Author’s pre-print manuscript of the following article

Intestinal α-Glucosidase Inhibitors in Achillea millefolium
Natural Product Communication, 2017, 12(8), 1259-1261.

Check the published version from the bellow link.
http://www.naturalproduct.us/index.asp
Intestinal \(\alpha\)-Glucosidase Inhibitors in \textit{Achillea millefolium}

Kota Noda, Eisuke Kato,\textsuperscript{1} and Jun Kawabata

Graduate School of Agriculture, Hokkaido University, Sapporo, Hokkaido 060-8589, Japan.

eikato@chem.agr.hokudai.ac.jp

Received: January XX, 2016; Accepted: XX, 2016

\textit{Achillea millefolium} is a plant used as a component of anti-diabetic preparation but the bioactive compounds responsible for its use are not known. Inhibition of intestinal \(\alpha\)-glucosidase is a preferable effect for prevention and treatment of diabetes, and \textit{A. millefolium} extract showed inhibitory activity against the enzyme. Activity-guided separation of the extract gave four mono- or di-caffeoylquinic acids as the isolate. Quantitation of these four caffeoylquinic acids and the activity of the isolates suggested that these are the major contributor for the \(\alpha\)-glucosidase inhibitory activity in the extract. However also, the presence of unidentified, minor, but potent \(\alpha\)-glucosidase inhibitor in the isolate was also suggested.

**Keywords:** diabetes, intestinal \(\alpha\)-glucosidase, maltase, glucosidase inhibitor, caffeoylquinic acid

Diabetes mellitus (DM) is a worldwide problem due to increase of the patient number year to year. DM is a chronic disease exhibiting high level of blood sugar due to either decreases in insulin production or sensitivity to insulin.

Intestinal \(\alpha\)-glucosidase is an essential enzyme for the decomposition and absorption of carbohydrates consumed as food. However, its inhibition can attenuate the rapid elevation of blood sugar level after meal consumption and therefore it is utilized for the treatment and prevention of DM.

\textit{Achillea millefolium}, commonly named yarrow, is a perennial herbal plant belonging to Asteraceae family. The plant is native to the regions of Northern Hemisphere including Eastern Asia to Europe. The plant is commonly consumed as an herbal tea or prepared as an essential oil. The traditional use of this plant includes the treatment of inflammatory and spasmodic gastro-intestinal disorders, for wound healing, skin inflammations or as an appetite-enhancing drug,\textsuperscript{1,2} and also as a component of antidiabetic preparation.\textsuperscript{3,4}

Recent scientific studies on this plant revealed sesquiterpenes as an active ingredient related to the antiphlogistic activity,\textsuperscript{5} di-caffeoylquinic acids as the choleretic substances,\textsuperscript{6} which are assumed to be related to anti-inflammatory properties and problems in digestive tracts. However, the active compound related to the antidiabetic property of \textit{Achillea millefolium} has not been reported.

Here, we searched for the intestinal \(\alpha\)-glucosidase inhibitor from the extract of \textit{Achillea millefolium}, and found several dicaffeoylquinic acids as the active component, which may contribute at least in part to the antidiabetic property of this plant.

The dried aerial part of \textit{Achillea millefolium} was extracted by aqueous methanol. The extract showed 55% inhibition at 1.6 mg/mL against rat intestinal \(\alpha\)-glucosidase and was separated with a guidance of the activity. The extract was partitioned between water, ethyl acetate and 1-butanol. The 1-butanol soluble part was further purified by DIAION HP-20 (Mitsubishi Chemical Co., Tokyo), and TOYOPEARL DEAE 650M (Tosoh Co., Tokyo) column chromatography. The obtained fractions were finally separated by HPLC using Inertsil ODS-3 (GL Science Co., Tokyo) column to obtain compound 1-4 (Scheme 1).

![Scheme 1. Isolation procedure of intestinal \(\alpha\)-glucosidase inhibitors from \textit{Achillea millefolium}](image-url)

Each compound was analyzed by \(^1\text{H}\)-NMR and mass spectrometry to determine the structure. \(^1\text{H}\)-NMR spectrum revealed that these are caffeoylquinic acids and MS analysis indicated that 1 is a mono-caffeoylquinic acid and 2-4 are di-caffeoylquinic acids. Comparison of the \(^1\text{H}\)-NMR spectrum with the possible structures revealed the structure to be chlorogenic acid (5-\(\text{O}\)-caffeoylquinic acid, 1), 3,4-di-
An inhibitory activity of 1-4 was next measured (Figure 2). Among the four caffeoylquinic acids, 2 showed the highest activity with 69% inhibition at 0.5 mM. Compounds 3 and 4 showed weaker activity compared to 2 and chlorogenic acid (1) showed the least activity.

In conclusion, a-glucosidase inhibitor in A. millefolium extract was investigated in relationship with the plant use as a component of anti-diabetic preparation. A. millefolium contained several caffeoylquinic acids (1-4) which are reported as a-glucosidase inhibitory compound. The mixture of these compounds showed comparable activity to the extract. However, the activity of 2 was much stronger than that in the previous reports. Therefore, we presume that there may be a more potent inhibitor in the extract of A. millefolium.

**Table 1: Content of the isolated compounds in Achillea millefolium and the extract.**

<table>
<thead>
<tr>
<th>Compounds</th>
<th>mg/g in A. millefolium</th>
<th>mM in 1.6 mg/mL extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.18</td>
<td>0.060</td>
</tr>
<tr>
<td>2</td>
<td>0.83</td>
<td>0.016</td>
</tr>
<tr>
<td>3</td>
<td>0.85</td>
<td>0.094</td>
</tr>
<tr>
<td>4</td>
<td>0.98</td>
<td>0.019</td>
</tr>
</tbody>
</table>

α-Glucosidase inhibitory activity of di-caffeoylquinic acids are shown in several articles. Reported IC_{50} values are 1-2 mM, depending on the assay procedure, which is in reasonable comparison with the current measured inhibitory activity for compound 3 and 4. In comparison, compound 2 in the current study shows quite high activity compared to the reported IC_{50} values (35% at 0.016 mM, Figure 3). Therefore, the fact suggests there may be a contribution of minor component included in 2 for the activity.

The α-glucosidase inhibitory activity of caffeoylquinic acids are known to highly depend on the number of caffeoyl groups attached to the quinic acid moiety. Tri-caffeoylquinic acids are reported to show much lower IC_{50} value compared to the di-caffeoylquinic acids. Since di- and tri-caffeoylquinic acids are related natural products, we analyzed the tested sample by MS to check if tri-caffeoylquinic acid is included. However no peaks were identified indicating the presence of tri-caffeoylquinic acid. In conclusion, α-glucosidase inhibitor in A. millefolium extract was investigated in relationship with the plant use as a component of anti-diabetic preparation. A. millefolium contained several caffeoylquinic acids (1-4) which are reported as α-glucosidase inhibitory compound. The mixture of these compounds showed comparable activity to the extract. However, the activity of 2 was much stronger than that in the previous reports. Therefore, we presume that there may be a more potent inhibitor in the extract of A. millefolium.
Compound data
chlorogenic acid (1): yellow powder; ESI-MS (negative): m/z 353 [M-H]⁻; ¹H-NMR (methanol-d₄, 500 MHz): 2.00-2.10 (2H, m), 2.14-2.25 (2H, m), 3.73 (1H, dd, J = 3.2, 8.5 Hz), 4.17 (1H, br s), 5.30-5.37 (1H, m), 6.27 (1H, d, J = 16.0 Hz), 6.78 (1H, d, J = 6.1 Hz), 6.95 (1H, dd, J = 2.1, 6.1 Hz), 7.05 (1H, d, J = 2.1 Hz), 7.56 (1H, d, J = 16.0 Hz) ppm. The data was compared with the commercial chlorogenic acid.

3,4-di-O-caffeyloyquinic acid (2): yellow powder; ESI-MS (negative): m/z 515 [M-H]⁻; ¹H-NMR (methanol-d₄, 500 MHz): 2.06-2.40 (4H, m), 4.30-4.40 (1H, m), 5.01 (1H, dd, J = 3.1, 8.4 Hz), 5.62-5.66 (1H, m), 6.25 (1H, d, J = 15.8 Hz), 6.29 (1H, d, J = 15.8 Hz), 6.73 (1H, d, J = 8.2 Hz), 6.77 (1H, d, J = 8.2 Hz), 6.88 (1H, br d, J = 8.2 Hz), 6.93 (1H, br d, J = 8.2 Hz), 7.03 (1H, br s), 7.04 (1H, br s), 7.55 (1H, d, J = 15.8 Hz), 7.58 (1H, d, J = 15.8 Hz) ppm.

3,5-di-O-caffeyloyquinic acid (3): white powder; ESI-MS (negative): m/z 515 [M-H]⁻; ¹H-NMR (methanol-d₄, 500 MHz): 2.15-2.35 (4H, m), 3.98 (1H, dd, J = 2.8, 7.3), 5.37-5.41 (1H, m), 5.42-5.45 (1H, m), 6.35 (1H, d, J = 16.0 Hz), 6.27 (1H, d, J = 16.0 Hz), 6.78 (2H, d, J = 8.2 Hz), 6.96 (1H, br d, J = 8.2 Hz), 6.98 (1H, dd, J = 1.6, 8.2 Hz), 7.07 (2H, br s), 7.58 (1H, d, J = 16.0 Hz), 7.62 (1H, d, J = 16.0 Hz) ppm.

References