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Chemical Components and Their *α*-Glucosidase Inhibitory Activity from the Leaves of *Ficus carica* Linn.

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Abstract: Nineteen compounds were purified from leaves of *Ficus carica* Linn. using various separation techniques such as recrystallization, column chromatography filled with silica gel, Sephadex LH-20, and MCI, and semi-preparative HPLC. By analysis of NMR, MS, and data comparison with those reported from literatures, their structures were identified as umbelliferone (**1**), psoralen (**2**), furopinnarin (**3**), 6,7-furano-hydrocoumaric acid (**4**), (*E*)-3-[5-(6-hydroxy) benzofuranyl] propenoic acid (**5**), (*E*)-3-(6-hydroxy-4-methoxy-5-benzofuranyl) propenoic acid (**6**), nodakenetin (**7**), oxypeucedanin hydrate (**8**), dihydrofurocoumarin (**9**), (*E*)-4-hydroxy-3,3,5-trimethy1-4-(3-oxobu-1-en-1-yl)-cyclohexan-1-one (**10**), dehydrovomifoliol (**11**), 4,5-dihydroblumenol A (**12**), blumenol A (**13**), 5-hydroxy-4′,7-dimethoxyisoflavone (**14**), cajanin (**15**), loliolide (**16**), indole-3-carboxaldehyde (**17**), 1H-indole-3-carboxylic acid (**18**), vitamin E quinone (**19**). Their *α*-glucosidase inhibitory activities were evaluated by IC_{50} values. Compounds **15** and **19** showed obvious *α*-glucosidase inhibitory activity with IC_{50} of 71.1±1.56 μM and 49.3 μM±1.21, respectively, and by the kinetics of enzymatic reaction, the inhibition type by which compound **15** acting against *α*-glucosidase was inferred as anticompetitive. Moreover, preliminary structure-activity relationship was recapticulated from the tested compounds.

Keywords: *Ficus carica* Linn.; coumarin; *α*-glucosidase inhibitor; enzyme kinetics.

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Chemical components from the leaves of *Ficus carica* Linn.

1. Plant Source

Leaves of *Ficus carica* Linn. were collected on June 10, 2021, at Yingri Fig Farm, Huadu District, Guangzhou City, Guangdong Province, China. The species was identified by associate professor Shaohuan Liu, Guizhou Medical University, China. A voucher specimen (20210610) was well-kept at the Herbarium of School Guizhou Medical University (GMB).

2. Previous Studies

Diabetes mellitus (DM) is a metabolic disease distinguished by hyperglycemia and dysfunctional carbohydrate utilization caused by defective or insufficient insulin secretion, which might eventually lead to dysfunction of eyes, kidneys, cerebral vessels and neurons [1]. It is one of the most common diseases and the patients diagnosed with it is expected to reach 10.4% of the world population by 2040 [2]. Among patients with diabetes mellitus, type 2 accounts for over 90% [3]. For the treatment of type 2 diabetes mellitus, *α*-glucosidase inhibitors are one of the most effective drugs which inhibit the *α*-glucosidase enzyme in the small intestinal mucosa in order to slow down the absorption of carbohydrates, therefore reducing postprandial hyperglycemia [4]. The *α*-glucosidase inhibitors available at present include acarbose, voglibose, and miglitol, however, these oral hypoglycemic agents are structurally complex and costly to prepare and may cause gastrointestinal side effects with prolonged use [5]. Therefore, there is still a need to search for new *α*-glucosidase inhibitors with low toxicity and cost.

Ficus carica Linn. is a deciduous shrub or tree, a member of the *Ficus* genus that belongs to Moraceae family. In China, *F. carica* was first introduced to Xinjiang in the Tang Dynasty and recorded in Zhu Su's *Materia Medica for Famine Relief* (*Jiuhuang Bencao*) in the Ming Dynasty [6]. Their leaves have important medicinal values due to their enrichment of a variety of biologically active phytochemicals such as flavonoids, terpenoids, coumarins, and phenols [7]. In traditional medicines, *F. carica* leaves are used as antidiabetic, insect repellent, and treatment to alleviate human contact dermatitis [8]. Moreover, recent studies have shown that the crude extract of *F. carica* leaves possesses anticancer, antimicrobial, anti-inflammatory, hepatoprotective, and antidiabetic activities [9-14].

3. Present Study

In our study, we evaluated the *α*-glucosidase inhibitory activity of the ethyl acetate extract of *F. carica* leaves, the result showed that at the concentration of 300 μg/mL, the *α*-glucosidase inhibition rate of the ethyl acetate extract was 78.78%, and its IC_{50} was measured as 79.26 μg/mL. While literatures and our preliminary results all displayed that the crude extract of *F. carica* leaves had antidiabetic activity, whether the compounds it contains confer the same effect is unknown. In this study, we tended to clarify its chemical components to find new agents with *α*-glucosidase inhibitory activity, thus an investigation on the phytochemistry of *F. carica* leaves has been performed.

The leaves of *F. carica* (10 kg) were air-dried and powdered and then put to extraction under reflux with 95% ethanol for 3 times, each for 2 h, and the exaction solvent was combined and concentrated to obtain crude extract. The crude extract was suspended in water and extracted with EtOAc to yield EtOAc part (310 g). The EtOAc part of *F. carica* leaves was purified by chromatographic separation, 19 compounds (Figure 1) were isolated and identified based on spectroscopic methods, and data comparison with those reported in the literatures (spectra were included in supporting information). The compounds were determined as umbelliferone (**1**), psoralen (**2**), furopinnarin (**3**), 6,7-furano-hydrocoumaric acid (**4**), (*E*)-3-[5-(6-hydroxy) benzofuranyl] propenoic acid (**5**), (*E*)-3-(6-hydroxy-4-methoxy-5-benzofuranyl) propenoic acid (**6**), nodakenetin (**7**), oxypeucedanin hydrate (**8**), dihydrofurocoumarin (**9**), (*E*)-4-hydroxy 3,3,5-trimethy1-4-(3-oxobu-1-en-1-yl)-cyclohexan-1-one (**10**), dehydrovomifoliol (**11**), 4,5-dihydroblumenol A (**12**), blumenol A (**13**), 5-hydroxy-4′,7-dimethoxyisoflavone (**14**), cajanin (**15**), loliolide (**16**), indole-3-carboxaldehyde (**17**), 1H-indole-3-carboxylic acid (**18**), and vitamin E quinone (**19**), among which compounds **1**-**9** were

coumarins, **10**-**13** were sesquiterpenoids, and **14**-**15** were flavonoids, compounds **3**-**6**, **9**-**13, 17**-**19** are reported for the first time from *F. carica*.

α-Glucosidase Inhibitory Assay: All compounds except **4** and **14** (due to their low yield) were assayed for their *α*-glucosidase inhibitory activities by utilizing the method reported [15-17]. To put it shortly, the assay was conducted in 96-well culture plates with 230 μL reaction system. First of all, 95 μL of phosphate buffered saline (PBS) solution (0.1 mol/L pH 6.8) was transferred into each well, afterward 5 μL of different concentrations of tested compounds (0.0625, 0.125, 0.25, 0.5, 1 mmol/L) or acarbose (0.00032, 0.00160, 0.008, 0.04, 0.2, 1, 5μmol/L) were added, after which 30 μL of 0.6 U/mL *α*-glucosidase was pipetted and mixed. The 96-well plates were incubated for 20 min at 37°C, followed by the addition of 20 μL of 800 mg/mL *p*-Nitrophenyl *α*-D-glucopyranoside (pNPG) for another 25 min incubation. By adding 80 μL of Na_2CO_3 (0.2 mol/L) to each well, the reaction was quenched. The absorbance was documented for each well at 405 nm by a microplate reader and the procedure was conducted in parallel for three times. The measured absorbance values were determined by Graphpad Prism software 9.5 to afford the IC_{50} values.

*Enzyme Kinetics of Compound **15** Against α-Glucosidase*: Although compound **19** (IC_{50} = 49.3 μM) exhibited more potent *α*-glucosidase inhibitory activity than compound **15 (**IC_{50} = 71.1 μM**) (**Table 1**),** compound **15** was chosen for further enzyme kinetics study due to its relatively high yield (22.3 mg, compound **19** was only 5.34 mg).

The pNPG solution was kept at the optimal concentration as 800 mg/mL, whereas the enzyme solution concentration was varied as 0, 0.3, 0.6, 1.2 and 2.4 U/mL, and appropriate concentrations of the sample test solution was selected to plot the lines using reaction rate as *y*-axis and enzyme concentration as *x*-axis. The characteristics of the lines were used to determine the reversibility of the test compound.

The inhibition type of compound **15** against *α*-glucosidase was determined by Lineweaver-Burk plot. Using the same protocols of *α*-glucosidase inhibitory assay described above, except that the *α*-glucosidase concentration was kept constant (0.6 U/mL), the enzymatic reaction rate was determined under different substrate concentrations (400, 800, 1200, 1600, and 2000 mg/mL) of the reaction system as the concentration of compound **15** increased from 0 to 200 μM (0, 50, 100, and 200 μM, respectively) A Lineweaver-Burk plot was drawn using the reciprocal of pNPG concentrations (1/[S]) as the horizontal coordinate and the reciprocal of the reaction rates (1/V) as the vertical coordinate to determine the type of inhibition. If a set of straight lines intersecting the *y*-axis is obtained, the inhibition type of the inhibitor is competitive; if a set of straight lines intersecting the *x*-axis is obtained, the inhibition type of the inhibitor is non-competitive; if a set of lines intersecting at the second quadrant is obtained, the inhibition type of the inhibitor is mixed competitive.

α-Glucosidase Inhibitory Activity: The IC_{50} values (inhibitor concentration at 50% inhibition) of the test samples (ethyl acetate extract of *F. carica* leaves, compounds **1**-**3**, **5**-**13**, **15**-**19**) were calculated using Graphpad prism 9.5 software. From the results (Table 1), IC_{50} of ethyl acetate extract of *F. carica* leaves was 79.26 ug/mL. Among the compounds evaluated, compounds **15** (IC_{50} = 71.1±1.56 μM) and **19** (IC_{50} = 49.3±1.21 μM) showed relatively strong inhibitory effects, compounds **5**-**6** and **17**-**18** showed moderate inhibitory effects with IC_{50} values ranging from 151.25 μM to 342.55 μM, whereas other compounds (**1**-**3**, **7**-**13**, and **16**) exhibited poor or no inhibitory effects on the enzyme, with IC_{50} values either near or above 1000 μM.

*Inhibitory Kinetics of Compound **15** Against α-Glucosidase*: The plots of the V vs. [*α*-glucosidase] at different concentrations of compound **15** were constructed to confirm the reversibility of compound **15**-mediated inhibition. The results were shown in Figure 2 (a), which displayed that the enzyme concentration and the reaction rate were in a good linear relationship, and with the increase of the concentration of compound **15**, the slope of the straight line gradually reduced, moreover, the four lines almost intersected at the zero point, indicating that compound **15** only reduced the catalytic

efficiency of the enzyme, and the inhibition process was reversible. The enzymatic reaction rate of compound **15** on *α*-glucosidase was measured at various concentrations (with the same concentration of *α*-glucosidase at 0.6 U/mL and pNPG at 400, 800, 1200, 1600, 2000 mg/mL, respectively), the kinetic curves were shown in Figure 2 (b). All the straight lines in Figure 2 (b) were almost parallel to each other. The reaction rate (Vm) and the Michaelis constant (Km) decreased as the concentration of the inhibitor increased, which implied that the compound **15** mediated *α*-glucosidase inhibition in an anticompetitive way, meaning it did not bind directly to the free enzyme, but only to the enzyme-substrate complex to interrupt the enzyme reaction.

Table 1. *In vitro α*-glucosidase inhibitory activities of the extract and isolates from *F. carica* leaves.

Compound	IC_{50} (μg/mL[a], μM[b])	Compound	IC_{50} (μg/mL[a], μM[b])
ethyl acetate extract	79.26±5.65	**11**	>1000
1	>1000	**12**	>1000
2	>1000	**13**	>1000
3	>1000	**15**	71.1±1.56
5	342.55±1.25	**16**	>1000
6	244.95±8.35	**17**	193.3±11.54
7	>1000	**18**	151.25±19.65
8	999.65±16.35	**19**	49.3±1.21
9	>1000	**Acarbose**[c]	0.002587±0.000117
10	>1000		

[a] *α*-glucosidase inhibitory activity of ethyl acetate extract is expressed in μg/mL.
[b] *α*-glucosidase inhibitory activity of compounds is expressed in μM.
[c] Acarbose was used as positive control.

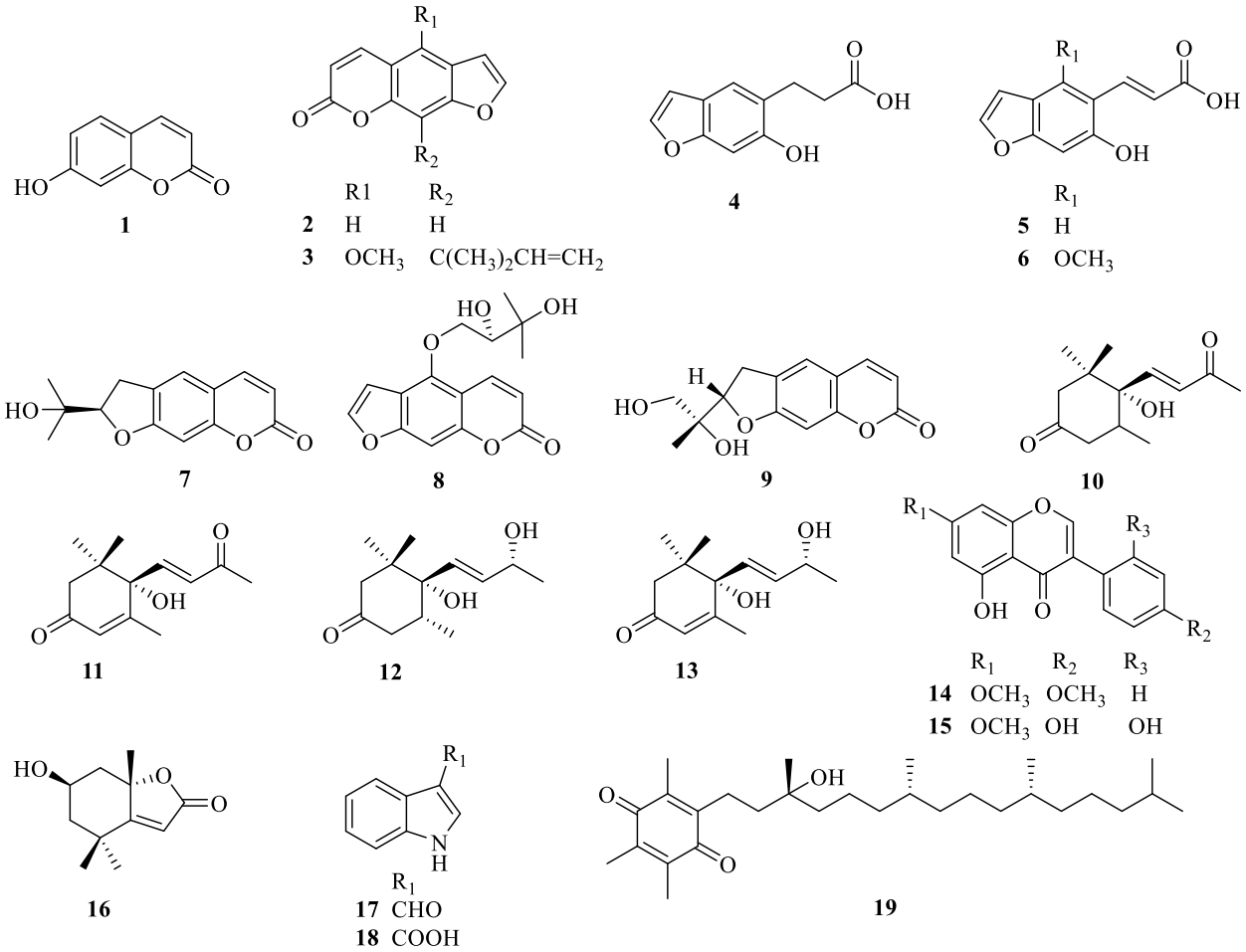

Figure 1. Compounds (**1-19**) isolated from the leaves of *F. carica*

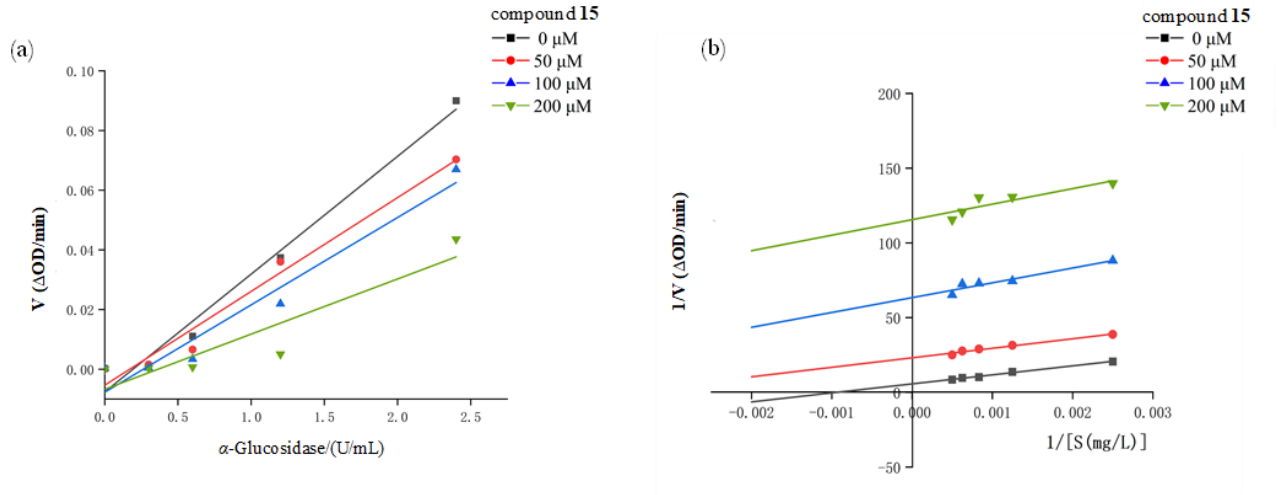

Figure 2. *α*-Glucosidase inhibition kinetics analysis of compound **15**. (a) Plots of V versus [*α*-glucosidase]. *c*(pNPG) = 800 mg/mL, *c* (compound **15**) = 0, 50, 100 and 200 μM, respectively. (b) Lineweaver–Burk plots. *c*(*α*-glucosidase) = 0.6 U/mL, and *c* (compound **15**) = 0, 50, 100 and 200 μM, respectively.

In conclusion, this study isolated 19 known compounds from *F. carica*, among which compounds **3-6**, **9-13**, **17-19** are reported for the first time from *F. carica*. Based on the results of the bioassay, the ethyl acetate extract of *F. carica* leaves having good *α*-glucosidase inhibitory activities could be partially attributed to propenoic acid (**5**), (E)-3-(6-hydroxy-4-methoxy-5-benzofuranyl) propenoic acid (**6**), cajanin (**15**), indole-3-carboxaldehyde (**17**), 1H-indole-3-carboxylic acid (**18**), and vitamin E quinone (**19**). The type of inhibition of the active compound (cajanin, **15**) with relatively high yield was determined to be anticompetitive by enzyme kinetics. Furthermore, preliminary structure-activity relationship was elucidated among the compounds evaluated. For coumarins (**1-9**), the compounds with open *α*-pyran ring (**5-6**) showed more potent activity than those with closed pyran ring, demonstrating that whether the *α*-pyran ring was open or closed is important for the activity. Moreover, from compounds **4-6**, whether the double bond on the C-3 side chain was important for the activity could be theoretically acquired, but it was a pity that compound **4** was not put to evaluation due to its low yield, and IC_{50} values of compounds **5** and **6** indicated that the introduction of a methoxy group at R_1 favors the activity. For sesquiterpenoids (**10-13**), none of them showed any inhibitory activities, suggesting that the skeleton does not favor this activity. For indoles (**17-18**), the aldehyde group at 3 position contributes more than the carboxyl group to the activity. Our results provide a reference for the utilization of the plant, and in-depth studies on the pharmacological mechanisms of the active compounds should be carried out in the future.

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Supporting Information

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