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School of Pharmacy & Pharmaceutical Sciences
Isfahan University of Medical Sciences

Original Article

1, 5-dicaffeoylquinic acid, an α-glucosidase inhibitor from the root of *Dorema ammoniacum* D. Don

Nikdokht Etemadi-Tajbakhsh[1], Mohammad-Ali Faramarzi[2], and Mohammad-Reza Delnavazi[1,*]

[1]Department of Pharmacognosy, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, I.R. Iran.
[2]Department of Medicinal Biotechnology, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, I.R. Iran.

Abstract

Background and purpose: *Dorema ammoniacum* D. Don (Apiaceae family) is a perennial plant whose oleo-gum resin is used as a natural remedy for various diseases, especially chronic bronchitis, and asthma. In the present study, hydromethanolic extract of *D. ammoniacum* root was subjected to phytochemical analyses and α-glucosidase inhibitory potentials of the isolated compounds were assessed.
Experimental approach: Silica gel (normal and reversed phases) and Sephadex® LH-20 column chromatographies were used for the isolation and purification of the compounds. Structures of the compounds were characterized by 1D and 2D nuclear magnetic resonance (NMR) techniques. All the isolated compounds were assessed for their *in vitro* α-glucosidase inhibitory activity in comparison with acarbose, a standard drug.
Findings/Results: Two phloroacetophenone glycosides; echisoside (**1**) and pleoside (**2**), along with dihydroferulic acid-4-O-β-D-glucopyranoside (**3**), and β-resorcylic acid (**4**), and two caffeoylquinic acid derivatives; chlorogenic acid (**5**) and 1, 5-dicaffeoylquinic acid (cynarin, **6**) were isolated. Among the isolated compounds, the α-glucosidase inhibitory effect of 1,5-dicaffeoylquinic acid was found as 76.9% of the acarbose activity at 750 µM (IC_{50} value of acarbose).
Conclusion and implications: Considerable α-glucosidase inhibitory effect of 1,5-dicaffeoylquinic acid makes it an appropriate candidate for further studies in the development of new natural antidiabetic drugs.

Keywords: α-glucosidase inhibitor; Caffeoylquinic acid; *Dorema ammoniacum* D. Don; Phloroacetophenone glycoside.

INTRODUCTION

Dorema ammoniacum D. Don (*D. ammoniacum*) from Apiaceae family is one of the seven *Dorema* species represented in the flora of Iran (1). The oleo-gum resin of this species (gum ammoniacum) is traditionally used for different medicinal purposes, especially as antispasmodic and expectorant for the treatment of chronic bronchitis and asthma (2). It has also been reported that gum ammoniacum is sold in Jordanian herbal markets as a traditional natural drug for the reduction of blood sugar (3). Previous biological studies have demonstrated antimicrobial, cytotoxic, anti-inflammatory, and anticonvulsant activity of gum ammoniac (4-7). In 2013, Adhami *et al*. reported the isolation of ammoresinol, dshamirone, and doremin A from this oleo-gum resin as compounds with acetylcholinesterase inhibitory effects (8). The root of this species has also been found to possess antioxidant activity in 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) methods, as well as considerable antimicrobial activity against *Bacillus subtilis, Pseudomonas aeruginosa*, and *Staphylococcus aureus* (9). There are some reports on the essential oil composition of *D. ammoniacum* root (9,10).

*Corresponding author: M.R. Delnavazi
Tel: +98-2164121213, Fax: +98-2166959096
Email: delnavazi@tums.ac.ir

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Website: http://rps.mui.ac.ir

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The gas chromatography-mass spectrometry (GC-MS) analysis of the essential oil of *D. ammoniacum* root from Kashan region (Isfahan province, Iran) resulted in the identification of β-bisabolene, hexadecanal and (*E*)-nerolidol with the relative percentages of 15.1, 13.2, and 11.3%, respectively, as the major compounds (9). In another study, Takallo *et al*. reported the presence of 3-n-butyl phthalide (62.49%), benzyl butanoate (6.57%), and liguloxide (5.15 %) in the essential oil of *D. ammoniacum* root collected from Rayen region (Kerman province, Iran) (10). In the present study, phenolic constituents of the hydromethanolic extract obtained from *D. ammoniacum* root were investigated. In view of the α-glucosidase inhibitory potentials reported for phenolic natural compounds in the literature (11,12), *in vitro* α-glucosidase inhibitory activity of the isolated compounds was also assessed.

MATERIALS AND METHODS

Plant material

The roots of *D. ammoniacum* were collected in June 2017 from Kashan district (Isfahan province, central Iran) was deposited at the research herbarium of Kashan Botanical Garden, Kashan, Iran under the voucher specimen (No. 1890-KBGH)

Extraction

The air-dried and comminuted roots (1.0 kg) were macerated with chloroform and 70% methanol in water (3 × 5 L each), successively at the lab temperature. The extract was concentrated with a rotary evaporator under 45 °C and dried using a vacuum oven.

Isolation and purification of compounds 1-6

Compound **1** (echisoside, 1.7 g) was obtained as a white precipitate following the addition of methanol (50 mL) to the hydromethanolic extract (50 g). The dried methanol soluble portion was then suspended in water (0.5 L) and partitioned with *n*-butanol (3 × 0.5 L). Four g of *n*-butanol fraction was subjected to Sephadex® LH-20 column chromatography with methanol to get nine fractions (A-I). Fraction D (2.1 g) was chromatographed on a silica gel (mesh 230-400, Merck) column using a gradient mixture of $CHCl_3$:MeOH (95:5 to 50:50) to give twenty fractions (D1-D20), of which fraction D6 yielded compound **2** (pleoside, 42 mg). Reversed-phase (RP) chromatography of fraction D9 (350 mg) on a RP-18 (mesh 230-400, Fluka) column using ACN-H_2O (2:8) yielded compound **3** (18 mg). Fraction G (360 mg) was divided into seven fractions (G1-G7) *via* Sephadex® LH-20 (GE Healthcare, USA) column chromatography (MeOH:H_2O, 8:2), among them fraction G2 was compound **4** (β-resorcylic acid, 21 mg). Compound **5** (chlorogenic acid, 12 mg) was isolated from fraction G3 (136 mg) on a RP-18 column using ACN-H_2O (1:9) as eluent. Column chromatography of fraction J (230 mg) on Sephadex® LH-20 (MeOH:H_2O, 8:2) afforded compound **6** (5-dicaffeoylquinic acid, cynarin, 24 mg).

Thin-layer chromatography (Pre-coated Si gel GF254 and Si gel 60 RP-18 F254s plates, Merck, Germany) was applied for the monitoring of column chromatography and fractions with similar spots under 254 and 366 nm UV wavelengths were combined. The structures of the isolated compounds were elucidated by proton nuclear magnetic resonance (^{1}H-NMR), carbon-13 (^{13}C)-NMR, heteronuclear single quantum correlation (HSQC), and heteronuclear multiple bond correlation (HMBC) spectral analysis (Bruker Avance 400 DRX, USA, 400 MHz for ^{1}H and 100 MHz for ^{13}C), as well as by comparison with published data.

α-Glucosidase inhibition assay

An *in vitro* colorimetric assay was used for the evaluation of α-glucosidase inhibitory potentials of the isolated compounds (13). Twenty µL of α-glucosidase enzyme (EC3.2.1.20, *S. cerevisiae*, 20 U/mg) solution, 20 µL of sample solution (750 µM), and 135 µL of potassium phosphate buffer (50 mM, pH 6.8) were added to a 96-well plate. After incubation of the plate at 37 °C for 10 min, 25 µL of *p*-nitrophenylglucopyranoside (Sigma-Aldrich, Switzerland) (4 mM) was added to the mixture and the plate was incubated for 20 min at 37 °C. Finally, the absorbance changes were recorded

at 405 nm by a spectrophotometer (Gen5, PowerWave XS2, BioTek, USA), and the percentage of enzyme inhibition was determined. Dimethyl sulfoxide (DMSO, 10% final concentration) and acarbose were used as a control and standard drug, respectively. Each sample was tested in triplicate.

Statistical analysis

Data were analyzed using Sigmaplot 11.0 software and expressed as mean ± SD.

RESULTS

Phytochemical analysis

The extraction of *D. ammoniacum* roots yielded 13.1 and 15.0% for chloroform and hydromethanolic extracts, respectively. Phytochemical analysis of the hydromethanolic extract obtained from *D. ammoniacum* roots using silica gel (normal and reversed-phase) and Sephadex® LH-20 column chromatography resulted in the isolation of six phenolic compounds (**1**-**6**). Structures of compounds were identified as echisoside (**1**), pleoside (**2**), dihydroferulic acid-4-O-β-D-glucopyranoside (**3**), β-resorcylic acid (4-hydroxy salicylic acid) (**4**), 5-O-caffeoylquinic acid (5-CQA, chlorogenic acid) (**5**), and cynarin (**6**) using ^{1}H-NMR, ^{13}C-NMR, HSQC, and HMBS spectral analysis, as well as by comparison with those reported in the literature (14-19) (Fig. 1).

Spectroscopic data of the isolated compounds

Echisoside (**1**)

^{1}H-NMR (DMSO-d_6, δ/ppm, *J*/Hz): 13.68 (1H, *s*, OH-6), 6.34 (1H, *d*, *J* = 2.0, H-3), 6.12 (1H, *d*, *J* = 2.0, H-5), 5.05 (1H, *d*, *J* = 7.6, H-1'), 4.17 (1H, *d*, *J* = 7.6, H-1"), 3.80 (3H, *s*, OCH_3), 2.90-3.75 (12H, overlapped signals, H-2'-6', H-2"-6"), 2.66 (3H, *s*, H-8); ^{13}C-NMR (DMSO-d_6, δ/ppm): 204.0 (C-7), 166.2 (C-4), 166.0 (C-6), 161.0 (C-2), 106.3 (C-1), 104.1 (C-1"), 100.9 (C-1'), 95.5 (C-5), 93.9 (C-3), 77.4 (C-3"), 77.2 (C-3'), 77.1 (C-5"), 76.0 (C-5'), 74.0 (C-2"), 73.6 (C-2'), 70.5 (C-4"), 70.1 (C-4'), 69.4 (C-6'), 61.5 (C-6"), 56.2 (OCH_3), 33.6 (C-8) (14).

Pleoside (**2**)

^{1}H-NMR (DMSO-d_6, δ/ppm, *J*/Hz): 13.69 (1H, *s*, OH-6), 6.28 (1H, *d*, *J* = 1.9, H-3), 6.13 (1H, *d*, *J* = 1.9, H-5), 5.00 (1H, *d*, *J* = 7.3, H-1'), 3.80 (3H, *s*, OCH_3), 3.1-3.5 (6H, overlapped signals, H-2'-6'), 2.66 (3H, *s*, H-8); ^{13}C-NMR (DMSO-d_6, δ/ppm): 204.0 (C-7), 166.1 (C-4 and C-6), 161.1 (C-2), 106.3 (C-1), 101.1 (C-1'), 95.4 (C-5), 93.9 (C-3), 77.8 (C-3'), 77.2 (C-5'), 73.6 (C-2'), 70.2 (C-4'), 61.1 (C-6'), 56.1 (OCH_3), 33.6 (C-8) (15).

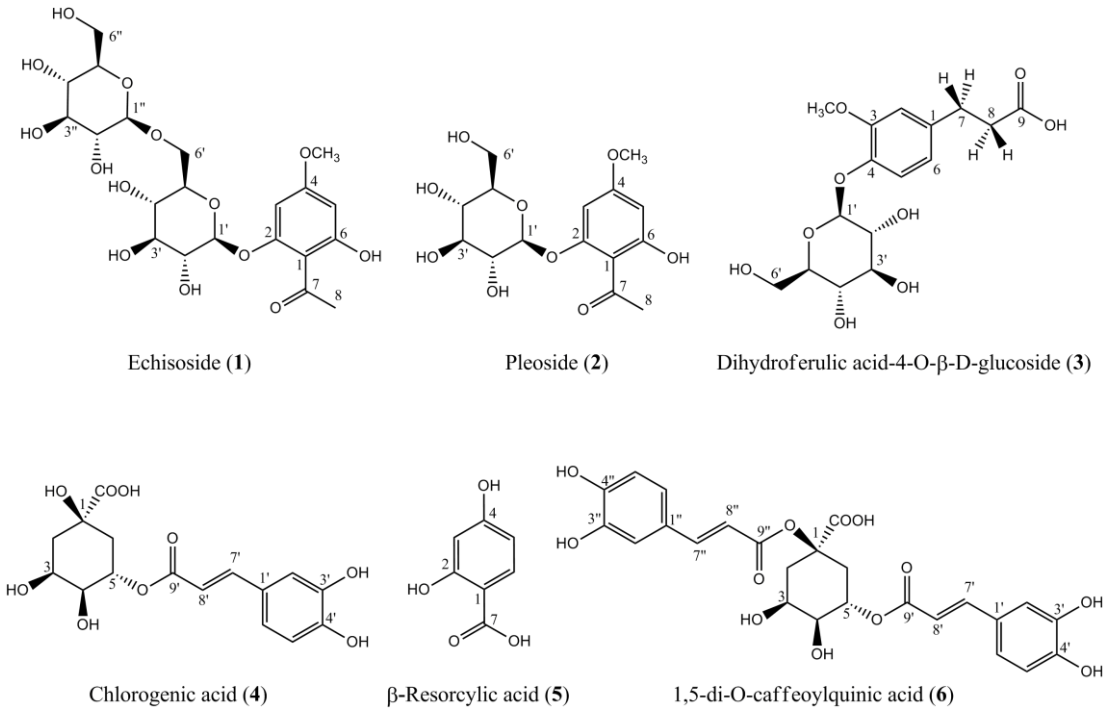

Fig. 1. Structures of the compounds isolated from *Dorema ammoniacum* root.

Table 1. α-Glucosidase inhibitory effects of the isolated compounds from *D. ammoniacum* root. Data represent mean ± SD, n = 3.

Samples [a]	**Inhibition (%)**
Echisoside (**1**)	13.5 ± 0.7
Pleoside (**2**)	17.2 ± 0.9
Dihydroferulic acid-4-O-β-D-glucopyranoside (**3**)	8.1 ± 0.9
Chlorogenic acid (**4**)	19.0 ± 1.0
β-Resorcylic acid (**5**)	13.6 ± 1.5
1,5-Dicaffeoylquinic acid (Cynarin) (**6**)	40.0 ± 1.0
Acarbose	52.0 ± 1.0

[a] Compounds were tested in a concentration of 750 µM, equimolar to IC_{50} value determined for acarbose.

Dihydroferulic acid-4-O-β-D-glucopyranoside (**3**)

^{1}H-NMR (DMSO-d_6, δ/ppm, *J*/Hz): 7.01 (1H, *d*, *J* = 8.3, H-5), 6.70 (1H, *d*, *J* = 2.2, H-2), 6.48 (1H, *dd*, *J* = 8.3, 2.2, H-6), 4.78 (1H, *d*, *J* = 7.0, H-1'), 3.70 (3H, *s*, OCH_3), 3.0-3.6 (6H, overlapped signals, H-2'-6'), 2.76 (2H, *m*, H-7), 2.56 (2H, *m*, H-8); ^{13}C-NMR (DMSO-d_6, δ/ppm): 175.4 (C-9), 159.1 (C-4), 156.6 (C-3), 130.3 (C-5), 122.5 (C-1), 107.0 (C-2), 102.06 (C6), 101.5 (C-1'), 77.6 (C-3'), 77.2 (C-5'), 73.8 (C-2'), 70.4 (C-4'), 61.3 (C-6'), 55.4 (OCH_3), 35.7 (C-7), 25.4 (C-8) (16).

5-O-Caffeoylquinic acid (*chlorogenic acid*) (**4**)

^{1}H-NMR (DMSO-d_6, δ/ppm, *J*/Hz): 7.43 (1H, *d*, *J* = 15.8, H-7'), 7.05 (1H, *d*, *J* = 1.5, H-2'), 6.98 (1H, *dd*, *J* = 8.1, 1.5, H-6'), 6.75 (1H, *d*, *J* = 8.1, H-5'), 6.20 (1H, *d*, *J* = 15.8, H-7'), 5.13 (1H, *m*, H-5), 3.90 (1H, *m*, H-3), 3.48 (1H, *br d*, *J* = 8.4, H-4), 1.6-2.0 (4H, overlapped signals, H-2,6); ^{13}C-NMR (DMSO-d_6, δ/ppm): 176.7 (C-7), 166.7 (C-9'), 148.9 (C-4'), 146.2 (C-7'), 145.1 (C-3'), 126.0 (C-1'), 121.7 (C-5'), 116.3 (C-6'), 115.2 (C-2'), 115.0 (C-8'), 75.0 (C-1), 74.1 (C-4), 71.9 (C-5,3), 39.8 (C-2), 38.2 (C-6) (17).

β-Resorcylic acid (*4-hydroxy salicylic acid*) (**5**)

^{1}H-NMR (DMSO-d_6, δ/ppm, *J*/Hz): 7.52 (1H, *d*, *J* = 8.7, H-6), 6.38 (1H, *dd*, *J* = 8.7, 2.2, H-5), 6.29 (1H, *d*, *J* = 2.2, H-3); ^{13}C-NMR (DMSO-d_6, δ/ppm): 191.2 (C-7), 165.8 (C-2), 164.0 (C-4), 133.8 (C-6), 111.4 (C-1), 109.2 (C-5), 102.9 (C-3) (18).

1,5-dicaffeoylquinic acid (**6**)

^{1}H-NMR (DMSO-d_6, δ/ppm, *J*/Hz): δ 7.47 (1H, *d*, *J* = 15.8, H-7'), 7.43 (1H, *d*, *J* = 15.9, H-7"), 7.11 (1H, *br s*, H-2'), 7.05 (1H, *br s*, H-2"), 6.94 (1H, *br d*, *J* = 8.1, H-6'), 6.92 (1H, *br d*, *J* = 8.1, H-6"), 6.75 (1H, *d*, *J* = 8.1, H-5'), 6.74 (1H, *d*, *J* = 8.1, H-5"), 6.22 (2H, *d*, *J* = 15.8, H-8'), 6.20 (2H, *d*, *J* = 15.9, H-8"), 5.27 (1H, *m*, H-5), 4.05 (1H, *m*, H-3), 3.54 (1H, *br d*, *J* = 8.2, H-4), 1.7-2.5 (4H, *m*, H-2,6); ^{13}C-NMR (DMSO-d_6, δ/ppm): 174.6 (C-7), 166.8 (C-9'), 165.7 (C-9"), 149.6 (C-4'), 148.8 (C-4"), 146.5 (C-3'), 145.8 (C3"), 145.4 (C7'), 144.4 (C7"), 126.2 (C1"), 125.8 (C1'), 121.8 (C-6'), 120.7 (C-6"), 116.6 (C-8"), 116.4 (C-5'), 116.3 (C-5"), 115.6 (C-2'), 115.6 (C-2"), 114.7 (C-8'), 82.1 (C-1), 72.6 (C-4), 70.9 (C-5), 69.1 (C-3), 37.9 (C-6), 35.3 (C-2) (19).

Evaluation of α-glucosidase inhibitory activity

The isolated compounds (**1**-**6**) were evaluated for their *in vitro* α-glucosidase inhibitory activity and compared with acarbose as the standard drug. The result of α-glucosidase inhibitory assay has been summarized in Table 1.

DISCUSSION

The structure of compound **2** was characterized as 2-O-β-D-g lucopyranosyl-4-O-methyl-phloroacetophenone (pleoside) based on ^{1}H-NMR and ^{13}C-NMR spectral data and comparing the data with those reported in the literature, as well (15). The ^{1}H-NMR spectrum of compound **2** showed two doublet signals at δ_H 6.28 and 6.12 with a coupling constant of 2.0 Hz, characteristic for a pair of *meta*-coupled aromatic protons, H-3 and H-5, respectively. A methyl singlet at δ_H 3.80 was assigned to the methoxy group. The presence of glucosyl moiety was confirmed by observation

of a series of overlapped signals at the range of 3.1-3.5 ppm, along with an oxymethine doublet at δ_H 5.00 (*J* = 7.2 Hz), characteristic for H-1' anomeric proton. An upfield methyl singlet at δ_H 2.66 was also assigned to H-8 of acetophenone skeleton. In the ^{13}C-NMR spectrum of compound **2**, thirteen signals were observed. A downfield quaternary carbon signal at δ_c 204.0 was assigned to ketone carbonyl at C-7. Resonances of aromatic ring carbons attached to phenolic hydroxy groups were observed at δ_c 166.0 (C-4 and C-6) and 161.1 (C-2). Signals appeared at 106.3, 95.4, and 93.9 were also assigned to C-1, C-5, and C-3, respectively. The signal of an anomeric carbon atom of glucosyl moiety (C-1') was found further downfield (δ_c 101.1) rather than the resonances of C-2' to C-6' (δ_c 61.0-78.0). Signals corresponding for methoxy and C-8 methyl carbons were also observed at δ_c 56.08 and 33.58, respectively.

The ^{1}H-NMR and ^{13}C-NMR spectra of compound **1** displayed close similarities with those of **2**. The diagnostic difference was the presence of one additional doublet (*J* = 7.6 Hz) oxymethine resonance at δ_H 4.18 and observation of more overlapped signals in sugar protons region (δ_H 3.0-3.5), in comparison with **2**. Therefore, the presence of a disaccharide residue was proposed for **1**, which was confirmed by its ^{13}C-NMR data. A comparison of the NMR data acquired for compound **2** with those published in the literature for acetophenone glycosides resulted in the elucidation of the structure of 2-O-[β-D-glucopyranosyl-(1"→6')-β-D-glucopyranosyl]-4-O-methyl-phloroacetophenone (echisoside) (14).

Compound **4** was isolated as a yellowish amorphous solid. This compound was identified as 5-O-caffeoyl quinic acid (chlorogenic acid) by ^{1}H-NMR and ^{13}C-NMR spectral analysis (17). The ^{1}H-NMR of compound **4** displayed an ABX system at 6.6-7.1 ppm, characteristic for a 1,2,4-trisubtituted benzene ring. Two doublet signals at δ_H 7.43 and 6.20 with the large splitting of 15.8 Hz, typical for *trans* orientated olefinic protons of an α-β unsaturated ester, were assigned to H-7' and H-8', respectively. Resonance of the oxymethine H-5 adjacent to caffeoyl moiety was found more downfield (δ_H 5.13), rather than H-3 (δ_H 3.90) and H-4 (δ_H 3.49) signals of the quinic acid skeleton. Overlapped signals appeared at the range of δ_H 1.6 to 2.0, were also assigned to aliphatic methylene protons of H-2 and H-6. The structure of chlorogenic acid for **4** was also supported by a comparison of ^{13}C-NMR data with literature data (17).

The ^{1}H-NMR spectrum of compound **6** was closely related to **4**, with an additional caffeoyl moiety. The partial similarity was observed between chemical shifts of H-3, H-4, and H-5 signals in **6** and **4**, which is indicative to substitution of second caffeoyl moiety at C-1 of the quinic acid skeleton. Therefore, the structure of cynarin was proposed for compound **6**. The ^{1}H-NMR and ^{13}C-NMR data of this compound were also identical to those reported for cynarin in the literature (19).

The structure of compound **3** was elucidated as dihydroferulic acid-4-O-β-D-glucopyranoside on the basis of ^{1}H-NMR and ^{13}C-NMR spectral analysis. In ^{1}H-NMR of compound **3**, signals at δ_H 7.01 (*d*, *J* = 8.3 Hz), 6.70 (*d*, *J* = 2.2 Hz) and 6.48 (*dd*, *J* = 8.3 Hz, 2.2, H-6), were assigned to aromatic protons of H-5, H-2, and H-6, respectively. The signal corresponding to an anomeric proton of glucosyl moiety (H-1') appeared as a doublet at δ_H 4.78 (*d*, *J* = 7.0 Hz). Six other protons of glucosyl moiety (H-2'-H-6') were also found as overlapped signals at the range of δ_H 3.0 to 3.6. A methyl signet at δ_H 3.70 was also assigned to the methoxy group. In comparison with cinnamic acid derivatives, two methylene multiplet signals at δ_H 2.76 and 2.56 were found in ^{1}H-NMR of compound **3**, instead of typical doublets signals of α/β vinylic methines, suggesting the saturation of C-7/C-8 bond in compound **3**. This was also confirmed by ^{13}C-NMR data analysis, trough the observation of C-7 and C-8 signals at δ_C 35.4 and 25.5. Other ^{13}C-NMR data were in agreement with those reported for dihydroferulic acid-4-O-β-D-glucopyranoside in the literature (16).

The ^{1}H-NMR of compound **5** showed three methine signals at δ_H 5.9-7.5, characteristic for a 1,2,4-trisubstituted benzene ring. In ^{13}C-NMR of **5**, seven carbon signals were observed including four quaternary carbons (δ_C 191.2, 165.8, 164.0, and 111.3) and three tertiary

carbons (δ_C 133.8, 109.2, and 102.9). Therefore, the structure of a dihydroxy benzoic acid was proposed for compound **5**. The positions of hydroxy groups were determined at C-2 and C-4 by HSQC and HMBC experiments. The key HMBC was found between H-6 (δ_H 7.42) and C-7 (191.2). Further ^2J and ^3J HMBC correlations were observed between H-6 (δ_H 7.52) and C-2 (165.8) and C-4 (164.0), H-5 (δ_H 6.38) and C-1 (111.4) and C-3 (102.9), H-3 (δ_H 6.29) and C-5 (109.2), C-1 (111.4) and C-4 (164.0). Accordingly, 4-hydroxy salicylic acid (β-resorcylic acid) was deduced for compound 5 (18).

All compounds (**1**-**6**) from the roots of *D. ammoniacum* are being reported for the first time. This is also the first report on the isolation of β-resorcylic acid (**5**) from the genus *Dorema* D. Don. Chlorogenic acid and some dicaffeoylquinic acid isomers (1,5-DCQA, 3,5-DCQA, and 4,5-DQCA) have been reported as potent free radical scavenging principles of the roots and aerial parts of *D. glabrum* by Delnavazi *et al*. (16). Echisoside and pleoside are also two phloroacetophenone glycosides, previously isolated from the roots of some *Dorema* species such as *D. glabrum*, *D. hyrcanum*, and *D. aitchisonii* (14,16,20,21). Inhibitory effects of echisoside, pleoside, chlorogenic acid, 4,5- and 1,5-dicaffeoylquinic acids on the proliferation of adenocarcinoma gastric cell line have been shown by Jafari *et al*. (22).

The presence and distribution of the isolated compounds **1-4** and **5** in some other *Dorema* species, especially *D. glabrum*, is indicative to close chemotaxonomic correlation between the members of *Dorema* genus from Apiaceae family. Among the mentioned compounds, echisoside, has only been reported from the genus *Dorema* (14,16,21), and can be considered as a chemotaxonomic marker of the plants classified in the genus *Dorema*. In *in vitro* α-glucosidase inhibitory assay, among the isolated compounds, α-glucosidase inhibitory effect of 1,5-dicaffeoylquinic acid was found as 76.9% of the acarbose activity in a concentration of 750 µM (IC_{50} value of acarbose, Fig. 2). Enzyme inhibition potentials of the other compounds were determined at the range of 15.5 to 36.5% of the acarbose activity (Table 1).

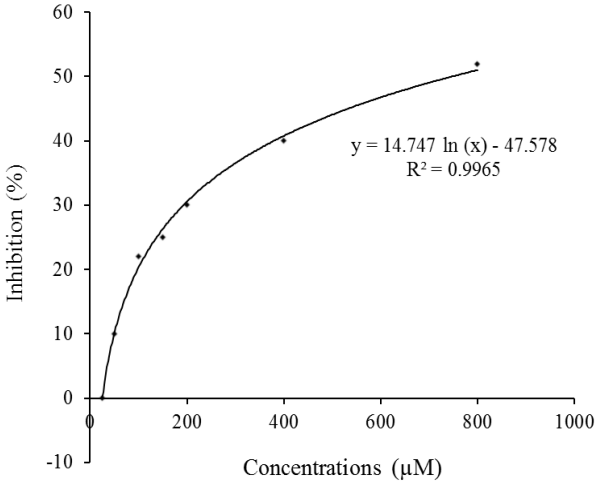

Fig. 2. Dose-response curve for acarbose in α-glucosidase inhibition assay.

Although myrciaphenone B, a phloroacetophenone glycoside from *Myrcia multiflora* DC. leaves has been reported to possess a potent inhibitory effect on maltase (IC_{50}: 440 µM) and sucrase (IC_{50}: 310 µM) (23), phloroacetophenone glycosides isolated from *D. ammoniacum* roots (echisoside and pleoside) showed a weak α-glucosidase inhibitory effect.

Comparing the inhibition activity of 1,5-dicaffeoylquinic acid (76.9%) and chlorogenic acid (36.5%) shows that the substitution of OH-1 group in chlorogenic acid by a caffeoyl moiety increases the α-glucosidase inhibitory effects. In an enzyme assay guided fractionation study on methanolic extract of 50 traditional Chinese herbs, Gao *et al*. reported 3,4-dicaffeoylquinic acid, 3,5-dicaffeoylquinic acid, and 4,5-dicaffeoylquinic acid as active principles of *Tussilago farfara* L. buds with the potent maltase inhibitory activity (IC_{50}: 0.89-0.91 mM) (24). The maltase inhibitory activity of quinic acid and caffeic was also reported less than 20% at 1 mM (24). Chen *et al*. demonstrated that yeast α-glucosidase inhibitory activity of the methyl ester derivatives of 3,4-dicaffeoylquinic acid and 3,5-dicaffeoylquinic acid isolated from the aerial parts of *Gynura divaricata* is about ten times more potent than their analogues (25). In another study on α-glucosidase inhibitory potentials of caffeoylquinic acid derivatives of Brazilian propolis, 1,3,5-dicaffeoylquinic acid was found to be more active than isolated

dicaffeoylquinic acids (3,4-DCQA and 3,5-DCQA) (26). The mechanism of α-glucosidase inhibitory activity of caffeoylquinic acids isolated from *Ilex kudingcha* was clarified by Xu and colleagues (27). They showed that caffeoylquinic acids (3,4-DCQA, 4,5-DCQA, and 3,5-DCQA) inhibit the α-glucosidase enzyme in a non-competitive mode, mainly through hydrophobic interaction which is led to decreasing the catalytic activity via altering the molecular conformation of the enzyme (27). Cynarin isolated in this work, is a polyphenol present in some functional foods such as artichoke and sunflower sprouts (28,29). Cynarin has been reported as an active constituent of sunflower sprouts with a potent inhibitory effect against the formation of advanced glycation end products (EC_{50}: 9.38 μg/mL) (29). The considerable α-glucosidase inhibitory activity of cynarin illustrates the possible beneficial impact of these functional foods for diabetic patients.

CONCLUSION

Phytochemical analyses of the hydromethanolic extract of *D. ammoniacum* root resulted in the isolation and identification of the six phenolic compounds, namely, echisoside (**1**), pleoside (**2**), dihydroferulic acid-4-O-β-D- glucopyranoside (**3**), β-resorcylic acid (**4**), chlorogenic acid (**5**), and 1, 5-dicaffeoylquinic acid (**6**) of which compound **6** was exhibited considerable α-glucosidase inhibitory effect. The results of this study showed the close chemotaxonomic relationship between *D. ammoniacum* and other *Dorema* speciesand suggesting 1,5-dicaffeoylquinic acid as a potent α-glucosidase inhibitor which has the great potential to be considered as an appropriate candidate in antidiabetic drug development researches.

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CONFLICT OF INTEREST STATEMENT

All authors declare no conflict of interest in this study.

AUTHORS' CONTRIBUTION

N. Etemadi-Tajbakhsh acquired and analyzed the experimental data. M.A. Faramarzi designed and analyzed the obtained data from the biological study. M.R. Delnavazi accomplished the concept, design, and data interpretation in the phytochemical study, the definition of intellectual content, literature search, manuscript preparation, manuscript editing, and manuscript review.

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