During the investigation, 4 different test sample formulas were prepared by dissolving dry extracts into deionized water to the final concentration of 1.25 mg/mL comprising: 1) GPE, 2) GIE, 3) GGRx1 (GPE: GIE, 1:1 (w/w)), and 4) GGRx2 (GPE: GIE, 2:1 (w/w)).

3.3 Chemical properties

3.3.1 Phytochemical contents

1) Determination of total phenolic compounds

Total phenolic compounds (TPC) of the extracts were determined by the Folin-Ciocalteu method previously described (Kulprachakarn et al., 2020). Approximately 20 µL of GPE, GIE, GGRx1, and GGRx2 extract was mixed with 0.15 mL of 10% (v/v) Folin-Ciocalteu reagent (Sigma-aldrich, USA), and the mixture was left for 3 min at the ambient temperature. Then, 0.1 mL of sodium carbonate at 7.5% (w/v) was added to the mixture and promptly measured. Utilizing a microplate reader (BMG Labtech, Ortenberg, Germany), the absorbance at a wavelength of 765 nm was determined. The calibration process utilized Gallic acid as a standard. TPC values were expressed as mg Gallic acid equivalent (GAE)/g sample.

2) Determination of total saponin content

The total saponin content (TSC) was prepared following a protocol previously reported, with minor adjustments (Medina-Meza et al., 2016). Briefly, 0.1 mL of each extract was added to 2.5 mL of reagent mixture (glacial acetic acid/sulfuric acid, 1:1 (v/v)). The mixture was then vigorously vortexed and reacted at 60°C in a water bath for 30 min and immediately cooled down in an ice bath after ending time. The absorbance of the sample was measured at a wavelength of 544 nm using a spectrophotometer (Shimadzu, Kyoto, Japan). Diosgenin was used as the standard for calibration. Total saponin values were expressed as mg Diosgenin equivalent (DIO)/g sample.

3.3.2 Antioxidant capacity

1) The 2,2-Diphenyl-1-picrylhydrazyl (DPPH) Free Radical Scavenging Assay

DPPH was measured following the procedure described previously, with slight modifications (Parklak et al., 2023). The DPPH stock solution was prepared by dissolving 40 mg of DPPH in 100 mL of methanol. The resulting absorbance at a wavelength of 517 nm was 0.70 ± 0.01. A baseline solution or 10 µL of sample extract was combined with 200 µL of a 0.1 mM DPPH reagent. Following that, the solution was kept for 30 min at ambient temperature in a dark environment. The determination of the mixture's absorbance at 517 nm was conducted using a microplate reader (BMG Labtech, Ortenberg, Germany). The values were expressed in terms of mg Trolox equivalent (TE)/g sample.

2) The 2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic Acid) (ABTS) Decolorization Assay

The antioxidant activity of all four extracts was evaluated using a modified method as described in previous studies (Parklak et al., 2023). The stock solution was prepared by mixing 7 mM ABTS reagent with 2.45 mM potassium persulfate ($K_8S_2O_8$) in a 1:1 (v/v) ratio. The combination was kept in the dark for 12 hours at room temperature. After a duration of 12 hours, the mixture was diluted with deionized water until the spectrophotometer detected an absorbance of 0.70 ± 0.02 at a wavelength of 734 nm. A total of 10 µL of GPE, GIE, GGRx1, and GGRx2 were mixed with 0.2 mL of ABTS radical cation stock solution and left to incubate in darkness for 30 min. The absorbance of the combination was measured at a wavelength of 734 nm. The value was expressed in terms of mg Trolox equivalent (TE)/g sample.

3) Ferric Reducing Antioxidant Power (FRAP) Assay

The antioxidant activity of individual GPE, GIE and their formulas was conducted following to the method previously described (Li et al., 2008). The FRAP reagent stock solution was produced by combining 300 mM acetate buffer, pH 3.6 (3.1 g sodium acetate trihydrate and 16 mL glacial acid diluted with an equal quantity of deionized water), 10 mM TPTZ (2,4,6-tris [2-pyridyl]-s-triazine) in 40 mM HCl, and 20 mM $FeCl_3.6H_2O$ in a ratio of 10:1:1, v/v/v. A total of 15 µL of samples were mixed with 0.29 mL of FRAP reagent and left at room temperature for 10 min. The microplate reader was used to measure the absorbance at a wavelength of 593 nm after a duration of 10 min. A standard

calibration curve of Iron (III) sulphate pentahydrate ($FeSO_4.7H_2O$) was plotted. The results were reported in terms of $FeSO_4.7H_2O$/g sample.

3.3.3 Anti-diabetic activity

The α-glucosidase inhibitory assay of GPE, GIE or another two formulas was assessed utilizing modified procedure that had been reported earlier (Bhatia et al., 2019). The α-glucosidase enzyme (20 µL, 1 U/mL in phosphate buffer, pH 6.9) was premixed with 20 µL of sample extract and incubated for 5 min at 37°C. Then, 20 µL of 1 mM p-nitrophenyl glucopyranoside (pNPG) in 50 mM of phosphate buffer (pH 6.9) was added to initiate the reaction. The mixture was incubated at 37°C for 20 min. The reaction was terminated by the addition of 50 µL of 1 mM sodium carbonate. The α-glucosidase activity was determined at 405 nm. The α-glucosidase inhibitory activity was calculated using the formula:

$$\text{Inhibitory activity} = [(X_C - X_S)/X_C] \times 100$$

where X_C is the absorbance of the negative control (100% enzyme activity) and X_S is the absorbance of the sample.

The absorbance values at 405 nm were determined as follows: 100% enzyme activity (solvent with enzyme), 0% enzyme activity (solvent without enzyme), sample (test sample with enzyme), and sample blank (test sample without enzyme), respectively. Acarbose, an anti-diabetic drug, was used as a positive control for assessing α-glucosidase inhibitory activity.

3.4 Statistical Analysis

The obtained data were evaluated using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test for comparing mean values, using SPSS 23 software. All results were presented as mean ± standard deviation. Additionally, Pearson correlation analysis assessed the relationship between certain phytochemical parameters (total phenols and total saponins), antioxidant activities (ABTS, DPPH, FRAP), and α-glucosidase inhibitory activity.

4. Results

The results of total phenolic compounds and total saponin contents of each test sample are presented in Figure 1A and 1B. Analysis of TPC revealed that the combination of GP:GI (1:1, w/w), denoted as GGRx1, exhibited the highest TPC value of 4.16 ± 0.21 mg GAE/g, while the combination of GP:GI (2:1, w/w) (GGRx2) showed a relative lower value of 3.59 ± 0.19 mg GAE/g. Both values, however, were significantly ($p < 0.05$) higher compared to those of single GPE or GIE, which had values of 2.36 ± 0.04 mg GAE/g and 3.15 ± 0.19 mg GAE/g, respectively (Figure 1A). Regarding total saponin contents, GGRx2 demonstrated the highest saponin value (2.21 ± 0.24 mg DIO/g), while the combination of GP:GI (1:1, w/w) exhibited a lower value of 1.90 ± 0.06 mg DIO/g. Both combined formulas were significantly ($p < 0.05$) higher in saponins when compared to GIE (0.66 ± 0.21 mg DIO/g extract) and GPE (1.61 ± 0.16 mg DIO/g extract), respectively (Figure 1B).

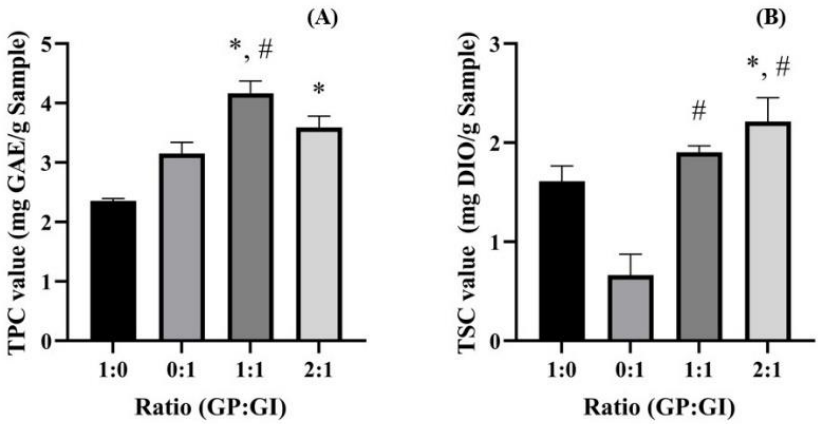

Figure 1 Phytochemical contents of different formulas prepared from *Gynostemma pentaphyllum* and *Gymnema inodorum* extract. (A) Total phenolic compounds (TPC), (B) Total saponin contents (TSC).
Note: $^{*}p < 0.05$ compared with GPE, $^{\#}p < 0.05$ compared with GIE

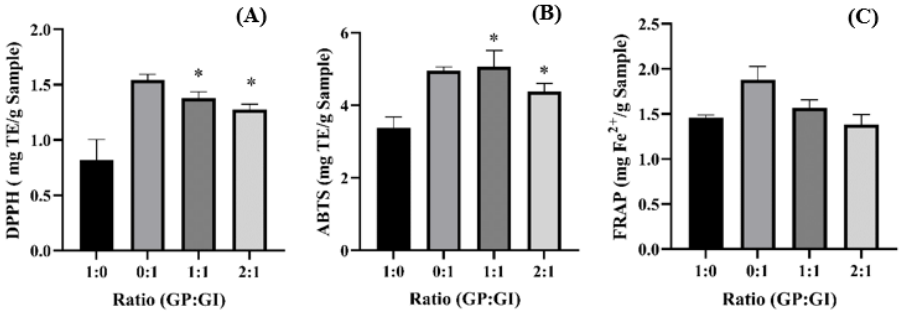

Figure 2 Antioxidant capacity of different formulas prepared from *Gynostemma pentaphyllum* and *Gymnema inodorum* extract determined by different antioxidant assays. (A) DPPH scavenging assay, (B) ABTS decolorization assay, (C) FRAP assay. Note: $^{*}p < 0.05$, compared with GPE. $^{\#}p < 0.05$, compared with GIE.

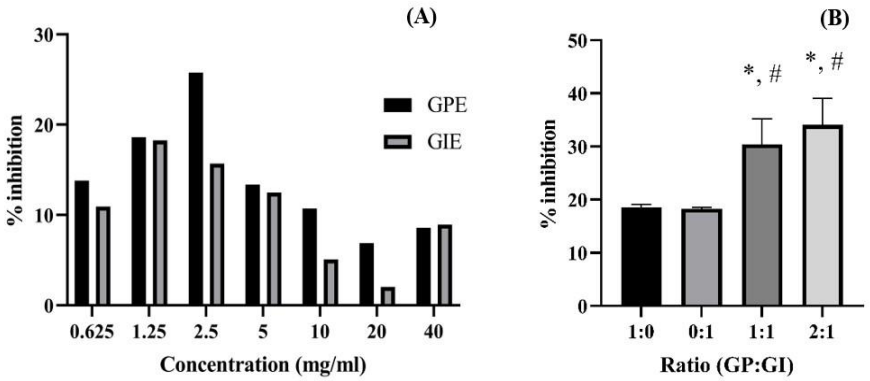

Figure 3 α-Glucosidase inhibitory activity of different formulas prepared from *Gynostemma pentaphyllum* and *Gymnema inodorum* extract. (A) Various concentrations (0.625-40 mg/mL) of GPE and GIE, (B) Single extract (GPE and GIE) and formulas (GGRx1 and GGRx2) at a concentration of 1.25 mg/mL.
Note: $^{*}p < 0.05$ compared with GPE, $^{\#}p < 0.05$ compared with GIE

Figure 2 depicts the results of the antioxidant activity of GPE, GIE, GGRx1, and GGRx2. For DPPH radical scavenging activity, GIE exhibited the highest value of 1.54 ± 0.05 mg TE/g extract, while GPE revealed a value of 0.82 ± 0.18 mg TE/g extract. The combination of GP and GI extracts, in this regard showed significantly ($p < 0.05$) higher antioxidant capacity compared with GPE alone, with values of 1.38 ± 0.05 mg TE/g and 1.28 ± 0.05 mg TE/g, for GGRx1 and GGRx2, respectively (Figure 2A). Regarding ABTS radical scavenging activity of the combined formula, the GP:GI (1:1, w/w) formula exhibited the highest activity at 5.06 ± 0.46 mg TE/g, followed by GP:GI (2:1, w/w) with 4.38 ± 0.23 mg TE/g. GIE and GPE alone revealed their ABTS scavenging activities as presented in Figure 2B, of 4.95 ± 0.11 and 3.37 ± 0.31 mg TE/g, respectively. In addition, in the analysis of ferric ion reducing antioxidant power (FRAP), GIE exhibited the highest value of 1.88 ± 0.15 mg Fe^{2+}/g, while GPE exhibited a value of 1.46 ± 0.03. GGRx1 showed a slightly higher level compared to single GPE, with 1.56 ± 0.09 mg Fe^{2+}/g. However, both GGRx1 and GGRx2 did not exhibit a significant difference ($p < 0.05$) compared to GPE, as shown in Figure 2C.

The results of alpha-glucosidase inhibitory activity (AGI) of each test sample are presented in Figure 3. Effects of various concentrations ranging from 0.625 to 40 mg/mL of GPE and GIE on enzymatic activity are presented in Figure 3A. Maximum inhibitory activity of 25.75% was found at 2.5 mg/mL GPE, followed by 18.60% at 1.25 mg/mL GPE. On the other hand, the highest ability of GIE for glucosidase inhibition was found to be 18.26% at a concentration of 1.25 mg/mL GIE. At a similar dose of 1.25 mg/mL, combined formulas GGRx1 and GGRx2 inhibited AGI by 30.40% and 34.04%, respectively. In addition, both formulas exhibited a significant ($p < 0.05$) increase in inhibitory activity compared to single GPE or GIE as shown in Figure 3B.

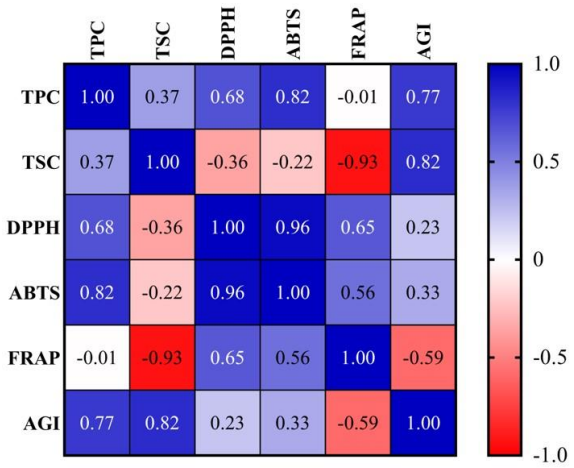

Figure 4 Pearson's correlations coefficient (*r*) represent the correlations among phytochemical content, antioxidant activity, and AGI of the extracts. Note: TPC = total phenolic compounds, TSC = total saponin contents, DPPH = DPPH scavenging property, ABTS = ABTS scavenging property, FRAP = Ferric reducing antioxidant power, AGI = alpha-glucosidase inhibitory activity.
The positive correlations are highlighted in blue. The negative correlations are highlighted with red.
The darker the color indicated the higher the correlation between the variables.

Figure 4 reports the correlations between phytochemical content, antioxidant activity and AGI of GPE, GIE, GGRx1 and GGRx2. The investigation revealed a positive association between TPC:DPPH, TPC:ABTS and TPC:AGI, with correlation coefficients of 0.68, 0.82 and 0.77, respectively. TSC exhibited opposite effects on DPPH and ABTS radical scavenging activities, as well as ferric ion reducing ability, with correlation values of -0.36, -0.22 and, -0.93, respectively. Additionally, AGI also showed a negative correlation of -0.59 with FRAP antioxidant activity.

5. Discussion

This study investigated the phytochemical content, antioxidant capacity and α-glucosidase inhibitory effects of aqueous extracts from the leaves of *Gynostemma pentaphyllum* and *Gymnema inodorum*. Our findings revealed that the combination of both extracts, referred to as GGRx1 and GGRx2 contained significantly higher levels of phytochemicals, including total phenolic and saponin compounds compared to the single GPE or GIE. Moreover, the antioxidant capacity of the GGRx1 and GGRx2 were notably higher than that of the GPE alone. Furthermore, the combination of GPE and GIE exhibited higher AGI compared to the monotherapy. Additionally, our study identified a positive correlation between TPC, antioxidant capacity and α-glucosidase inhibition. Notably, this study represents the first investigation into the combined effects of GP and GI extracts on phytochemical content, antioxidant activity and alpha-glucosidase inhibitory activity *in vitro*.

Medicinal plants have received increased attention due to the presence of various phytochemicals such as alkaloids, tannins, saponins, flavonoids, phenols, steroids, and carotenoids. According to our results, aqueous extracts of the leaves of *G. pentaphyllum* and *G. inodorum* contain certain amounts of phenolic compounds. The findings are consistent with previous- reports of the total phenolic content of *G. pentaphyllum* (8.11 ± 0.25 mg GAE/g) (Šamec et al., 2016) and *G. inodorum* (18.115 ± 0.626 mg GAE/g) (Wongklom et al., 2023). On the other hand, the presence of saponins in GIE was found to be higher compared to GPE. Thus, combining GPE and GIE extracts may lead to an increased diversity of such bioactive phytochemical compounds. Solvent interactions or matrix effects regarding the diversity of phytochemicals in one plant extract may enhance the extraction or stability of phenolic compounds from another plant extract, leading to higher phenolic content in the combined extract (Dai, & Mumper, 2010).

Antioxidants found in medicinal plants have been extensively researched due to their diverse biological activities. Our study found that the DPPH, ABTS, and FRAP antioxidant activities of GGRx1 were higher than those of GPE alone. According to Nuchuchua and colleagues, who reported that *G. inodorum* extract exerts its antioxidant activities via

the ability of active compounds to transfer hydrogen atoms and electrons (Nuchuchua et al., 2024). The results obtained in this study revealed weak antioxidant capacity of the aqueous GP extract, which is in agreement with lower antioxidant capacity of aqueous GP extract in comparison with methanolic extract of the same plant (Šamec et al., 2016). Nonetheless, previously published data on combining *Gymnema sylvestre* and *Combretum micranthum* extracts in a 1:1 ratio demonstrate enhanced antioxidant properties compared to monotherapy (Ibrahim et al., 2017). Accordingly, this present study revealed that the combination of GPE and GIE in a 1:1 ratio significantly enhanced scavenging activity against DPPH and $ABTS^{\bullet+}$ radicals compared to individual GPE. This suggests that the combination between the two plant extracts could be beneficial for treating pathological damage associated with oxidative stress. The combined formulas of GPE and GIE offer potential in addressing free radical-induced diseases compared to single-agent therapy.

Alpha-glucosidase, an enzyme that facilitates the breakdown of α-1,4 linked polysaccharides into glucose molecules, provides energy for cellular functions by accelerating glucose metabolism (Kashtoh, & Baek, 2022). A substance with α-glucosidase inhibitory activity, so called α-glucosidase inhibitors (AGIs), thus capable of inhibiting the absorption of carbohydrates from the gut and may be used in the treatment of patients with type 2 diabetes or those who have impaired glucose tolerance. According to the results, the aqueous extracts of *G. pentaphyllum* and *G. inodorum* exert α-glucosidase inhibitory activity, with the highest percentage of inhibition observed at a concentration of 2.5 mg/mL and 1.25 mg/mL for GPE and GIE, respectively. These findings are in line with the in vitro observation on α-glucosidase inhibitory effects of ethanolic extract of *G. inodorum* (Srinuanchai et al., 2021) or *G. pentaphyllum* (Wang et al., 2019). Previous studies have demonstrated the alpha-glucosidase inhibitory effects of ethanolic *G. pentaphyllum* extract (50%, 42.8 µg/mL) (Megalli et al., 2006) and methanolic *G. inodorum* extract (40%, 200 µg/mL) (Trang et al., 2021). Unlike previous studies using organic solvents, this investigation employed hot water for sample extraction, mimicking the typical preparation of tea and ensuring safety for potential clinical applications. The results indicate that both *G. pentaphyllum* and *G. inodorum* extract demonstrated moderate inhibitory effect on α-glucosidase activity with increasing extract concentrations. Consequently, by combining two plant extracts, the GGRx1 and GGRx2 formulas demonstrated a significant increase of inhibitory activity against alpha-glucosidase up to 87%. The results suggest a synergistic benefit of combining the two extracts compared to single therapy. These findings are consistent with prior research showing that the combination of *G. inodorum* and other plant extract can improve its biological activities compared with its own (Tiamyom et al., 2019). Furthermore, prior investigations into the sub-chronic toxicity study of *G. pentaphyllum* extract have demonstrated possible modification effects on hematological and blood chemistry parameters (Chiranthanut et al., 2013). Consequently, since the combined GPE and GIE extracts exhibited more potent α-glucosidase inhibition compared to each extract individually, lower total concentrations could achieve the desired biological activity. This property of the combination therapy is advantageous for human therapeutic applications by maximizing efficacy while minimizing potential toxicity risks.

Our results demonstrated a positive correlation among total phenolic content (TPC), antioxidant capacities (ABTS and DPPH assays) and α-glucosidase inhibitory (AGI) activity, suggesting that phenolic and flavonoid compounds contribute significantly to the antioxidant effects through their ability to donate hydrogen atoms for neutralizing free radicals. These observations align with the well-established roles of phenolic compounds as potent DPPH radical scavengers and α-glucosidase inhibitors, which have been extensively documented (Dai, & Mumper, 2010; Zhang et al., 2013). Quercetin, kaempferol and their derivatives have been shown to possess antioxidant and antidiabetic activities (Chayarop et al., 2011; Lawal et al., 2015).

Furthermore, saponins available in *G. pentaphyllum* are potent compounds associated with anti-inflammatory and antioxidant properties (Zhao et al., 2012). Conversely, this study found a negative correlation between total saponin content (TSC) and antioxidant capacities determined by DPPH, ABTS and FRAP assay. Though positive correlation between TSC and α-glucosidase inhibitory (AGI) activity was found. This contradictory evidence may result from interactions among the various active compounds present in the reaction mixtures. In contradiction, the negative correlation between TSC and antioxidant capacity or between AGI and FRAP were found in this study. This evidence may be attributed to the interaction among various active compounds present in the reaction mixture.

Therefore, the present findings indicated that the combined GPE and GIE may exhibit superior anti-diabetic and antioxidant effects compared to either extract alone. This enhanced therapeutic potential likely arises from synergistic inhibition of carbohydrate absorption in the intestine to mitigate hyperglycemia, as well as free radical scavenging activities. Notably, a lower combined dosage of the extracts was required to achieve the same effect as higher individual dosages of GPE or GIE. Additionally, the interaction between the two plant extracts demonstrated synergistic effects regarding total phenolic content (TPC), total saponin content (TSC), DPPH and ABTS radical scavenging activities, and glucose inhibition activity (GIA). Such a finding certainly corroborates the findings with previously reported (Li et al., 2021). The synergistic effects observed from the interactions between the extracts in this study could be ascribed to the diverse array of phytochemical compounds present, as well as the inherent adaptability and complexity of such interactions between the constituents of the individual extracts.

While many studies have revealed the potential antidiabetic effects of GPE or GIE individually, research investigating formulations combining GPE and GIE to enhance their inhibition of α-glucosidase for managing diabetes mellitus is still lacking. Moreover, recent evidence shows that patients with Type 2 diabetes experience increased free radical production and impaired antioxidant defenses, leading to various complications. Supplementation with antioxidants could therefore provide therapeutic benefits for these patients (Unuofin, & Lebelo, 2020). The results demonstrated that the combined GPE and GIE extracts exhibited higher antioxidant potential than extract alone, suggesting they may help improve antioxidant status in Type 2 diabetes. This study indicates that formulating GPE and GIE together can synergistically enhance antioxidant capacity and may aid in preventing hyperglycemia. One limitation of this study is that only crude extracts were utilized, and tests were conducted solely *in vitro*. Future research should aim to isolate and characterize the specific active compounds present, as well as investigate potential synergistic interactions between different plant extracts and their combined effects in more physiologically relevant models.

6. Conclusion

This study demonstrates that combining aqueous extracts from *Gynostemma pentaphyllum* and *Gymnema inodorum* synergistically enhances their antioxidant and anti-diabetic properties. The phytochemical contents of the combined extracts correlate with increased antioxidant activity and inhibition of the α-glucosidase digestive enzyme. Our findings suggest that this combined extract formulation shows promise as a therapeutic intervention for managing blood glucose levels and oxidative stress in individuals with hyperglycemia. However, further research is necessary to identify the specific active molecules conferring these effects and evaluate the therapeutic potential in preclinical *in vivo* models before potential clinical applications can be assessed.

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