The α-amylase and α-glucosidase inhibitory activities of the dichloromethane extracts and constituents of Ferulago bracteata roots

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ABSTRACT

Context: Ferulago (Apiaceae) species have been used since ancient times for the treatment of intestinal worms, hemorrhoids, and as a tonic, digestive, aphrodisiac, or sedative, as well as in salads or as a spice due to their special odors.

Objectives: This study reports the α-amylase and α-glucosidase inhibitory activities of dichloromethane extract and bioactive compounds isolated from Ferulago bracteata Boiss. & Hausskn. roots.

Materials and methods: The isolated compounds obtained from dichloromethane extract of Ferulago bracteata roots through bioassay-guided fractionation and isolation process were evaluated for their in vitro α-amylase and α-glucosidase inhibitory activities at 5000–400 μg/mL concentrations. Compound structures were elucidated by detailed analyses (NMR and MS).

Results: A new coumarin, peucedanol-2′-benzoate (1), along with nine known ones, osthole (2), imperatorin (3), bergapten (4), prantschimgin (5), grandivitinol (6), suberosin (7), xanthotoxin (8), felamidin (9), umbelliferone (10), and a sterol mixture consisted of stigmasterol (11), β-sitosterol (12) was isolated from the roots of F. bracteata. Felamidin and suberosin showed significant α-glucosidase inhibitory activity (IC50 0.42 and 0.89 mg/mL, respectively) when compared to the reference standard acarbose (IC50 4.95 mg/mL). However, none of the tested extracts were found to be active on α-amylase inhibition.

Discussion and conclusions: The present study demonstrated that among the compounds isolated from CH2Cl2 fraction of F. bracteata roots, coumarins were determined as the main chemical constituents of this fraction. This is the first report on isolation and characterization of the bioactive compounds from root extracts of F. bracteata and on their α-amylase and α-glucosidase inhibitory activities.

INTRODUCTION

Ferulago W. Koch. (Apiaceae) is represented by approximately 50 taxa throughout the world and 35 taxa in Turkey, 18 of which are endemic. Therefore, Anatolia is considered to be the gene center of this genus (Güner et al. 2012). Ferulago bracteata Boiss. & Hausskn. is also an endemic perennial species, grown only in Gaziantep, Southeastern Anatolia, Turkey (Pşmen 1972; Troia et al. 2012).

Ferulago species have been used in folk medicine for their aphrodisiac, digestive, tonic, sedative, antiseptic, carminative, and vermifuge properties as well as for the treatment of hemorrhoids, ulcers, snake bites, spleen diseases and headaches (Boulus 1983; Demetzos et al. 2000). Other than medicinal usage, they have been consumed as salad or spice due to their special odor, also as food for goats and deer (Erdurak 2003).

Previous phytochemical studies indicated that coumarins are the most common metabolites on Ferulago species (Jimenez et al. 2000). Coumarins have various biological activities such as anticancer (Lee et al. 2011; Luo et al. 2011), anti-inflammatory (Kwon et al. 2011; Huang et al. 2012), anticoagulant (Aoyama et al. 1992; Hirsh et al. 2001), antiadipogenic (Shin et al. 2010), antitubercular (Chiang et al. 2010), antihyperglycemic (Fort et al. 2000; Tchamadeu et al. 2010), antiviral (Venugopala et al. 2013), antifungal (Chou et al. 2007; Wang et al. 2009), antibacterial (Rosselli et al. 2009), antihypertensive (Crichton & Waterman, 1978; Gantimur et al. 1986), anticonvulsant (Luszczki et al. 2009), antioxidant (Kim et al. 2008; Basile et al. 2009), neuroprotective (Wang et al. 2012), and antidiabetic (Marles & Farnsworth 1995; Patel et al. 2012). This is the first report of isolation and structure elucidation study on the roots of F. bracteata to afford a new coumarin, peucedanol-2′-benzoate (1), along with nine known ones (2–10) and a sterol mixture (11–12). The α-amylase and α-glucosidase inhibitory activities of isolated coumarins were also evaluated. Coumarins may be a potential source of new antidiabetic agents and may also be used by peripheral tissues by improving insulin resistance and increasing glucose uptake (Zhang et al. 2017). Peucedanol 7-O-β-D-glucopyranoside (Lee et al. 2004), coumarin (1,2-benzyoprynone) (Pari & Rajarajeswari 2009) umbelliferone (Ramesh & Pugalendi 2005), imperatorin (Adebajo et al. 2009), psoralen, 5-methoxypsoralen, 8-methoxypsoralen, isooxypeucedanin, pabulenol, oxypeucedanin methanolate, oxypeucedanin hydrate (Shalaby et al. 2014), isobergapten, pimpinellin, isopimpinellin, sphondin, scopoletin, and isoflavone.
phellopterin, byakangelicin and daucosterol (Zhang et al. 2017) were isolated from various plants belonging to the Apiaceae family and were found to be antidiabetic. So, this may be a significant approach in the treatment of type 2 diabetes. This study is a first report on the isolation and characterization of the bioactive compounds from root extracts of F. bracteata and also reports α-amylase and α-glucosidase inhibitory activities of this species.

Materials and methods

General

NMR spectra were recorded on a Varian Mercury Plus at 400 MHz for 1H NMR and 100 MHz for 13C NMR by using TMS as the internal standard. The solvents were CDCl3. HR-ESI-MS was performed on Agilent 6530 Accurate Mass Q-TOF LC/MS. UV spectra were measured using Thermo Scientific Multiskan GO microplate and cuvette spectrophotometer. IR spectra were run on a Bruker VERTEX 70v FT-IR Spectrophotometer. Column chromatographies were performed on Silica gel 60 (0.063-0.200 mm, Merck) and Sephadex LH-20 (Fluka). TLC was carried out on pre-coated Kieselgel 60 F254 aluminum sheets (Merck).

Plant material

The roots of F. bracteata were collected in July 2014 from Gaziantep (C6 Gaziantep: Nurdag Antep road 22. km, calcareous cliff rocks, 1642 m east of Turkey, Coordinates: 37° 11’ 226N, 36° 57’ 792 E) and identified by Prof. Dr. Hayri Duman, a plant taxonomist in the Department of Biological Sciences, Faculty of Art and Sciences, Gazi University. A voucher specimen (No AEF 26676) is stored in the Herbarium of Faculty of Pharmacy, Ankara University, Turkey.

Extraction and isolation

Air-dried roots of F. bracteata (450 g) were powdered and macerated three times with methanol for 8 h in a water bath not exceeding 45°C (4 x 2 L) using a mechanical mixer at 300 rpm. Combined extracts were filtered and concentrated to dryness (60.94 g), then dispersed in methanol:water (1:9) and fractionated with ethyl acetate to give compounds 1 (220 mg). Fr. C was applied to silica gel column eluting with n-hexane-ethyl acetate (85:15) and Sephadex LH-20 column eluting with ethyl acetate to give compounds 3 (125 mg) and 4 (130 mg). Eluting with n-hexane-ethyl acetate (90:10) over silica gel column of Fr. D gave compound 5 (400 mg) and Fr. E gave compounds 1 (320 mg), 6 (150 mg), and 7 (330 mg). Fr. F was eluted with 25% ethyl acetate in n-hexane and rechromatographed with 25% ethyl acetate in n-hexane on silica gel column to obtain compound 8 (110 mg). Fr. G was fractioned by column chromatography over silica gel using n-hexane-ethyl acetate mixtures (70:30 and 90:10) consecutively to obtain compound 9 (325 mg). Fr. H was submitted on a silica gel column using n-hexane-ethyl acetate (65:35) and the resulting fraction was chromatographed on silica gel column using n-hexane-ethyl acetate (90:10) to obtain compound 10.

Peucedanol-2'-benzoate (1)

White powder; IR νmax (KBr) cm–1: 1702, 1623, 1565; UV λmax (CH2Cl2) nm (log ε): 350 (4.20); 1H NMR (400 MHz, CDCl3) and 13C NMR (100 MHz, CDCl3) spectroscopic data, see Table 1; HR-ESI-MS at m/z 367.1999 [M-H]⁺ (Calculated for C21H19O6 367.1181).

Antidiabetic activity

α-Amylase inhibitory activity

α-Amylase inhibitory activity was established in accordance with the reported method (Nampoothiri et al. 2011) with slight modifications. One percent starch solution (100 μL) in 20 mM sodium phosphate buffer (pH 6.9 with 6 mM sodium chloride) and sample solutions (100 μL) were incubated at 25°C for 10 min in 24-well microplate. After incubation, 100 μL α-amylase solution (0.5 mg/mL) was added to each well and the reaction mixtures were incubated at 25°C for 10 min. In order to stop the reaction after the incubation, dinitrosalicylic acid color reagent (200 μL) was added and then the microplate was incubated in a boiling water bath for 5 min and cooled at room temperature. 50 μL was taken from each well and then was added to 96-well microplate. The reaction mixture was diluted by adding 200 μL distilled water and absorbance was measured at 540 nm. Each assay for all samples was carried out in triplicate. Percentage inhibitions of all

| Table 1. 1H NMR (400 MHz) and 13C NMR (100 MHz) data of 1 in CDCl3. |  |  |
|---|---|---|
| No | δH (J in Hz) | δC |
| 2 | 161.38 |  |
| 3 | 112.37 |  |
| 4 | 143.62 |  |
| 5 | 123.22 |  |
| 6 | 124.55 |  |
| 7 | 163.49 |  |
| 8 | 98.01 |  |
| 9 | 155.84 |  |
| 10 | 112.71 |  |
| 1' | 29.67 |  |
| 2' | 82.93 |  |
| 3' | 29.67 |  |
| 4' | 21.38 |  |
| 5' | 105.40 |  |
| 6' | 131.04 |  |
| 7' | 129.39 |  |
| 8' | 128.27 |  |
| 9' | 129.39 |  |
samples were calculated using the equation at following:

\[
\text{Inhibition (\%)} = \left( 1 - \frac{\Delta A_{\text{sample}}}{\Delta A_{\text{control}}} \right) \times 100
\]

**\(\alpha\)-Glucosidase inhibitory activity**

\(\alpha\)-Glucosidase inhibitory activity was established by using a 96-well microtiter plate in accordance with the described method (Tao et al. 2013) with slight modifications. \(p\)-Nitro-phenyl-\(\alpha\)-\(D\)-glucopyranoside (\(p\)-NPG) was used as the substrate and was prepared in 0.1 M potassium phosphate buffer (pH 6.8). \(\alpha\)-Glucosidase (0.1 Unit/mL, enzyme solution) was dissolved in the same buffer. Samples were dissolved in dimethyl sulfoxide (DMSO) and all samples (20 µL) together with the enzyme solution (20 µL) were mixed in the plate. Afterwards, the substrate (40 µL) was added for initiation of the reaction and the mixture was incubated at 37°C for 40 min. After incubation is complete, 0.2 M sodium carbonate (80 µL) in phosphate buffer (pH 6.8) was added to all wells in order to quench the reaction. The amount of released \(p\)-nitrophenol (pNP) was measured at 405 nm using a 96-well microplate reader. Each assay for all samples was carried out in triplicate. Percentage inhibition of all samples was calculated using the following equation:

\[
\text{Inhibition (\%)} = \left( 1 - \frac{\Delta A_{\text{sample}}}{\Delta A_{\text{control}}} \right) \times 100
\]

**Statistical analysis**

All results are expressed as mean ± SE and differences between means were statistically analyzed using one-way analysis of

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Figure 1. Chemical structures of compounds 1–12.
ANOVA followed by Bonferroni’s complementary analysis, with p < 0.05 considered to indicate statistical significance.

Results and discussion

Methanol extract of the roots of F. bracteata was fractionated using solvents with different polarities (n-hexane, dichloromethane, ethyl acetate, and n-butanol) and the obtained fractions were evaluated for their α-amylase and α-glucosidase inhibitory activities. The active dichloromethane extract was subjected to column chromatography over silica gel and Sephadex LH-20. As a result, a new coumarin, peucedanol-2′-benzoate (1), together with nine known ones, osthole (2) (Sajjadi et al. 2009), imperatorin (3) (Muller et al. 2004), bergapten (4) (Stevenson et al. 2003), prantschimgin (5) (Sajjadi et al. 2015), grandivitinol (6) (Abyshev et al. 1977), suberosin (7) (Tabanca et al. 2016), xanthotoxin (8) (Stevenson et al. 2003), felamidin (Kilic et al. 2006) (9), umbelliferone (10) (Singh et al. 2010), and a sterol mixture consisted of stigmasterol (11), β-sitosterol (12) (Woldeyes et al. 2012) (Figure 1) were isolated and identified.

Peucedanol-2′-benzoate (1) was isolated as a white powder with the molecular formula of C₂₁H₂₀O₆ as determined by the HR-ESI-MS at m/z 367.1999 [M + H]⁺ (Calcd for C₂₁H₂₀O₆ 367.1181). The IR spectrum of 1 showed absorption bands for C = O groups (1702 cm⁻¹) and -CH = CH- bonds (1623, 1565 cm⁻¹). The ¹H NMR spectrum of compound 1 displayed two AB type system protons at δH 6.26 and 7.64 (each 1 H, d, J = 9.4 Hz) which was attributed to the H-3 and H-4 protons of the coumarin nucleus. The two single aromatic proton signals at δH 7.28 and 6.80 were assigned to H-5 and H-8 protons.

The ¹³C NMR spectrum revealed the presence of 9 carbons resonances including four methine [δC 112.37 (C-3), 143.62 (C-4), 123.22 (C-5), 98.01 (C-8)], three oxygenated quaternary [δC 161.38 (C-2), 163.49 (C-7), 155.84 (C-9)], and two non-oxygenated quaternary carbons [δC 124.55 (C-6), 112.71 (C-10)] for coumarin skeleton. Two tertiary methyl groups at δH 1.72 (3 H, s, H-4′), 1.71 (3 H, s, H-5′) and at δC 22.16 (C-4′), 21.38 (C-5′) with the hydroxyl group; an oxygenated methine at δH 5.16 (1 H, dd, J = 9.2/7.3 Hz, H-2′) and at δC 89.12 (C-2′); and a methylene at δH 3.38 (2 H, m, H-1′) and at δC 29.67 (C-1′) confirmed the 2′,3′-dihydroxy-3′-methyl butylo moiety. HMBC correlation (Figure 2) between H-1′ (δH 3.38) and C-6 (δC 124.55) suggested that it was attached to C-6 position. Characteristic signals of a benzoyl moiety were also exhibited, including a pair of 2 H at δH 7.73 (H-3″, H-7″) and 7.32 (H-4″, H-6″) and 1 H at 7.51 (H-5″) in the ¹H NMR spectrum and aromatic carbons at δC 131.04 (C-2″), 129.39 (C-3″, C-7″), 128.27 (C-4″, C-6″), 132.86 (C-5″) with a carbonyl carbon at δC 165.40 (C-1″) in the ¹³C NMR spectrum. The linkage of the benzoyl group to the 2′,3′-dihydroxy-3′-

![Figure 2. Significant HMBC (→) correlations of compound 1.](image-url)
methyl butyl moiety was deduced from the downfield shifted signal of H-2' (δH 5.16) and C-2' (δC 89.12). Thus, the structure of the compound 1 was characterized as peucedanol-2'-benzoate.

The extracts and compounds 1–10, obtained via bioassay-guided fractionation and isolation process, were evaluated for their in vitro α-amylase and α-glucosidase inhibitory activities. The IC50 values and inhibitory effects (%) were given in Table 2 and Figure (3,4). Acarbose was used as a reference standard for both assays. Dichloromethane extract showed significant activity against α-glucosidase (IC50 0.95 mg/mL) and among the tested compounds felamidin (IC50 0.42 mg/mL) possessed the best inhibitory activity which were more potent than acarbose (IC50 4.95 mg/mL). Suberosin, osthole, imperatorin, prantschimgin, peucedanol-2’-benzoate also showed α-glucosidase inhibitory activity (IC50 0.89, 0.95, 1.23, 1.86, 3.24 mg/mL, respectively) which had lower effect than felamidin but stronger than acarbose. Among the tested compounds bergapten, xanthotoxin, and umbelliferone (IC50 6.12, 5.38, and 9.32 mg/mL, respectively) showed lower activity than reference compound acarbose. Grandivitinol (IC50 20.01 mg/mL) possessed the worst inhibitory activity. However, none of the extracts showed meaningful α-amylase inhibitory activity, while acarbose indicated 82.28% inhibition at a concentration of 1 mg/mL. These results indicate that felamidin was 11 times more effective than acarbose against α-glucosidase.

To our knowledge, no previous studies have been reported on α-glucosidase and α-amylase inhibitory activities of *F. bracteata* and the isolated coumarins prantschimgin, peucedanol-2’-benzoate, felamidin, grandivitinol, and suberosin. Also, this is the first report on the phytochemical analysis of *F. bracteata*.

Our results are similar to the previous studies performed on related coumarins. Shalaby et al. (2014) found that imperatorin (at 1000 μg/mL α-glucosidase inhibition% was found to be 69.66 ± 3.67 and we found an inhibition of 88.97 ± 0.88% at a concentration of 5000 μg/mL) showed appreciable antidiabetic activity. Comparing these results with previous studies in which α-glucosidase IC50 value of umbelliferone was found to be 7.79 ± 0.11 μg/mL, we have found a higher inhibitory activity with 9.32 mg/mL (Ramith et al. 2014). Comparing these results with a previous study in which α-glucosidase IC50 value of umbelliferone was 0.547 mg/mL at 0.5 mg/mL, the inhibitory activity that we found was higher (Ayyasamy & Rajamanickam 2015). Luo et al. (2012) reported that α-glucosidase inhibitory activity of bergapten, xanthotoxin, and imperatorin has been too low to compare to that of acarbose. In our study, it was determined that bergapten and xanthotoxin showed similar effects, however, imperatorin was more effective than acarbose. In spite of the advances in biomedical science and the introduction of new treatment ways, diabetes mellitus has been a major cause of end-stage renal disease, new-onset blindness, and cardiovascular diseases, all of which cause excess mortality and morbidity in people with diabetes (Islam et al. 2013). Medical herbs are generally rich in phenolic compounds, such as phenolic acids, flavonoids, tannins, stilbenes, lignans, coumarins, and lignins (Celik et al. 2011). One of the therapeutic approach for treating diabetes is to reduce the postprandial hyperglycemia and this is made by delaying the absorption of glucose via the inhibition of the carbohydrate-hydrolyzing enzyme α-glucosidase in the digestive tract. Inhibitors of intestinal α-glucosidase have been utilized in the treatment of non-insulin-dependent diabetes mellitus and represented at the large ratio of antidiabetic drug market (Yin et al. 2008). The tested extracts and isolated compounds are rich in phenolic compounds, as well as in coumarins, which may contribute to its in vitro antidiabetic effect. In addition, this study suggests that the glucose-lowering effect of these plants can be due, at least in part, to the inhibition of α-glucosidase. Also, investigations are warranted to define the active principles and elucidate other possible mechanism(s) of action. Natural products are still considered as potential sources for drug exploration and play a significant role in drug development programs. Furthermore, many medicinal plants could be rich sources of...
biochemical activities that are importantly free from undesired adverse effects and show powerful pharmacological activities.

Conclusions

In conclusion, the present study demonstrated that among the compounds isolated from CH2Cl2 fraction of *Ferulago bracteata* roots, coumarins were determined the main chemical constituents of this fraction. The most potent compounds found were felaminid and suberosin, respectively. Thus, *F. bracteata* along with its isolated compounds could be used for further studies on the development of novel preventive or therapeutic agents for the treatment of diabetes.

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Disclosure statement

The authors report no declarations of interest.

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