Phenolic Compounds from *Cotinus Coggygria* Scop. with Alpha Glucosidase Inhibition

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**SUMMARY**

Diabetes mellitus (DM), characterized by hyperglycemia, is one of the serious metabolic disorders. Inhibiting the key enzymes in digestion of dietary starch such as α-amylase and α-glucosidase, is one of the methods in treatment of DM. Antidiabetic drugs that inhibit carbohydrate-hydrolyzing enzymes have undesired side effects therefore, there is a huge interest in search of medicinal plants. *Cotinus coggygria* Scop. (Anacardiaceae) is used in Turkish folk medicine to treat DM. Therefore the bioguided fractionation and isolation studies were carried on *C. coggygria*. The ethyl acetate fraction possessed significant α-glucosidase inhibitory effect with 8.2 µg/mL IC\textsubscript{50} value but no meaningful α-amylase inhibitory effect. Gallocatechin (1), methyl gallate (2), myricetin-3-O-α-rhamnoside (3), myricetin-3-O-β-galactoside (4) and 1, 2, 3, 4, 6-penta-O-galloyl-β-glucose (5) were isolated from this active fraction. Compound 5 showed significant α-glucosidase inhibitory activity with 1.5 µg/mL IC\textsubscript{50} value, when compared to acarbose (IC\textsubscript{50} = 3364.2 µg/mL) which used as positive control.

**Key Words:** α-Amylase inhibition, α-glucosidase inhibition, anacardiaceae, cotinus coggygria scop., diabetes mellitus, hyperglycemia

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**ÖZ**

Diabetes mellitus (DM), hiperglisemi ile karakterize edilen önemli bir metabolik bozukluktur. DM’yi tedavi etme yollarından bir tanesi diyetle alınan nişastanın sindiriminde rol alan α amilaz ve α glukozidaz gibi antidiyabetik ilaçların etkileri, yan etkileri bulunması sebebiyle tıbbi bitkilerle ilgi artmıştır. *Cotinus coggygria* Scop. (Anacardiaceae) Türk halk tıbbında diyabeti tedavi etmek amacıyla kullanılmaktadır. Bu nedenle *C. coggygria* üzerinde biyolojik aktivite kontrollü fraksiyonlama ve izolasyon çalışmalari yürütülmüştür. Etil asetat fraksiyonu 8.2 µg/mL IC\textsubscript{50} değeri ile güçlü alfa glukozidaz inhibitory etki göstermekte olup aynı aktivite gösteren antidiyabetik ilaçlar arası bir alfa amilaz inhibitör etkisi göstermiştir. Etkili fraksiyonun gallokateşin (1), metil gallat (2), nirsititin-3-O-a-ramnozit (3), nirsititin-3-O-β-galaktozit (4) ve 1, 2, 3, 4, 6-penta-O-gallil-β-glukoz (5) bileşikleri izole edilmiştir. Pozitif kontrol olarak kullanılan acarboz (IC\textsubscript{50} = 3364.2 µg/mL) ile kıyaslandığında 5 numaralı bileşik 1.5 µg/mL IC\textsubscript{50} değeriyle güçlü alfa glukozidaz inhibitör etki göstermiştir.

**Anahtar Kelimeler:** α-Amilaz inhibitörü, α-glukozidaz inhibitörü, anacardiaceae, cotinus coggygria scop., diabetes mellitus, hiperglisemi

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INTRODUCTION

Cotinus genus is represented by eight species on worldwide and one species in Turkey (The Plant List; Davis et al., 1982). Cotinus coggygria Scop. (Anacardiaceae), commonly known as "smoke tree", is generally seen as small trees or large shrubs (Matic et al., 2016) and grows widely in South Russia, South and Central Europe, Latakia, Caucasus, Crimea and Turkey (Davis et al., 1982). It has been reported to be well growth in areas where there is much slope and erosion, and to be used in the prevention of erosion. Also, its wood has been used in leather and fabric dyeing since ancient times (Gültekin et al., 2007).

In Turkish folk medicine, the decoction of C. coggygria leaves are used against DM (Baytop, 1999; Kültür, 2007; Arıtuluk and Ezer, 2012). Several parts like shoots, flowers, leaves and stem of C. coggygria contain biologically active constituents which are mainly polyphenols, flavonoids and tannins (Antal et al., 2010; Matic et al., 2016). Some scientific results showed that C. coggygria possess some significant pharmacological activities such as an antioxidant (Savikin et al., 2009; Marcetic et al., 2013), antimicrobial (Marcetic et al., 2013; Fraternale and Ricci, 2014), anti-inflammatory (Marcetic et al., 2013), anticaner (Noh et al., 2015), antigenotoxic, hepatoprotective (Matic et al., 2013), antidiabetic (Cha et al., 2009), gastroprotective (Pavlov et al., 2013a, 2013b), wound healing (Aksoy et al., 2016) and antiviral (Jing et al., 2012) activities.

DM is a severe metabolic sickness, characterised by hyperglycaemia, results in dysfunction and the loss of many organs. The prevalence of this health problem has increased worldwide in recent years (Madeswaran et al., 2014). Retarding absorption of glucose to the bloodstream is one of the methods in treatment of diabetes. The inhibition of the key enzymes in digestion of dietary starch such as α-amylase and α-glucosidase has increased worldwide in recent years (Madeswaran, 2012) activities.

The current study describes the bioguided fractionation and isolation assay for C. coggygria. As a result of this study five phenolic compounds were isolated as well as the assessment of their α-glucosidase and α-amylase inhibition effects.

The ethyl acetate fraction, due to the significant α-glucosidase inhibitory effect (IC50 = 8.2 µg/mL), was selected for isolation to yield five phenolic compounds. They were identified as gallochalcone (1) (Davis et al., 1996), methyl gallate (2), myricetin-3-O-α-rhamnoside (3) (Rashed et al., 2014), myricetin-3-O-β-galactoside (4) (Tahrouch et al., 2000) and 1,2,3,4,6-penta-O-galloyl-β-glucose (5) (Matic et al., 2016) (Figure 1.)

MATERIALS AND METHODS

Plant material

The leaves of C. coggygria Scop. was collected from O lur/Ormanagzi Village (Erzurum) on May 2014, and was identified by Assist. Prof. Songül Karakaya (Department of Pharmacognosy, Faculty of Pharmacy, Atatürk University, Erzurum, Turkey). A voucher specimen (AUEF 1004) has been kept in the herbarium of the Faculty of Pharmacy, Atatürk University, Erzurum, Turkey.

Extraction and Isolation

The leaves of the plant material (500 g) were dried in the shade and powdered. They were extracted 2 times with 70% methanol (MeOH) at 40 °C (2 × 2 L). Evaporation of the MeOH gave 197 g of MeOH extract which was dissolved in water (H2O) and partitioned with petroleum ether, dichloromethane, ethyl acetate and n-butanol, respectively (petroleum ether fraction: 0.08 g, dichloromethane fraction: 0.41 g, ethyl acetate fraction: 22.96 g, n-butanol fraction: 54.99 g). 54.05 g of aqueous fraction was remained.

The ethyl acetate fraction (22.96 g) was chromatographed on a silica gel (Silica gel 60, 0.063-0.200 mm, Merck) column eluting with chloroform (CHCl3):MeOH mixtures (100:0 → 0:100) to yield three subfractions (Frs. A-C). Fraction B (10.12 g) was subjected to vacuum liquid chromatography (VLC) on reversed-phase material (Lichroprep RP-18, 25-40 µm, Merck), using MeOH:H2O mixtures (0:100 → 100:0) to give three subfractions (Fr. B-1, Fr. B-2 and Fr. B-3). Fr. B-1 (0.69 g) was applied to VLC using reversed-phase material employing MeOH:H2O mixtures (0:100 → 100:0) to give two subfractions (Fr. B-1-1 and Fr. B-1-2). Fr. B-1-1 was precipitated to obtain compound 1 (121 mg). Fr. B-2 (1.09 g) was fractioned by column chromatography over reversed-phase material using MeOH:H2O mixtures (0:100 → 100:0) and four subfractions (Fr. B-2-1, Fr. B-2-2, Fr. B-2-3 and Fr. B-2-4) were obtained. Fr. B-2-2 (134.5 mg) was submitted to silica gel column chromatography (CC) eluting with CHCl3:MeOH mixtures (100:0 → 95:5) to yield compound 2 (47.2 mg). Fr. B-2-4 (823.1 mg) was applied to a Sephadex LH-20 (Fluka) column and eluted with MeOH to give compound 3 (6.7 mg). Fr. C (2.15 g) was subjected to a column of Sephadex LH-
20 by eluting with MeOH to give two subfractions (Fr. C-1 and Fr. C-2). Fr. C-1 (0.4 g) was fractioned by column chromatography over reversed-phase material using MeOH:H₂O mixtures (0:100 → 100:0) and two subfractions (Fr. C-1-1 and Fr. C-1-2) were obtained. Fr. C-1-2 (39.4 mg) was subjected to Sephadex LH-20 using 100% MeOH to yield two subfractions (Fr. C-1-2-1 and Fr. C-1-2-2). Purification of Fr. C-1-2-2 (36.3 mg) by Sephadex LH-20 CC using MeOH gave compound 4 (9.9 mg). Fr. C-2 (0.79 g) was purified by VLC on reversed-phase material, eluted with MeOH:H₂O (0:100 → 100:0) to give compound 5 (48.1 mg). Their structures were identified by means of spectral methods [1D- and 2D-NMR (Varian Mercury Plus 400 MHz, USA), ESI-MS (Waters LC/MS Micromass ZQ Mass Spectrometer)].

Enzyme Inhibition Assays

Alpha Amylase Inhibition Assay

Alpha amylase inhibition activity was designated according to described method (Nampoothiri et al., 2011) with slight modifications. All reagents, conditions, and calculations were the same as described in our previous publication (Güvenalp et al., 2017).

**Alpha Glucosidase Inhibition Assay**

Alpha glucosidase inhibition activity was carried out in compliance with described method (Tao et al., 2013) with slight modifications. All reagents, conditions, and calculations were the same as described in our previous publication (Güvenalp et al., 2017).

**RESULTS**

The MeOH extract showed inhibitory effect on α-glucosidase, but no significant α-amylase inhibitory effect. Thereby, on the following studies just α-glucosidase inhibitory effect screening was performed on subfractions and pure compounds. As shown in Table 1, 1,2,3,4,6-penta-O-galloyl-β-glucose showed significant α-glucosidase inhibitory effect with 1.5 µg/mL IC₅₀ value, when compared to acarbose (reference compound). On the other hand, none of the other compounds showed α-glucosidase inhibitory activity in all tested concentrations. The assays showed that the significant activity of the ethyl acetate fraction arises from the compound 5.

<table>
<thead>
<tr>
<th>Extracts/Fractions/Compounds</th>
<th>IC₅₀ value (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MeOH extract</td>
<td>47.4 ± 60</td>
</tr>
<tr>
<td>Petroleum ether fraction</td>
<td>44.5 ± 30</td>
</tr>
<tr>
<td>Dichloromethane fraction</td>
<td>144.1 ± 80</td>
</tr>
<tr>
<td>Ethyl acetate fraction</td>
<td>8.2 ± 10</td>
</tr>
<tr>
<td>n-Butanol fraction</td>
<td>49.4 ± 50</td>
</tr>
<tr>
<td>Gallocatechin (1)</td>
<td>ND</td>
</tr>
<tr>
<td>Methyl gallate (2)</td>
<td>ND</td>
</tr>
<tr>
<td>Myricetin-3-O-α-rhamnose (3)</td>
<td>ND</td>
</tr>
<tr>
<td>Myricetin-3-O-β-galactoside (4)</td>
<td>ND</td>
</tr>
<tr>
<td>1,2,3,4,6-penta-O-galloyl-β-glucose (5)</td>
<td>1.5 ± 40</td>
</tr>
<tr>
<td>Acarbose</td>
<td>3364.2 ± 120</td>
</tr>
</tbody>
</table>

*a*Data were expressed as mean ± S.D. (n=3). *b*Acarbose, an antidiabetic agent used as a positive control. ND: Not Determined
DISCUSSION

The inhibition mechanism of the galloyl glucoses against α-glucosidase could be the tannin-protein interaction. It changes configuration of the enzyme, and thus, decreases the enzyme activity by precipitation (Toda et al., 2000, 2001). The glucose core with one or more galloyl groups is necessary to interact with and stimulate the receptor target (Ren et al., 2006). Therefore it should be stated that compound 2 was inactive against α-glucosidase because of lack of a sugar moiety. Compounds 3 and 4 were found to be inactive. The glycosylation at C-3 position of flavonoids may reduce the inhibitory effect of α-glucosidase. Steric hindrance weakens the linkage interaction between α-glucosidase and flavonoids (Islam et al., 2013; Zeng et al., 2016). Compound 1 was also inactive against α-glucosidase. A gallate group linked to the 3-position of flavan-3-ols is critical for α-glucosidase inhibition and non-gallated catechins are poor enzyme inhibitors (Yilmazer-Musa et al., 2012).

CONCLUSION

In conclusion, the present study has given supporting evidence to verify the ethnomedical use of C. coggygria Scop. against DM. 1,2,3,4,6-penta-O-galloyl-β-glucose (5) was the most effective constituent of the species, also more potent than the reference drug (acarbose). On the other hand, to the best of our knowledge, gallocatechin (1) is reported from C. coggygria Scop. for the first time. These results indicate that C. coggygria could be a good natural source for α-glucosidase inhibition which is very important in treatment of DM.

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.
REFERENCES


