



Comparison of inhibitory activities of 50 *Salvia* species against α -Glucosidase

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Abstract

Objectives Type 2 diabetes is a common metabolic disease affecting millions of people worldwide. α -Glucosidase inhibitors can be used as one of the therapeutic approaches to decrease the postprandial glucose levels through the inhibition of carbohydrate hydrolysis. Medicinal plants are one of the main sources of α -glucosidase's natural inhibitors. In this study, we report the inhibitory effects of 50 different accessions of 32 *Salvia* species against α -glucosidase.

Methods To estimate the relative potency of the crude extracts, the inhibitory activities of the 80% methanol of the plants extracts were determined in three different concentrations (1000, 500 and 250 $\mu\text{g/ml}$) and compared to that of acarbose as the positive control.

Results *S. multicaulis*, *S. santolinifolia*, *S. dracocephaloides*, and *S. eremophila* were stronger inhibitors than acarbose ($p < 0.05$) with IC_{50} values in the range of 26.23–92.35 $\mu\text{g/mL}$. According to the LC-PDA-ESIMS and NMR analysis of crude extracts of the studied *Salvia* species, 8 phytochemicals including luteolin-7-*O*-glucoside (**1**) luteolin-7-*O*-glucuronide (**2**), apigenin-7-*O*-glucoside (**3**), apigenin-7-*O*-glucuronide (**4**), Hispidulin-7-*O*-glucuronide (**5**), hispidulin-7-*O*-glucoside (**6**), rosmarinic acid (**7**), carnosol (**8**) and carnosic acid (**9**) were identified as the most common α -glucosidase inhibitors. The above compounds constituted the major compounds in the active *Salvia* species in the range of 1.5–95.0%. Among them rosmarinic acid (39–95%) was detected in almost all potent α -glucosidase inhibitor species. Therefore, it can be considered as a biochemical marker in the antidiabetic *Salvia* species in addition to the other minor compounds.

Conclusions Considering the high α -glucosidase inhibitory potential of the four- out of fifty *Salvia* species, they are suggested for further *in vivo* antidiabetic tests as potential medicinal plants.

Keywords Sage · *Salvia* · α -glucosidase · Enzyme inhibition · Diabetes mellitus

Introduction

Diabetes mellitus is a chronic metabolic disorder that is known as one of the major global-health threatening disease [1]. The absolute insulin deficiency and a decreased responsiveness of the tissues to the secreted insulin can induce the type 1 and type 2 diabetes, respectively [2]. Uncontrolled type 2 diabetes is more common and can cause many complications such as cardiovascular disease, retinopathy, neuropathy and renal function recession [2]. Up to now, clinicians have introduced several medicinal approaches to treat diabetes, among which suppression of

the carbohydrate hydrolases is one of the main strategies via decreasing the postprandial hyperglycemia. α -Glucosidase is one of the enzymes with oligosaccharides hydrolyses function to yield glucose. On the other hand, the oligosaccharides themselves are resulted from the action of α -amylase on polysaccharides. Therefore, glucose is the end product of both enzymes catalytic actions on the carbohydrates and inhibition of α -glucosidase enzyme can decrease the carbohydrate digestion. As a result, the lower glucose uptake into the blood is achieved when the carbohydrate hydrolyzing enzymes; α -amylase and α -glucosidase lose their functions [3]. To inhibit α -glucosidase enzyme activity as a therapeutic approach, various synthetic inhibitors such as acarbose, miglitol and voglibose are effective [4]. However, to avoid their severe side effects, discovery of safer synthetic or natural alternative hydrolyzing-enzyme inhibitory medicines are developed [4].

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Traditional medicinal plants contain various active constituents such as triterpenoids [5–7], flavonoids and phenolic acids made them an important candidate as α -glucosidase inhibitors [8]. Among the various medicinal plants that exhibit antidiabetic properties [9], *Salvia* species are remarkable herbs, since they are important medicinal plants of the Lamiaceae family and are very popular in the folk medicine of different countries, from Americas to Asia. Previous studies on the α -glucosidase inhibitory activities and the significant properties of the *Salvia* species including *S. officinalis* L. [10], *S. nemorosa* L. [11], *S. atropatana* Bunge [11], and *S. mirzayanii* Rech. F. & Esfand [12] showed that these species can be considered as important candidates for further studies in diabetes treatments. Iran is one of the hubs for *Salvia* diversity in the Middle East [13], some of which are used as antidiabetic medicinal herb in the Iranian traditional medicine [13]. Considering their application in the folk medicine of different countries, several researchers investigate the antidiabetic properties of various *Salvia* species. For instance, ethanol extracts of six Iranian *Salvia* species (*S. hydrangea* DC., *S. hypoleuca* Benth., *S. officinalis* L., *S. reuterana* Boiss., *S. verticillata* L. and *S. virgata* Jacq.) were examined for their α -amylase properties. Among the studied species, the extracts of *S. verticillata* and *S. virgata* inhibited the enzyme activity [14]. Petroleum ether extract of an endemic sage of Iran; *S. mirzayanii* inhibited α -glucosidase, significantly [12]. The methanol (MeOH) extracts of different Iranian sages; *S. nemorosa* L., *S. atropatana* Bunge, *S. limbata* C.A. Mey., *S. syriaca* L. [11] *S. multicaulis* Vahl. [11] and *S. santolinifolia* Boiss. [15], inhibited α -glucosidase with moderate to high potential.

Although the antidiabetic effects of a few Iranian *Salvia* species has been screened, the activities such as α -glucosidase inhibition potential of the remaining Iranian *Salvia* species and characterizing their active phytochemicals remain to be explored. In our previous study, we reported the antioxidant potentials, total phenol contents and total flavonoid contents of the aerial parts of different *Salvia* species [16]. Most of the extracts exhibited high contents of phenols and flavonoids. Previous studies revealed that flavonoids and polyphenols are among the most potent natural antidiabetic agents [17, 18]. Therefore, in the present paper we report the α -glucosidase inhibitory potential of the above mentioned 50 Iranian *Salvia* species, and detect their bioactive constituents with interpreting of our earlier HPLC–PDA-MS and NMR analyses [16].

Material and methods

Chemicals and instruments

HPLC-grade methanol and acetonitrile were purchased from Merck (Darmstadt, Germany). Distilled water was prepared, using a Milli-Q system (Millipore, Bedford, MA, USA).

HPLC–PDA-MS fingerprints were measured with a Shimadzu system equipped with a SPD-M20A photo diode array detector (DAD) and a heated-electrospray ionization source (ESI).

The ^1H and APT ^{13}C NMR spectra of the extracts (in DMSO-D₆) were run on a 300 MHz Bruker Avance III. Acarbose, α -glucosidase (EC 3.2.1.20) and *p*-nitrophenyl- α -D-glucopyranoside (PNPG) were purchased from Sigma-Aldrich (Germany) and all the solvents were obtained from Merck chemical companies.

Plant material and extract preparation

The areal parts of 50 *Salvia* samples belonging to 32 different species, were collected, dried and extracted with 80% MeOH, as reported in our previous study [16]. The botanical characteristics; the collection dates and locations, the random identification code (1–50), and the herbarium voucher numbers were presented in Table 1 of that published earlier [16].

α -Glucosidase inhibition assay

α -Glucosidase (from *Saccharomyces cerevisiae*) inhibitory activity of the extracts were measured using previously described method by Park et al. [19, 20], with minor modifications. Briefly, 5 μL of 80% MeOH extracts of each of the sage extract were incubated in 96-well microplates, in three different concentrations (1000, 500 and 250 $\mu\text{g}/\text{ml}$). Then, they were diluted with 90 μL of 0.1 mM potassium phosphate buffer (pH 6.8), followed by adding 20 μL of α -glucosidase enzyme (0.25 U/mL) in phosphate buffer solution. After 10 min of incubation of the plates in the dark condition at 37 °C, 15 μL of 2.5 mM substrate (*p*-nitrophenyl- α -D-glucopyranoside) in buffer solution was added into the mixture. Then the plates were incubated for further 30 min in the same condition, before quenching the reaction by addition of 80 μL of a 0.2 M Na_2CO_3 into each well. The absorbance (A) of each of the well's solution was measured at λ 405 nm using a microplate reader (Bio-Rad Model 680). Acarbose was used as the standard drug and the percentage of α -glucosidase inhibitory activity was calculated with the following formula.

$$\% \text{Inhibition} = (A_{\text{control}} - A_{\text{extract}}) / A_{\text{control}} \times 100$$

where A_{extract} is the absorbance of the sample and A_{control} is the absorbance of the control. The IC_{50} , was expressed as the concentration (in $\mu\text{g}/\text{mL}$) of the sample that inhibited 50% of the enzyme activity, using Curve Expert software from linear regression curves.

Table 1 α -Glucosidase inhibitory activity (%) of the *Salvia*'s 80% MeOH extracts

Plant Name	α -glucosidase inhibitory (%)			Plant Code No. ^c
	1000 ($\mu\text{g/mL}$)	500 ($\mu\text{g/mL}$)	250 ($\mu\text{g/mL}$)	
<i>S. atropatana</i>	65.57 \pm 0.77	62.39 \pm 2.26	16.49 \pm 2.22	8
<i>S. atropatana</i>	4.71 \pm 0.85	NA ^a	NA	38
<i>S. atropatana</i>	46.86 \pm 4.72	22.78 \pm 3.45	7.95 \pm 3.08	46
<i>S. bracteata</i>	51.71 \pm 2.66	33.50 \pm 1.48	7.37 \pm 1.00	3
<i>S. bracteata</i>	65.19 \pm 0.75	48.29 \pm 2.19	22.99 \pm 1.80	28
<i>S. candidis-sima</i>	39.10 \pm 1.88	9.68 \pm 2.16	NA	35
<i>S. ceratophylla</i>	— ^b	4.25 \pm 2.82	NA	20
<i>S. compressa</i>	41.57 \pm 4.00	26.87 \pm 0.52	NA	18
<i>S. compressa</i>	57.15 \pm 4.67	42.33 \pm 2.54	22.91 \pm 4.28	50
<i>S. dracocephaloides</i>	66.35 \pm 2.04	47.68 \pm 1.46	28.48 \pm 0.55	7
<i>S. dracocephaloides</i>	89.10 \pm 1.45	85.25 \pm 0.29	71.85 \pm 0.27	25
<i>S. eremophila</i>	94.02 \pm 1.08	86.30 \pm 1.13	96.06 \pm 0.87	22
<i>S. grossheimii</i>	10.83 \pm 2.21	NA	NA	12
<i>S. hydrangea</i>	7.83 \pm 1.73	NA	NA	19
<i>S. indica</i>	10.17 \pm 4.12	NA	NA	26
<i>S. lachnocalyx</i>	35.96 \pm 1.95	14.09 \pm 2.81	NA	14
<i>S. limbata</i>	29.71 \pm 3.43	2.78 \pm 1.64	NA	10
<i>S. limbata</i>	19.83 \pm 2.01	2.21 \pm 0.50	NA	33
<i>S. macrochlamys</i>	56.45 \pm 2.70	21.39 \pm 4.39	NA	6
<i>S. macrosiphon</i>	NA	NA	NA	15
<i>S. macrosiphon</i>	11.31 \pm 1.98	NA	NA	39
<i>S. macrosiphon</i>	34.55 \pm 2.83	NA	NA	44
<i>S. mirzayanii</i>	57.43 \pm 4.59	36.21 \pm 2.45	NA	2
<i>S. multicaulis</i>	91.96 \pm 1.07	77.79 \pm 1.22	70.29 \pm 2.80	29
<i>S. multicaulis</i>	94.27 \pm 1.53	88.47 \pm 1.08	80.13 \pm 1.85	31
<i>S. nemorosa</i>	70.15 \pm 2.93	29.78 \pm 2.73	20.89 \pm 0.92	23
<i>S. nemorosa</i>	35.73 \pm 3.68	22.14 \pm 4.74	9.59 \pm 0.78	32
<i>S. nemorosa</i>	23.55 \pm 3.76	4.51 \pm 1.35	NA	43
<i>S. nemorosa</i>	28.65 \pm 1.51	9.41 \pm 0.32	7.13 \pm 1.37	49
<i>S. pachystachys</i>	49.78 \pm 2.82	28.79 \pm 1.68	NA	13
<i>S. palaestina</i>	36.12 \pm 0.78	16.34 \pm 2.28	NA	36
<i>S. reuterana</i>	43.49 \pm 1.00	25.22 \pm 4.38	8.89 \pm 3.18	47
<i>S. reuterana</i>	—	NA	NA	27
<i>S. russellii</i>	—	49.50 \pm 2.51	29.56 \pm 0.58	4
<i>S. sahendica</i>	60.22 \pm 3.89	24.28 \pm 0.68	NA	5
<i>S. sahendica</i>	39.57 \pm 1.12	16.56 \pm 1.67	9.47 \pm 1.25	45
<i>S. santolinifolia</i>	94.35 \pm 1.63	91.68 \pm 2.08	96.18 \pm 2.28	21
<i>S. santolinifolia</i>	85.85 \pm 3.39	84.09 \pm 2.48	81.80 \pm 1.89	48
<i>S. sclarea</i>	61.49 \pm 3.38	19.71 \pm 2.23	NA	11
<i>S. sclareopsis</i>	48.87 \pm 3.88	NA	NA	1
<i>S. sharifii</i>	9.84 \pm 2.04	NA	NA	17
<i>S. spinosa</i>	44.96 \pm 3.31	5.85 \pm 1.99	NA	34

Table 1 (continued)

Plant Name	α -glucosidase inhibitory (%)			Plant Code No. ^c
	1000 ($\mu\text{g/mL}$)	500 ($\mu\text{g/mL}$)	250 ($\mu\text{g/mL}$)	
<i>S. spinosa</i>	—	21.65 \pm 1.51	NA	41
<i>S. syriaca</i>	NA	NA	NA	16
<i>S. syriaca</i>	—	NA	NA	40
<i>S. urmiensis</i>	20.39 \pm 1.11	20.65 \pm 3.10	NA	30
<i>S. verticillata</i>	—	13.80 \pm 1.49	NA	9
<i>S. verticillata</i>	26.82 \pm 2.02	NA	NA	24
<i>S. verticillata</i>	—	52.21 \pm 4.10	29.20 \pm 3.83	42
<i>S. xanthocheila</i>	26.57 \pm 2.82	6.32 \pm 2.99	NA	37
Acarbose	87.88 \pm 0.57	76.69 \pm 0.23	66.03 \pm 1.83	

Values are presented as mean \pm SE of three experiments

^a NA: Not active at the tested concentration

^b —: Color and absorbance limitation at high concentration

^c Plant Code No.: the random codes were devoted to each species to avoid any systematic error in determination of the same species from different locations

LC–MS, ¹H NMR and APT ¹³C NMR analysis

LC–MS and NMR spectroscopy analyses of the plant extracts were performed previously to detect the metabolites present in the plant extracts[16].

Statistical analysis

Each experiment was repeated three or four times. The results express as Mean \pm standard error (SE). Statistical significance was determined using SPSS software and accepted at $P < 0.05$. The IC_{50} values were calculated with the Curve-Expert software, version 1.6.5, for Windows.

Result

In vitro α -glucosidase inhibition

The *in vitro* α -glucosidase inhibitory activity percentage (%) of the 80% MeOH extracts of the sage samples in three different concentrations (250, 500 and 1000 $\mu\text{g/mL}$) were measured and compared to those for acarbose as the standard drug (Table 1 and Fig. 1). The highest inhibitory activity was found in *S. santolinifolia* (S21) with 91.68 \pm 2.08 percent. While *S. limbata* (S33) had the lowest inhibitory activity with 2.21 \pm 0.50 percent. Plant extracts including *S. multicaulis* (S29 and S31), *S. santolinifolia* (S21 and S48), *S. dracocephaloides* (S25), *S. eremophila* (S22) were stronger than that obtained for acarbose ($p < 0.05$). Therefore, The IC_{50} values of the strongest plant extracts were evaluated and shown in Table 2.

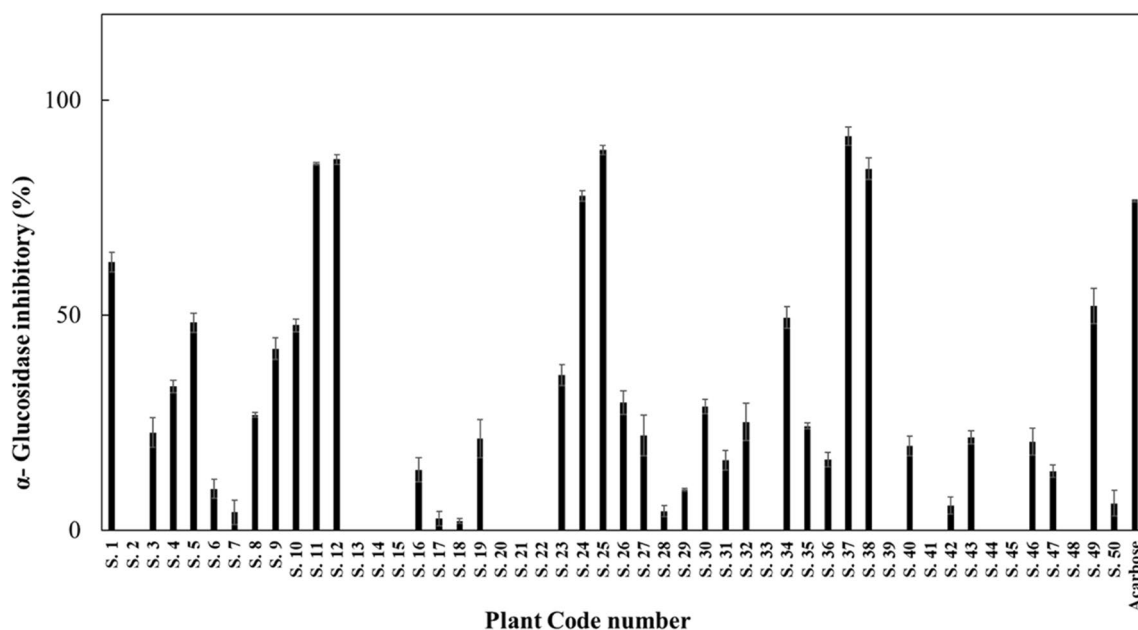


Fig. 1 Bar graph showing the α -glucosidase inhibitory activity of *Salvia*'s 80% MeOH extracts of the fifty crude extracts at concentration of 500 ($\mu\text{g/mL}$). Results shown are the average value of three replicates with mean \pm SD ($n=3$)

Table 2 α -Glucosidase inhibitory potential of the most active plant extracts expressed as half maximal inhibitory concentration (IC_{50})

Plant Code No	Plant Name	IC_{50} ($\mu\text{g/mL}$)
25	<i>S. dracocephaloides</i>	92.35 ± 4.99
22	<i>S. eremophila</i>	33.28 ± 0.22
29	<i>S. multicaulis</i>	30.76 ± 0.86
31	<i>S. multicaulis</i>	78.03 ± 4.23
21	<i>S. santolinifolia</i>	26.23 ± 2.05
48	<i>S. santolinifolia</i>	63.35 ± 5.24
	Acarbose	182.65 ± 1.63

^a Values represent as means \pm S.E.M

α -Glucosidase kinetic studies

S. santolinifolia (S21), as the most effective α -glucosidase inhibitor, was selected for the study of its enzyme kinetic. Lineweaver–Burk plot of α -glucosidase inhibitory activity of the 80% MeOH extract of *S. santolinifolia* were examined at 0, 1 and 2 mg/mL stock solution, with different substrate concentrations of PNPG (1–5 mM) (Fig. 2) [21]. Data analysis revealed that increasing the concentration of the plants' extract, decreases the V_{max} and also K_m by 2.1, 0.41 and 0.20 mM, respectively (Fig. 2).

HPLC fingerprints of the potent *Salvia* extracts

To characterize the bioactive chemical constituents of the most potent α -glucosidase inhibitors, *S. multicaulis* (S29 and

S31), *S. santolinifolia* (S21 and S48), *S. dracocephaloides* (S25) and *S. eremophila* (S22), their HPLC fingerprints were reconsidered (Fig. 3).

In the previous publication we have characterized 13 phytochemicals in the plants' extracts using LC-UV-ESIMS, ^1H - and ^{13}C NMR spectral data [16]. In the present report we have detected 8 of those compounds in the enzyme inhibitory-active extracts using reconsideration of their spectroscopic data. They include luteolin-7-*O*-glucoside (1) luteolin-7-*O*-glucuronide (2), apigenin-7-*O*-glucoside (3), apigenin-7-*O*-glucuronide (4), hispidulin-7-*O*-glucuronide (5), hispidulin-7-*O*-glucoside (6), rosmarinic acid (7), carnosol (8), and carnosic acid (9) (Figures 3 and 4). In addition, in *S. multicaulis* (S29) HPLC chromatogram, the peaks at Rt 23.8 and 25.4 min. presented the same pattern of UV spectrum as recorded for compound 7, but in their –ESIMS, two peaks at m/z 555, 493 and 717, 563 were compatible for the molecular formula $\text{C}_{27}\text{H}_{24}\text{O}_{13}$ and $\text{C}_{36}\text{H}_{30}\text{O}_{16}$ of salvianolic acid A derivative and salvianolic acid B, respectively. In addition, three peaks at Rt 48.0, 48.4, 49.3 min. were due to abiatane diterpenoids based on their UV (λ_{max} 222; 222, 293; 222 nm, respectively), ^1H NMR (Fig. 4) and MS spectral data with –ESIMS peaks at m/z 361, 343 and 345, respectively.

Discussion

In the present paper, the α -glucosidase inhibition effects of the *Salvia* species are measured and then their active phytochemicals are characterized.

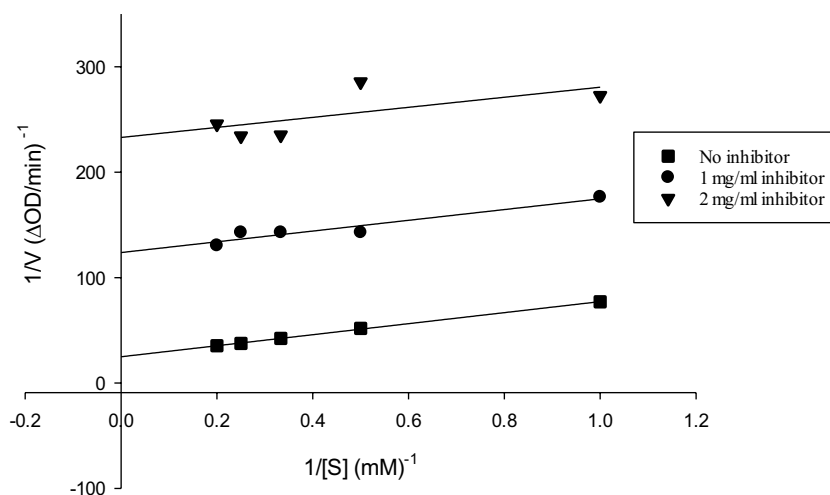


Fig. 2 Lineweaver–Burk plot analysis of the inhibition kinetics of α -glucosidase inhibitory effects by the extract of 80% MeOH *Salvia santolinifolia* as inhibitor. [α -Glucosidase]=0.25 U/mL; in 0, 1 and 2 mg/mL inhibitor. pH 6.8, at room temperature

In addition to the best *Salvia* species that showed better enzyme inhibition power than that of the standard drug (Table 2), some of them exhibited strong and medium enzyme inhibitory activity that can be concluded according to their inhibition %. Some of the tested extracts like *S. atropatana* (S8), *S. russellii* (S4) and *S. verticillata* (S42) showed 50% inhibition between 250 and 500 μ g/mL concentrations, while the others like *S. bracteata*, (S3, 28), *S. compressa* (S18), *S. dracocephaloides* (S7) *S. macrochlamys* (S6), *S. mirzayanii* (S2) *S. nemorosa* (S23), *S. pachystachys* (S13), *S. sahendica* (S5) and *S. sclarea* (S11) displayed 50% α -glucosidase inhibition in the range of 500–1000 μ g/mL. The rest of plant extracts were suggested as less active exhibiting less than 50% inhibition with 1000 μ g/mL concentration. Our results confirmed the enzyme inhibitory activities reported for some of the species in the earlier investigations [11, 12, 14, 15].

The total phenolic content (TPC), total flavonoid content (TFC) and their antioxidant activities of the under studied sages were reported in our previous paper [16]. Unlike the positive correlation between antioxidant activity, TPC and TFC, we found low positive relationship between TPC, TFC, antioxidant activity and α -glucosidase inhibition (R^2 is in the range of 0.22–0.25, excluding non-active species). These results suggest that the enzyme inhibitory activities of the *Salvia* extracts depend on the quality of polyphenols and flavonoids rather than their quantities [14]. Another reason is that some of the sages like *S. grossheimii* consisted other types of phytochemicals like triterpenoids that inhibited the enzyme [5, 7].

Also, the kinetic study of the 80% MeOH extract of *S. santolinifolia* (S21) suggested the uncompetitive mechanism for the α -glucosidase inhibition, but two of its major constituents, rosmarinic acid (7), carnosol (8), and carnosic acid

(9) were reported to be a competitive and non-competitive inhibitors, respectively [22, 23].

HPLC–UV fingerprints of the most potent α -glucosidase inhibitor species were evaluated to characterize their bioactive chemical constituents (Fig. 3). The identified compounds are belonging to hydroxycinnamic acid derivatives, flavonoids and phenolic diterpenoids categories. Among the identified compounds, compound 7 is a key biomarker of the *Salvia* species and constituted as the most abundant compounds, ranging from 39.2–95.1% in the active extracts of *Salvia* species. Carnosol and carnosic acid are the two bioactive substances that were identified as the major constituents of *S. eremophila* and *S. santolinifolia*. Compounds 2 (11.9%, 16.7%) was also found in *S. multicaulis* (S29 and S31), respectively (Table 3). The remaining compounds are less distributed in the plant extracts. However, all of them are α -glucosidase inhibitors. Compound 1 and 6 were previously isolated from *S. dracocephaloides* [16, 24–26].

Most of the detected compounds in the potent enzyme inhibitor extracts have been reported with effective roles in controlling the blood glucose levels. For instance compound 1 exhibited *in vitro* and *in vivo* antidiabetic effects [27, 28]. It (1) was also isolated from aerial parts of *S. chloroleuca* and showed potent α -glucosidase inhibitory effect [29]. Compound 3 showed antidiabetic effects and enhanced adiponectin secretion and caused phosphorylation of insulin receptor- β , and GLUT4 translocation [30]. Compound 4 was a moderate α -glucosidase with IC_{50} value of 543.28 ± 11.41 μ g/mL [31]. Finally, compound 7 which was the major phenolic compound in all the extracts and was reported as an important antidiabetic agents *in vivo* [32] and *in vitro* [33] tests. As a chemical marker, rosmarinic acid was isolated from *S. multiorrhiza* and reported to be stronger α -glucosidase inhibitor than acarbose [34].

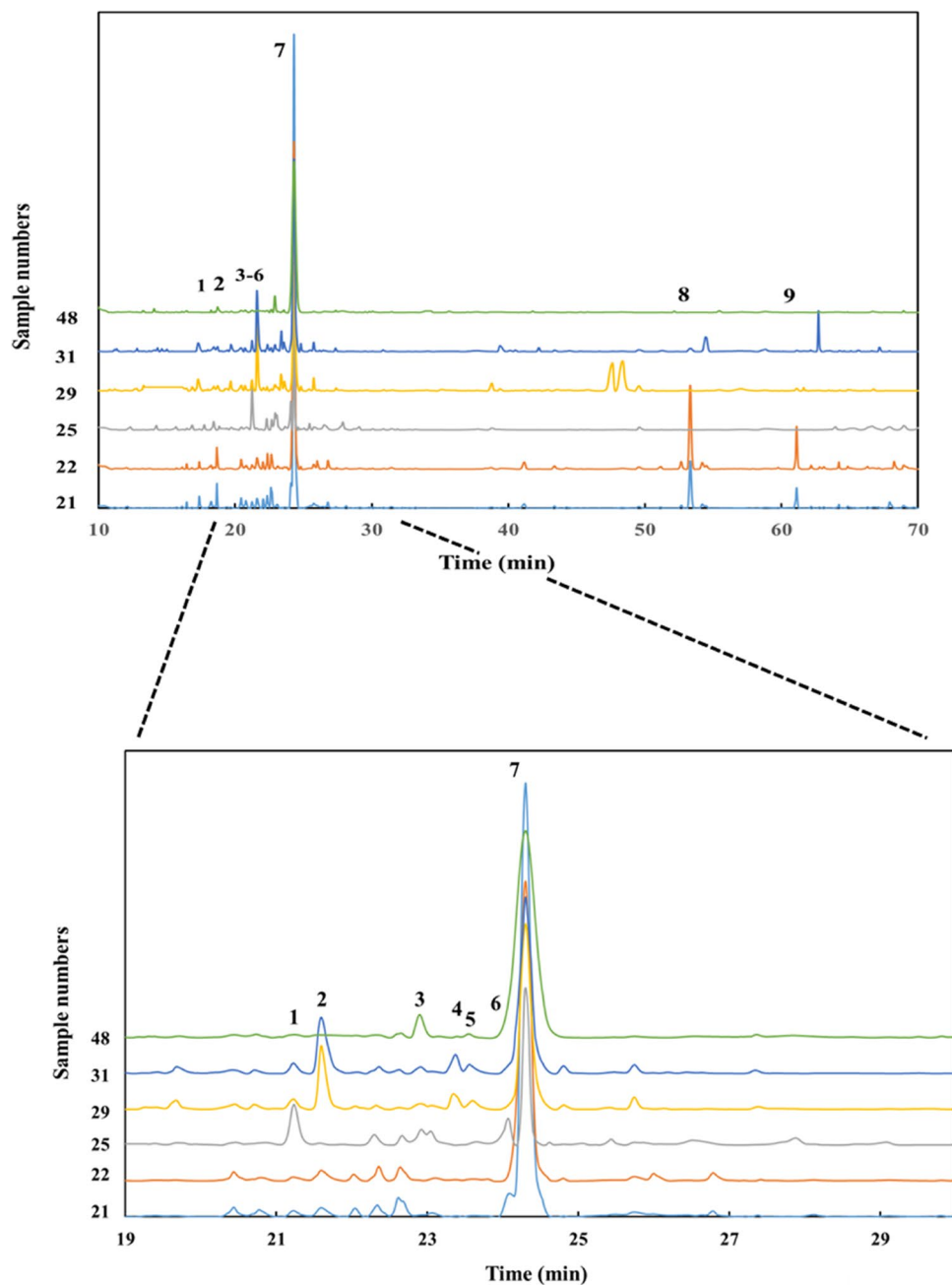


Fig. 3 Chromatographic fingerprints of 6 *Salvia* samples; *S. santolinifolia* (S21 and 48), *S. eremophila* (S22), *S. dracocephaloides* (S25), and *S. multicaulis* (S29 and S31). More details are visualized

in the zoom plot. Peak numbers refer to components listed in Table 3. Mobile phase: water (formic acid 0.1%) and acetonitril (formic acid 0.1%). Detection is recorded at average absorbance of λ 190–600 nm

Hence, rosmarinic acid is likely to be the most important potent α -glucosidase inhibitor in the examined *Salvia* species. Carnosol (8), is another chemical marker of some *Salvia* species which was reported to be stronger α -glucosidase inhibitor than the standard drug, acarbose [35]. Moreover, the oral administration of 8 by normal mice reduced their postprandial blood glucose levels [35]. The last significant peak belongs to carnosic acid (9) that showed both α -amylase and α -glucosidase inhibitory

activity, significantly. The mechanisms of action, *in silico* and *in vivo* studies suggested compound 9 as a potent anti-diabetic diterpenoid [23]. Among the tested samples the plant extracts that were rich in carnosol, carnosic acid and rosmarinic acid showed the lowest IC_{50} s. The presence of these compounds were deduced based on their ESIMS [16] and 1H NMR analyses (Fig. 4) [16].

Screening α -glucosidase inhibition-potential of medicinal plants is an alternative way to find new drugs with lower side

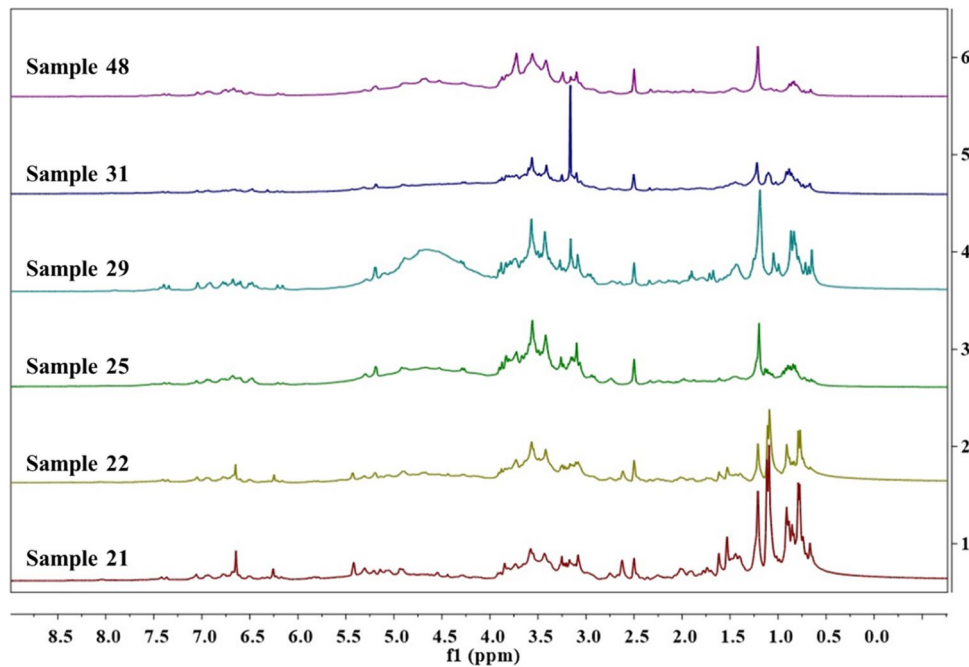


Fig. 4 ^1H NMR spectrum of the *Salvia* extracts that have the significant α -glucosidase inhibitory activity including *S. santolinifolia* (S21 and 48), *S. eremophila* (S22), *S. dracocephaloides* (S25), and *S. multicaulis* (S29 and S31)

Table 3 The HPLC area percentage of the major α -glucosidase inhibitors detected in the bioactive plant extracts

Peak No	RT (min)	Putative Annotation	Area % in plant
1	21.27	Luteolin-7- <i>O</i> -glucoside (cynaroside)	S25 (13.39%)
2	21.57	Luteolin-7- <i>O</i> -glucuronide	S29 (10.43%) S31 (16.67%)
3	22.80	Apigenin-7- <i>O</i> -glucoside (cosmosiin)	S48 (4.38%)
4	23.40	Apigenin-7- <i>O</i> -glucuronide	S25 (4.46%) S31 (4.84%)
5	23.57	Hispidulin-7- <i>O</i> -glucuronide	S25 (1.5%) S31 (1.99%)
6	24.10	Hispidulin-7- <i>O</i> -glucoside (Homoplantaginin)	S21 (2.37%) S25 (7.96%)
7	24.33	Rosmarinic acid	S21 (72.66%), S22 (58.33%), S25 (39.16%), S29 (44.44%), S31 (63.36%), S48 (95.61%)
8	53.3	Carnosol	S21 (8.31%), S22 (14.88%)
9	61.15	Carnosic acid	S21 (2.91%), S22 (5.93%)

effects compared to those of common medicines with the same mechanisms of action such as acarbose, voglibose and miglitol. One of these class of compounds are polyphenols that play a key role as antioxidants, which enable them to neutralize the harmful free radicals and to stop damaging the cells and reduce the risk of harmful cardiovascular, cancer and diabetes disease [36]. Via the carbohydrate hydrolyzing-enzyme inhibition, polyphenols prevent the breakdown of starch to the simple sugars, resulting in decreasing the blood glucose levels after meal consumption. In addition, they can stimulate the secretion of insulin to keep the blood sugar

levels in a steady state [37]. Furthermore, polyphenol-rich diets could improve the glucose tolerance and insulin sensitivity to decrease the risk of type 2 diabetes, however they have unpleasant taste [38]. Since *Salvia* species are a rich source of novel bioactive polyphenolics we may consider them as an alternative medicine for treatment of diabetes after further detailed clinical tests. In addition to the crude extracts of the plant material whose chemical constituents are characterized in this research, synthetic or biotechnological approaches can afford the antidiabetic natural compounds to perform the required *in vivo* diabetic tests may be

suggested for development of new drugs. The above mentioned approaches may be a way to overcome the limitations such as the lack of enough plant material, or their active constituents. While application of crude standardized medicinal plant preparations can reduce the cost of purified natural products as antidiabetic medicines.

Conclusion

In conclusion, the α -glucosidase inhibitory activity of 50 different accessions of 32 *Salvia* species were evaluated to introduce the potent medicinal herbs for diabetes treatments. Among the tested extracts, six species showed better enzyme inhibition than acarbose, while three species were comparable and ten are moderate α -glucosidase inhibitors according to the inhibition potency of the standard drug. Among them eight *Salvias*; *S. russellii*, *S. bracteata*, *S. compressa*, *S. dracocephaloides*, *S. macrochlamys*, *S. pachystachys*, *S. sahendica* and *S. sclarea* are reported as potential antidiabetic plants for the first time. Among the characterized bioactive compounds in the potent plants' extracts, rosmarinic acid was both the main and the most common constituent, therefore it may be considered for further clinical tests. Finally, we suggest the *Salvia* species as appropriate antidiabetic medicinal plants, and their bioactive substances such as carnosol, the flavonoid derivatives of apigenin and luteoline for further *in vivo* and clinical tests. The presence of salvianolic acids and abietane diterpenoids in the extract of *S. multicaulis* (S29) together with its high α -glucosidase activity suggest these derivatives of rosmarinic acids and diterpene phenols may play the key role in the antidiabetic activity of the sage plants.

Declarations

Conflict of Interest The authors declared that they have no conflict of interest. The financial support of the present research was provided by the research council of Shiraz University of Medical Sciences, Shiraz, Iran (grant no. 17131). Amir Reza Jassbi is the mentor and founder of a startup company "Aryana Phytochemistry of Tirazis".

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