



Research article

First identification of *N-trans-feruloyl*tyramine as a key α -glucosidase inhibitor from *Piper sarmentosum* fruits

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Abstract

Importance of the work: *Piper sarmentosum* is used as an herb and in traditional medicine for diabetes treatment. This was the first report of the isolation of α -glucosidase inhibitors from *P. sarmentosum* fruits.

Objectives: To isolate and identify the bioactive compounds from the fruits of *P. sarmentosum* and to evaluate their α -glucosidase inhibition.

Materials and Methods: The CH₂Cl₂ and acetone extracts were separated and purified using various chromatographic techniques. The structures of the compounds were determined based on spectroscopic data (nuclear magnetic resonance and mass spectrometry) and compared with other reported data. The levels of α -glucosidase inhibitory activity of the isolated compounds were assessed using a colorimetric method, with acarbose serving as a positive control.

Results: In total, 10 known compounds were identified: sarmentamide A (1), sarmentamide B (2), deacetylsarmentamide B (3), (3*S*,4*R*)-3,4,5-trihydroxypentanoic acid 1,4-lactone (4), *N-trans-feruloyl*tyramine (5), (1*E*,3*S*)-1-cinnamoyl-3-hydroxypyrrolidine (6), sarmentosine (7), chaplupyrrolidone A (8), piperlotine-A (9) and coumaric acid (10). The phenylpropanamides 1, 3 and 5 produced weak-to-moderate inhibitory activity against both sucrase and maltase, with half maximal inhibitory concentration (IC₅₀) values in the range 0.28–9.48 mM. Phenylpropanamide 6 inhibited only sucrase (IC₅₀ value of 0.93 mM), whereas phenylpropanamide 2 and lactone 4 inhibited only maltase (IC₅₀ values of 6.16 mM and 9.26 mM, respectively). Alkyl amide 7 and the phenylpropanamides 8 and 9, as well as phenylpropanoid 10 produced no inhibition.

Main finding: The phenylpropanamides contained more α -glucosidases than the alkyl amide, lactone and phenylpropanoid. *P. sarmentosum* fruits could be regarded as a potential source of α -glucosidase inhibitors. Further investigation into the inhibitory mechanisms could pave the way for their appropriate utilization in pharmaceutical applications.

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The fraction DS5 (4.3 g) was isolated with SephadexLH-20 column chromatography (CC) using a MeOH-to-CH₂Cl₂ ratio of 1:1 to produce three subfractions (5A–5C). The subfraction 5B was isolated using SephadexLH-20 CC with a MeOH-to-CH₂Cl₂ ratio of 1:1 to produce three subfractions (5B1–5B3). The subfraction 5B2 was purified using silica gel CC with a hexane-to-CH₂Cl₂-to-EtOAc ratio of 3:1:1 to produce four subfractions (5B21–5B24). The subfraction 5B22 was purified using silica gel CC with an acetone-to-CH₂Cl₂ ratio of 2:98 to produce compound 1 (10 mg).

The fraction DS6 (5.6 g) was isolated using SephadexLH-20 CC with a MeOH-to-CH₂Cl₂ ratio of 1:1 to produce three subfractions (6A–6C). The subfraction 6B was separated using SephadexLH-20 CC with a MeOH-to-CH₂Cl₂ ratio of 1:1 to produce three subfractions (6B1–6B3). The subfraction 6B2 was purified using silica gel CC with an EtOAc-to-CH₂Cl₂ ratio of 1:1 and a step gradient to produce two subfractions (6B21–6B22). The subfraction 6B22 was purified using flash reverse phase silica gel CC with a gradient solvent system (MeOH-to-H₂O ratio of 30:70 through to 100:0) to produce compound 2 (0.27 mg).

The subfraction 6B3 was isolated using SephadexLH-20 CC with a MeOH-to-CH₂Cl₂ ratio of 1:1 to produce three subfractions (6B3A–6B3C). The subfraction 6B3B was separated using silica gel CC with a MeOH-CH₂Cl₂ step gradient to produce two subfractions (B1–B2). The subfraction B2 was purified using flash reverse phase silica gel CC with a gradient solvent system to produce compound 3 (6 mg).

The subfraction 6C was isolated using SephadexLH-20 CC with a MeOH-to-CH₂Cl₂ ratio of 1:1 to produce three subfractions (6C1–6C3). The subfraction 6C1 was separated using flash reverse phase silica gel CC with a gradient solvent system (MeOH-to-H₂O ratio of 30:70 through to 100:0 to produce subfraction D22A. The subfraction D22A was purified using silica gel CC with a CH₂Cl₂-to-MeOH-to-EtOAc ratio of 4.0:0.5:0.5 to produce subfraction D22A2 and further separated using flash reverse phase silica gel CC with a gradient solvent system (MeOH-to-H₂O ratio of 30:70 through to 100:0) to produce compound 4 (5 mg).

The subfraction 6C2 was isolated using SephadexLH-20 CC with a MeOH-to-CH₂Cl₂ ratio of 1:1 to produce subfraction 6C2A. The subfraction 6C2A was purified using silica gel CC with a MeOH-CH₂Cl₂ step gradient to produce compound 5 (5.5 mg).

The fraction DS7 (4.75 g) was fractionated using SephadexLH-20 CC with a MeOH-to-CH₂Cl₂ ratio of 1:1 to produce three subfractions (DS7A–DS7C). The fraction DS7B was fractionated using SephadexLH-20 CC with a MeOH-to-CH₂Cl₂ ratio of 1:1 to produce three subfractions

(10B1–10B3). The subfraction B2 was purified using silica gel CC with a MeOH-CH₂Cl₂ step gradient to produce two subfractions (B21–B22). The subfraction B22 was separated using SephadexLH-20 CC with a MeOH-to-CH₂Cl₂ ratio of 1:1 to produce compound 6 (0.69 g).

The acetone extract (6.30 g) was fractionated using a method similar to that applied to the CH₂Cl₂ extract, resulting in eight fractions (ES1–ES8). The fraction ES5 (0.65 g) was separated using SephadexLH-20 CC with a MeOH-to-CH₂Cl₂ ratio of 1:1 to produce three subfractions (5A–5C). The subfraction 5C was rechromatographed using SephadexLH-20 CC with a MeOH-to-CH₂Cl₂ ratio of 1:1 to produce two subfractions (5CA–5CB). The subfraction 5CB was separated using flash reverse phase silica gel CC with a gradient solvent system (MeOH-to-H₂O ratio of 20:80 through to 100:0) to produce compound 8 (0.015 g).

The fraction ES7 (2.5 g) was separated using SephadexLH-20 CC with a MeOH-to-CH₂Cl₂ ratio of 1:1 to produce five subfractions (7A–7E). The subfraction 7B was isolated using SephadexLH-20 CC with a MeOH-to-CH₂Cl₂ ratio of 1:1 to produce four subfractions (BA–BD). The subfraction BB was purified using silica gel CC with a MeOH-to-CH₂Cl₂ ratio of 2:98 to produce three subfractions (BB1–BB3). The subfraction BB2 was separated using flash reverse phase silica gel CC with a gradient solvent system (MeOH-to-H₂O ratio of 20:80 through to 100:0) to produce substance 7 (6 mg). The subfraction BC was isolated using flash reverse phase silica gel CC with a gradient solvent system (MeOH-to-H₂O ratio of 20:80 through to 100:0 to produce compounds 7 (8 mg) and 9 (4 mg). The subfraction A11E was separated using SephadexLH-20 CC with a MeOH-to-CH₂Cl₂ ratio of 1:1 to produce three subfractions (E1–E3). The subfraction E2 was separated using flash reverse phase silica gel CC with a gradient solvent system (MeOH-to-H₂O ratio of 20:80 through to 100:0) to produce compound 10 (12 mg).

Sarmentamide A (1) colorless gum; ¹H NMR (CDCl₃, 500 MHz) δ_H 7.29 (m, 5H), 7.18 (m, 1H), 6.15 (td, *J* = 1.8, 6.1 Hz, 1H), 4.41 (dd, *J* = 1.8, 4.0 Hz, 1H), 3.30 (t, *J* = 7.8 Hz, 2H), 3.01 (t, *J* = 7.8 Hz, 2H) (Tuntiwachwuttikul et al., 2006).

Sarmentamide B (2) brown gum; [α]_D²⁴ +65.8 (c = 0.12, MeOH); ¹H NMR (CDCl₃, 500 MHz) δ_H 7.67 (d, *J* = 15.5 Hz, 1H), 7.55 (m, 2H), 7.40 (m, 3H), 6.63 (d, *J* = 15.5 Hz, 1H), 5.11 (brd, *J* = 4.5 Hz, 1H), 4.07 (dd, *J* = 4.5, 14.0 Hz, 1H), 4.35 (brd, *J* = 4.5 Hz, 1H), 3.95 (dd, *J* = 4.5, 14.0 Hz, 1H), 3.87 (dd, *J* = 4.5, 12.0 Hz, 1H), 3.15 (brd, *J* = 13.3 Hz, 1H), 2.05 (s, 6H); ¹³C NMR (CDCl₃, 125 MHz) δ_C 170.5x2, 165.7, 143.0, 134.8, 130.0, 128.8, 128.1, 117.8, 73.5, 71.4, 50.3, 49.6, 21.1x2 (Tuntiwachwuttikul et al., 2006).

Deacetylsarmentamide B (3) colorless gum; $[\alpha]_D^{24} +7.8$ ($c = 0.18$, MeOH); ^1H NMR (CDCl_3 , 500 MHz) δ_{H} 7.62 (m, 2H), 7.59 (d, $J = 15.5$ Hz, 1H), 7.38 (m, 3H), 6.92 (d, $J = 15.5$ Hz, 1H), 4.17 (brd, $J = 3.1$ Hz, 1H), 4.11 (brd, $J = 3.8$ Hz, 1H), 3.92 (dd, $J = 4.1$, 11.4 Hz, 1H), 3.66–3.70 (m, 2H), 3.58 (d, $J = 13.3$ Hz, 1H); ^{13}C NMR (CDCl_3 , 125 MHz) δ_{C} 166.4, 143.6, 134.8, 129.9x2, 129.2x2, 131.1, 119.4, 74.7, 73.5, 53.7, 53.1 (Damsud et al., 2013).

(3*S*,4*R*)-3,4,5-Trihydroxypentanoic acid 1, 4-lactone (4) colorless gum; $[\alpha]_D^{24} +6.8$ ($c = 0.8$, MeOH); ^1H NMR (CDCl_3 , 500 MHz) δ_{H} 4.41 (dt, $J = 2.3$, 4.4 Hz, 1H), 4.35 (brq, $J = 3.5$ Hz, 1H), 3.65–3.76 (dd, $J = 3.3$, 12.4 Hz, 2H) 2.90 (dd, $J = 6.8$, 18.0 Hz, 1H), 2.36 (dd, $J = 2.5$, 18.0 Hz, 1H); ^{13}C NMR (CDCl_3 , 125 MHz) δ_{C} 89.4, 68.9, 61.2, 38.2 (Fernández et al., 1990).

N-trans-Feruloyltyramine (5) colorless gum; ^1H NMR (CDCl_3 , 500 MHz) δ_{H} 7.40 (d, $J = 15.7$ Hz, 1H), 7.09 (d, $J = 2.0$ Hz, 1H), 7.02 (d, $J = 8.3$ Hz, 2H), 6.92 (dd, $J = 2.0$, 8.2 Hz, 1H), 6.77 (d, $J = 8.2$ Hz, 1H), 6.69 (d, $J = 8.3$ Hz, 2H), 6.37 (d, $J = 15.7$ Hz, 1H), 3.47 (t, $J = 7.0$ Hz, 2H), 2.76 (t, $J = 7.0$ Hz, 2H) (King and Calhoun, 2005).

(1*E*,3*S*)-1-Cinnamoyl-3-hydroxypyrrolidine (6) colorless gum; $[\alpha]_D^{24} +35.1$ ($c = 0.11$, MeOH); ^1H NMR (CDCl_3 , 500 MHz) δ_{H} trans 7.62 (m, 2H), 7.60 (d, $J = 15.5$ Hz, 1H), 7.38 (m, 3H), 6.91 (d, $J = 15.5$ Hz, 1H), 4.45 (m, 1H), 3.72 (m, 2H), 3.57–3.71 (m, 2H), 2.00 (m, 2H); ^{13}C NMR (CDCl_3 , 125 MHz) δ_{C} 143.4, 167.3, 136.6, 131.0, 129.9, 129.1, 119.6, 71.7, 55.9, 45.2, 33.5 cis 7.62 (m, 2H), 7.60 (d, $J = 15.5$ Hz, 1H), 7.38 (m, 3H), 6.97 (d, $J = 15.5$ Hz, 1H), 4.51 (m, 1H), 3.82 (m, 2H), 3.57–3.71 (m, 2H), 2.08 (m, 2H); ^{13}C NMR (CDCl_3 , 125 MHz) δ_{C} 167.3, 143.4, 136.6, 131.0, 129.9, 129.1, 119.6, 70.1, 55.4, 45.9, 35.0 (Shi et al., 2017).

Sarmentosine (7) colorless gum; ^1H NMR (CDCl_3 , 500 MHz) δ_{H} 6.92 (td, $J = 6.9$, 15.3 Hz, 1H), 6.92 (td, $J = 6.9$, 15.3 Hz, 1H), 6.86 (d, $J = 1.5$ Hz, 1H), 6.72 (m, 2H), 6.31 (td, $J = 1.3$, 15.7 Hz, 1H), 6.12 (td, $J = 1.5$, 15.3 Hz, 1H), 6.02 (td, $J = 6.7$, 15.7 Hz, 1H), 5.92 (s, 2H), 3.50 (t, $J = 6.9$, 2H), 3.48 (t, $J = 6.9$, 2H), 2.34 (m, 4H), 1.94 (m, 2H), 1.84 (m, 2H) (Likuitwitayawuid et al., 1987).

Chaplupyrrolidone A (8) colorless gum; $[\alpha]_D^{24} -6.7$ ($c = 0.335$, CH_2Cl_2); ^1H NMR (CDCl_3 , 500 MHz) δ_{H} 7.29 (dd, $J = 0.5$, 6.1 Hz, 1H), 7.19–7.26 (m, 5H), 6.18 (dd, $J = 0.5$, 6.1 Hz, 1H), 6.12 (brs, 1H), 3.25 (m, 2H), 2.97 (t, $J = 7.7$ Hz, 2H) (Damsud et al., 2013).

Piperlotine-A (9) colorless gum; ^1H NMR (CDCl_3 , 500 MHz) δ_{H} 7.65 (d, $J = 15.5$ Hz, 1H), 7.48 (d, $J = 8.8$ Hz, 2H), 6.89 (d, $J = 8.8$ Hz, 2H), 6.60 (d, $J = 15.5$ Hz, 1H), 3.83 (s, 3H),

3.62 (t, $J = 6.8$ Hz, 2H), 3.59 (t, $J = 6.8$ Hz, 2H), 2.00 (m, 2H), 1.89 (m, 2H) (Li et al., 2007).

Coumaric acid (10) colorless gum; ^1H NMR (CDCl_3 , 500 MHz) δ_{H} 7.78 (d, $J = 15.5$ Hz, 1H), 7.55 (m, 3H), 7.40 (m, 2H), 6.45 (d, $J = 15.5$ Hz, 1H) (Kalinowska et al., 2007).

α -Glucosidase inhibitory activity

The α -glucosidase inhibitory activity against rat intestinal maltase and sucrase was assessed following the method described by Worawalai et al. (2019). The isolated compounds (1 mg/mL in dimethyl sulfoxide, 10 μL) were mixed with 30 μL of 0.1 M phosphate buffer (pH 6.9), 20 μL of substrate solution (2 mM maltose and 20 mM sucrose in 0.1 M phosphate buffer), 80 μL of glucose assay kit (SU-GLLQ2, Human) and 20 μL of crude enzyme solution. Then, the reaction mixture was incubated at 37°C for 10 min for maltose and 40 min for sucrose. Enzymatic activity was assessed by measuring the absorbance of quinoneimine formed at 500 nm using a Bio-Rad 3550 microplate reader. The percentage inhibition was calculated using the formula $(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}} \times 100$, where A_{sample} is the absorbance with the sample and A_{control} is the absorbance without the sample. The IC_{50} value was determined from a plot of percentage inhibition against sample concentration, with acarbose serving as the positive control. Assays were performed in triplicate.

Results and Discussion

The dried fruits of *P. sarmentosum* were individually extracted using the procedures outlined in Fig. 2 and yielded hexane, CH_2Cl_2 and acetone extracts. In total, 10 compounds were obtained after applying chromatographic techniques for separation (Fig. 3). Their structures were determined based on their spectroscopic data and comparisons with those already reported to be sarmentamide A (1) and sarmentamide B (2) (Tuntiwachwuttikul et al., 2006); deacetylsarmentamide B (3) (Damsud et al., 2013); (3*S*,4*R*)-3,4,5-trihydroxypentanoic acid 1,4-lactone (4) (Fernández et al., 1990); *N*-trans-feruloyltyramine (5) (King and Calhoun, 2005); (1*E*,3*S*)-1-cinnamoyl-3-hydroxypyrrolidine (6) (Shi et al., 2017); sarmentosine (7) (Likuitwitayawuid et al., 1987); chaplupyrrolidone A (8) (Damsud et al., 2013); piperlotine-A (9) (Li et al., 2007); and coumaric acid (10) (Kalinowska et al., 2007), as shown in Fig. 4.

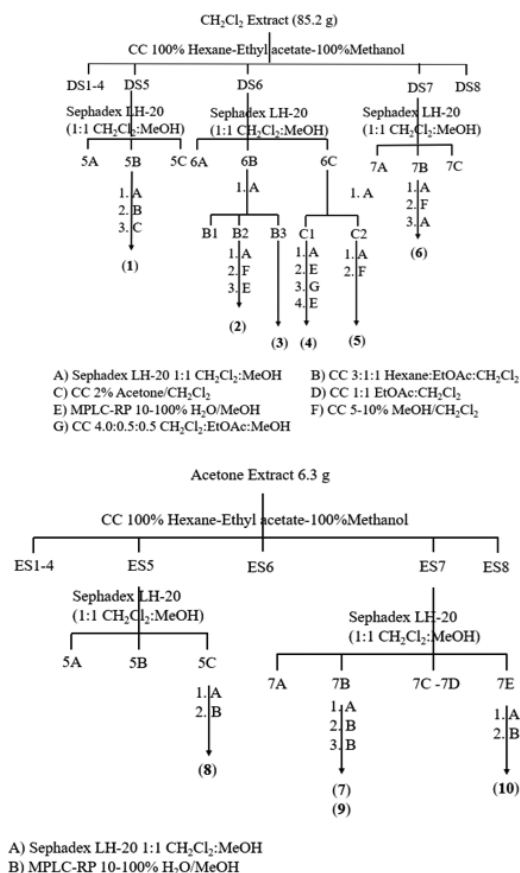


Fig. 3 Chromatographic separation of CH₂Cl₂ and acetone extracts

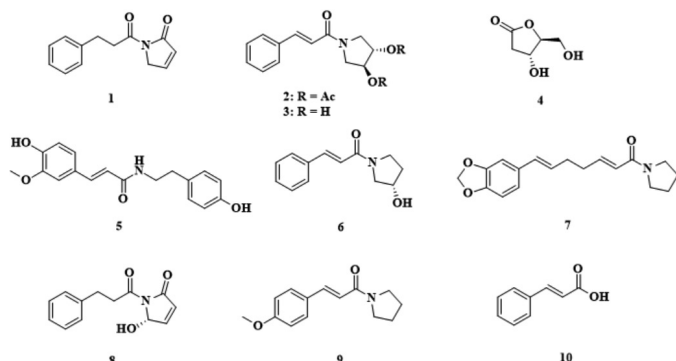


Fig. 4 Structures of 10 isolated compounds from fruits of *Piper sarmentosum*

All 10 isolated compounds were evaluated for α -glucosidase inhibition. As shown in Fig. 5, phenylpropanamide 5 had the highest inhibitory activity levels against both sucrase and maltase, with IC₅₀ values of 0.50 and 0.28 mM, respectively. In addition, phenylpropanamides 1 and 3 were weakly inhibited sucrase and maltase reductase in rats, with IC₅₀ values in the range 1.15–9.47 mM. Phenylpropanamide 2 and lactone 4 produced only maltase inhibition, with IC₅₀ values of

6.16 and 9.26 mM, respectively, while phenylpropanamide 6 produced only sucrase inhibition, with an IC₅₀ value of 0.93 mM. Alkylamide 7, phenylpropanamides 8 and 9, and phenylpropanoid 10 produced no inhibitory activity on sucrase and maltase.

Structure-activity relationship studies revealed that phenylpropanamide 1, which lacks a hydroxy group in the pyrrolidone ring, inhibited both sucrase and maltase with IC₅₀ values of 9.47 mM and 1.75 mM, respectively. In contrast, phenylpropanamide 8, which has a hydroxy group in the pyrrolidone ring, produced no inhibition, indicating that the hydroxyl group on the pyrrolidone ring did not affect inhibition. Phenylpropanamide 2, which has two hydroxy groups on the pyrrolidine ring, inhibited maltase with an IC₅₀ value of 6.16 mM. In contrast, phenylpropanamide 6, with only one hydroxy group on the pyrrolidine ring, produced no inhibition. These results suggested that the number of hydroxy groups on the pyrrolidine ring enhanced inhibition. Additionally, phenylpropanamide 3, with two acetate groups on the pyrrolidine ring, produced greater maltase inhibition (IC₅₀ = 1.32 mM) than phenylpropanamide 2 (IC₅₀ = 6.16 mM), indicating the crucial role of the acetate groups on the pyrrolidine ring in α -glucosidase inhibition.

Li et al. (2007) reported that phenylpropanamide 1 from *Piper lolot* leaves produced strong antiplatelet aggregation activity with an IC₅₀ value of 15.2 μ g/mL. Furthermore, phenylpropanamide 5, isolated from the fibrous roots of *Liriope muscari* (Liliaceae), produced mean ($\pm$ SD) 2,2-diphenyl-1-picrylhydrazyl inhibition (7.2 ± 0.6) and mean 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid inhibition of 32.7 ± 1.6 at 25 μ g/mL (Li et al., 2012). Additionally, phenylpropanamide 5 from the roots of *Solanum melongena* produced inhibitory activity against α -glucosidase with an IC₅₀ value of 500.6 μ M (Liu et al., 2011).

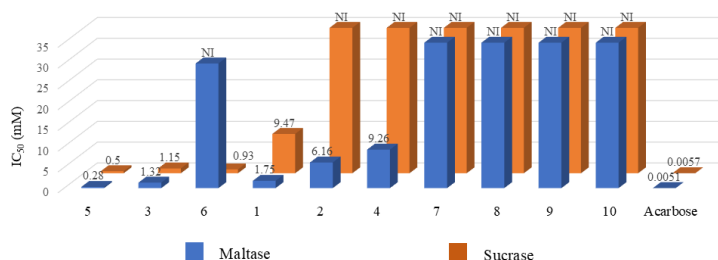


Fig. 5 α -Glucosidase inhibition of 10 isolated compounds (x axis) from fruits of *P. sarmentosum*, where NI = percentage inhibition less than 30% at most concentrations tested and IC₅₀ = half maximal inhibitory concentration

In conclusion, the current study identified and reported on 10 known compounds from *P. sarmentosum* fruits. Furthermore, the study demonstrated the potent α -glucosidase inhibitory potential of the phenylpropanamides of *P. sarmentosum*. Structure-activity relation investigation indicated that the hydroxyl and acetate groups on the pyrrolidine ring affected the activity of the α -glucosidases. Further research is necessary to determine their potential as glucosidase inhibitors for pharmaceutical applications.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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