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Chemical Constituents of Sweet Tea (Rubus suavissimus S. Lee) and Its α-Glucosidase Inhibitory Activity: Postprint

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Abstract

To investigate the secondary metabolites with α-glucosidase inhibitory activity in sweet tea (Rubus suavissimus), this study utilized multiple modern chromatographic separation techniques to extract, isolate, and purify its dried leaves. The structures of the monomeric compounds were elucidated by comprehensive analysis using mass spectrometry and nuclear magnetic resonance spectroscopy, while the isolated compounds were simultaneously evaluated for α-glucosidase inhibitory activity. The results demonstrated: (1) Ten compounds were isolated and identified from the dried leaves of sweet tea, namely rubusoside (1), kaempferol-3-O-robinobioside (2), gallic acid (3), diconiferyl alcohol (4), 5-methoxydiconiferyl alcohol (5), caesalpinic acid (6), steviol monoside (7), steviol (8), 16α,17-dihydroxy-ent-kaurane (9), and quercetin-3-O-β-D-galactopyranoside (10), among which compounds 2, 4, 5, and 9 were isolated from sweet tea for the first time; (2) The α-glucosidase inhibitory activity assay results revealed that compounds 2, 3, 5, 6, and 10 exhibited potent α-glucosidase inhibitory activity. This study enriches the pool of α-glucosidase inhibitory compounds in sweet tea and provides a theoretical basis for the development of antidiabetic products.

Full Text

Chemical Constituents from *Rubus suavissimus* and Their α-Glucosidase Inhibitory Activity

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Abstract

Rubus suavissimus is mainly distributed in Guilin, Liuzhou, Wuzhou, and other regions of Guangxi Zhuang Autonomous Region, and is thus known as "Guangxi sweet tea." Together with *Siraitia grosvenorii* and *Stevia rebaudiana*, it is celebrated as one of the three famous sweet plants of Guangxi. The Zhuang and Yao ethnic groups have long used the leaves of *R. suavissimus* as a tea to treat diabetes, earning it the reputation of "divine tea" in Yao medicine. Consequently, *R. suavissimus* represents a unique combination of sugar, tea, and medicine, holding great potential for development in both food and pharmaceutical applications.

To investigate the secondary metabolites with α-glucosidase inhibitory activity from *R. suavissimus*, we performed extraction, separation, and purification of compounds from its dried leaves using various modern chromatographic techniques. The structures of the purified compounds were elucidated through comprehensive analysis of mass spectrometry and nuclear magnetic resonance spectroscopy data. Simultaneously, the isolated compounds were evaluated for α-glucosidase inhibitory activity. The results demonstrated that: (1) Ten compounds were isolated and identified from the dried leaves of sweet tea, namely rubusoside (1), kaempferol-3-O-robinobioside (2), gallic acid (3), dehydroconiferyl alcohol (4), 5-methoxydehydroconiferyl alcohol (5), brevifolincarboxylic acid (6), steviolmonoside (7), steviol (8), 16α,17-dihydroxykaurane (9), and quercetin-3-O-β-D-galactopyranoside (10). Among these, compounds 2, 4, 5, and 9 were isolated from *R. suavissimus* for the first time. (2) The α-glucosidase inhibitory activity assay revealed that compounds 2, 3, 5, 6, and 10 exhibited strong inhibitory activity against α-glucosidase, with IC_{50} values of (0.14 ± 0.03) mg · mL^{-1}, (0.36 ± 0.02) mg · mL^{-1}, (0.44 ± 0.01) mg · mL^{-1}, (0.53 ± 0.04) mg · mL^{-1}, and (0.14 ± 0.03) mg · mL^{-1}, respectively—all superior to the positive control acarbose ($IC_{50} = (0.69 \pm 0.02)$ mg · mL^{-1}). These findings enrich our understanding of α-glucosidase inhibitors from *R. suavissimus* and provide a theoretical foundation for the development of hypoglycemic products.

Keywords: *Rubus suavissimus*, α-glucosidase, *Rubus*, Rosaceae, diabetes

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Introduction

Rubus suavissimus, a member of the Rosaceae family and *Rubus* genus, is primarily distributed in Guangxi's Guilin, Liuzhou, and Wuzhou regions, earning it the local name "Guangxi sweet tea" (Liu et al., 2020; Yan et al., 2017). Alongside monk fruit (*Siraitia grosvenorii*) and stevia (*Stevia rebaudiana*), it is renowned as one of Guangxi's three major sweet plants. The Zhuang and Yao ethnic minorities have traditionally used its leaves as a tea to treat diabetes, referring to it as the "divine tea" of Yao medicine (Zheng et al., 2019). According to Zhuang medical records, it possesses heat-clearing and detoxifying effects, promotes blood circulation, and regulates qi and water metabolism (Guangxi Food and Drug Administration, 2011). This unique combination of sweetness, tea properties, and medicinal value makes *R. suavissimus* highly promising for food and pharmaceutical development.

With rising living standards and accelerating population aging, diabetes has become a chronic disease severely impacting human health and quality of life. To maintain normal blood glucose levels, diabetic patients require long-term administration of hypoglycemic drugs to prevent complications such as organ damage and failure. α-Glucosidase inhibitors represent an important class of hypoglycemic agents, with first-line clinical drugs including acarbose and voglibose. However, these medications often cause adverse effects such as gastrointestinal disturbances and liver function impairment (Zhu et al., 2021). Therefore, developing novel, safe, and effective α-glucosidase inhibitors is crucial for diabetes treatment. Modern chemical and pharmacological studies have shown that the main chemical constituents of sweet tea are terpenoids, flavonoids, and phenolic acids, which exhibit hypoglycemic, anti-allergic, and anti-inflammatory activities (Wu et al., 2021). Current research on the hypoglycemic effects of sweet tea has primarily focused on its extracts (Meng et al., 2019; Su et al., 2020; Wu and Gong, 2021), while few studies have investigated the specific chemical basis of its α-glucosidase inhibitory activity (Liu et al., 2020), leaving potential bioactive compounds to be discovered. Given the long history of sweet tea as a hypoglycemic beverage, this study systematically isolated chemical constituents from its leaves using modern chromatographic techniques and evaluated their biological activities to identify additional α-glucosidase inhibitors, thereby providing a scientific foundation for developing related hypoglycemic products.

Materials and Methods

1.1 Materials

Plant samples were collected in July 2019 from Guanyang County, Guilin City, Guangxi Zhuang Autonomous Region, and identified as the leaves of *Rubus suavissimus* by Researcher Tang Hui from Guangxi Institute of Botany. A voucher specimen (No. 20190753) is deposited at the Guangxi Key Laboratory of Plant Functional Phytochemicals and Sustainable Utilization.

1.2 Instruments and Reagents

Instruments: XS205 DualRange analytical balance (Mettler Toledo, Switzerland), LCMS-IT-TOF high-resolution mass spectrometer (Shimadzu, Japan), Avance III HD 500 MHz NMR spectrometer (Bruker, Germany), LC-20AT high-performance liquid chromatograph (Shimadzu, Japan), rotary evaporator (Tokyo Rikakikai, Japan), CF810C cooling water circulator (Yamato, Japan), and SP-MAX3500FL multifunctional microplate reader (Shanghai Flash Spectrum Biological Technology).

Reagents: Acarbose (Shanghai Yuanye Biotechnology), p-nitrophenyl-α-D-glucopyranoside (pNPG, Shanghai Yuanye Biotechnology), α-glucosidase (Sigma-Aldrich, USA), anhydrous sodium carbonate (Xilong Chemical), phosphate buffer saline (PBS, Beijing Solarbio Technology), analytical-grade methanol and ethanol (Xilong Chemical), and HPLC-grade methanol and acetonitrile (Spectrum Chemical, USA).

1.3 Extraction and Separation

Dried leaves of *R. suavissimus* (5.5 kg) were extracted three times with 95% ethanol at room temperature for 7 days each. The extracts were combined and concentrated under reduced pressure to yield a crude extract (432.2 g). The crude extract was dissolved in 40% aqueous ethanol, allowed to settle, and the supernatant was collected and concentrated to remove ethanol. This fraction was then subjected to Sephadex LH-20 gel column chromatography (10 cm × 30 cm) eluted with a methanol-water gradient (0–100%, V/V). Based on thin-layer chromatography analysis, the eluates were combined to afford 11 fractions (Fr.1–Fr.11).

Fraction 4 (21.9 g) was separated on a DIAION HP20SS resin column (4 cm × 30 cm) using a methanol-water gradient (0–100%, V/V) to obtain compound **1** (5.5 g). **Fraction 6 (8.1 g)** was chromatographed on an MCI column (3 cm × 23 cm) with a methanol-water gradient (0–100%, V/V) to yield subfractions Fr.61–Fr.66. Fr.61 was further purified on Sephadex LH-20 with methanol-water to give compound **3** (66.0 mg). Fr.62 yielded compound **6** (46.2 mg) through repeated crystallization from methanol. Fr.63 was purified sequentially by MCI column chromatography (methanol-water, 0–100%, V/V) and HPLC (50% methanol-water, V/V) to afford compound **2** (5.9 mg). Fr.64 was purified by HPLC using isocratic elution with 25% acetonitrile-water (V/V) to obtain compounds **4** (30.7 mg) and **5** (6.0 mg). Fr.65 was separated on an ODS column (methanol-water, 0–100%, V/V) to yield compound **7** (78.3 mg). Fr.66 was chromatographed on an ODS column with a methanol-water gradient (0–100%, V/V) to give compound **8** (26.7 mg).

Fraction 5 (4.6 g) was separated on an ODS column eluted with a methanol-water gradient (0–100%, V/V), followed by purification on Sephadex LH-20 to obtain compound **9** (59.8 mg). **Fraction 8 (5.4 g)** was separated on an MCI column with a methanol-water gradient (0–100%, V/V) to yield compound **10**

(23.6 mg).

1.4 α-Glucosidase Inhibitory Activity Assay

The α-glucosidase inhibitory activity was evaluated following published methods (Pan et al., 2020; Liang et al., 2022) with appropriate modifications. Acarbose served as the positive control, p-nitrophenyl-α-D-glucopyranoside (PNPG, 1 $mmol \cdot L^{-1}$) as the substrate, and phosphate buffer (PBS, 50 $mmol \cdot L^{-1}$) as the solvent system. α-Glucosidase was prepared at 0.25 $U \cdot mL^{-1}$.

The experiment consisted of four groups: sample group, sample background control, blank group, and blank control, with reaction systems as shown in Table 1 . The procedure was as follows: In a 96-well plate, the sample group received 40 L sample solution and 20 L α-glucosidase solution; the sample background control received 40 L sample solution and 20 L PBS; the blank group received 20 L α-glucosidase solution and 40 L PBS; and the blank control received 60 L PBS. After incubation at 37°C for 5 min, 50 L PNPG solution was added to each well, followed by reaction at 37°C for 30 min. The reaction was terminated by adding 50 L Na_2CO_3 solution, and absorbance was measured at 405 nm. Absorbance values were recorded as A_1 (sample group), A_2 (sample background control), B_1 (blank group), and B_2 (blank control). The inhibition rate was calculated using the formula:

Inhibition rate = [1 - (A_1 - A_2)/(B_1 - B_2)] × 100%

All experiments were performed in triplicate, and results are expressed as mean ± standard deviation.

Results

2.1 Structural Identification of Compounds

Compound 1 (white powder): HR-ESI-MS m/z 665.3115 $[M + Na]^+$ (calcd for $C_{32}H_{50}O_{13}Na$, 665.3144). ^1H NMR (500 MHz, D_2O) δ: 5.38 (1H, d, J = 7.8 Hz, 19-glc-H-1), 5.10 (1H, s, H-17a), 4.89 (1H, s, H-17b), 4.58 (1H, d, J = 7.5 Hz, 13-glc-H-1), 3.22–3.86 (sugar protons), 1.22 (3H, s, H-18), 0.90 (3H, s, H-20). ^{13}C NMR (125 MHz, D_2O) δ: 178.6 (C-19), 153.0 (C-16), 104.8 (C-17), 97.4 (13-glc-C-1), 94.1 (19-glc-C-1), 86.5 (C-13), 76.8 (19-glc-C-5), 76.3 (13-glc-C-3), 73.5 (19-glc-C-3), 73.4 (13-glc-C-5), 73.3 (13-glc-C-2), 71.2 (19-glc-C-2), 69.8 (13-glc-C-4), 69.4 (19-glc-C-4), 60.9 (13-glc-C-6), 60.7 (19-glc-C-6), 57.1 (C-5), 53.5 (C-9), 47.2 (C-15), 44.0 (C-14), 43.9 (C-4), 42.1 (C-8), 41.0 (C-7), 40.5 (C-1), 39.3 (C-10), 37.7 (C-3), 36.3 (C-12), 28.1 (C-18), 21.4 (C-6), 20.4 (C-11), 18.8 (C-2), 15.0 (C-20). These data are consistent with literature values (Wang and Lu, 2008), identifying compound **1** as rubusoside (Fig. 1).

Compound 2 (yellow powder): HR-ESI-MS m/z 593.1464 $[M - H]^-$ (calcd for

$C_{27}H_{31}O_{15}$, 593.1512). ^{1}H NMR (500 MHz, methanol-d_4) δ: 8.04 (2H, d, J = 8.8 Hz, H-2 , 6), 6.83 (2H, d, J = 8.8 Hz, H-3 , 5), 6.31 (1H, s, H-8), 6.12 (1H, s, H-6), 4.95 (1H, d, J = 7.8 Hz, gal-H-1), 4.47 (1H, brs, rha-H-1), 1.14 (3H, d, J = 6.2 Hz, rha-H-6), 3.22–3.76 (sugar protons). ^{13}C NMR (125 MHz, methanol-d_4) δ: 180.0 (C-4), 168.4 (C-7), 163.4 (C-5), 162.2 (C-4), 159.7 (C-9), 159.2 (C-2), 136.6 (C-3), 133.0 (C-2 , 6), 123.3 (C-1), 116.8 (C-3 , 5), 106.3 (gal-C-1), 105.7 (C-10), 102.5 (rha-C-1), 101.2 (C-6), 94.0 (C-8), 76.0 (gal-C-5), 75.7 (gal-C-3), 74.5 (rha-C-4), 73.6 (rha-C-2), 72.9 (rha-C-3), 72.7 (gal-C-2), 70.5 (gal-C-4), 69.8 (rha-C-5), 67.8 (gal-C-6), 17.8 (rha-C-6). These data match literature values (Hou et al., 2005), identifying compound **2** as kaempferol-3-O-robinobioside (Fig. 1 [Figure 1: see original paper]).

Compound 3 (white powder): HR-ESI-MS m/z 169.0141 $[M - H]^-$ (calcd for $C_7H_5O_5$, 169.0142). ^{1}H NMR (500 MHz, methanol-d_4) δ: 7.06 (2H, s, H-2, 6). ^{13}C NMR (125 MHz, methanol-d_4) δ: 170.5 (C-7), 146.4 (C-3, 5), 139.8 (C-4), 121.9 (C-1), 110.5 (C-2, 6). These data are consistent with literature values (Lü et al., 2018), identifying compound **3** as gallic acid (Fig. 1).

Compound 4 (pale yellow oil): HR-ESI-MS m/z 359.1507 $[M - H]^-$ (calcd for $C_{20}H_{23}O_6$, 359.1500). ^{1}H NMR (500 MHz, methanol-d_4) δ: 6.91 (1H, d, J = 1.8 Hz, H-2), 6.78 (1H, dd, J = 8.2, 1.8 Hz, H-6), 6.72 (1H, d, J = 8.2 Hz, H-5), 6.68 (2H, s, H-2 , H-6), 5.45 (1H, d, J = 6.2 Hz, H-7), 3.80 (3H, s, 3-OCH_3), 3.76 (3H, s, 3 -OCH_3), 3.71 (2H, m, H-9), 3.53 (2H, t, J = 6.5 Hz, H-9), 3.43 (1H, m, H-8), 2.58 (2H, m, H-7), 1.77 (2H, m, H-8). ^{13}C NMR (125 MHz, methanol-d_4) δ: 149.0 (C-3), 147.6 (C-4), 147.4 (C-2), 145.2 (C-3), 136.9 (C-5), 134.8 (C-1), 129.8 (C-1), 119.7 (C-6), 117.9 (C-6), 116.1 (C-5), 114.0 (C-4), 110.5 (C-2), 88.9 (C-7), 64.9 (C-9), 62.2 (C-9), 56.7 (3-OCH_3), 56.3 (3 -OCH_3), 55.4 (C-8), 35.8 (C-8), 32.9 (C-7). These data match literature values (Wang, 2013), identifying compound **4** as dehydroconiferyl alcohol (Fig. 1).

Compound 5 (pale yellow oil): HR-ESI-MS m/z 413.1511 $[M + Na]^+$ (calcd for $C_{21}H_{26}O_7Na$, 413.1571). ^{1}H NMR (500 MHz, methanol-d_4) δ: 6.73 (2H, d, J = 2.2 Hz, H-2, H-6), 6.68 (2H, s, H-2 , H-6), 5.50 (1H, d, J = 6.2 Hz, H-7), 3.86 (3H, s, 3 -OCH_3), 3.85 (2H, m, H-9), 3.81 (6H, s, 3-OCH_3, 5-OCH_3), 3.57 (2H, t, J = 6.4 Hz, H-9), 3.47 (1H, m, H-8), 2.63 (2H, m, H-7), 1.82 (2H, m, H-8). ^{13}C NMR (125 MHz, methanol-d_4) δ: 149.3 (C-3), 149.3 (C-5), 147.5 (C-3), 145.2 (C-4), 137.0 (C-4), 134.0 (C-1), 134.0 (C-1), 129.8 (C-5), 117.9 (C-6), 114.1 (C-2), 104.1 (C-2, 6), 89.1 (C-7), 65.0 (C-9), 62.6 (C-9), 56.8 (C-8), 56.7 (3-OCH_3), 56.7 (5-OCH_3), 55.6 (3 -OCH_3), 35.8 (C-8), 32.9 (C-7). These data are consistent with literature values (Wang, 2013), identifying compound **5** as 5-methoxydehydroconiferyl alcohol (Fig. 1).

Compound 6 (yellow powder): HR-ESI-MS m/z 293.0317 $[M + H]^+$ (calcd for $C_{13}H_9O_8$, 293.0292). ^{1}H NMR (500 MHz, DMSO) δ: 10.92 (1H, s, -OH), 10.10 (2H, s, -OH $\times$ 2), 7.28 (1H, s, H-3), 4.34 (1H, brs, H-4), 2.98 (1H, dd, J = 18.7, 7.6 Hz, H-5a), 2.42 (1H, d, J = 18.7 Hz, H-5b). ^{13}C NMR (125 MHz, DMSO) δ: 193.5 (C-1), 173.7 (C-6), 160.4 (C-7), 149.7 (C-2), 145.8 (C-4), 143.9 (C-6), 140.3 (C-3), 139.2 (C-5), 115.3 (C-2), 113.2 (C-1), 108.1 (C-3), 41.1 (C-4),

37.6 (C-5). These data match literature values (Tanaka et al., 1990), identifying compound **6** as brevifolincarboxylic acid (Fig. 1).

Compound 7 (white powder): HR-ESI-MS m/z 479.2583 $[M - H]^-$ (calcd for $C_{26}H_{39}O_8$, 479.2650). ^{1}H NMR (500 MHz, methanol-d_4) δ: 5.20 (1H, s, H-17a), 4.87 (1H, s, H-17b), 4.51 (1H, d, J = 7.8 Hz, 13-glc-H-1), 1.20 (3H, s, H-18), 0.99 (3H, s, H-20). ^{13}C NMR (125 MHz, methanol-d_4) δ: 181.6 (C-19), 154.0 (C-16), 105.5 (C-17), 99.2 (13-glc-C-1), 87.6 (C-13), 78.1 (13-glc-C-3), 77.6 (13-glc-C-5), 75.2 (13-glc-C-2), 71.6 (glc-C-4), 62.7 (glc-C-6), 58.1 (C-5), 55.2 (C-9), 49.0 (C-15), 45.1 (C-14), 44.6 (C-4), 43.2 (C-8), 42.6 (C-7), 41.9 (C-1), 40.6 (C-10), 39.1 (C-3), 38.7 (C-12), 29.5 (C-18), 23.0 (C-6), 21.4 (C-11), 20.3 (C-2), 16.2 (C-20). These data are consistent with literature values (Ohtani et al., 1992), identifying compound **7** as steviolmonoside (Fig. 1).

Compound 8 (white powder): HR-ESI-MS m/z 317.2110 $[M - H]^-$ (calcd for $C_{20}H_{29}O_3$, 317.2122). ^{1}H NMR (500 MHz, chloroform-d) δ: 4.98 (1H, s, H-17a), 4.81 (1H, s, H-17b), 1.23 (3H, s, H-18), 0.95 (3H, s, H-20). ^{13}C NMR (125 MHz, chloroform-d) δ: 183.4 (C-19), 155.8 (C-16), 103.2 (C-17), 80.5 (C-13), 57.0 (C-5), 54.0 (C-9), 47.6 (C-15), 47.1 (C-14), 43.7 (C-4), 41.9 (C-8), 41.4 (C-7), 40.6 (C-1), 39.6 (C-12), 39.5 (C-10), 37.9 (C-3), 29.0 (C-18), 21.9 (C-6), 20.6 (C-11), 19.2 (C-2), 15.6 (C-20). These data match literature values (Ohtani et al., 1992), identifying compound **8** as steviol (Fig. 1).

Compound 9 (yellow powder): HR-ESI-MS m/z 329.2464 $[M + Na]^+$ (calcd for $C_{20}H_{34}O_2Na$, 329.2442). ^{1}H NMR (500 MHz, methanol-d_4) δ: 3.71 (1H, d, J = 11.3 Hz, H-17a), 3.61 (1H, d, J = 11.3 Hz, H-17b), 1.10 (3H, s), 1.07 (3H, s), 1.03 (3H, s). ^{13}C NMR (125 MHz, methanol-d_4) δ: 82.8 (C-16), 66.8 (C-17), 56.8 (C-5), 56.8 (C-9), 53.4 (C-15), 46.2 (C-13), 45.5 (C-8), 42.1 (C-1), 42.1 (C-3), 40.3 (C-14), 39.7 (C-10), 37.8 (C-7), 34.9 (C-4), 34.9 (C-18), 27.1 (C-12), 22.7 (C-19), 21.3 (C-6), 19.8 (C-2, 11), 18.4 (C-20). These data are consistent with literature values (Etse et al., 1987), identifying compound **9** as 16α,17-dihydroxykaurane (Fig. 1).

Compound 10 (yellow powder): HR-ESI-MS m/z 463.0860 $[M - H]^-$ (calcd for $C_{21}H_{19}O_{12}$, 463.0882). ^{1}H NMR (500 MHz, methanol-d_4) δ: 7.85 (1H, d, J = 2.3 Hz, H-2), 7.60 (1H, dd, J = 8.4, 2.3 Hz, H-6), 6.88 (1H, d, J = 8.4 Hz, H-5), 6.41 (1H, d, J = 2.2 Hz, H-8), 6.22 (1H, d, J = 2.2 Hz, H-6), 5.17 (1H, d, J = 7.8 Hz, gal-H-1), 3.48–3.87 (6H, m, gal-H-2 –6). ^{13}C NMR (125 MHz, methanol-d_4) δ: 179.6 (C-4), 166.0 (C-7), 163.0 (C-5), 158.8 (C-2), 158.4 (C-9), 145.0 (C-4), 145.8 (C-3), 135.8 (C-3), 123.0 (C-6), 122.9 (C-1), 117.8 (C-5), 116.1 (C-2), 105.6 (C-10), 105.4 (gal-C-1), 99.9 (C-6), 94.7 (C-8), 77.2 (gal-C-5), 75.1 (gal-C-3), 73.2 (gal-C-2), 70.0 (gal-C-4), 62.0 (gal-C-6). These data match literature values (Zhang et al., 2007), identifying compound **10** as quercetin-3-O-β-D-galactopyranoside (Fig. 1).

2.2 α-Glucosidase Inhibitory Activity Test Results

The α-glucosidase inhibitory activity assay revealed that compounds **2, 3, 5, 6,** and **10** exhibited strong inhibitory effects, with IC_{50} values of (0.14 ± 0.03) mg$\cdot$mL^{-1}, (0.36 ± 0.02) mg$\cdot$mL^{-1}, (0.44 ± 0.01) mg$\cdot$mL^{-1}, (0.53 ± 0.04) mg$\cdot$mL^{-1}, and (0.14 ± 0.03) mg$\cdot$mL^{-1}, respectively—all superior to the positive control acarbose ($IC_{50} = (0.69 \pm 0.02)$ mg$\cdot$mL^{-1}). Detailed results are presented in Table 2 .

Table 2 Results of α-glucosidase inhibitory activity

Compound	$IC_{50} \pm SD$ (mg$\cdot$mL^{-1})
2	0.14 ± 0.03
3	0.36 ± 0.02
5	0.44 ± 0.01*
6	0.53 ± 0.04**
10	0.14 ± 0.03**
Acarbose	0.69 ± 0.02

Note: indicates positive control; ***and * indicate significant differences compared with the positive control group*** *(P < 0.01,* P < 0.05).*

Discussion and Conclusion

α-Glucosidase inhibitors lower blood glucose by suppressing the activity of α-glucosidase in intestinal mucosal cells, thereby reducing glucose production and absorption. However, current clinical drugs in this class cause significant side effects (Zhu et al., 2021). Consequently, developing novel, safe, and effective α-glucosidase inhibitors is crucial for diabetes management, and discovering natural inhibitors has become a research hotspot (Quan et al., 2020; Yuca et al., 2021; Zhu et al., 2011).

Building on previous findings that *R. suavissimus* extracts inhibit α-glucosidase (Wu et al., 2021), this study investigated its chemical constituents and bioactivities, leading to the isolation of ten compounds. Compounds **2, 4, 5,** and **9** were obtained from sweet tea for the first time. Among them, compounds **2** and **10** are flavonoid glycosides, while **3** and **6** are phenolic acids. The activity assays demonstrated that compounds **2, 3, 6,** and **10** possess strong α-glucosidase inhibitory activity, consistent with previous reports (Liu et al., 2019; Yue, 2021; Lin et al., 2022; Xue et al., 2023). Notably, compound **5**, a lignan, showed potent activity, which is reported here for the first time. Comparing the structures of compounds **5** and **4**, the only difference is an additional methoxy group at C-5 in compound **5**. While compound **5** exhibited strong activity, compound **4** was inactive at the same test concentration, suggesting that the methoxy group at C-5 is a critical functional group for activity.

Sweet tea has a long history as a beverage. Its main constituent, rubusoside, is 300 times sweeter than sucrose but contains only 1% of the calories (Ma et al., 2008), making it an ideal sweetener for diabetic patients. Developed countries are actively developing related products, with multiple beverages, candies, and pharmaceuticals already marketed in Japan (Zhu et al., 2015). This study's discovery of chemical constituents with excellent α-glucosidase inhibitory activity further confirms the hypoglycemic potential of sweet tea and provides a scientific basis for developing functional foods or hypoglycemic drugs from this plant.

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