Chemical Constituents from Turnip and Their Effects on α-Glucosidase

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Abstract: Brassica rapa var. rapa (turnip) is an important crop in Qinghai-Tibet Plateau (QTP) with anti-hypoxic effect. Turnip is rich in glucosinolates, isothiocyanates and phenolic compounds with diverse biological activities, involving anti-oxidant, anti-tumor, anti-diabetic, anti-inflammatory, anti-microbial, hypolipidemic, cardioprotective, hepatoprotective, nephroprotective and analgesic properties. In this study, the ethyl acetate (EtOAc) and butanol parts of Brassica rapa were first revealed with inhibitory effects on α-glucosidase, whereas the water part was inactive. Subsequent bioassay-guided isolation on the EtOAc and butanol parts yielded 12 compounds, involving three indole derivatives, indole-3-acetonitrile (1), 4-methoxyindole-3-acetonitrile (2) and indole-3-aldehyde (3) two flavonoids, liquiritin (4) and licochalcone A (5) two phenylpropanoids, sinapic acid (6) and caffeic acid (7) two phenylethanol glycosides, 2-phenylethyl β-glucopyranoside (8) and salidroside (9) and three other compounds, syringic acid (10) adenosine (11) and (3β, 20E)-ergosta-5,20-di-en-3-ol (12). Licochalcone A (5) and caffeic acid (7) showed α-glucosidase inhibitory activity with IC₅₀ values of 62.4 ± 8.0 μM and 162.6 ± 3.2 μM, comparable to the positive control, acarbose (IC₅₀ = 142 ± 0.02 μM). Docking study suggested that licochalcone A (5) could well align in the active site of α-glucosidase (docking score = -52.88) by forming hydrogen bonds (Gln1372, Asp1420, Gln1372, Arg1510), hydrophobic effects (Tyr1251, Tyr1251, Trp1355, Phe1560, Ile1587, Trp1355, Phe1559, Phe1559) and π-π stacking interaction (Trp1355). This study provides valuable information for turnip as a new resource in searching anti-diabetic candidates.

Keywords: Brassica rapa; turnip; α-glucosidase; licochalcone A; docking study

1 Introduction

Brassica rapa var. rapa (turnip) is an important crop with medicinal and edible purposes, which is widely consumed for protecting hypoxia in Qinghai-Tibet Plateau (QTP) [1]. Phytochemical investigation on turnip revealed that glucosinolates, isothiocyanates, flavonoids and volatiles were the main constituents [2]. Turnip showed a variety of bioactivities, involving anti-oxidant [3], anti-tumor [4], anti-diabetic [5], anti-inflammatory [6], anti-microbial [7], hypolipidemic, cardioprotective [8], hepatoprotective [9], nephroprotective [10] and analgesic effects [11]. Diabetes is a kind of metabolic disorder characterized by high levels of blood glucose, which affects about 425 million people all over the world [12]. According to the previous investigation, the ethanol extract of turnip showed anti-diabetic potency on type 2 diabetic mice, whereas the active constituents were still unclear [13]. In this study, the ethyl acetate (EtOAc) and butanol parts of turnip were first revealed with inhibitory effects on α-glucosidase, whereas the water part was inactive. As a continuous search for anti-diabetic candidates from natural resources, bioassay-guided...
fractionation on the EtOAc and butanol parts of turnip yielded 12 compounds. Herein, we reported their isolation and biological effects.

2 Materials and Methods

2.1 General Experimental Procedure

Silica gel (200-300 mesh) from Qingdao Makall Chemical Company (Qingdao, China) and Sephadex LH-20 (20-50 μm) from Pharmacia Fine Chemical Co., Ltd. (Uppsala, Sweden) were used for column chromatography (CC). Thin layer chromatography (TLC) spots were visualized on silica gel GF254 plates (Qingdao Haiyang, Qingdao, China) by heating after spraying with 10% H2SO4 in EtOH. Shimadzu HPLC system (Shimadzu, Kyoto, Japan) equipped with LC-20AR pumps and a model SPD-M20A UV detector, and supplied with a YMC-Pack Ph column (5 μm, 10 × 250 mm) was used for purification. HRESIMS data were obtained on a LCMS-IT-TOF mass spectrometer (Shimadzu, Kyoto, Japan). 1H and 13C NMR spectra were recorded on an AV-600 spectrometer (Bruker, Bremerhaven, Germany) and chemical shifts were given in δ.

2.2 Plant Materials

The tubers of Brassica rapa var. rapa were collected from Huodeng Village, Lajing Township, Lanping County, Nujiang Prefecture, Yunnan Province in March 2016, which were taxonomically identified by Prof. Yong-Ping Yang. A voucher specimen (KTRG-B-43) was deposited in Kunming Botanical Garden, Kunming Institute of Botany, Chinese Academy of Sciences.

2.3 Extraction and Isolation

The air-dried turnip tubers (2 kg) were powdered and extracted with 70% ethanol (20 L × 3) under reflux for three times. The combined extracts were condensed under reduced pressure to give a residue which was dissolved in water and partitioned with EtOAc and butanol. The EtOAc part (18.5 g) was subjected on silica gel column chromatography (Si CC) (8 × 60 cm) and eluted with CHCl3-MeOH gradient to give five fractions (Frs. A1-A5). Fr. A2 (4.0 g) was purified by repeated Si CC (PE-EtOAc, PE-acetone) and sephadex LH-20 CC (CHCl3-MeOH, 1:1) to yield compound 12 (9.5 mg). Fr. A3 (3.5 g) was purified by repeated Si CC, sephadex LH20 CC and HPLC to generate compounds 6 (8.0 mg), 7 (9.8 mg) and 10 (7.3 mg). The butanol (75.5 g) part was separated on Si CC and eluted with CHCl3-MeOH-H2O gradient to give six fractions (Frs. B1-B6). Fr. B2 (6.0 g) was purified by Si CC, sephadex CC and HPLC to generate compounds 6 (8.0 mg), 7 (9.8 mg) and 10 (7.3 mg). Similarly, compounds 4 (10.8 mg), 8 (8.8 mg), 9 (12.0 mg) and 11 (12.0 mg) were obtained from Fr. B4 (7.8 g) by repeated Si CC and HPLC purification. The separation procedure was briefly indicated in Fig. 1.
2.4 Bioassay in Vitro

Inhibitory activity against α-glucosidase was performed in accordance with our previous reports [14,15].

2.5 Docking Study

The crystal structure the C-terminal domain of human intestinal α-glucosidase (PDB ID: 3TOP) complexed with acarbose was retrieved from RCSB Protein Data Bank, and was used as molecular target. The docking calculation was performed using Yinfo Cloud Computing Platform (http://cloud.yinfotek.com), a friendly and versatile web server for bio-medicinal, material, and statistical researches. The active site of the enzyme was defined from the co-crystallized ligands from PDB files. The default docking protocols were applied for prediction the binding energies and the interactions between the ligands and protein.

3 Results

The total extraction of turnip was divided into EtOAc, butanol and water parts, and evaluated for their effects on α-glucosidase. The EtOAc extract showed significant activity (75.0 ± 15.4%), obviously higher than the butanol part (13.0 ± 2.5%). Whereas, the water part was inactive to α-glucosidase at the tested concentration (200 μg/mL). Therefore, the EtOAc and butanol parts were subjected to the following isolation.

All the isolates were determined by comparing their spectroscopic data with those reported in literatures. As a result, compounds 1-12 were deduced to be indole-3-acetonitrile (1) [16], 4-methoxyindole-3-acetonitrile (2) [17], indole-3-aldehyde (3) [18], liquiritin (4) [19], licochalcone A (5) [20], sinapic acid (6) [21], caffeic acid (7) [22], 2-phenylethyl β-glucopyranoside (8) [23], salidroside (9) [24], syringic acid (10) [25], adenosine (11) [26] and (3β, 20E)-ergosta-5,20(22)-dien-3-ol (12) [27] as shown in Fig. 2.
Compounds 1-12 were assayed for their α-glucosidase inhibitory activities in vitro. As shown in Tab. 1, compounds 5 and 7 showed obvious activity with inhibition rates of 118.9 ± 10.2% and 76.9 ± 6.8%, at the concentration of 1 mM. The dose-response study provided the IC$_{50}$ values of 62.4 ± 8.0 μM for 5 and 162.6 ± 3.2 μM for 7. The isopentenylated chalcone, licochalcone A (5), was more potent than the flavon, liquiritin (4), suggesting that the chalcone skeleton is preferable for maintaining activity. Sinapic acid (6) and caffeic acid (7) are two cinnamic acid analogues, and the main difference is the substitution on phenyl ring (hydroxy or methoxyl). Caffeic acid (7) showed obviously higher activity (76.9 ± 6.8% vs 5.8 ± 0.5%) than sinapic acid (6) indicated the importance of free hydroxyl group in the structure.

**Table 1: Inhibitory activities of compounds 1-12 (1 mM)**

| Compounds | Inhibition rates | Compounds | Inhibition rates |
|-----------|-----------------|-----------|-----------------|
| 1         | 2.5 ± 0.2       | 7         | 76.9 ± 6.8   |
| 2         | 2.2 ± 0.2       | 8         | 7.6 ± 1.9    |
| 3         | 2.1 ± 0.5       | 9         | 8.8 ± 0.8    |
| 4         | -15.0 ± 3.6     | 10        | -1.2 ± 0.2   |
| 5         | 118.9 ± 10.2    | 11        | 1.7 ± 0.9    |
| 6         | 5.8 ± 0.5       | 12        | -1.5 ± 0.5   |

*Acarbose was used as the positive control with an IC$_{50}$ value of 142 ± 20.0 μM.

The docking pose of licochalcone A (5) with 3TOP was shown in Fig 3. Licochalcone A (5) could well align in the active site of α-glucosidase (docking score = -52.88) by forming hydrogen bonds with Gln1372, Asp1420, Gln1372, Arg1510, hydrophobic effects with Tyr1251, Tyr1251, Trp1355, Phe1560, Ile1587, Tyr1251, Tyr1251, Trp1355, Trp1355, Phe1559, Phe1559, and π-π stacking interaction with Trp1355.

**Figure 3:** Docking pose (A) and interactions (B) of 5 (blue) at the binding site of α-glucosidase. Hydrogen bonds were represented by the green lines, and hydrophobic effects were showed as gray lines, and π-π stacking interaction was denoted in yellow line. The Grid Score was -52.88 suggesting strong affinity between 5 and α-glucosidase.
4 Conclusion

In this study, both the EtOAc and butanol extracts of turnip were revealed with anti-diabetic potency in vitro, from which two compounds (5 and 7) with α-glucosidase inhibitory effects were obtained. This investigation provides valuable information for turnip as a new resource in searching anti-diabetic candidates.

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Conflicts of Interest: The authors declare that they have no conflicts of interest to report regarding the present study.

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