ABSTRACT: The aim of this study was to isolate α-glucosidase inhibitory and tyrosinase constituents from 70% ethanol extracts of Angelica dahurica roots by bio-assay guided method. Six compounds were isolated and identified in active fractions Fr.5, Fr.6 and Fr.7. Compound 4 and 5 were isolated from A. dahurica for the first time. Their chemical structures were determined by spectroscopic methods, including NMR, MS, GC-MS analyses. Compound 3 and 4 showed stronger inhibitory activity on α-glucosidase activity with inhibition rates of 98.41% and 99.05%. Compound 1, 3 and 4 displayed inhibitory effects on tyrosinase activity, while compound 5 had activating effect.

KEY WORDS: Angelica dahurica roots, α-glucosidase inhibitory activity, and tyrosinase activity, active constituents

INTRODUCTION

Angelica dahurica, belonging to Umbelliferae family, is a wildly grown species of angelica native to Siberia, Russia Far East, Mongolia, Northeastern China, Japan, Korea, and Taiwan (China Pharmacopoeia Committee, 2015). The root of the plant is widely used in areas such as food, pharmacy, alternative medicine and natural therapy (Figure 1). Phytochemical research showed that coumarins, volatile oil, alkaloids, sterols, neolignan glycoside and polysaccharides were the main chemical composition (You et al., 2002; Liang et al., 2005; Wang et al., 2007; Zhao et al., 2007; Deng et al., 2015; Koutelidakis et al., 2016; Wang et al., 2017). Pharmacological investigation revealed that A. dahurica had anti-inflammatory (Lee et al., 2017; Wang et al., 2016), antibacterial (Xie et al., 2016), anticaner (Zheng et al., 2016), antioxidant (Pervin et al., 2014), exciting central nervous effects and inhibitory effects on tyrosinase, a copper-containing enzyme that catalyzes the tyrosine oxidation to produce of melanin and other pigments in plants and animals (Li et al., 1991; Doris et al., 2004).

α-Glucosidase inhibitors have been used as agents in the treatment for diabetes mellitus type 2 that work by preventing the digestion of carbohydrates such as starch and table sugar (Laar, 2008; Hao. et al., 2017; Zhang. et al., 2012). It has been known that a number of antidiabetic medicinal plants can be an important source of α-glucosidase inhibitors (Benalla et al.,
At present, the studies on *A. dahurica* mainly focused on evaluating the volatile oil (Wang et al., 2010; Zhu and Sheng, 2010), whether or not natural α-glucosidase inhibitors are present in *A. dahurica* is practically unknown. Thus, in the present study, we investigated the inhibitory effects of 70% ethanol extracts of *A. dahurica* roots on the activities of α-glucosidase and tyrosinase with bio-assay guiding method.

**FIGURE 1. Angelica dahurica Materia.**

### MATERIALS AND METHODS

**Materials and reagents**

*A. dahurica* roots were collected in Henan Yuzhou Jindi Chinese Medicine Decoction Pieces Co., LTD, (Kaifeng, Henan, China) in April 2013. The plant samples were identified by a plant scientist, Professor Changqin Li of Institute of Chinese Materia Medica, Henan University. A voucher specimen was deposited in the Institute of Chinese Materia Medica, Henan University.

α-Glucosidase (EC 3.2.1.20), 4-N-trophenyl-α-D-glucopyranoside (PNPG, 026k1516), acarbose (Lot 20120523) and dimethyl sulfoxide (DMSO) were purchased from Sigma (St. Luis, MO, USA). Levodopa (L-3-(3, 4-Dihydroxyphenyl)alanine, alfaesar (10102261) and tyrosinase were obtained from Worthington Biochemical Corporation (Lakewood, NJ, USA) 32 M13706), 8-methoxypsoralen was purchased from Chengdu Herbpurify Co. (Chengdu, Xichuan, China), LTD, H-035-130627). D101 macroporous resin was obtained from Tianjing Haiguang Chemical Co., LTD (Tianjin, China). Sephadex LH-20 was purchased from Pharmacia (Burlington, MA, USA). C-18 was obtained from Merck (Kenilworth, NJ, USA).

**Plant Material**

Samples extraction and isolation

*A. dahurica* roots powders (2.8 kg) were extracted with 70% (v/v) ethanol at room temperature for 3 times, for 3 d each time. An extract was obtained after removing ethanol. The extract (210 g) was suspended in distilled water and then chromatographed on D101 macroporous resin, and successively eluted with distilled water, 20% methanol, 40% methanol, 60% methanol and methanol to obtain eight fractions from Fr.1 to Fr.8.

Fr.5 (2.7 g) was chromatographed on a silica gel H and then chromatographed on Sephadex LH-20 (acetone) to obtain Fr.5.1, Fr.5.2 and Fr.5.3. Fr.5.1 was separated on a silica gel H with petroleum ether/chloroform (from 20:1 to 7:3), and further chromatographed on Sephadex LH-20 and eluted with mixture of petroleum ether/chloroform/methanol (9:9:2) to give compound 1 (15.2 mg) and 2 (5.1 mg). Fr.6 (3.2 g) was subjected to a silica gel H medium-pressure liquid chromatography of preparation and eluted with mixture of petroleum ether/chloroform (1:1) to obtain Fr. 6.1 and Fr.6.2. Fr.6.1 was purified by silica gel H with chloroform/acetone (20:1), and further chromatographed on Sephadex LH-20 (chloroform/methanol = 1:1) to yield compound 5 (12.0 mg). Fr.6.2 was chromatographed on Sephadex LH-20 (methanol), and further chromatographed on ODS to obtain compound 3 (7.7 mg) and compound 4 (8.5 mg). Fr.7 (5.2 g) was chromatographed on a silica gel H and eluted with chloroform/methanol (v:v 80:1-7:3) to obtain Fr.7.1: Fr.7.1 was separated on silica gel H and Sephadex LH-20 to give compound 6 (4.6 mg).

**In vitro α-glucosidase inhibitory activity**

The α-glucosidase inhibitory activities of the fractions and compounds of *A. dahurica* roots could be assayed in a 96-well plate by the method described previously (Chang et al., 2014; Zhang et al., 2014). The samples were dissolved in dimethyl sulfoxide (DMSO) and diluted with 112 μL phosphate buffer (pH 6.8) and α-glucosidase (20 μL, 0.2 U/L) were mixed and incubated at 37 °C for 15 min. Then, substrate solution (20 μL, 2.5 mM PNPG prepared in the same buffer) was added. The reaction was terminated by adding 80 μL of 0.2 M Na₂CO₃ solution after incubated at 37 °C for 15 min. Enzymatic inhibition data were expressed as IC₅₀ values (concentration of inhibitor required for 50% inhibition against α-glucosidase).

All reactions were carried out with three replications. Acarbose was used as positive control. The inhibitory rates (%) were calculated according to the following formula:

\[
\text{Inhibitory rates (\%)} = \left[1 - \frac{OD_{\text{test}} - OD_{\text{blank}}}{OD_{\text{control}} - OD_{\text{blank}}} \right] \times 100\%
\]

**Tyrosinase activity**

Tyrosinase assay was carried out according to the method described in the literatures (Ding, 2012). 45 μL of phosphate buffer (pH 6.8), 5 μL of DMSO and 25 μL of 0.2 U/mL tyrosinase were mixed together and incubated at 30°C for 10 min. Then 25 μL of levodopa was added into mixture. After the mixture was incubated at 30°C for 5 min, the absorbance was then measured at 492 nm by microplate reader. The activation
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rates (%) were calculated according to the following formula:

\[
\text{Activation rates} = \left( \frac{\text{OD}_{\text{sample}} - \text{OD}_{\text{substrate} + \text{enzyme}}}{\text{OD}_{\text{substrate} + \text{enzyme}} - \text{OD}_{\text{substrate}}} \right) \times 100\%
\]

**Statistical analysis**

All measurements were made in triplicate. The data are presented as mean±SD. One-way analysis of variance was analyzed using SPSS software, version 19.0. A difference of P<0.05 was considered as significant.

**RESULTS AND DISCUSSIONS**

**In vitro α-glucosidase inhibitory activity**

α-Glucosidase inhibitory activity of *A. dahurica* fractions was screened to determined the active constituent by α-glucosidase inhibitory model in vitro. The results demonstrated that the *A. dahurica* fractions had certain inhibitory activity, the screening inhibition rates were showed in Fig. 2. The inhibition rates of Fr.5, Fr.6 and Fr.7 (94.44%, 100.82% and 91.5%, respectively) were higher than that of Fr.1, Fr.2, Fr.3, Fr.4 and Fr.8, and also higher that of acarbose (59.62%) as positive control.

Therefore, various chromatography methods were used including silica gel, Sephadex LH-20, C18 reversed-phase and recrystallization for further separation and purification of Fr.5, Fr.6 and Fr.7. Six compounds were isolated from Fr.5, Fr.6 and Fr.7. α-Glucosidase inhibitory activity showed in Fig. 3, compound 3 and 4 that from Fr.6 were exhibited much higher inhibitory activity on α-glucosidase activity with the inhibition rates 98.41 and 99.05 (%), respectively, and were stronger than that of acarbose (1% was 61.23%). Compound 1, 5, and 6 were isolated from Fr.5, Fr.6 and Fr.7, respectively, and were showed weaker inhibitory activity on α-glucosidase. Among the furan-type coumarins possessing a side chains at C-4 or C-9 (3, 4 and 5), 3 and 4 had the same side chain- 2-hydroxy-3-methyl-3-butanyl-oxy, the position of the side chain was different, but the α-glucosidase inhibitory activity was no different. The data suggested that 2-hydroxy-3-methyl-3-butanyl-oxy side chain in furan-type coumarins was important for the α-glucosidase inhibitory activity, which was supported by the weak activity of 5 possessing an 2,3-dyhydroxy-3-methyl-3-butyl-oxy side chain.

Currently, the structure types α-glucosidase inhibitor from plants were diversity, for example, terpenes, alkaloids, quinines, flavonoids, phenols, phenylpropanoids, steroids and organic acids, esters, alcohols, allyls (Yin et al., 2014). The study on α-glucosidase inhibitory activity of furan-type coumarins was few, it was for the first time to report α-glucosidase inhibitory activity of compound 3, 4 and 5. For studying the glycosidases action mechanisms, glycosidase inhibitors are the important tools, for some degenerative diseases, glycosidase are also prospective therapeutic agents, for example, diabetes, viral attachment and cancer (Kim et al., 2004; Kuntz et al., 2008; Bhat et al., 2011). Therefore, based on this in vitro study, we recommend that the A. dahurica might have beneficial effects in managing type II diabetes mellitus.

**Activation of tyrosinase activity**

As showed in Table 1, Fr.1-Fr.7 at all three doses had activating effects on tyrosinase, but these effects were weaker than that of 8-methoxypsoralen as positive control. At the concentrations of 2 mg/mL and 1 mg/mL, the tyrosinase activation rates of Fr.8 were 89.07 and 79.79%, respectively, which were stronger than that of 8-methoxypsoralen (82.06% and 69.02%) as positive control (P<0.05). The

**FIGURE 2. Inhibitory effect of *A. dahurica* fractions on α-glucosidase activity**

**FIGURE 3. The α-glucosidase inhibitory effect of six compounds isolated from *A. dahurica*.** Compound 1= palmitic acid, Compound 2= 2′-deoxymeranzin hydrate, Compound 3= pabulenol, Compound 4= isogosferol, Compound 5= heraclenol and Compound 6= stearic acid.

**TABLE 1. The activation rate of *A. dahurica* fractions on tyrosinase.** 8-Methoxypsoralen as positive control; a: P <0.05 Compared with positive control.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Concentration (mg/mL)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>0.25</td>
</tr>
<tr>
<td>Fr.1</td>
<td>17.03±0.36 a</td>
</tr>
<tr>
<td>Fr.2</td>
<td>4.11±0.33 a</td>
</tr>
<tr>
<td>Fr.3</td>
<td>7.78±0.13 a</td>
</tr>
<tr>
<td>Fr.4</td>
<td>21.35±0.62 a</td>
</tr>
<tr>
<td>Fr.5</td>
<td>17.56±0.44 a</td>
</tr>
<tr>
<td>Fr.6</td>
<td>6.79±0.60 a</td>
</tr>
<tr>
<td>Fr.7</td>
<td>32.03±0.79 a</td>
</tr>
<tr>
<td>Fr.8</td>
<td>89.07±0.91 a</td>
</tr>
<tr>
<td>8-methoxypsoralen</td>
<td>82.06±0.85</td>
</tr>
</tbody>
</table>
activation rates of Fr.1-Fr.7 were ranged between 3.45% and 32.03%, and lower than that of 8-methoxypsoralen (P<0.05). These results showed that tyrosinase activation rates of eight fractions were increased with the increasing concentration from 0.25 to 2.0 (mg/mL).

As shown in Table 2, compound 1 was isolated from Fr.5 that exhibited inhibitory effect on tyrosinase activity. At the three concentrations, the tyrosinase activation rates of compound 5 were 59.56%, 17.69% and 8.99%, respectively, while compound 3 (-30.01%, -28.96% and -28.79%) that isolated from Fr.6. displayed inhibitory effects on tyrosinase. Compound 4 (-15.95% and -4.35) that isolated from Fr.6. displayed inhibitory effects on tyrosinase at the concentrations of 2 mg/mL and 1 mg/mL. Interestingly, although the chemical structures of compounds 3, 4 and 5 were quite similar, they differed substantially with respect to their activation effect on tyrosinase. The results suggested that the 2-hydroxy-3-methyl-3-butenyl-oxy side chain was not important for the tyrosinase activation activity, but 2,3-dihydroxy-3-methyl-3-buty-oxy side chain was not important for the tyrosinase activation activity.

Hu et al. (2013) showed that A. dahurica extracts had better inhibitory effects on tyrosinase. However, Ding found that at the concentrations of 100, 50 and 10 (mg/mL) of A. dahurica ethanol extracts, the activation rates on tyrosinase were 180.2, 175.3 and 150.2 (%) respectively (Ding, 2012). Liu et al., Li and Zhu studied the effects of 90% ethanol extracts of A. dahurica on tyrosinase. Our results together with those reported in the studies mentioned above confirm that the different extraction methods, concentrations and producing area will have different influences of A. dahurica on tyrosinase.

TABLE 2. The activation rates of compounds from A. dahurica on tyrosinase 8-Methoxypsoralen as positive control; a: P <0.05 Compared with positive control. Compound 1= palmitic acid, Compound 3= pabulenol, Compound 4= isogosferol and Compound 5= heracelenol.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Concentration (mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2</td>
</tr>
<tr>
<td>Compound 1</td>
<td>-6.16±0.62</td>
</tr>
<tr>
<td>Compound 3</td>
<td>-30.01±0.59</td>
</tr>
<tr>
<td>Compound 4</td>
<td>-15.95±0.23</td>
</tr>
<tr>
<td>Compound 5</td>
<td>59.56±0.63</td>
</tr>
<tr>
<td>8-Methoxypsoralen</td>
<td>92.68±0.96c</td>
</tr>
</tbody>
</table>

Identification of the compounds
By correlating melting points and spectral data of 1H-NMR, 13C-NMR, and MS from the literature values, compounds 1-6 were identified as palmitic acid (1) (Liu et al., 2007), 2'-deoxymeranzin hydrate (2) (Zhang et al., 2007), pabulenol (3) (Zhao et al., 2012), isogosferol (4) (Zhao and Yang, 2014), heracelenol (5) (Zhao and Yang, 2014), and stearic acid (6) (Zhang et al., 2010). Compound 4 and 5 were isolated from A. dahurica for the first time. The chemical structures of compounds 1-6 were shown in Fig. 3.

FIGURE 4. Chemical structures of six compounds isolated from A. dahurica roots.

The spectroscopic, 1H and 13C-NMR spectroscopic data and other physical data of the isolated compounds 1-6 were indicated below.

Palmitic Acid (1)
Colorless crystal with C15H30O2 molecular formula; mp 61-63°C; EI-MS (70 eV) m/z: 256[M]+. 1H-NMR (400MHz, CDCl3) δ: 0.86 (3H, t, H-16), 1.22-1.30 (24H, m, H-4-15), 1.68 (2H, m, H-3), 2.35 (2H, t, H-2), 3.50 (1H, s, OH). 13C-NMR (100MHz, CDCl3) : 178.65 (C-1), 34.32-24.21 (C-2-14), 22.67 (C-15), 14.22 (C-16).

2'-Deoxymeranzin hydrate (2)
White powder; mp 61-63°C; EI-MS (70 eV) m/z: 262[M]+. 1H-NMR (400MHz, CDCl3) δ: 1.69 (6H, s, H-4', H-5'), 1.99 (1H, t, J=3.5 Hz, H=16.5 Hz, H-2), 3.03 (1H, t, J=3.5 Hz, J=16.5 Hz, H-1'), 3.93 (3H, s, OCH3), 6.84 (1H, d, J=8.4 Hz, H-6), 7.31 (1H, d, J=8.4 Hz, H-5), 7.62 (1H, d, J=9.5 Hz, H-4), 6.24 (1H, d, J=9.5 Hz, H-3). 13C-NMR (100MHz, CDCl3) : 161.42 (C-8), 112.84 (C-16), 143.70 (C-3), 126.92 (C-6), 107.85 (C-6), 158.38 (C-7), 117.50 (C-8), 152.96 (C-9), 56.85 (OCH3), 33.2 (C-1'), 33.2 (C-2'), 70.05 (C-3' ), 44.39 (C-4'), 18.3 (C-5').

Pabulenol (3)
White crystal with C24H30O5 molecular formula; mp137-138°C; EI-MS (70 eV) m/z: 286[M]+. 1H-NMR (400MHz, CDCl3) δ: 1.82 (3H, s, H-3''), 4.31 (1H, dd, J=9.8, 8.8 Hz, H-2''),
4.54 (1H, dd, J=8.8, 2.4 Hz, H-1’b), 4.61 (1H, dd, J=9.8, 2.4 Hz, H-1’a), 4.99 (1H, brs, H-4’b), 5.16 (1H, brs, H-4’a), 6.37 (1H, d, J=9.6 Hz, H-3), 6.83 (1H, brs, H-3’), 7.39 (1H, s, H-5), 7.69 (1H, brs, H-2’), 7.76 (1H, d, J=9.6 Hz, H-4). 13C-NMR (100MHz, CDCl3) δ: 161.42 (C-2), 112.84 (C-3), 139.70 (C-4), 149.22 (C-5), 158.38 (C-7), 94.50 (C-8), 152.96 (C-9), 107.85 (C-10), 145.09 (C-11), 105.20 (C-12), 70.05 (C-1’), 119.39 (C-2’), 139.95 (C-3’), 18.3 (C-4’), 75.7 (C-5’).

Isogosferol (4)
White crystal with C16H18O5, EI-MS m/z: 286[M]⁺. 1H-NMR (400MHz, CDCl3) δ: 1.82 (3H, s, H-3’'), 4.39 (1H, dd, J=9.6, 7.0 Hz, H-1’’b), 4.46 (1H, dd, J=9.6, 2.0 Hz, H-1’’a), 4.53 (1H, dd, J=7.0, 2.0 Hz, H-2’’), 5.06 (3H, brs, H-4’’a), 5.20 (3H, brs, H-4’’b), 6.27 (1H, d, J=10.0 Hz, H-3), 6.97 (1H, brs, H-3’), 7.15 (1H, s, H-8), 7.60 (1H, brs, H-2’’), 8.18 (1H, d, J=10.0 Hz, H-4). 13C-NMR (100MHz, CDCl3) δ: 160.47 (C-2), 115.04 (C-3), 144.50 (C-4), 114.01 (C-5), 126.32 (C-6), 148.24 (C-7), 132 (C-8), 143.60 (C-3’), 112.8 (C-4’’), 19.0 (C-5’).

Heraclenol (5)
White powder, mp 116-117°C, EI-MS m/z: 304[M]⁺. 1H-NMR (400MHz, CDCl3) δ: 1.30 (3H, s, H-3’’), 3.83 (1H, dd, J=7.6, 2.9 Hz, H-2’’), 4.42 (1H, dd, J=10.6, 7.8 Hz, H-1’’b), 4.73 (1H, dd, J=10.2, 2.7 Hz, H-1’’a), 6.37 (1H, d, J=9.5 Hz, H-3), 6.87 (1H, d, J=2.2 Hz, H-3’’), 7.35 (1H, s, H-5), 7.60 (1H, d, J=2.2 Hz, H-2’’), 7.78 (1H, d, J=9.6 Hz, H-4). 13C-NMR(100MHz, CDCl3) δ: 162.83 (C-2), 117.89 (C-3), 145.82 (C-4), 113.20 (C-5), 127.41 (C-6), 148.61 (C-7), 131.86 (C-8), 141.21 (C-9), 115.12 (C-10), 147.14 (C-11), 108.10 (C-12), 76.55 (C-1’), 78.71 (C-2’), 72.21 (C-3’), 30.32 (C-4’’), 18.96 (C-5’).

Stearic acid (6)
White powder, mp 67-69°C, EI-MS m/z: 284[M]⁺. 1H-NMR (400MHz, CDCl3) δ: 0.87 (3H, t, H-18), 1.20 (28H, m, H-4-17), 1.62 (2H, m, H-3), 2.34 (2H, t, H-2), 3.48 (1H, s, OH). 13C-NMR(100MHz, CDCl3) δ: 179.92 (C-1), 34.22 (C-2), 32.00 (C-3), 29.79-29.21 (C-4-15), 24.84 (C-16), 22.82 (C-17), 14.21 (C-18).

CONCLUSION
By using bio-assay guiding method, six compounds were isolated and identified from active fractions, i.e. Fr.5, Fr.6 and Fr.7, of A. dahurica roots. Among them, pabulenol and isogosferol had relatively strong inhibitory effects on α-glucosidase and were the active components of Fr.6. Pabulenol and isosferol might be the potential inhibitors of on α-glucosidase for treatment of type 2 diabetes. Palmitic acid, pabulenol and isogosferol possessed inhibitory effects on tyrosinase whereas heraclenol had activating effect on tyrosinase. Palmitic acid, pabulenol and isogosferol might be potential medicines for reducing melanin deposition in the skin. The above studies could provide theoretical basis for its application in α-glucosidase glucosidase inhibitors and tyrosinase.

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REFERENCES
Activity of Angelica dahurica roots


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