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ORIGINAL PAPER

α-Glucosidase inhibitors from the aerial part of *Thymus fedtschenkoi*: isolation, kinetic and molecular docking study

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Abstract

Thymus fedtschenkoi Ronniger from Lamiaceae family is an endemic Iranian plant and used as traditional remedy by local people. The present study, evaluated α-glucosidase inhibitory potentials of *T. fedtschenkoi* fractions and its compounds. The aerial part of *T. fedtschenkoi* was extracted with methanol/water and fractioned by *n*-hexane, chloroform, and *n*-butanol solvents, successively. The *n*-butanol fraction with the highest α-glucosidase inhibitory effect was further investigated with open column chromatography. The structures of isolated compounds were elucidated by ^{1}H-NMR and ^{13}C-NMR spectral analyses. In addition, antidiabetic potentials of the fractions and the isolated compounds were assessed through an in vitro α-glucosidase inhibitory test and kinetic and molecular docking studies were done for the isolated compounds. Eight phenolic compounds including 3, 4-di-*O*-feruloyl quinic acid (**1**), luteolin-7-*O*-rutinoside (**2**), luteolin (**3**), rosmarinic acid methyl ester (**4**), rosmarinic acid (**5**), apigenin-7-*O*-glucoside (**6**), luteolin-7-*O*-glucuronide (**7**), and luteolin-7-*O*-glucoside (**8**) were isolated. According to the results, compound **5** was the most potent α-glucosidase inhibitor with IC_{50} value of 43.38 ± 0.05 µM which was about 17 times lower than the IC_{50} value of the acarbose as reference compound (750.0 ± 1.0 µM). The present study showed a good potency for the *n*-butanol fraction of *T. fedtschenkoi* and its compounds to inhibit α-glucosidase enzyme.

Keywords *Thymus fedtschenkoi* · α-Glucosidase · Phenolic compounds · Diabetes

Introduction

Diabetes as a chronic metabolic disorder seriously affected the well-being throughout the world with economic and social consequences; such that according to the International Diabetes Federation (IDF) data, diabetes is responsible for one death every eight seconds in 2019. It is estimated that the number of affected persons with diabetes is going to reach 700 million by 2045. In addition, based on various epidemiological studies diabetes is related to other pathological conditions such as renal failure, blindness, neurodegeneration and major depressive disorder (Chatterjee et al. 2017; Duarte-Silva et al. 2021; Groenewegen et al. 2021; Huang et al. 2020; IDF Diabetes Atlas 2019; Saeedi et al. 2019; Zhu et al. 2021).

Diabetes types 1 and 2 along with gestational diabetes mellitus are the main subcategories of diabetes in which the type 2 diabetes mellitus (T2D) responsible for the 90% of the total cases. T2D is generally known for abnormal insulin secretion and postprandial hyperglycemia. One of the promising ways for diabetes treatment is using inhibitors of the enzymes like α-glucosidase and α-amylase which resulting in reduction in postprandial hyperglycemia by delaying the digestion of carbohydrates. On the other hand, these carbohydrase inhibitors can be useful in the management of the weight gain and obesity. Considering this application and the adverse effects of available α-glycosidase inhibitors (acarbose, miglitol, etc.) in the market, searching plant extracts and natural compounds targeting α-glucosidase is a mainstream way in drug discovery for manage of diabetes

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and related disorders (Lankatillake et al. 2021; Rathod and Yadav 2021; Sharma et al. 2021; Zhu et al. 2021).

The genus *Thymus* L. from the Lamiaceae family has about 215 species all over the world. This genus originated from the Mediterranean zone and spreads throughout Europe, Greenland, Northern America, and Asia. Many plants of this genus have aromatic nature and widely used in food, cosmetic and medicinal purposes. Some of *Thymus* plants are important medicines in various traditional medicines and their infusions and decoctions were applied as carminative, digestive, antispasmodic, anti-inflammatory, emmenagogue, and tonic agents. Many studies focused on essential oils in *Thymus* plants which generally contained two well-known compounds named thymol and carvacrol. However nonvolatile compounds like flavonoids, simple phenylpropanoids, lignans, tannins, etc. could be the responsible substances for biological activities of these medicinal herbs. Thymus species showed a variety of activities in either in vivo or in vitro pharmacological researches e.g., as antimicrobial, antioxidant, antitumor, anti-inflammatory, anti-hypertensive, antidiabetic effects (Li et al. 2019; Salehi et al. 2019; Tohidi et al. 2019).

As mentioned above, *Thymus* species have a place in diabetes treatments. *T. spicata* var. *spicata, T. fallax* and *T. kotschyanus* have been used for treatment of diabetes in Iran and Turkey folk medicines (Li et al. 2019; Ozturk et al. 2018; Salteh and Amani 2020). In addition, some modern researches reveal the antidiabetic properties of *Thymus* species. Various studies reported good antidiabetic effect for *T. quinquecostatus*, *T. praecox*, and *T. argaeus* (Li et al. 2019). Taleb et al. (2017) ran a clinical trial for aqueous extract of *T. kotschyanus* in patients with type II diabetes. As a result, intake of *T. kotschyanus* infusion prepared from 10 g dried herb twice a day for three months along with drugs led to the better control of glucose levels and improved function of pancreatic beta cells.

Thymus fedtschenkoi Ronniger is a perennial subshrub wildly grown in the rocky slopes of the Irano-Turanian floristic region in Iran and Turkey. This plant which is closely related to *T. kotschyanus*, is used by indigenous people as antitussive, expectorant and antiseptic in treatment of common cold and sore throat (Hosseini et al. 2021). There are only a few studies conducted on this plant and the antimicrobial and antifungal activity is the only reported biological effect in the literature (Alinezhad et al. 2011; Aminkhani et al. 2019; Delazar et al. 2011). The essential oil of *T. fedtschenkoi* was analyzed in several studies. In many of these studies, thymol or carvacrol was the main compound of the essential oil (Abousaber et al. 2002; Aminkhani et al. 2019; Choi et al. 2002; Delazar et al. 2011; Tohidi et al. 2017). Nevertheless, other reports showed that linalool could be the main part of the essential oil of *T. fedtschenkoi* depending on the different phenological stages and environmental factors (Khorshidi et al. 2014; Rustaiee et al. 2011).

Therefore, on the one hand because of the importance of finding active natural products for diabetes management and on the other hand as a result of previous studies in *Thymus* species, this study was conducted to evaluate the potentials of *T. fedtschenkoi* fractions and its compounds in inhibiting α-glucosidase, in order to use in diabetes treatment.

Experimental

Materials

Plant materials

The aerial part of *Thymus fedtschenkoi* Ronniger was collected from Mishu-dagh Mountains (Shanjan, Shabestar, East-Azerbaijan, Iran) before its flowering stage in May 2017. The collected plant was authenticated by taxonomist Dr. Yousef Ajani and a voucher specimen was deposited in the herbarium of Research Institute of Forests and Rangelands (TARI), Tehran, Iran (Code Number: 107145).

Methods

Extraction and fractionation

The collected plant (1 kg) was macerated at room temperature with 70% methanol in water (5 L) for 72 h after shade drying and grounding. The process was repeated four times with fresh solvent. After maceration, the obtained dried hydroalcoholic extract was dispersed in minimum volume of water and fractionated successively with *n*-hexane, chloroform and *n*-butanol by liquid–liquid fractionation method. All of the fractions were concentrated and dried by rotary evaporator and vacuum oven (40 °C).

Isolation of compounds

The *n*-butanol part (10 g) was fractionated on a sephadex LH-20 column eluting with methanol to obtain 16 fractions (B1–B16). Open column chromatography of B3 with silica gel (230–400 mesh, Merck, Germany) using $CHCl_3$–MeOH–H_2O (8.5:1:0.5 to 6.5:3:0.5) as gradient solvent resulted in isolation of compounds **1** (15.2 mg) and **2** (9.1 mg). Fraction B7 (230 mg) was moved on silica gel column and eluted with $CHCl_3$–MeOH (9.5:0.5) to get compounds **2** (13.1 mg) and **3** (21.3 mg). Compounds **5** (16 mg) and **6** (15 mg) from fraction B9 (80 mg), compound **7** (23.6 mg) from fraction B11 (160 mg), and compound **8** (15.1 mg) from fraction B13 (230 mg) isolated by the same method used for fraction B3. All collected fractions

were checked by TLC under UV at 254 and 366 nm following ammonia vapor exposure in each step. The structures of the isolated compounds were elucidated using ^{1}H-NMR and ^{13}C-NMR spectral analyses, as well as comparing with published data.

α-Glucosidase inhibition assay

The inhibitory activity of the fractions and isolated compounds against α-Glucosidase were assayed with the previously published method (Adib et al. 2019; Peytam et al. 2020; Akocak et al. 2021; Markus et al. 2022; Taslimi et al. 2021). The α-glucosidase enzyme (EC3.2.1.20, *Saccharomyces cerevisiae*, 20 U/mg) was purchased from Sigma-Aldrich and sample solutions from the fractions and pure compounds were prepared at 500 µg/mL and 750 µM concentrations, respectively. The studied enzyme solution (1 U/mL, 20 µL), potassium phosphate buffer (50 mM, pH 6.8, 135 µL) and sample solution (20 µL) were added to a 96-well plate and incubated at 37 °C for 10 min. In next step after adding 25 µL *p*-nitrophenyl-α-D-glucoside (PNPG, purchased from Sigma-Aldrich) at 4 mM concentration, the plate was incubated for 20 min at 37 °C. At last, the absorbance of each sample was measured with a spectrophotometer (Gen5, PowerWave XS2, BioTek) at 405 nm and the percentage of enzyme inhibition was calculated in comparison with the negative control. The 10% Dimethyl sulfoxide solution and Acarbose were used as negative and positive control, respectively. All experiments were performed in triplicate, and results were analyzed using Sigma plot 11.0 software and expressed as mean ± SD.

Enzyme kinetic assay

For revealing the inhibition type of the most potent compound, α-glucosidase enzyme activity was computed in the absence and presence of different concentrations of the tested compound (0, 55, 120 and 185 µM) along with substrate (PNPG) at concentrations 1 to 10 mM (Adib et al. 2019; Peytam et al. 2020). The type of inhibition determined through applying a line weaver Burk plot. In addition, the Michaelis–Menten constant (K_m) value was calculated from plot between reciprocal of the substrate concentration (1/[*S*]) and reciprocal of enzyme rate (1/*V*) at different inhibitor concentrations, and the experimental inhibitor constant (K_i) value was resulted from secondary plots of the inhibitor concentrations [*I*] versus K_m.

Molecular docking study

Molecular docking of the isolated compounds in the active site of the α-glucosidase protein was conducted with Auto Dock 4 software and Auto Dock Tools (ADT 1.5.6) using the hybrid Lamarckian Genetic Algorithm (LGA). In human's intestine, Maltase-glucoamylase (MGAM) catalyzes the hydrolysis of the disaccharides. Therefore, the N-terminal subunit of maltase-glucoamylase enzyme was used for docking to better understanding of the isolated compounds-enzyme interactions. The three-dimensional (3D) crystal structure of human intestinal α-glucosidase (PDB code 2QMJ) with a resolution of 1.90 ˚A was taken from the RCSB Protein Data Bank (https://www.rcsb.org). The 3D structures of selected compounds prepared by Hyperchem.8.0.10 software. The cubic grid box was built by 50 ˚A size in dimensions ($x = -20.808$, $y = -6.586$, $z = -5.074$) in accordance with the position of the co-crystalized ligand (acarbose). All other parameters were left to default settings of AutoDock4. For validation of results, the root mean-square deviation (RMSD) was determined in comparison to re-docking of original inhibitor, acarbose, that co-crystallized with enzyme (2QMJ) and the RMSD less than 2.0 ˚A was used as threshold. The most favorable conformation by lowest free energy of binding was chosen and their target residues were determined. The result was pictured using Discovery Studio Visualizer (Biovia, D.S. Discovery Studio Modeling Environment 2015) (Askin et al. 2021; Güleç et al. 2022; Osmaniye et al. 2022; Setiawansyah et al. 2022).

Results

Isolation and structural elucidation

In consequence of chromatographical analyzing of the *n*-butanol fraction obtained from *T. fedtschenkoi* aerial part, eight phenolic compounds were isolated which were identified as: 3, 4-di-*O*-feruloyl quinic acid (**1**), luteolin-7-*O*-rutinoside (**2**), luteolin (**3**), rosmarinic acid methyl ester (**4**), rosmarinic acid (**5**), apigenin-7-*O*-glucoside (**6**), luteolin-7-*O*-glucuronide (**7**), and luteolin-7-*O*-glucoside (**8**) (Fig. 1). Molecular structures of the isolated compounds were determined with ^{1}H-NMR and ^{13}C-NMR spectral data. The elucidated structures were confirmed through comparison with published data (Chirikova et al. 2019; He et al. 2014; Hyun et al. 2015; Orhan et al. 2012; Lu and Yeap Foo 2000; Sevindik et al. 2015; Shu et al. 2013).

Spectroscopic data of isolated compounds

3, 4-Di-O-feruloyl quinic acid ($C_{27}H_{28}O_{12}$) (**1**); ^{1}H-NMR (DMSO-d_6, 400 MHz): δ 7.55 (2H, *d*, *J* = 15.8 Hz, H-7′ and H-7″), 7.32 (1H, *br s*, H-2′), 7.30 (1H, *br s*, H-2"), 7.11 (1H, *br d*, *J* = 7.8 Hz, H-6′), 7.09 (1H, *br d*, *J* = 7.8 Hz, H-6″), 6.81 (1H, *d*, *J* = 7.8 Hz, H-5′), 6.80 (1H, *d*, *J* = 8.1 Hz, H-5″), 6.48 (1H, *d*, *J* = 15.8 Hz, H-8′), 6.44 (1H, *d*,

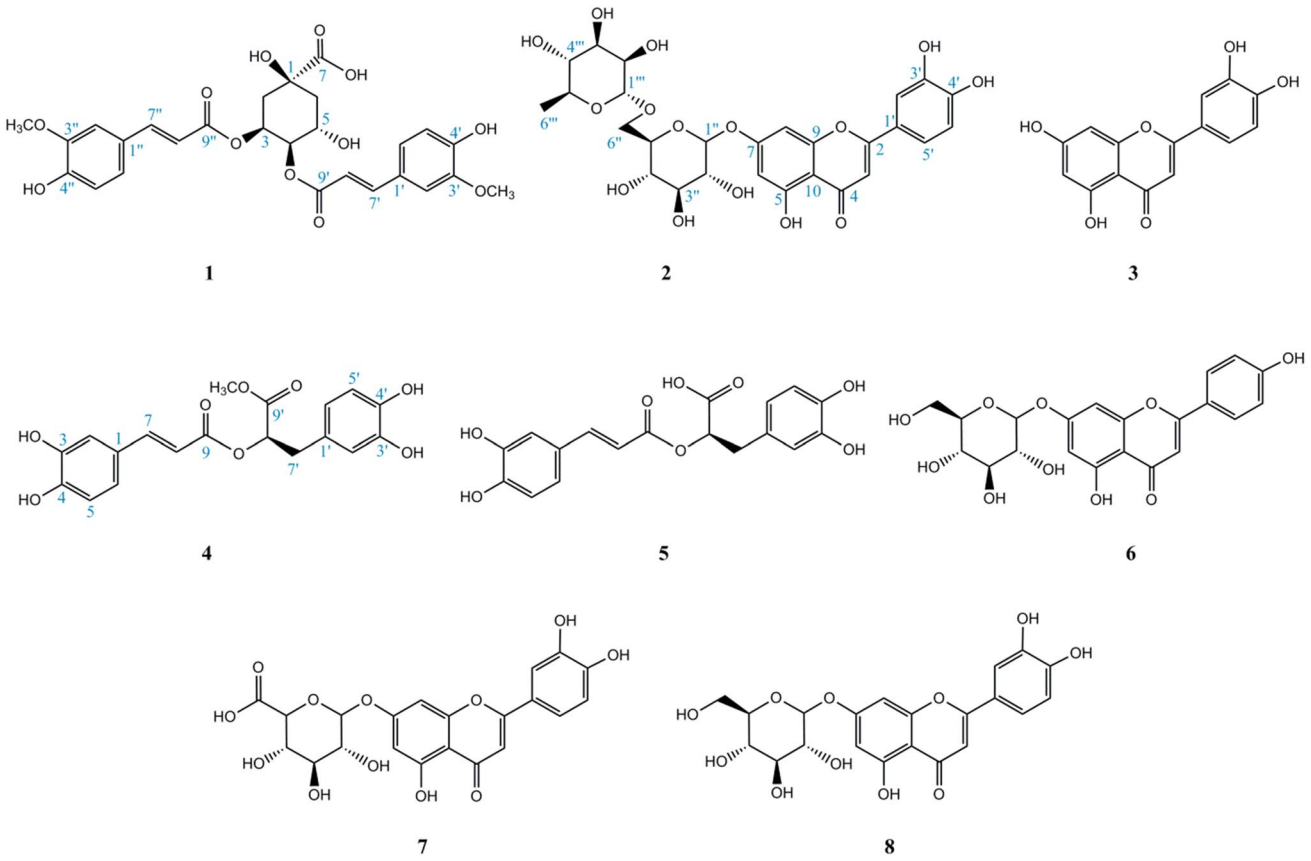

Fig. 1 Structures of the compounds isolated from *T. fedtschenkoi* aerial part (**1**–**8**)

J = 15.8 Hz, H-8″), 5.31 (1H, *br d*, J = 11.1 Hz, H-4), 4.80 (1H, *m*, H-3), 4.16 (1H, *m*, H-5), 1.5–2.0 (4H, *m*, H-2 and H-6). ^{13}C-NMR (DMSO-d_6, 100 MHz): δ 179.62 (C-7), 166.90 (C-9′), 166.48 (C-9″), 149.74 (C-4′), 149.65 (C-4″), 148.42 (C-7′), 148.39 (C-7″), 145.13 (C-3′), 144.96 (C-3″), 126.16 (C-1′), 126.09 (C-1″), 123.65 (C-6′), 123.46 (C-6″), 115.95 (C-5′), 115.95 (C-5″), 115.75 (C-8′), 115.64 (C-8″), 111.36 (C-2′), 111.31 (C-2″), 73.46 (C-1), 70.85 (C-3), 70.66 (C-5), 69.63 (C-4), 56.12 (OCH_3), 56.8 (OCH_3), 36.88 (C-2), 36.55 (C-6) (Chirikova et al. 2019).

Luteolin-7-O-rutinoside ($C_{27}H_{30}O_{16}$) (**2**); ^{1}H-NMR (DMSO-d_6, 400 MHz): δ 7.37 (1H, *br d*, J = 8.0 Hz, H-6′), 7.37 (1H, *br s*, H-2′), 6.81 (1H, *d*, J = 8.0 Hz, H-5′), 6.72 (1H, *s*, H-3), 6.67 (1H, *br s*, H-8), 6.42 (1H, *br s*, H-6), 5.05 (1H, *d*, J = 7.3 Hz, H-1″), 4.55 (1H, *br s*, H-1‴), 3.0–4.0 (11H, *overlapped signals*, H2″ to H6″ and H2‴ to H5‴), 1.08 (3H, *d*, J = 6.0 Hz, H-6‴). ^{13}C-NMR (DMSO-d_6, 100 MHz): δ 181.01 (C-4), 164.77 (C-2), 162.46 (C-7), 161.25 (C-5), 158.24 (C-9), 148.60 (C-4′), 147.48 (C-3′), 125.72 (C-1′), 118.35 (C-6′), 116.45 (C-5′), 113.83 (C-2′), 105.21 (C-1′), 103.87 (C-3), 101.59 (C-1″), 101.01 (C-1‴), 101.1 (C-6), 95.63 (C-8), 76.85 (C-5″), 76.11 (C-3″), 73.49 (4‴), 72.29 (C-2″), 70.94 (C-2‴), 70.74 (C-3‴), 70.36 (C-4″), 68.89 (C-5‴), 65.14 (C-6″), 18.23 (C-6‴) (Shu et al. 2013).

Luteolin ($C_{15}H_{10}O_6$) (**3**); ^{1}H-NMR (DMSO-d_6, 400 MHz): δ 12.95 (1H, *br s*, OH-5), 7.40 (1H, *br d*, J = 8.1 Hz, H-6'), 7.39 (1H, *br s*, H-2'), 6.88 (1H, *d*, J = 8.1 Hz, H-5'), 6.65 (1H, *s*, H-3), 6.43 (1H, *br s*, H-8), 6.17 (1H, *br s*, H-6). ^{13}C-NMR (DMSO-*d6*, 100 MHz): δ 182.11 (C-4), 164.65 (C-7), 164.33 (C-2), 161.93 (C-5), 157.74 (C-9), 150.19 (C-4'), 146.20 (C-3'), 121.91 (C-1'), 119.45 (C-6'), 116.46 (C-5'), 113.79 (C-2'), 104.12 (C-10), 103.30 (C-3), 99.29 (C-6), 94.30 (C-8) (Hyun et al. 2015).

Rosmarinic acid methyl ester ($C_{19}H_{18}O_8$) (**4**); ^{1}H-NMR (DMSO-d_6, 400 MHz): δ 7.49 (1H, *d*, J = 15.8 Hz, H-7), 7.07 (1H, *br s*, H-2), 7.02 (1H, *br d*, J = 8.1 Hz, H-6), 6.77 (1H, *d*, J = 8.1 Hz, H-5), 6.65 (1H, *br s*, H-2′), 6.64 (1H, *d*, J = 8.0 Hz, H-5′), 6.50 (1H, *br d*, J = 8.0 Hz, H-6′), 6.26 (1H, *d*, J = 15.8 Hz, H-8), 5.11 (1H, *dd*, J = 6.5 and 5.5 Hz, H-8′), 3.64 (3H, *s*, $COOCH_3$), 2.95 (2H, *m*, H-7′). ^{13}C-NMR (DMSO-d_6, 100 MHz): δ 170.37 (C-9′), 166.32 (C-9), 149.29 (C-4), 146.80 (C-7), 146.09 (C-3), 145.43 (C-3′), 144.57 (C-4′), 127.06 (C-1′), 125.64 (C-1), 122.15 (C-6), 120.49 (C-6′), 117.11 (C-2′), 116.20 (C-5), 115.35 (C-5′), 115.36 (C-2), 113.21 (C-8), 73.23

(C-8′), 52.43 (COO<u>C</u>H$_3$), 36.62 (C-7′) (Sevindik et al. 2015).

Rosmarinic acid ($C_{18}H_{16}O_8$) (**5**); ^{1}H-NMR (DMSO-d_6, 400 MHz): δ 7.42 (1H, *d*, *J* = 15.8 Hz, H-7), 7.06 (1H, *br s*, H-2), 6.98 (1H, *br d*, *J* = 8.0 Hz, H-6), 6.76 (1H, *d*, *J* = 8.0 Hz, H-5), 6.68 (1H, *br s*, H-2′), 6.62 (1H, *d*, *J* = 8.0 Hz, H-5′), 6.51 (1H, *br d*, *J* = 8.0 Hz, H-6′), 6.22 (1H, *d*, *J* = 15.8 Hz, H-8), 4.96 (1H, *dd*, *J* = 9.2 and 1.3 Hz, H-8′), 3.01 (1H, *br d*, *J* = 13.9 Hz, H-7′b), 2.84 (1H, *dd*, *J* = 13.5 and 9.2 Hz, H-7′a). ^{13}C-NMR (DMSO-d_6, 100 MHz): δ 172.13 (C-9′), 166.51 (C-9), 149.09 (C-4), 146.18 (C-7), 145.76 (C-3), 145.39 (C-3′), 144.31 (C-4′), 128.78 (C-1′), 125.86 (C-1), 121.79 (C-6), 120.36 (C-6′), 117.13 (C-2′), 116.35 (C-5), 115.87 (C-5′), 115.42 (C-2), 114.31 (C-8), 74.50 (C-8′), 37.00 (C-7′) (Sevindik et al. 2015).

Apigenin-7-O-glucoside ($C_{21}H_{20}O_{10}$) (**6**); ^{1}H-NMR (DMSO-d_6, 400 MHz): δ 7.88 (2H, *d*, *J* = 8.1 Hz, H-2′ and H-6′), 6.89 (2H, *d*, *J* = 8.1 Hz, H-3′ and H-5′), 6.81 (1H, *s*, H-3), 6.79 (1H, *br s*, H-8), 6.40 (1H, *br s*, H-6), 5.06 (1H, *d*, *J* = 7.0 Hz, H-1″), 3.1–3.9 (6H, H-2″ to H-6″). ^{13}C-NMR (DMSO-d_6, 100 MHz): δ 182.36 (C-4), 164.72 (C-2), 163.31 (C-7), 161.63 (C-5), 161.50 (C-4′), 157.39 (C-9), 129.06 (C-6′ and C-2′), 121.45 (C-1′), 116.42 (C-3′ and C-5′), 105.76 (C-10), 103.54 (C-3), 100.30 (C-1″), 99.95 (C-6), 95.38 (C-8), 77.48 (C-5″), 76.59 (C-3″), 73.39 (C-2″), 69.91 (C-4″), 60.95 (C-6″) (He et al. 2014).

Luteolin-7-O-glucorunide ($C_{21}H_{18}O_{12}$) (**7**); ^{1}H-NMR (DMSO-d_6, 400 MHz): δ 7.41 (1H, *br s*, H-2′), 7.39 (1H, *br d*, *J* = 8.0 Hz, H-6′), 6.86 (1H, *d*, *J* = 8.0 Hz, H-5′), 6.79 (1H, *br s*, H-8), 6.71 (1H, *s*, H-3), 6.42 (1H, *br s*, H-6), 5.09 (1H, *d*, *J* = 7.3 Hz, H- 1″), 3.65 (1H, *d*, *J* = 9.7 Hz, H-5″), 3.2–3.6 (3H, H-2″ to H-4″). ^{13}C-NMR (DMSO-d_6, 100 MHz): δ 182.25 (C-4), 173.01 (C-6"), 166.88 (C-2), 163.42 (C-7), 161.51 (C-5), 157.37 (C-9), 152.09 (C-4′), 146.88 (C-3′), 120.55 (C-1′), 119.53 (C-6′), 116.63 (C-5′), 113.75 (C-2′), 105.68 (C-10), 102.98 (C-3), 100.03 (C-1″ and C-6), 95.86 (C-8), 76.87 (C-3″), 74.21 (C-5″), 73.38 (C-2″), 72.38 (C-4″) (Lu and Yeap Foo 2000).

Luteolin-7-O-glucoside (cynaroside) ($C_{21}H_{20}O_{11}$) (**8**); ^{1}H-NMR (DMSO-d_6, 400 MHz): δ 7.73 (1H, *br s*, H-2′), 7.58 (1H, *br d*, *J* = 8.5 Hz, H-6′), 6.81 (1H, *d*, *J* = 8.5 Hz, H-5′), 6.63 (1H, *s*, H-3), 6.45 (1H, *br s*, H-8), 6.14 (1H, *br s*, H-6), 4.75 (1H, *d*, *J* = 7.5 Hz, H-1″), 3.1–3.8 (6H, H-2″ to H-6″). ^{13}C-NMR (DMSO-d_6, 100 MHz): δ 181.76 (C-4), 164.52 (C-2), 162.89 (C-7), 161.09 (C-5), 156.88 (C-9), 150.36 (C-4′), 145.87 (C-3′), 120.98 (C-1′), 119.09 (C-6′), 116.00 (C-5′), 113.31 (C-2′), 105.30 (C-10), 103.00 (C-3), 99.97 (C-1″), 99.55 (C-6), 95.38 (C-8), 77.12 (C-5″), 76.37 (C-3″), 73.08 (C-2″), 69.58 (C-4″), 60.63 (C-6″) (Shu et al. 2013).

α-Glucosidase inhibition assay

The inhibitory activity of the fractions and isolated compounds (**1**–**8**) were measured against α-glucosidase enzyme in contrast to standard inhibitor (acarbose). As shown in Table 1, *n*-butanol fraction demonstrated the most α-glucosidase inhibitory activity among the tested fractions with the 67.4 ± 2.1% inhibition in concentration of 500 µg/ml. Among the tested compounds, rosmarinic acid (**5**) showed the strongest α-glucosidase inhibition (IC_{50} = 43.38 ± 0.05 µM) and the compounds **4**, **6**, **8**, **3** and **7** were in the next order. In fact, except of **1** and **2**, other isolated compounds demonstrated more potency than acarbose as a standard drug with IC_{50} value of 750.0 ± 1.0 µM (Table 1).

Enzyme kinetic study

The kinetic of rosmarinic acid (**5**) as the most potent among the isolated compounds was studied. The Lineweaver–Burk and related plots showed that the K_m value increased, while V_{max} remained constant through the presence of rosmarinic acid in various concentrations. In addition, the K_i value of rosmarinic acid was calculated as 43 µM (Fig. 2).

Molecular docking study

The interactions between the isolated compounds and α-glucosidase enzyme were examined using ADT (version 1.5.6). As formerly demonstrated, there is a significant homology between catalytic domains of *S. cerevisiae* α-glucosidase (G13) and human maltase-glucoamylase

Table 1 α-Glucosidase inhibitory effects of the fractions and isolated compounds from *T. fedtschenkoi* aerial part (mean ± SD)

Samples	Inhibition[a] (%)	IC_{50} value (µM)
n-Hexane fraction	*Trace*	–
Chloroform fraction	8.2 ± 0.7	–
n-Butanol fraction	67.4 ± 2.1	–
Residual aqueous fraction	12.7 ± 0.8	–
3, 4-Di-*O*-feruloylquinic acid (**1**)	–	> 750
Luteolin-7-*O*-rutinoside (**2**)	–	> 750
Luteolin (**3**)	–	110.70 ± 1.5
Rosmarinic acid methyl ester (**4**)	–	68.3 ± 2.5
Rosmarinic acid (**5**)	–	43.38 ± 0.05
Apigenin-7-*O*-glucoside (**6**)	–	96.33 ± 0.11
Luteolin-7-*O*-glucuronide (**7**)	–	745.39 ± 0.09
Luteolin-7-*O*-glucoside (**8**)	–	108.58 ± 0.05
Acarbose (positive control)	–	750.0 ± 1.0

[a]Fractions were tested in concentration of 500 µg/ml. A dash (–) indicates to not determined

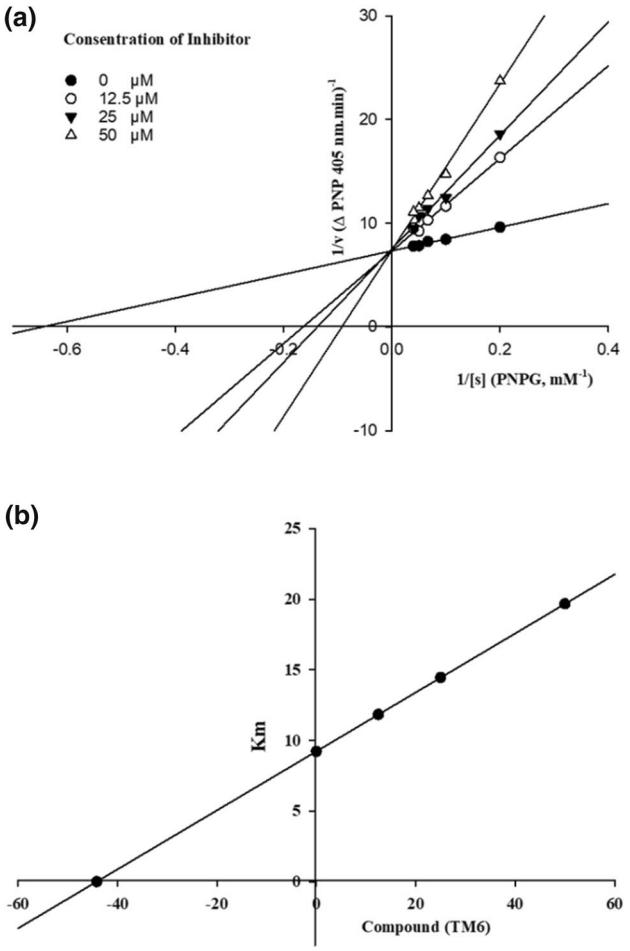

Fig. 2 **a** The Lineweaver–Burk plot in the absence and presence of compound **5** at different concentrations (µM); **b** the secondary plot between Km and various concentrations of compound **5**

enzyme (GH31). In result, the crystal structure of N-terminal domain of human intestinal α-glucosidase (PDB code: 2QMJ) was used for docking simulations (Rigden 2002; Setiawansyah et al. 2022). The structures of standard drug (acarbose) and compound **5** (rosmarinic acid) as the most potent inhibitor of the isolated compounds were superimposed in the active site of enzyme (Fig. 3). In addition, the docking poses of acarbose and the isolated compounds were shown in Fig. 4.

The -log of free binding energy and related data for the isolated compounds were briefed in Table 2. According to the resulted poses, acarbose interacted with the active site of enzyme through hydrogen bonds with residues ASP203, THR205, TYR299, MET444, ASP542, and GLN603. There are also van der waals interactions for acarbose with residues ARG202, THR204, TYR214, TRP406, ASP443, PHE450, LYS480, ARG526, PHE575, ALA576, and TYR605. The docked pose of rosmarinic acid (**5**) showed a slightly different alignment in the interaction site of enzyme, whereas the hydrogen bond interactions with residues ASP203, THR205, and GLN603 are common interacted residues between rosmarinic acid (**5**) and acarbose. The TYR299, SER448, PHE450, PHE575, GLY602, and TYR605 are residues which linked with rosmarinic acid via van der waals interactions. Rosmarinic acid (**5**) also has pi–pi interaction via its aromatic rings with residues TRP406, ASP542, and ALA576.

Discussion

Diabetes is one of the major health concerns worldwide and characterized by hyperglycemia resulted from inefficient metabolism of carbohydrates and insulin secretion. One of the strategies to control diabetes is reducing postprandial hyperglycemia via inhibiting enzymes like α-glucosidase

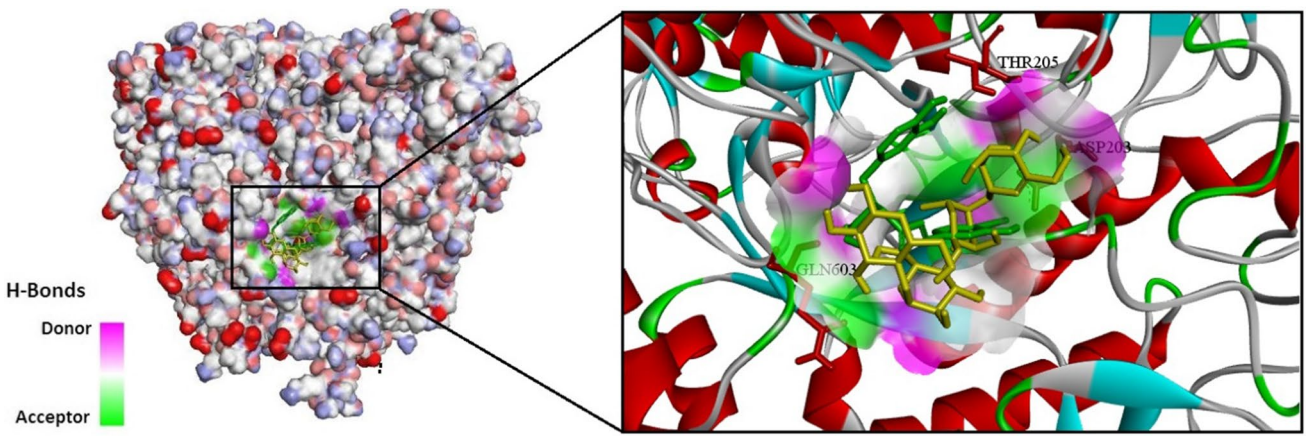

Fig. 3 Acarbose (yellow) and rosmarinic acid (green) as the most potent compound superimposed in the active site pocket

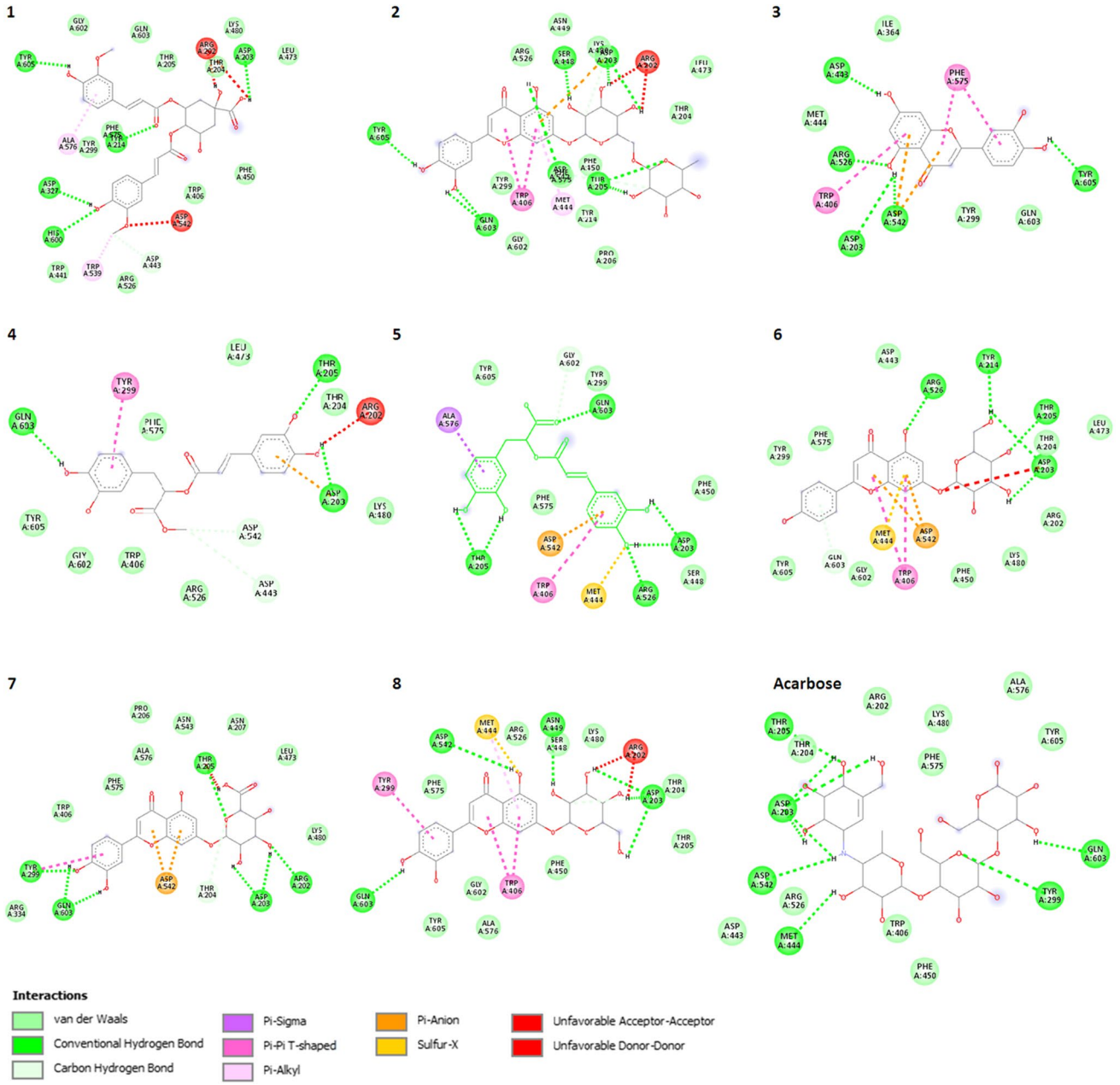

Fig. 4 Docking poses of the acarbose and the isolated compounds (**1**–**8**) in the enzyme active site

(Ruiz-Vargas et al. 2019; Uysal et al. 2019). In the current survey, the potential α-glucosidase inhibitory of *T. fedtschenkoi* various fractions assessed. Bioassay guided isolation of the *n*-butanol fraction resulted in five flavone derivatives (**2**, **3** and **6**–**8**) in addition to three caffeic acid derivatives (**1, 4** and **5**). This is the first report of all of these compounds from the aerial part of *T. fedtschenkoi*. This study also reports the isolation of 3, 4-di-O-feruloylquinic acid (**1**) from Lamiaceae family for the first time. This compound has just been detected in some limited natural sources such as some *Coffea* species (Rubiaceae), *Dacus carota* (Apiaceae), *Panax vietnamensis* (Araliaceae), *Artemisia annua* and *Lychnophora ericoides* (Asteraceae) (Chirikova et al. 2019; Gobbo-Neto and Lopes 2008; Perrone et al. 2008; Viacava et al. 2020; Zhao et al. 2015).

The most effective α-glucosidase inhibitory activity was observed for rosmarinic acid. It was not a surprising result as for this compound, the antidiabetic properties were well established in numerous previous reports (Istifli 2021; Ruiz-Vargas et al. 2019; Swain and Puttaswamy 2020; Uysal et al. 2019; Zhu et al. 2019). In fact, rosmarinic acid is one of the effective natural compounds not only in α-glucosidase

Table 2 The energy of the docked compounds against the active site of the N-terminal domain of human intestinal α-glucosidase (PDB code: 2QMJ)

Ligands	− Log FBE (kcal/mol)	Residues with polar interaction
3,4-Di-*O*-feruloylquinic acid (**1**)	− 4.65	ASP203-TYR214-ASP327-HIS600-TYR605
Luteolin-7-*O*-rutinoside (**2**)	− 6.56	ASP203-THR205-SER448-ASP542-GLN603-TYR605
Luteolin (**3**)	− 6.11	ASP203-ASP443-ARG526-ASP542-TYR605
Rosmarinic acid methyl ester (**4**)	− 5.39	ASP203-THR205-GLN603
Rosmarinic acid (**5**)	− 3.99	ASP203-THR205-ARG526-GLN603
Apigenin-7-*O*-glucoside (**6**)	− 6.72	ASP203-THR205-TYR214-ARG526
Luteolin-7-*O*-glucuronide (**7**)	− 5.64	ARG202-ASP203-THR205-TYR299-GLN603
Luteolin-7-*O*-glucoside (**8**)	− 6.45	ASP203-ASN449-ASP542-GLN603
Acarbose (positive control)	v4.69	ASP203-THR205-TYR299-MET444-ASP542-GLN603

FBE free binding energy

inhibition but also in other pathways which could be effective in diabetes treatment. Some studies found rosmarinic acid in diabetes management as effective as standard drugs like acarbose (Singh et al. 2012; Zhu et al. 2021). Considering these reports, rosmarinic acid is possibly one of the main active substances in the *n*-butanol fraction and it could make *T. fedtschenkoi* preparations as effective phytomedicine for diabetes treatment.

Luteolin, as a flavone, showed beneficial effects in diabetes, and its α-glucosidase inhibitory activity was established by copious reports (Cheng et al. 2014; Dao et al. 2021; Jia et al. 2019; Kim et al. 2000; Park et al. 2016; Yan et al. 2014). In fact luteolin and its glycosides like daidzein are well known as strong α-glucosidase inhibitors (Lodhi and Kori 2021). In the present study, among the isolated luteolin derivatives, luteolin-7-*O*-glucoside (cynaroside) had lowest IC_{50} against α-glucosidase. Kim et al. (Kim et al. 2000) and Asghari et al. (Asghari et al. 2015) studied the α-glucosidase inhibition of cynaroside. In correlation with current study, they reported good inhibition for cynaroside too. The compounds **2** (luteolin-7-*O*-rutinoside) and **7** (luteolin-7-*O*-glucuronide) showed weak inhibitory effects with IC_{50} values higher than 750 µM. However, this is the first report of α-glucosidase inhibitory activity of compound **2**, but the glucuronide derivative of luteolin was assessed by Asghari et al. (2015) and controversially, the compound **7** showed good inhibitory effect even better than cynaroside.

As shown in Fig. 4, the residues ASP203, THR205, and GLN603 are common interacted sites between all docked compounds and probably they are important residues for interaction of substrate with N-terminal domain of human intestinal α-glucosidase. The flavonoid glycosides such as compounds **2**, **7**, and **8** interacted with ASP203 and THR205 via their glycoside hydroxyls (OH in positions 3″, 4″ or even 6″). On the other hand, luteolin (**3**), as a non-glycoside flavonoid, bond to ASP203 via OH in position 5 of A-ring instead of glycoside hydroxyls and there is no reaction between luteolin and THR205. On the other hand, all of the isolated glycoside flavonoids except to compound 6, linked to GLN603 by OH of B-ring. Compound **6** and **3** (luteolin) have van der Waals reaction via B-ring with residue GLN603. This is in accordance with previous reports which claimed that flavonoids can bond to the active site residues of α-glucosidase through the hydroxyl groups in B-ring. Aromatic rings and specific distributing of electron clouds of flavonoid facilitate the donating of hydrogens in 3′ or 4′ OH groups (Liu et al. 2020; Singh et al. 2018; Xu 2010). Rosmarinic acid (**5**) reacted with residues ASP203 and THR205 by OH groups in phenyl lactic and p-coumaryl parts (3, 4, 3′, and 4′ positions) as an alternative of glycosidic hydroxyls in flavonoids, and it linked to residue GLN603 with carboxyl group of phenyl lactic part (9′ position).

The compound **6**, as an apigenin glycoside showed the second lowest IC_{50} (96.33 ± 0.11 µM) in α-glucosidase inhibition. Furthermore, the results of other studies demonstrated that apigenin-7-*O*-glucoside (**6**) is a strong α-glucosidase inhibitor (Jia et al. 2019; Villa-Rodriguez et al. 2018). Considering that apigenin-7-*O*-glucoside could be effective in diabetes treatment through improving insulin resistance, and increasing glucose uptake by HepG2 cells, this plant metabolite is a potential lead compound for antidiabetic drug development surveys (Jia et al. 2019).

Conclusions

Analyzing the *n*-butanol fraction of *T. fedtschenkoi* resulted in the isolation and identification of five flavonoids, alongside with 3, 4-di-*O*-feruloylquinic acid, rosmarinic acid and rosmarinic acid methyl ester as caffeic acid derivatives. In in vitro enzyme inhibition assay, rosmarinic acid (**5**) demonstrated a potent competitive inhibition toward α-glucosidase enzyme with the lowest IC_{50} value (43.38 ± 0.05 µM). Furthermore, apigenin-7-*O*-glucoside (**6**) and cynaroside (**7**) were showed strong inhibition of α-glucosidase. The present study suggests these phenolic compounds as appropriate

natural candidates in antidiabetic drug development research and also highlights the beneficial potentials of *T. fedtschenkoi* as a helpful herbal supplement for diabetic patients. However, further pharmacological and toxicological studies followed by clinical trial are needed to establish the efficacy and safety of this medicinal plant in management of diabetes mellitus type II.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11696-022-02511-7.

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