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Phenolics-Enhancing *Piper sarmentosum* (Roxburgh) Extracts Pre-Treated with Supercritical Carbon Dioxide and its Correlation with Cytotoxicity and α-Glucosidase Inhibitory Activities

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ABSTRACT

Piper sarmentosum was found to have various phytochemicals such as polyphenols and flavonoids but also contains asarone isomers, which were reported as carcinogenic in several preclinical studies. Therefore, reducing asarone isomers from this functional food is important while retaining its functional properties. This study compares the total phenolics, total flavonoids, vitexin and naringenin contents, cytotoxicity, and anti-hyperglycaemic activity between various solvent extracts using a two-step extraction with SC-CO_2 pre-treatment and a single-step conventional solvent extraction without SC-CO_2 pre-treatment. The findings showed that phenolic content was significantly enhanced in ethanol, 50% ethanol, and water extracts pre-treated with SC-CO_2, significantly correlated with α-glucosidase inhibitory activity. SC-CO_2 pre-treated extracts enhanced the viability of two normal fibroblasts NIH/3T3 and CCD-18Co cell lines. It is concluded that SC-CO_2 extraction offers a rapid pre-treatment step to produce safer extracts with better quality and efficacy.

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INTRODUCTION

Carbon dioxide in supercritical fluid extraction (SFE) has been used as a treatment to lessen or enhance the extraction of natural products using organic solvents (Arumugham et al., 2022; Voung & Roach,

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2014). The fundamental benefit of this method is that by adjusting pressure and temperature settings, the physicochemical properties of the solvent may be changed to increase extraction selectivity (Fayaz et al., 2021). SFE was proven to be beneficial in the removal of toxic and undesirable compounds such as aristolochic acid from Aristolochia plants (Liang et al., 2010), phthalate plasticiser residue in *Ganoderma lucidum* (Li et al., 2018), and many other undesirable substances from food and herbal-based supplement products (Kobori et al., 2013; Shinde & Mahadik, 2019). Previously, we established a condition to reduce potentially carcinogenic asarone isomers from *Piper sarmentosum* leaves by removing 80.42%–100% of α-asarone, 91.26%–100% of β-asarone, and 89.24%–100% of γ-asarone by using supercritical carbon dioxide (SC-CO_2) extraction while applying the Box–Behnken experimental design at optimised pressure (P) = 81.16 bar, temperature (T) = 50.11°C, and time (t) = 80.90 min (Hamil et al., 2022).

Piper sarmentosum Roxburgh (Piperaceae), a creeping terrestrial shrub native to Malaysia, Cambodia and Thailand, is widely used in medicinal as well as culinary applications. In traditional medicines, all plant parts are utilised in treating health problems. Leaves and roots of *P. sarmentosum* are usually used to alleviate headaches, toothache, pleurisy, flu, rheumatism, cough and asthma (Ismail et al., 2018). Apart from that, the whole *P. sarmentosum* parts are used as an antispasmodic, expectorant, antiflatulence, and appetite enhancer, as well as for treating diabetes in Thailand (Rahman et al., 2016). The roots and leaves were also reported as carminative, stomachic and able to reduce ostalgia (Muhamad & Mustafa, 2010; Ridtitid et al., 1998). *Areca catechu* and *P. sarmentosum* roots can be taken together to relieve asthma and cough (Seyyedan et al., 2013). Furthermore, taken together with ginger or nutmeg, it may alleviate toothache and pleurisy. Anti-pyretic and stomachic activities have also been established from the plant leaves in Chinese traditional medicine (Sun et al., 2020).

Traditional and modern medicinal knowledge of the plant has led to scientific investigations of its pharmacological potential. One of the pharmacological properties reported was in treating diabetes or hyperglycaemia. Krisanapun et al. (2012) reported that the aerial part of *P. sarmentosum* water extract (5 and 10 mg/mL) substantially reduced glucose absorption, which was similar to positive control (sodium fluoride). The improvement of glucose metabolism and reduction of its absorption were credited with the hypoglycaemic activity. In another study, oral administration of aqueous extract (125 mg/kg) significantly reduced blood sugar after 7 days of treatment (Peungvicha et al., 1998). Hussan et al. (2013) also reported that 28 days of administration of *P. sarmentosum* leaves water extract (125 mg/kg) in streptozotocin-induced diabetic rats successfully decreased blood sugar levels. In addition, there was an improvement in glomeruli, suggesting that the extract exerted a nephroprotective effect.

SC-CO_2 is a new, simple, yet rapid method in *P. sarmentosum* pre-treatment to remove carcinogenic asarones from the raw leaves selectively. The pre-treated leaves can be re-extracted using solvents to extract beneficial phenolics, flavonoids and other phytochemicals. However, no study is available on the phytochemical and bioactivity profiles of the SC-CO_2 pre-treated *P. sarmentosum* extracts. Therefore, this work focused on evaluating the phytochemical contents, cytotoxicity and anti-hyperglycaemic activity, including α-glucosidase and α-amylase-inhibitory assays between three solvent extracts, namely ethanol, 50% ethanol and water extracts with their extracts after being treated with SC-CO_2.

MATERIALS AND METHODS

Plant Material

Fresh *P. sarmentosum* leaves were collected from Batu Gajah, Perak, Malaysia. The plant was identified by a botanist at the School of Biological Sciences, USM, Penang, Malaysia (voucher specimen number 11481). The plant materials were washed thoroughly and dried at 40°C for five days. The thoroughly dried leaves were ground into fine powder and stored in a 2–7°C container before further use.

Treatment of *P. sarmentosum* Leaves Using SC-CO_2

Dried powdered leaves (50 g) were extracted using a 1-litre SC-CO_2 extractor (Separex, France). The experimental parameters were set according to the method established by Hamil et al. (2022): pressure = 81.16 bar, temperature = 50.11°C and dynamic extraction time = 80.90 min. The flow rate was maintained at 30 g/min, and the static extraction time was fixed at 30 min. The residue of the leaves was subsequently extracted with conventional solvents for analysis.

Conventional Extraction of *P. sarmentosum* Leaves

Three types of extraction solvents, ethanol (EM), 50% ethanol (EWM) and water (WM), were used in the present study. First, powdered material (10 g) was macerated with one of the stated solvents or solvent mixtures at 50°C for 24 hrs. The raw material to solvent ratio was fixed at (1:20). The samples were prepared in triplicate. The same extraction methods were performed on the SC-CO_2 treated leaves material for each type of solvent used, namely ethanol maceration residue (EM-R), 50% ethanol maceration residue (EWM-R) and water maceration residue (WM-R). The extracts were concentrated, and their yields were calculated using Equation 1:

$$\text{Yield (\%)} = \frac{\text{Dried extract weight}}{\text{Original sample weight}} \times 100 \qquad [1]$$

Determination of Total Phenolics Content

A colorimetric assay was performed to determine total phenolic content (TPC) as Ghasemzadeh et al. (2010) described with slight modification. *P. sarmentosum* extracts (100 µg/mL) were prepared in methanol. Next, distilled water (1 mL) was added, followed by Folin-Ciocalteu phenol reagent (100 µL). The samples were incubated for 5 min in dark conditions before adding 20% sodium carbonate (200 µL). Further incubation was performed in dark conditions for 60 min, and absorbance was measured at 750 nm. Gallic acid prepared in the range of 0.20–200 µg/mL was used to construct the standard calibration curve. Total phenolics were calculated based on the linear regression and expressed as mg of gallic acid equivalent (mg GAE/g).

Determination of Total Flavonoids Content

The aluminium chloride ($AlCl_3$) method was used to determine total flavonoid content (TFC), as described by Asha et al. (2010). *P. sarmentosum* extracts (500 µL) were mixed with 1.5 mL of methanol. Then, 10% $AlCl_3$ and 1M potassium acetate (100 µL each) were added. Absorbance was measured at 415 nm after 30 min incubation against a blank containing all reagents and samples except $AlCl_3$. Quercetin in the 3.125–100 µg/mL range was used to construct the standard calibration curve. Total flavonoids were calculated from the linear regression and expressed as mg of quercetin equivalent (mg QE/g).

HPLC Analysis of Vitexin and Naringenin

A gradient HPLC system for the separation of vitexin and naringenin was performed based on the validated method described by Hamil et al. (2016) using an Agilent 1100 HPLC system and ZORBAX Eclipse Plus C-18 column (Agilent, USA). The mobile phase consisted of A (0.1% formic acid) and B (0.1% formic acid in acetonitrile). The mobile phase composition (A: B) was initially set at 85:15 before gradually increasing solvent B to 75% for 10 min, remaining constant for 1 min, and then changing to 85:15 in the final 5 min. An injection volume (10 µL) and flow rate were set at 1 mL/min. The temperature and detector were operated at 30°C and 330 nm.

Preparation of Reference Markers and Samples. Vitexin and naringenin were dissolved in HPLC grade methanol (Merck, USA) and mixed to obtain a 1 mg/mL solution. The mixture was then serially diluted (1.56–50 µg/mL) to produce calibration curves. *P. sarmentosum* extracts (2 mg/mL) were prepared in their respective solvents and filtered through 0.45-µm syringe filters prior to analysis.

Quantification Analysis of Vitexin and Naringenin. 10 µL of each extract was injected, and peak areas corresponding to the reference markers were recorded. The linear equations

for each marker were used to calculate the concentration of vitexin and naringenin in the samples, and the findings were given as the average of wt/wt% using Equation 2 (n=3):

$$\text{wt/wt\%} = \frac{\text{mass of marker}}{\text{mass of extract}} \times 100 \quad [2]$$

Cytotoxicity Study

Cytotoxic activities of *P. sarmentosum* extracts were determined using mouse embryo fibroblast (NIH/3T3) and human colon fibroblast (CCD-18Co) normal cell lines using the method proposed by Tajudin et al. (2012). The cells were seeded in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% foetal bovine serum (FBS) with 1% penicillin/streptomycin. Following a 24-hour incubation, different concentrations of *P. sarmentosum* extracts (0–100 µg/mL) were added and further incubated for 72 hrs. Next, 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reagent (20 µL) was added and subsequently incubated for 4 hrs. After that, the growth medium was removed, 100% of dimethyl sulfoxide (100 µL) was added and incubated for 15 min. Cell viability was quantified using an M200 ELISA microplate reader (Tecan, Switzerland) at 570 nm and 630 nm. Graphs of the cell's proliferation percentage against the concentration of the extracts were used to determine IC_{50}. Doxorubicin was used as a positive control.

Determination of α-Glucosidase Inhibition Activity

An α-glucosidase inhibition assay was evaluated according to Feng et al. (2011) with some modifications. Baker's Yeast α-glucosidase and P-Nitrophenyl-α-D-glucopyranoside (PNPG) were purchased from Sigma (USA). 20 µL of yeast α-glucosidase (0.8 U/mL) in 0.1 M phosphate buffer (pH 6.8) were added to 120 µL *P. sarmentosum* extracts (50–2000 µg/mL). The mixture was incubated for 15 min. Next, 20 µL of PNPG (0.005 M in 0.1M phosphate buffer at pH 6.8) was added and further incubated at 37°C for 15 min. Eighty µL of 0.05 M sodium carbonate was added to the mixture to stop the reaction, and the absorbance was read at 405 nm. Control samples were prepared without plant extracts, and acarbose was used as a positive control. The percentage of inhibition was calculated according to Equation 3 (n=3):

$$\text{Inhibition (\%)} = \frac{\text{Abs (Control)} - \text{Abs (Extract)}}{\text{Abs (Control)}} \times 100 \quad [3]$$

The IC_{50} values were determined from plots of percentage inhibition versus log inhibitor concentration.

Determination of α-Amylase Inhibition Activity

An α-amylase inhibition assay was adapted from Bharathi et al. (2014) with slight modification. Different concentrations (100–5000 µg/mL) of *P. sarmentosum* extracts were prepared in 1 mL DMSO. 500 µL of α-amylase solution (0.5 mg/mL) in 0.02 M sodium phosphate buffer (pH 6.9) were mixed with 500 µL of the extracts. The mixture was incubated at 25 °C for 10 min. Next, 500 µL of 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9) was added and incubated at 25°C for 10 min. After that, 3, 5 dinitrosalycylic acid (DNSA) colour reagent (1 mL) was added to stop the reaction. After a 5-minute incubation in a boiling water bath, the tubes were cooled to room temperature. Distilled water (10 mL) was finally added, and absorbance was measured at 540 nm. Acarbose was used as a positive control. The percentage of inhibition by α-amylase was calculated using Equation 4 (n=3):

$$\text{Inhibition (\%)} = \frac{\text{Abs (Control)} - \text{Abs (Extract)}}{\text{Abs (Control)}} \times 100 \qquad [4]$$

The IC_{50} values were determined from plots of the percentage of inhibition versus log inhibitor concentration.

Statistical Analysis

Statistical analyses were conducted using the SPSS 22.0 software package (IBM, USA). Analysis of variance (ANOVA) was performed to estimate significant differences between samples, and unpaired *t*-tests were used to compare two groups (SC-CO_2-treated *vs.* untreated). Correlation coefficients between α-glucosidase and cytotoxicity assay with metabolites profile including total phenolics, flavonoids, vitexin and naringenin were calculated using Pearson correlation analysis. Statistical significance was determined at $p<0.05$ in all the analyses.

RESULTS AND DISCUSSION

Enhancement of Phenolics and Flavonoids in SC-CO_2 Pre-Treated Extracts

The total phenolic and flavonoid content between EM, EWM, and WM and their SC-CO_2 pre-treated extracts (EM-R, EWM-R, and WM-R) are shown in Table 1. All extracts found total phenolics in a range of 39.29–107.91 mg/g gallic acid equivalent (GAE). Comparatively, total phenolics in EM-R, EWM-R and WM-R were significantly enhanced at $p<0.05$. The highest enhanced total phenolics was obtained in EM-R with 107.91 mg/g compared to EM with 105.52 mg/g. The enhancement in EWM-R (75.56 mg/g) and WM-R (43.26 mg/g) was observed compared to non-treated EWM and WM with 68.11 and 39.29 mg/g, respectively. Total flavonoids ranged between 0.30–1.36 mg/g QE for ethanol, 50%

ethanol, and water extracts. Enhancement of total flavonoids in SC-CO_2 pre-treated extracts was observed in EM-R and EWM-R; however, the differences were not significant.

SC-CO_2 has been proven to enhance the extraction of metabolites from plant materials. It was used as a pre-treatment to remove non-polar compounds from elderberry (Seabra et al., 2010). Subsequent re-extraction of the residue material with 50% ethanol showed an enhanced concentration of phenolic contents. Comparing the two-step extraction to the standard single-step extraction, the total phenolics recovered were substantially higher. The enhanced extraction efficiency of phenolic compounds was attributed to the effective removal of non-polar components by CO_2 and the breakage of the cell walls in plant tissues, which was caused by high pressure (Vatai et al., 2009). Another study reported that an optimised SC-CO_2 pre-treatment removed 80.1% of caffeine from cocoa powder. Besides that, the residual powder re-extracted with solvent retained polyphenols and theobromine in the sample at 84.7% and 94.1%, respectively.

The study reported a significant enhancement in the antioxidant activity of SC-CO_2-treated cocoa powder compared to the untreated sample (Kobori et al., 2013). Vuong and Roach (2014) also reported that functional polyphenolics such as catechins were maintained and preserved in green tea after decaffeination using SC-CO_2. Our previous study successfully produced an asarones-free (α-, β- and γ-asarones) ethanol extract using the two-step extraction technique (Hamil et al., 2022). The present study showed that SC-CO_2 pre-treatment could retain and enhance the quantity of other beneficial components in the sample material, such as polyphenols and flavonoids, apart from removing unwanted compounds.

Table 1
Total phenolics and flavonoids content in P. sarmentosum *extracts. Results displayed as mean ± standard deviation, (n = 3)*

Sample	Total Phenolics (mg/g GAE) ± SD	Total Flavonoids (mg/g QE) ± SD
EM	105.52 ± 0.39	0.30 ± 0.11
EM-R	107.91 ± 0.08*	0.51 ± 0.23
EWM	68.11 ± 0.16	0.75 ± 0.05
EWM-R	75.56 ± 0.32*	0.78 ± 0.24
WM	39.29 ± 0.12	1.36 ± 0.28
WM-R	43.26 ± 0.19*	1.07 ± 0.24

Note. The symbol asterisk (*) indicates statistically significant ($p<0.001$) compared to their non-treated extracts

Quantification of Vitexin and Naringenin in *P. sarmentosum* Extracts

Figure 1 shows the concentration of reference markers in *P. sarmentosum* extracts. The peak of naringenin was eluted at 9.40 min, whereas vitexin was observed at 5.80 min. All extracts contained vitexin with a concentration ranging from 0.40%–0.58%. In 50%

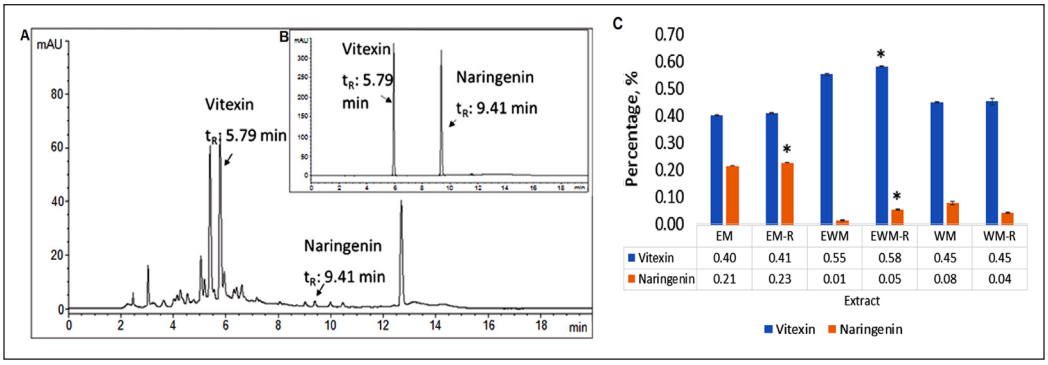

Figure 1. (A) HPLC chromatogram of *P. sarmentosum* ethanol extract, (B) chromatogram of the vitexin and naringenin standards, and (C) amount of vitexin and naringenin in SC-CO_2 pre-treated and non-treated extracts. The symbol asterisk (*) indicates statistically significant ($p<0.01$) compared to their non-treated extracts

ethanol extracts, EWM-R showed a significant increase in vitexin ($p<0.01$). Vitexin was not significantly enhanced in EM-R and WM-R. Naringenin was observed in all *P. sarmentosum* extracts. It was significantly increased in EM-R and EWM-R with 0.23% and 0.05%, respectively. WM-R also shows an increase in naringenin concentration, but there was no significant difference.

Our study's findings were slightly lower than those reported by Ugusman et al. (2012). The authors reported that the vitexin concentration in *P. sarmentosum* water extract was 0.52%. They also reported that naringenin was absent in the extract. On the other hand, vitexin was reported at 0.23% in aqueous extract using a high-speed extractor machine at 80 °C for 3 hours (Azmi et al., 2021). Naringenin was previously reported from *P. sarmentosum* leaves methanol extract; however, data on the quantification of the chemical compound was not available (Subramaniam et al., 2003). *P. sarmentosum's* flavonoids vary due to both internal and external factors. Some internal factors contributing to phytoconstituent variation are chemotypic, ecotypic, ontogenetic and genotypic. Externally, factors such as growth conditions, collection time, age of plants and storage conditions may affect their chemical compositions (Hussain et al., 2009a). Additionally, the type of solvents also contributed to the extraction efficacy of flavonoids. Generally, flavonoids have poor aqueous solubility (Zhao et al., 2019). They will likely have higher solubility in ethanol, methanol and ethyl acetate. Ethanol used in our study has the highest efficacy in extracting naringenin. Vitexin showed better extraction efficacy in 50% ethanol, possibly due to the presence of a glycoside side chain. Sugar moiety in flavonoids increases the compound's polarity, thus possessing higher solubility in polar substances (Ko et al., 2014).

Biological Assay of *P. sarmentosum* Extracts

Cytotoxic Activity of *P. sarmentosum* Extracts using MTT Assay. The cytotoxic activity of *P. sarmentosum* SC-CO_2 pre-treated and non-treated ethanol, 50% ethanol and water

extracts were determined on normal colon fibroblast (CCD-18Co) and normal fibroblast (NIH/3T3) cell lines (Figure 2). From the experiment, it was found that the percentage viability of both cell lines was enhanced with SC-CO_2 pre-treated *P. sarmentosum* extracts. At 100 µg/mL, all SC-CO_2 pre-treated extracts showed higher cell viability than the non-treated extracts. For NIH/3T3 cells, EWM-R showed significantly higher cell viability at 81.29% compared to EWM at 68.08%. As for the CCD-18Co cell, EM-R and WM-R showed significant cell viability at 80.21% and 96.85% compared to their non-treated counterparts (54.63% and 73.09%), respectively. The positive control, doxorubicin, showed IC_{50} at 1.0 µg/mL against NIH/3T3 cell lines and 1.0 µg/mL against CCd18-Co and cell lines, respectively.

Cytotoxicity screening on normal cell lines provides significant information on the substances' potential toxicity and safety profiles. According to the United States National Cancer Institute (NCI), a plant extract is regarded to have an active cytotoxic effect if its IC_{50} value is 20 g/mL or below (Boik, 2001). In a previous study, chloroform extract of *P. sarmentosum* showed IC_{50} at 64.43 µg/mL against normal human umbilical vascular endothelial cells (HUVEC) (Hussain et al., 2009b). The present study found that SC-CO_2 pre-treated extracts showed higher cell viability than non-treated extracts at different concentrations. However, all the extracts did not exhibit cytotoxic effects up to 100 µg/mL and thus can be considered safe for animal and human consumption.

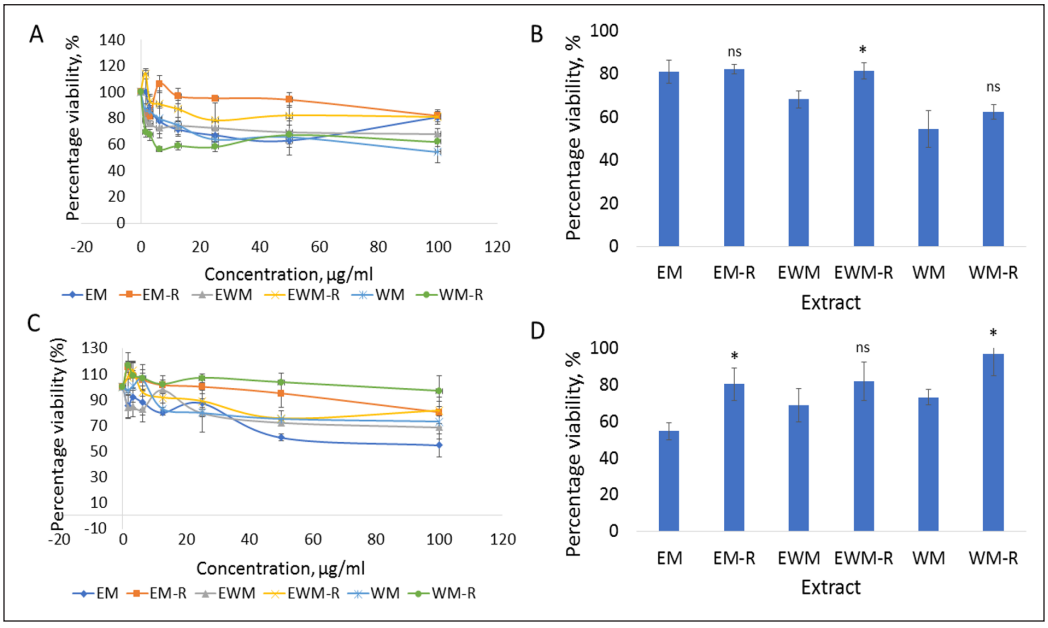

Figure 2. Percentage viability of NIH/3T3 cells treated with *P. sarmentosum* extracts in a dose-dependent manner (A), 100 µg/mL (B), percentage viability of CCD-18Co cells treated with *P. sarmentosum* extracts in a dose-dependent manner (C) and 100 µg/mL (D). The symbol asterisk (*) indicates statistically significant ($p<0.05$), and ns indicates not statistically significant ($p>0.05$) compared to their non-treated extracts

α-Glucosidase Inhibition Assay

The *in-vitro* α-glucosidase inhibition assay demonstrated that the ethanol extract exhibited considerable inhibitory activity among all extracts tested. The percentage inhibition of EM-R at 1000 µg/mL was significantly increased (73.38%) compared to EM (57.08%) at $p<0.001$ (Figure 3). There was an activity enhancement in EWM-R and WM-R compared to their respective non-treated extracts; however, the inhibition was lower than that of ethanol extracts. The IC_{50} values of EM and EM-R were 1236.21 ± 8.40 µg/mL and 869.72 ± 9.39 µg/mL, respectively. The IC_{50} of the positive control (acarbose) was recorded at 181.03 ± 2.98 µg/mL. The chemical markers naringenin and vitexin also showed significant α-glucosidase inhibition activity, with IC_{50} values of 87.35 ± 1.05 µg/mL and 76.49 ± 2.32 µg/mL, respectively (Table 2).

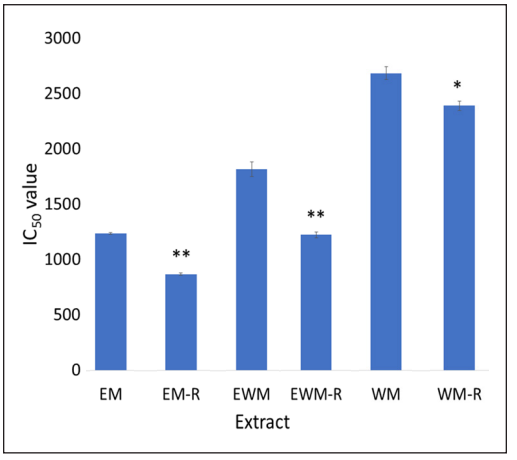

Figure 3. IC_{50} values for *in vitro* α-glucosidase inhibition assay of *P. sarmentosum* extracts. The symbol asterisk (*) indicates statistically significant ($p<0.01$), and the symbol asterisk (**) indicates statistically significant ($p<0.001$) compared to their non-treated extracts

Table 2
IC_{50} values for in vitro α-glucosidase inhibition assay of chemical markers in P. sarmentosum *and positive control acarbose. Results are displayed as mean ± standard deviation (n=3)*

Sample	IC_{50} value (µg/mL) ± SD
Vitexin	76.49 ± 2.32
Naringenin	87.35 ± 1.05
Acarbose	181.03 ± 2.98

Flavonoids are well-known as potent α-glucosidase inhibitors. Chen et al. (2013) reported that a strong α-glucosidase inhibitory effect (IC_{50} 61.30 µg/mL) was obtained from *Microctis folium* extract. Isovitexin, isorhamnetin 3-O-β-D-rutinoside and vitexin were discovered as main flavonoids in the plant, which contributed to the activity. Another study demonstrated that 25 mg/kg naringenin administration in T2D rats significantly inhibited α-glucosidase activity. They mentioned that carbohydrate absorption was successfully delayed, thus decreasing the postprandial blood glucose levels (Priscilla et al., 2014). In a recent study, Daud et al. (2021) reported that phytochemical constituents, including phenolics, flavonoids, terpenoids, as well as saponins and tannins in *P. sarmentosum* extract may associated with the potent α-glucosidase inhibition activity. Our study showed a strong negative correlation between IC_{50} of α-glucosidase and phenolics content ($p<0.01$). Naringenin also exhibited significant correlation ($p<0.05$) with the activity (Table 3). Therefore, this class of compounds *P. sarmentosum* extracts may contribute to the extracts' α-glucosidase inhibition activity.

Table 3
Correlation among the phytoconstituents and activities observed in P. sarmentosum *extracts*

Variables	Phenolics	Flavonoids	Vitexin	Naringenin	NIH/3T3	CCD-18Co	α-glucosidase
Phenolics	1.00						
Flavonoids	-0.65	1.00					
Vitexin	-0.29	0.18	1.00				
Naringenin	0.80*	-0.60	-0.76*	1.00			
NIH/3T3	0.87*	-0.72	0.02	0.55*	1.00		
CCD-18Co	-0.43	0.44	0.16	-0.36	-0.21	1.00	
α-glucosidase	-0.94**	0.74	0.02	-0.62*	-0.94	0.25	1.00

Note. The symbol asterisk (*) indicates statistically significant ($p<0.05$); the symbol asterisk (**) indicates statistically significant ($p<0.01$)

α-Amylase Inhibition Assay

In this assay, all the extracts showed no inhibition towards α-amylase enzyme up to 5000 µg/mL. The positive control (acarbose) showed inhibition with an IC_{50} value of 325.39 ± 1.32 µg/mL. Concentration above 5000 µg/mL led to high turbidity and poor solubility. Hence, higher concentrations were not determined as they would not produce reliable results. According to Salehi et al. (2013), α-amylase inhibition activity did not correlate with the extracts' phenolic content. In another study, a combination of quercetin-myricetin, hyperin-avicularin and kaempferol-quercetin did not exhibit synergistic effects against α-amylase inhibition activities (Wang et al., 2010).

The present result was corroborated by a study conducted by Wongsa et al. (2012). Their study reported that *P. sarmentosum* extract did not inhibit α-amylase but showed 70% inhibition activity against α-glucosidase. Similarly, *Phyllanthus amarus*, *Euphorbia hirta*, and *Lagerstroemia speciosa* extracts also showed no inhibition against α-amylase with mild to moderate α-glucosidase inhibition despite high levels of tannins, terpenoids and phenolic acids (Binh et al., 2016). It can be postulated that although *P. sarmentosum* extracts showed the presence of vitexin and naringenin, these flavonoids did not contribute to the α-amylase inhibition activity of the extracts. A good herbal medicine for early management and treatment of hyperglycaemia should have good inhibition activity against α-glucosidase and moderate α-amylase inhibitory activity. Inhibition of α-amylase in excess is not favourable as it could cause upset and discomfort in the stomach (Cheplick et al., 2010).

CONCLUSION

Using an optimised SC-CO_2 extraction method for the removal of toxic asarone isomers from *P. sarmentosum*, two-step extraction successfully demonstrated the feasibility of using supercritical fluid extraction as a pre-treatment step to enhance total phenolics

content significantly and produced better inhibition against α-glucosidase compared to the conventional solvent extraction method. This study found that solvents that produced the total phenolic content enhancement showed a significant correlation with α-glucosidase inhibition activity, with ethanol showing the best activity, followed by 50% ethanol and water extracts. SC-CO_2 pre-treatment also enhances the viability of NIH/3T3 and CCD-18Co cell lines, thus improving the safety profile of the extracts. It is concluded that SC-CO_2 extraction offers a rapid pre-treatment step to produce safer extracts with better quality and efficacy.

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