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Original Article

Chemical Constituents and *A*-Glucosidase Inhibitory Activity of *Syzygium Yunnanense*

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Abstract:
Type 2 diabetes represents a pervasive global public health challenge, necessitating the discovery of novel, safe, and efficacious antidiabetic agents. This study presents the first phytochemical investigation of Syzygium yunnanense, isolating 23 secondary metabolites. Compounds 2, 6, and 8, whose α-glucosidase inhibitory activity is reported for the first time in this work, exhibited stronger in vitro inhibition than the positive control quercetin, with stable target binding validated via molecular docking. These results establish a scientific foundation for S. yunnanense as a promising antidiabetic medicinal resource. The antibacterial activity was also tested.

Keywords: Chemical constituents, α-glucosidase, inhibition, molecular docking, Syzygium yunnanense

Introduction

DIABETES is a chronic non-communicable disease that seriously threatens human health[1]. According to the latest statistics from the International Diabetes Federation, there are currently 415 million diabetic patients worldwide, and this number is projected to reach nearly 642 million by 2040. Type 2 diabetes mellitus (T2DM) accounts for approximately 95% of all diabetes cases. As one of the major chronic diseases threatening public health in China, T2DM creates an urgent demand for the development of anti-diabetic drugs. *Syzygium*, a large genus of the Myrtaceae family, comprises more than 1200 species worldwide, with 74 species distributed in China. Plants of this genus possess great value in traditional applications and modern pharmacological research for diabetes treatment. Multiple *Syzygium* species have been used to alleviate hyperglycemia in traditional Asian medicine, and their leaves, stem barks, fruits and other parts are commonly applied for blood glucose regulation. For centuries, *Syzygium* genus plants and their extracts have been used for diabetes therapy in Asian countries such as such as India, Indonesia, Malaysia and the Philippines[2,3]. Modern pharmacological studies have further demonstrated that the extracts of several *Syzygium* species, such as *S. cumini*, *S.*

zeylanicum, *S. aromaticum* and *S. caryophyllatum*, can effectively reduce blood glucose levels in diabetic rats[4-7].

Although the chemical constituents of some *Syzygium* species have been elucidated[8-12], a great number of species remain under-explored, especially those native to China. Up to now, our research group has systematically studied several *Syzygium* species growing in Yunnan, including *S. austroyunnanense*, *S. fluviatile*, *S. samarangense* and *S. brachyantherum*. A variety of phloroglucinols, resorcinols and anacardic acid derivatives were isolated from these plants. A series of α-glucosidase inhibitors with stronger activity than the positive control quercetin was identified. These findings suggest that the above compounds are likely the main constituent basis responsible for the anti-hyperglycemic effects of *Syzygium* genus plants[13-20]. *Syzygium yunnanense*, an evergreen tree, is locally named "Xiangguo (fragrant fruit)" owing to its low-moisture fruits with characteristic rose aroma[21]. To date, no phytochemical study on *S. yunnanense* has been reported. As part of our ongoing search for bioactive natural products with antidiabetic and antibacterial potential from *Syzygium* plants, we conducted a phytochemical investigation on the ethyl acetate-soluble fraction of the methanolic extract from the branches and leaves of *S. yunnanense*, collected in Xishuangbanna, Yunnan Province, China. This investigation led to the isolation of 23 compounds, which were subsequently evaluated for their *α*-glucosidase inhibitory and antibacterial activities.

Results and Discussion

Structural Identification

In this study, various chromatographic separation methods and modern spectroscopic techniques were applied to conduct the first phytochemical investigation on the stems and leaves of *S. yunnanense*, which led to the isolation and identification of 23 compounds (Figure 1). These compounds were characterized as resorcinol derivatives: 5-[(*Z*,*Z*,*Z*)-Heptadeca-8,11,14-trienyl] resorcinol (**1**)[22], 5-[(*Z*,*Z*)-heptadeca-8,11-dienyl]resorcinol (**2**)[23], 5-[(*Z*)-heptadec-8-enyl]resorcinol (**3**)[24,] adipostatin A (**4**)[25], 5-tricosylresorcinol (**5**)[26], methyl 2,4-dihydroxy-3,6- dimethylbenzoate (**9**)[27], methyl 2,6-dihydroxy-3,4-dimethylbenzoate (**10**)[28]; anacardic acid derivatives: 2-[(*Z*,*Z*)-heptadeca-8,11-dienyl]-6-hydroxybenzoic acid (**6**)[29], 2-[(*Z*)-heptadeca-10-enyl]-6-hydroxybenzoic acid (**7**)[30], 2-pentadecyl-6-hydroxy benzoic acid (**8**)[31]; triterpenoid derivatives: 2,4-ethyl-lophenol (**11**), citrostadienol (**12**)[32], 3-*epi*-oleanolic acid (**13**)[33,34], arjunolic acid (**14**)[35], oleanolic acid (**15**)[36], 3-*epi*-ursolic acid (**16**)[33], ursolic acid (**17**)[36], betulonic acid (**18**)[37], 3-*epi*-betulinic acid (**19**)[37], alphitolic acid (**20**)[38], methyl betulinate (**21**)[39] and flavonoid derivatives: demethoxymatteucinol (**22**)[40] and 4,6-dihydroxy-3,5-dimethyl-2-methoxychalcone (**23**)[41].

α-Glucosidase inhibitory activity

Compounds **2**, **3**, **5**–**8** were tested for their inhibitory effects against *α*-glucosidase with quercetin as positive control. As shown in Figure 2, compounds **2**.

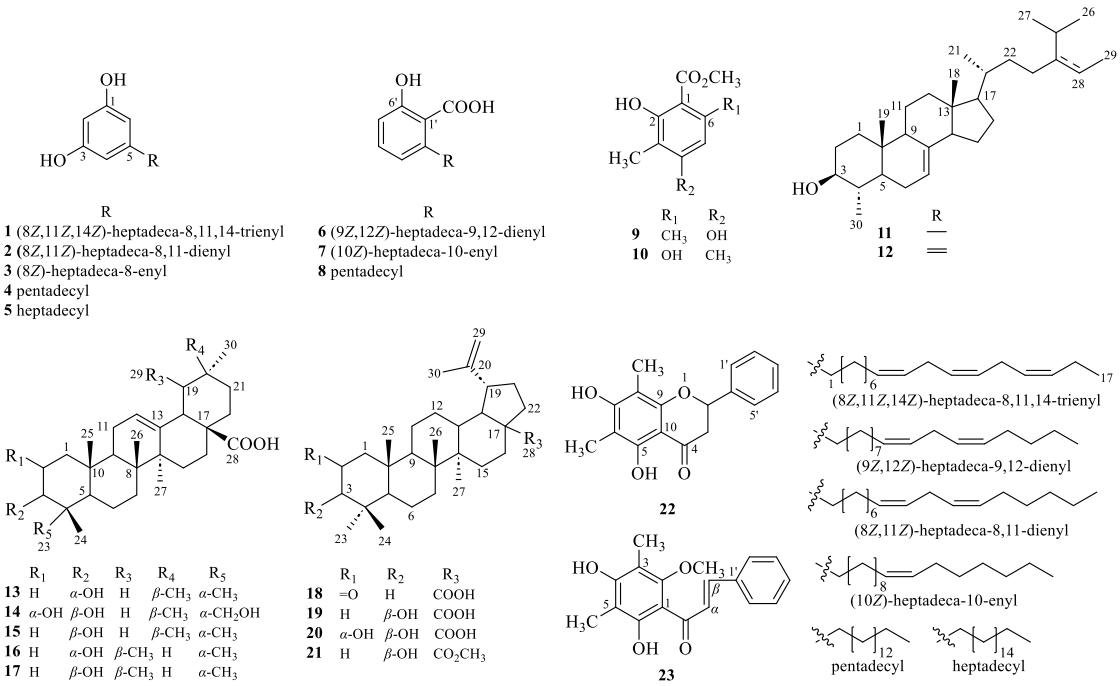

Figure 1. Chemical structures of compounds **1**–**23**

3, **6** and **8** exhibited stronger inhibitory effects than the positive control (IC_{50} = 6.87 μM), with IC_{50} values from 1.23 to 3.16 μM. Compounds **5** and **7** showed no significant inhibition (IC_{50} > 40 μM). Compounds **1** and **4** have been previously reported as α-glucosidase inhibitors in our earlier studies on two other *Syzygium* species[15, 29], and the α-glucosidase inhibitory activity of compound **3** has also been documented by another research group[42]. Therefore, this study is the first to reveal the α-glucosidase inhibitory activity of compounds **2**, **6** and **8**. A preliminary structure-activity relationship analysis suggested that a higher number of double bonds in the aliphatic chain may contribute to enhanced inhibitory activity.

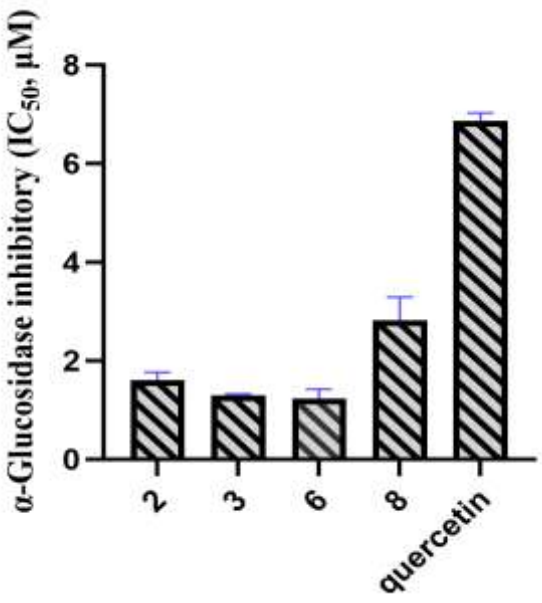

Figure 2. α-Glucosidase inhibitory activity of compounds

Molecular docking of active compounds with α-glucosidase

The predicted binding affinity of compound **2** was −7.1 kcal/mol, indicating that compound **2** was well accommodated within the active pocket of *α*-glucosidase. A conventional hydrogen bond was formed with His280, which may contribute to the ligand-enzyme recognition. In addition, the aromatic ring of compound **2** engaged in π–π stacking with Phe303, and further π–alkyl interactions were observed with the same residue (Phe303). Molecular docking revealed that compound **6** bound to *α*-glucosidase with a binding affinity of −7.1 kcal/mol. It formed conventional hydrogen bonds with Arg315 at the enzyme active pocket. Electrostatic π-interactions including T-shaped π–π stacking with Phe303 further reinforced binding. The long alkyl tail of compound **6** produced π-alkyl hydrophobic interactions with Lys156. Compound **8** exhibited a binding free energy of −6.7 kcal/mol against *α*-glucosidase. It formed hydrogen bonds with Gln353 and Asp352, a π-anion interaction with Glu411, and a π-alkyl interaction between its long alkyl tail and Tyr158. Multiple synergistic intermolecular interactions enable stable binding of compound **8** at the enzyme active site. Therefore, combined hydrogen-bonding, electrostatic and hydrophobic interactions jointly supported their stable binding and potential *α*-glucosidase inhibitory activity.

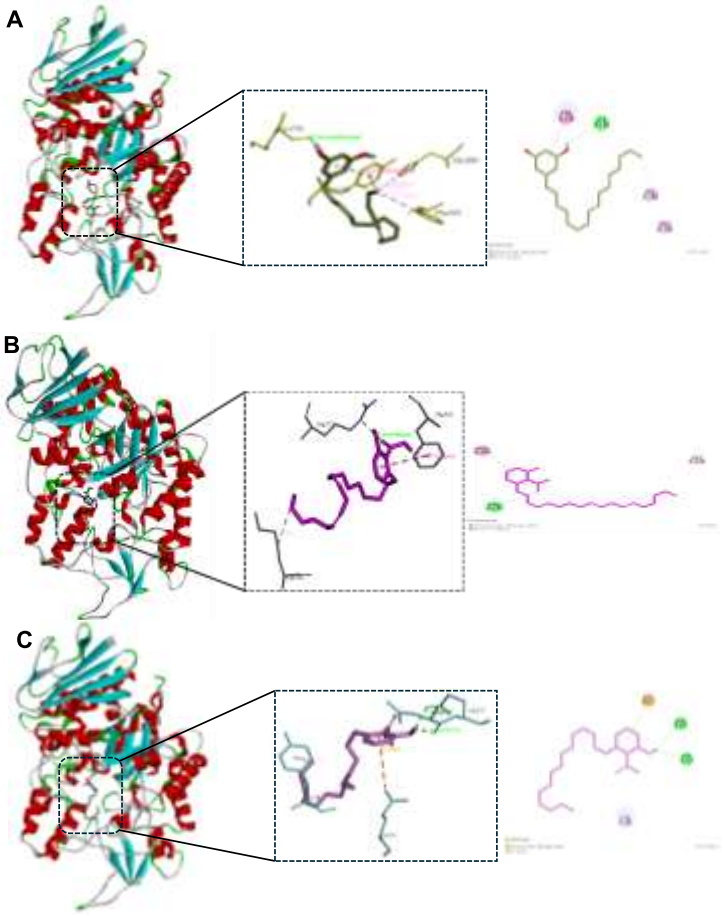

Figure 3. 3D and 2D Docking simulation key interactions of 2 (A), 6 (B), and 8 (C) in the active site of *α*-glucosidase. In the 2D docking result diagram, green dashed lines indicate hydrogen bond interactions, orange dashed lines indicate Pi-Anion interaction, π–π stacking interactions are shown by deep purple dashed lines and π–alkyl interactions are shown by light purple dashed lines.

Antibacterial activity

Compounds **1**–**7**, **22** and **23** were subjected to activity screening against methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococcus* (VRE, ATCC 51299), *Candida albicans* (*C. albicans*), *Staphylococcus aureus (S. aureus)* and *Escherichia coli* (*E. coli*). The results demonstrated that none of them possessed remarkable inhibitory activity against the five strains mentioned above, with MIC values exceeding 100 μg/mL (**Table 1**).

Table 1. MIC of the test samples and positive controls (μg/mL)

compounds	**MRSA**	**VRE ATCC51299**	***C. albicans***	***S. aureus***	***E. coli***
1 ~ **7**, **22** and **23**	> 100	> 100	> 100	> 100	> 100
vancomycin	2	> 100	—	1	—
ampicillin	> 100	2	—	2	—

"—"indicates that the mean was not detected.

Materials and Methods

Plant Material

Syzygium yunnanense were collected in Xishuangbanna, Yunnan Province, in September 2018, which was authenticated by Mr. Yu Chen, Kunming Institute of Botany, Chinese Academy of Sciences. A voucher specimen (No. 20180902) has been deposited at the College of Chemistry and Chemical Engineering, Yunnan Normal University.

Extraction and Isolation

The air-dried powder of branches and leaves of *S. yunnanense* (10.0 kg) was extracted with industrial-grade methanol (15 L × 2d × 3) to afford a black crude extract (1.3 kg). The crude extract was suspended in distilled water (3.8 L) and sequentially subjected to four-times liquid-liquid extraction with an equal volume of petroleum ether, ethyl acetate and n-butanol, sequentially, to yield petroleum ether (20 g), ethyl acetate (250 g) and n-butanol (650 g) fractions. The ethyl acetate fraction was fractionated via silica gel CC eluting with petroleum ether/ethyl acetate (1:0 to 0:1, v/v) to obtain eight subfractions (Fr.A-Fr.H). Subfraction Fr.C (30 g) was further separated by silica gel CC (200–300 mesh) with a gradient of petroleum ether/acetone (80:1 to 0:1, v/v). to give two subfractions Fr.C1 (3 g) and Fr.C2 (6 g). Fr.C1 was first purified by Sephadex LH-20 CC eluted with methanol, followed by repeated silica gel CC (petroleum ether/acetone) and subsequent Sephadex LH-20 ($CHCl_3$/MeOH, 1:1, v/v) purification. Final isolation by HPLC (93% MeOH/H_2O) afforded compounds **17** (1.6 mg, t_R = 6.2 min), **6** (18 mg, t_R = 21 min), **8** (4.9 mg, t_R = 24 min) and **7** (6.7 mg, t_R = 27 min). Fr.C2 was separated via Sephadex LH-20 ($CHCl_3$/MeOH, 1:1, v/v) and further purified by HPLC (51% MeOH/H_2O, v/v) to give compound **9** (19 mg, t_R = 40 min). Compounds **12** (2 mg, t_R = 22.5 min) and **11** (3 mg, t_R = 25 min) were isolated from Fr.D1 by HPLC (98% MeOH/H_2O, v/v). Based on the identical procedure, compounds **22** (2.8 mg, t_R = 7.5 min), **23** (80 mg, t_R = 17 min) and **10** (4.3 mg, t_R = 33 min) were separated by HPLC (70% MeOH/H_2O, v/v, v/v). **13** (8.5 mg, t_R = 7.7 min), **15** (18 mg, t_R = 10 min) and **21** (4.6 mg, t_R = 12.7 min) by HPLC (90%MeOH/H_2O, v/v) from Fr.D2 by using similar isolation procedure. Fr.E was sequentially subjected to Sephadex LH-20 CC ($CHCl_3$/MeOH 1:1), silica gel CC and another round of Sephadex LH-20 CC, and was further divided into six subfractions (Fr.E1–Fr.E6). Fr.E2 was isolated by semi-Pre HPLC (90%MeOH/H_2O) to give **18** (4.8 mg, t_R = 7.2 min) and **16** (5.6 mg, t_R = 10.0 min). Fr.E4 was isolated by HPLC (88% MeOH/H_2O) to give **14** (4.1 mg, t_R = 14 min) and **19** (15.6 mg, t_R = 18.5 min). Fr.E5 was purified by semi-Pre HPLC (80%

MeOH/H_2O) to provided **1** (56.2 mg, tR = 9 min), **2** (45.4 mg, t_R = 12 min), **3** (3.7 mg, t_R = 21.5 min), **5** (4.1 mg, t_R = 22.5 min) and **4** (17.1 mg, t_R = 25.5 min). Fr.F (2 g) was fractionated by silica gel CC using petroleum ether/acetone (8:1, v/v) as eluent; followed by Sephadex LH-20 ($CHCl_3$/MeOH, 1:1, v/v) to afford compound **20** (8 mg).

α-Glucosidase Inhibitory Activity Assay

The α-glucosidase inhibitory assay was performed by referring to the method reported previously[15]. A mixture containing 20 μL of test samples at five different concentrations, 10 μL of α-glucosidase solution (0.025 U/mL), 10 μL of 0.1 M phosphate buffer (pH 6.9), and 20 μL of 1 mM *p*-nitrophenyl α-D-glucopyranoside solution was incubated in 96 well plates at 37 °C for 50 min. After incubation, the optical density (OD) at 405 nm was measured using a microplate reader (SpectraMax M2, Molecular Devices Corp., operated with SoftmaxPro v.4.6 software, Sunnyvale, CA, USA). The OD values were compared with that of the positive control (quercetin), where 20 μL of buffer solution replaced the test sample. The α- glucosidase inhibitory activity was expressed as percent inhibition, calculated as described below. The IC_{50} (concentration required for 50% inhibition) was determined by the Reed–Muench method[43].

Molecular Docking

Molecular docking simulations were performed using AutoDock Vina to investigate the binding modes of the isolated compounds and the positive control (quercetin) with α-glucosidase. The crystal structure of isomaltase from *Saccharomyces cerevisiae* (PDB ID: 3A4A, resolution 1.60 Å), retrieved from the RCSB Protein Data Bank[44], was employed as the template, which shares 75% sequence identity with the target protein. This template was selected due to its high sequence identity and its widespread use as a reference model for docking studies of α-glucosidase inhibitors. Prior to docking, the protein and ligand structures were prepared using AutoDock Tools (ADT), including the addition of polar hydrogens, assignment of Gasteiger charges, and removal of water molecules and co-crystallized ligands. A grid box with dimensions of 40 × 40 × 40 $Å^3$ was defined, centered at coordinates (x = 19.288, y = –6.841, z = 22.517), to encompass the active site. The binding affinities of the test compounds and quercetin were calculated and expressed as binding energy (kcal/mol). Subsequently, the docking results were analyzed and visualized in both 2D and 3D formats using Discovery Studio to illustrate the key intermolecular interactions between the ligands and the enzyme.

Antibacterial Activity Screening

The minimum inhibitory concentration (MIC) was determined following the standard guidelines of the Clinical and Laboratory Standards Institute (CLSI, 2019). The target compounds or antibiotics were subjected to twofold serial dilution in tryptic soy broth (TSB) and then mixed with an equal volume of bacterial suspension (approximately 2×10^5 CFU/mL) in 96-well plates. Following incubation at 37 °C for 24 h, the MIC was defined as the lowest concentration of the test agent that produced visible inhibition of bacterial growth. Vancomycin and ampicillin served as positive controls.

Conclusions

In conclusion, this study represents the first phytochemical investigation of the ethyl acetate fraction from the branches and leaves of *S. yunnanense*, leading to the isolation of 23 known compounds. For the first time, compounds **2**, **6**, and **8** were identified as natural α-glucosidase inhibitors. Molecular docking initially revealed their interaction with α-glucosidase. Collectively, our findings not only enrich the chemical knowledge of *S. yunnanense* but also provide a scientific basis for its further development and

utilization as a potential hypoglycemic food source or antidiabetic agent.

Supplementary Materials: The following supporting information can be downloaded. Supporting information includes the NMR data of compounds **1**–**23** and their 1D NMR spectrums.

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Conflicts of Interest: The authors declare no conflict of interest.

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