Alpha-amylase inhibitory activity and phytochemical study of *Zhumeria majdae* Rech. f. and Wendelbo

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ABSTRACT

Background: *Zhumeria majdae* (Lamiaceae) is an endemic species growing in the South parts of Iran especially Hormozgan province. The plant is so-called Mohrekhosh locally and widely used for medicinal purposes including stomachache and dysmenorrhea. **Objective:** In order to separation and identification of the main flavonoid glycosides of the plant (aerial parts including leaves, stems, flowers, and fruits were used) and evaluation of its alpha-amylase inhibitory (AAI) activity, methanolic extract was prepared and fractionated to botanolic portion. **Materials and Methods:** Isolation of the main compounds of the butanol extract of the plant have been performed using different column chromatography methods such as high-performance liquid chromatography (C_{18} column) and Sephadex LH-20 as well. The isolated compounds were identified by Hydrogen-1 nuclear magnetic resonance and Carbon-13 nuclear magnetic resonance spectra and comparison with those reported in previous literature. Moreover, inhibitory activity of the butanolic extract of the plant against alpha-amylase enzyme was examined in different concentrations (15–30 mg/mL), where acarbose used as a positive control. **Results:** Three flavonoid glycosides: Linarin (1), hispidulin-7-O-(4-O-acetyl-rutinoside) (2), hispidulin-7-O-rutinoside (3) were successfully identified in the extract. The activity of alpha amylase enzyme was dose-dependently suppressed by the butanol extract. The extract exhibited the highest inhibition at 30 mg/mL toward enzyme (77.9 ± 2.1%), while acarbose inhibited the enzyme at 20 mg/mL by 73.9 ± 1.9%. The inhibitory concentrations of 50% for the extract and acarbose were calculated at 24.5 ± 2.1 and 6.6 ± 3.1 mg/mL, respectively. **Conclusion:** *Z. majdae* contains glycosylated flavones and could be a good candidate for anti-diabetic evaluations in animal and clinical trials due to possessing AAI activity.

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INTRODUCTION

Today, diabetes is one of the main systemic illnesses in a significant proportion of the population worldwide.[1] In the treatment of diabetes and some other metabolic disorders (like obesity and periodontal diseases), alpha-amylase inhibitory (AAI) are playing a significant role. AAIs originate from both plants and synthetic resources, of which medicinal plants are applied as therapeutic or functional food resources. Recently, there have been phytochemicals and plant species found as AAIs, particularly whose constituents belong to the phenolic compounds.[2] Undoubtedly, traditional medicine may bring some evidences for more investigations on the application of medicinal plants in treatment of diabetes.[3] For this reason, some researchers have focused on finding the anti-diabetic constituents from natural origin.[4] Among Iranian medicinal plants, *Zhumeria majdae,* belonging to the Lamiaceae family, is an endemic species in the South parts of Iran especially Hormozgan province. The plant is locally named Mohrekhosh, and its flowering aerial parts have widely been used for medicinal purposes including stomachache and dysmenorrhea.[5,6] So far, antinociceptive, anti-inflammatory, and acute toxicity of *Z. majdae* extracts (aqueous infusion and ethanolic maceration of the

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aerial parts of the plant) have been reported in mice and rats.[5] The authors revealed that both the extracts possessed anti-nociceptive activity against acetic acid-induced writhing, which was partially blocked by naloxone. Furthermore, both extracts exhibited considerable activity against acute inflammation, induced by acetic acid in mice. Furthermore, efficacy of the extracts was the same as baclofen and dexamethasone in chronic inflammation test. Moreover, the plant extracts had anti-inflammatory effects in both acute and chronic inflammation models.[5]

In another study on this plant, the gas chromatography and gas chromatography mass spectrography analysis of the essential oil (obtained from aerial parts of the plant) were reported, and the major compounds identified as linalool (53.28%) and camphor (26.15%). Furthermore, the authors demonstrated that the oil possessed antibacterial activity against *Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Staphylococcus epidermidis, Bacillus subtilis,* and *Pseudomonas aeruginosa*. Moreover, the oil and methanolic extract reduced the stable free radical 2, 2-diphenyl-1-picrylhydrazyl with an inhibitory concentrations of 50% (IC_{50}) of 20.5 ± 1.6 and 26.1 ± 1.5, respectively.[7] On the other side, the methanolic extract (80%) of this plant has been studied for its antiviral activity resulting in inhibitory effects against herpes simplex type 1.[8] Literature reviews showed that there is no report on isolation and identification of the glycosylated flavonoids as the main active components of the polar extracts of this plant, and also there is no paper on its anti-diabetic activity, although there have been some reports of anti-diabetic and/or AAI activity of several Lamiaceae plants. Therefore, in the present study, we aimed to evaluate AAI activity of the methanolic and butanolic extracts of this plant and also the main flavonoid constituents thereof.

MATERIALS AND METHODS

Experimental

Precoated silica gel 60F254 plates (Merck TM) were used for thin layer chromatography. Spots were detected by spraying anisaldehyde-H_2SO_4 reagent followed by heating. Hydrogen-1 nuclear magnetic resonance (^{1}H-NMR) and Carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectra were measured on a Bruker Avance TM 500 DRX (500MHz for ^{1}H and 125MHz for ^{13}C) spectrometer with tetramethylsilane as an internal standard; chemical shifts are given in δ (ppm).

Plant material

Zhumeria majdae Rech. f. and Wendelbo was collected from Hormozgan province during summer 2012. The plant was identified by Mr. Yousef Ajani (one of the authors) and a voucher specimen (1651) was deposited at the Herbarium of the Institute of Medicinal Plants, Iranian Academic Centre for Education, Culture and Research.

Isolation process

The aerial parts of the plant (including leaves, stems, flowers and fruits) were dried at shade and extracted (730 kg) with methanol at room temperature three times, each of which was about 24 h by percolation method. The extract was concentrated by rotary evaporator and dried by freeze drier. The methanolic extract (130 g) was then washed by petroleum ether to remove most parts of chlorophyll and fatty acids. Afterward, 100 g of the washed extract was dispersed in distilled water and decanted by butanol three times. The butanolic extract was concentrated to 20 g dried fraction, of which 1 g was submitted to Sephadex LH_{20} CC with aqueous methanol (50%) to gain 10 fractions B1-B10.

Fraction B7 (132 mg) was subjected to high performance liquid chromatography with a semi-preparative C18-reverse phase column eluted by water: Methanol (45:55) for 110 min, and then water: Methanol (25:75) for more 70 min. Photodiod-array detector recorded three pure peaks at 310 nm belonging to the compounds 1 (9.4 mg; Rf = 0.64 on reverse-phase-thin layer chromatography with aqueous: Methanol, 25:75), 2 (10.2 mg; Rf = 0.0.59 on RP-TLC with aqueous: Methanol, 25:75), and 3 (6.4 mg; Rf = 0.42 on RP-TLC with aqueous: Methanol, 25:75), respectively.

Alpha-amylase inhibition

The AAI assay was performed by some modification in the method proposed by Giancarlo *et al.*,[9] The starch solution (1% w/v) was obtained by boiling and stirring 1 g of potato starch in 100 mL of sodium phosphate buffer for 30 min. The enzyme (EC 3.2.1.1) solution (50 unit/1 mL) was prepared by mixing 0.01 g of α-amylase in 10 mL of sodium phosphate buffer (pH 6.9) containing 0.0006 mM sodium chloride. The extracts were dissolved in dimethyl sulfoxide (DMSO) to get concentrations as 20, 25, and 30 mg/mL. The color reagent was a solution containing 0.1 g of 3, 5-dinitrosalicylic acid plus 2.99 g sodium potassium tartrate in 0.16 g sodium hydroxide and phosphate buffer (10 mL).

Fifty microliter of each plant extract and 150 μL of starch solution, as well as 10 μL of enzyme were mixed all together in a 96 well plate and incubated at 37°C for 30 min. Then, 20 μL of sodium hydroxide and 20 μL of color reagent were added and the closed plate placed into a 100°C water bath. After 20 min, the reaction mixture was removed from the water bath and cooled, thereafter α-amylase activity was determined by measuring the absorbance of the mixture at 540 nm in Elisa stat fax 2100 (Awareness Technology Inc.,). Blank samples were used to correct

the absorption of the mixture, in which the enzyme was replaced with buffer solution. Furthermore, a control reaction was used, in which the plant extract was replaced with 50 μ of DMSO to determine the maximum enzyme activity. Acarbose solutions (the concentrations: 5, 10, 15 mg/mL) were used as a positive control. The inhibition percentage of α-amylase was assessed by the following formula:

$$I\alpha\text{-Amylase}\% = 100 \times \frac{(\Delta A_{Control} - \Delta A_{Sample})}{\Delta A_{Control}}$$

$$\Delta A_{Control} = A_{Control} - A_{Blank}$$

$$\Delta A_{Sample} = A_{Test} - A_{Blank}$$

Statistical analysis was performed using the IBM Corporation, 2012. (http://www-01.ibm.com/software/analytics/spss/). The IC_{50} values were estimated by the nonlinear curve and presented as their respective 95% confidence limits. Probit analysis of variance was used to assess the presence of significant differences ($P < 0.05$) between the extracts.

Table 1: ^{13}C-NMR data of the compounds, linarin (1), hispidulin -7-O-[4-O-rutinoside] (2), hispidulin -7-O-rutinoside (3) in DMSO-d_6

Carbon number	1	2	3
2	163.99	164.46	164.51
3	103.33	102.62	102.62
4	182.34	182.27	182.28
5	152.14	152.10	152.13
6	132.69	132.65	132.62
7	156.39	156.28	156.40
8	94.41	94.34	94.28
9	152.48	152.48	152.52
10	105.82	105.74	105.80
1′	122.6	121.00	120.98
2′	128.39	128.56	128.59
3′	114.69	116.07	116.12
4′	162.39	161.30	161.35
5′	114.69	116.07	116.12
6′	128.39	128.56	128.59
1″	99.88	99.88	100.33
2″	73.19	73.19	73.12
3″	76.42	76.41	76.42
4″	69.25	69.21	69.39
5″	75.32	75.23	75.61
6″	65.48	65.46	65.80
1‴	100.10	100.06	100.27
2‴	70.30	70.28	70.71
3‴	68.17	68.15	70.36
4‴	73.79	73.77	71.95
5‴	65.71	65.70	68.27
6‴	17.16	17.13	17.70
6-OCH3	60.26	60.24	60.29
4′-OCH3	55.54		
−CH_3COO	20.71	20.79	
	169.92	169.96	

DMSO=Dimethyl sulfoxide; ^{13}C-NMR=Carbon-13 nuclear magnetic resonance

RESULTS AND DISCUSSION

Isolation of the main compounds of the plant *Z. majdae* via chromatographic process resulted in identification of three flavonoid glycosides, named linarin (1),[10] hispidulin-7-O-(4-O-acetyl-rutinoside) (2), hispidulin-7-O-rutinoside (3)[11,12] based on the spectroscopic spectra (^{1}H-NMR, ^{13}C-NMR) compared to the known standard compounds [Figure 1], which reported in the literature. ^{13}C-NMR data of the compounds 1–3 in DMSO-d_6 is reported in Table 1. ^{1}H-NMR of the compounds 1–3 is also indicated at Table 2. The compound 1, linarin, has recently been isolated from the aerial parts of *Linaria reflexa* Desf. (Scrophulariaceae), which employed in North African folk medicine for treatment of certain skin diseases.[10] The NMR data of the present study are in good agreement with those reported by Cheriet *et al.*,[10] However, this is the first report of the isolation of this compound from *Zhumeria* genus and Lamiaceae family. The aglycone of the two other flavonoids 2 and 3 is a well-known methoxylated flavone named hispidulin. The mentioned

Table 2: ^{1}H-NMR data of the compounds, linarin (1), hispidulin -7-O-[4-O-rutinoside] (2), hispidulin -7-O-rutinoside (3) in DMSO-d_6

Carbon number	1	2	3
3	6.95 (s, 1H)	6.84 (s, 1H)	6.84 (s, 1H)
8	6.99 (s, 1H)	6.96 (s, 1H)	6.92 (s, 1H)
2′	8.05 (d, J=8.9, 1H)	7.93 (d, J=8.7, 1H)	7.93 (d, J=8.8, 1H)
3′	7.15 (d, J=8.9, 1H)	6.94 (d, J=8.8, 1H)	6.95 (d, J=8.9, 1H)
5′	7.15 (d, J=8.9, 1H)	6.94 (d, J=8.8, 1H)	6.95 (d, J=8.9, 1H)
6′	8.05 (d, J=8.9, 1H)	7.93 (d, J=8.7, 1H)	7.93 (d, J=8.8, 1H)
1″	5.16 (d, J=7.1, 1H)	5.15 (d, J=7.1, 1H)	5.11 (d, J=7.4, 1H)
1‴	4.60 (brs, 1H)	4.58 (brs, 1H)	4.56 (brs, 1H)
6‴	0.85 (d, J=6.2, 3H)	0.83 (d, J=6.2, 3H)	1.02 (d, J=6.0, 2H)
6-OCH3	3.78 (s, 3H)	3.78 (s, 3H)	3.78 (s, 3H)
4′-OCH3	3.83 (s, 3H)		
CH_3COO	1.96 (s, 3H)	1.95 (s, 3H)	
5-OH	12.93 (s, 1H)	13.01 (s, 1H)	12.97 (s, 1H)

^{1}H-NMR=Hydrogen-1 nuclear magnetic resonance; DMSO=Dimethyl sulfoxide

aglycone has previously been reported from *Scoparia dulcis* Linn. (Scrophulariaceae) and was found to be inactive against HIV-1/IIIB in MT-4 cells, whereas the same test on the aqueous extract of the plant was positive.[13] However, it showed other pharmacological activities. For instance, it is a benzodiazepine receptor ligand with positive allosteric properties abled to traverse the blood-brain barrier and exhibit anticonvulsive effects.[14] Furthermore, hepatoprotective activity has been reported from this compound.[15] So far, different glycosides of the aglycone "hispidulin" have been reported from other plant species. For example, chromatographic separation of the butanolic fraction of the methanol extract of *Cirsium japonicum* var. ussuriense resulted in the isolation of hispidulin 7-neohesperidoside together with the known cirsimaritin 4'-glucoside and acacetin 7-rutinoside.[12] However, to the best of our knowledge, hispidulin-7-O-(4-O-acetyl-rutinoside) (2), hispidulin-7-O-rutinoside (3) have not yet been separated from *Zhumeria* or other relative genus in Lamiaceae family.

Table 3: AAI activities and IC_{50} values of the tested extracts from *Zhumeria majdae*

Plant species	Extract	Concentration (mg/mL)	AAI percentage ±SD	IC_{50} (mg/mL)
Zhumeria majdae	Butanol	20	20.0±1.8	24.6±2.1
		25	40.8±2.2	
		30	77.9±2.1	
	Methanol	20	66.7±2.5	22.0±2.7
		25	26.5±1.5	
		30	7.0±2.8	

The inhibitory concentration of 50% (IC_{50}) for acarbose was calculated at 6.6±3.1 mg/mL as the positive control. AAI=Alpha-amylase inhibitory; SD=Standard deviation

The results of AAI activity has been summarized in Table 3. The activity of alpha amylase enzyme was dose-dependently suppressed by the butanol extract. The butanol fraction exhibited the highest inhibition at 30 mg/mL toward enzyme (77.9 ± 2.1%), while acarbose inhibited the enzyme at 20 mg/mL by 73.9 ± 1.9%. The IC_{50} for the butanolic fraction and methanolic extract were calculated at 24.5 ± 2.1 and 22.0 ± 2.7 mg/mL, respectively, in compared to acarbose (6.6 ± 3.1 mg/mL) as the positive standard. Although both the methanolic extract and butanolic fraction of *Z. majdae* exhibited moderate AAI activity, we had no chance to test the efficacy of the isolated flavonoids due to the trace amounts of them. A literature review revealed that

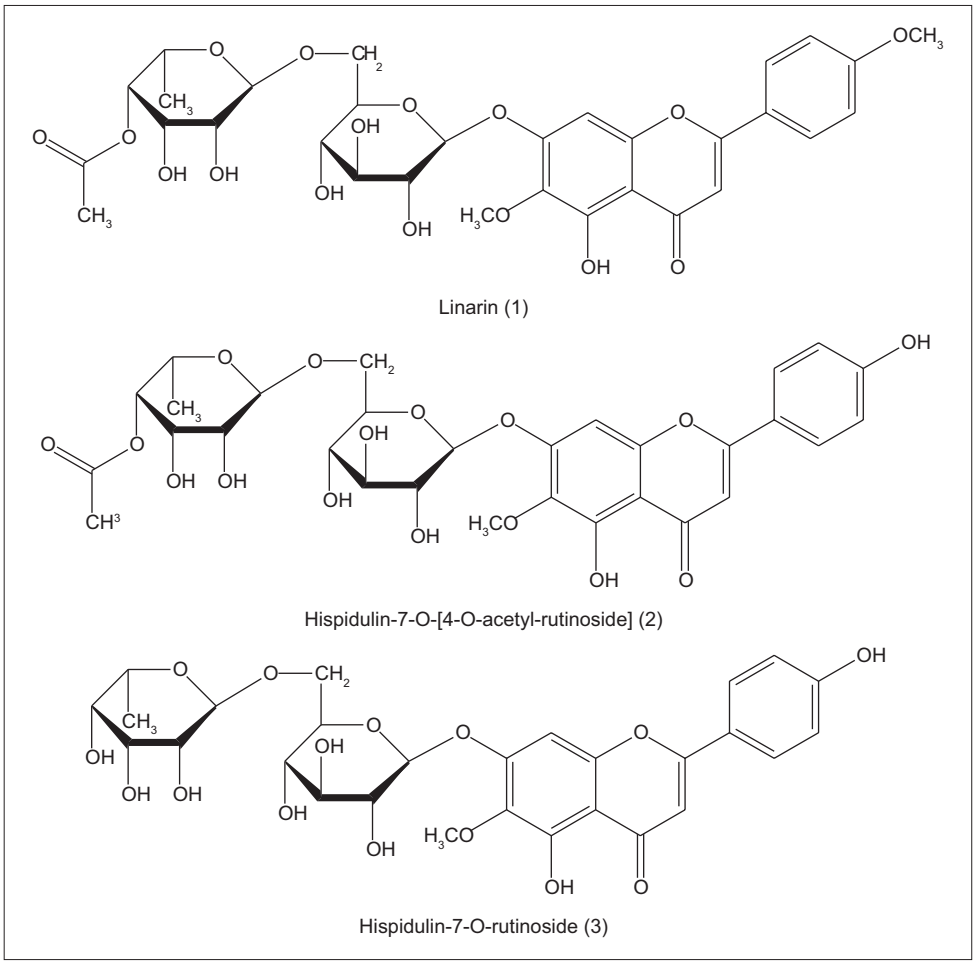

Figure 1: Chemical structures of the compounds 1–3 isolated from *Zhumeria majdae*

some Lamiaceae genera like *phlomis, Satureja, Salvia,* and *Hymenocrater* are moderately AAIs, while some other are potent anti-diabetic herbal medicines.[16] Therefore, more investigations on *Z. majdae* are recommended to evaluate its anti-diabetic activity in animals via different mechanism of action.

CONCLUSION

Taking together, *Z. majdae* contains glycosylated flavones especially acetylated ones, and also could be a good candidate for anti-diabetic evaluations in animal and clinical trials due to possessing AAI activity moderately.

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