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RESEARCH ARTICLE

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 significative α-glucosidase inhibitory effect with 8.2 µg/mL IC_{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_{50} value, when compared to acarbose (IC_{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*

Cotinus Coggygria Scop.'tan Elde Edilen Alfa Glukozidaz Etkili Fenolik Bileşikler

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 alfa amilaz ve alfa glukozidaz gibi anahtar enzimleri inhibe etmektir. Karbonhidratları hidrolize eden enzimleri inhibe eden antidiyabetik ilaçların istenmeyen yan etkileri bulunması sebebiyle tıbbi bitkilere olan 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 çalışmaları yürütülmüştür. Etil asetat fraksiyonu 8.2 µg/mL IC_{50} değeri ile güçlü alfa glukozidaz inhibitör aktivite gösterirken anlamlı bir alfa amilaz inhibitör etki göstermemiştir. Etkili fraksiyondan gallokateşin (1), metil gallat (2), mirsetin-3-O-α-ramnozit (3), mirsetin-3-O-β-galaktozit (4) ve 1, 2, 3, 4, 6-penta-O-galloil-β-glukoz (5) bileşikleri izole edilmiştir. Pozitif kontrol olarak kullanılan akarboz (IC_{50} = 3364.2 µg/mL) ile kıyaslandığında 5 numaralı bileşik 1.5 µg/mL IC_{50} değeriyle güçlü alfa glukozidaz inhibitör etki göstermiştir.

Anahtar Kelimeler: *α-Amilaz inhibisyonu, α-glukozidaz inhibisyonu, 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, Caucasia, 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), anticancer (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 provides to control blood glucose level (Hamid et al., 2015). Nowadays used anti-diabetic drugs that inhibit carbohydrate-hydrolysing enzymes have undesired side effects therefore, there is a huge interest in search of herbal ingredients (Huang et al., 2015).

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 (IC_{50} = 8.2 μg/mL), was selected for isolation to yield five phenolic compounds. They were identified as gallocatechin (**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 Olur/Ormanagzı 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 (H_2O) 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 ($CHCl_3$):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:H_2O 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:H_2O 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:H_2O 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 $CHCl_3$: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:H_2O 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:H_2O (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 IC_{50} 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 1. Alpha glucosidase inhibitory activity of MeOH extract, fractions and isolated compounds derived from *C. coggygria* Scop.

Extracts/Fractions/Compounds	IC_{50} value (μg/mL)
MeOH extract	47.4 ± 60[a]
Petroleum ether fraction	44.5 ± 30
Dichlorometane fraction	144.1 ± 80
Ethyl acetate fraction	8.2 ± 10
n-Butanol fraction	49.4 ± 50
Gallocatechin (**1**)	ND
Methyl gallate (**2**)	ND
Myricetin-3-*O*-α-rhamnoside (**3**)	ND
Myricetin-3-*O*-*β*-galactoside (**4**)	ND
1,2,3,4,6-penta-*O*-galloyl-β-glucose (**5**)	1.5 ± 40
Acarbose[b]	3364.2 ± 120

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

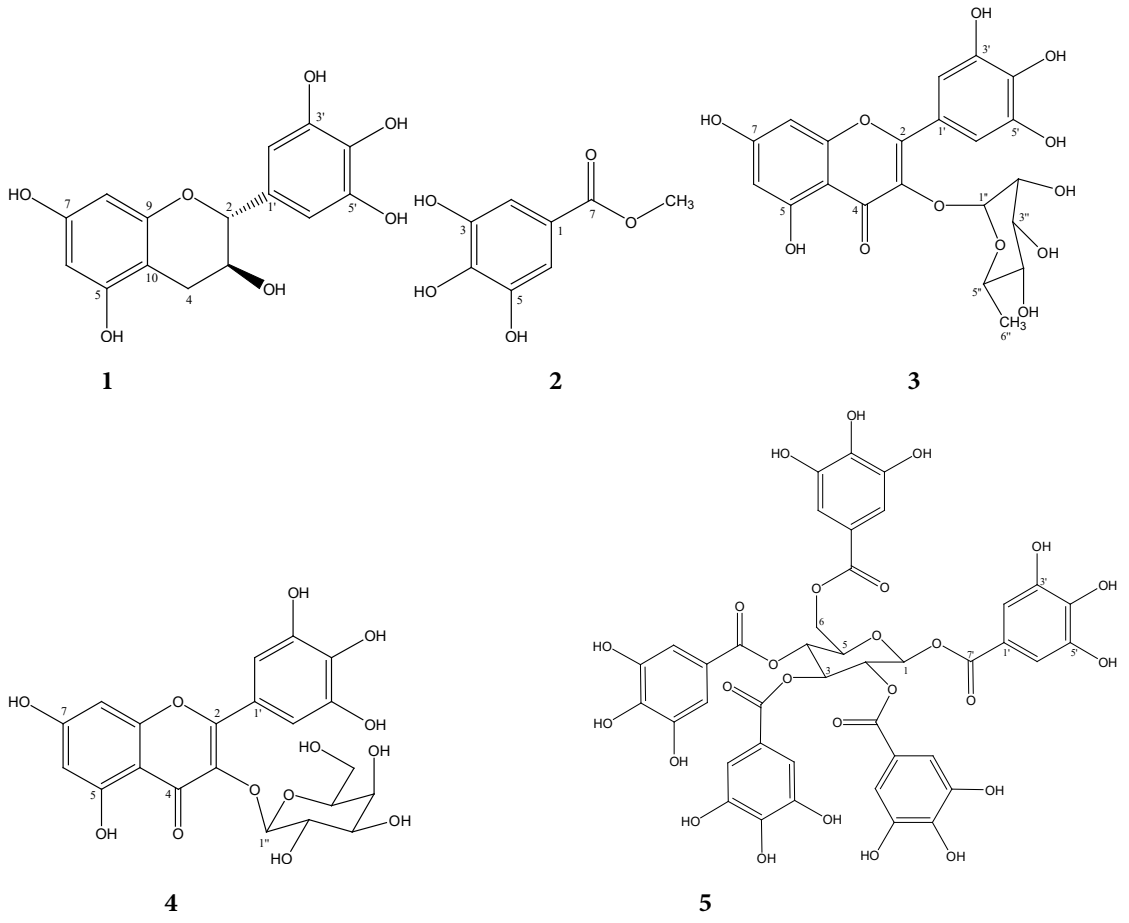

Figure 1. Chemical structures of compounds 1-5.

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.

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