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Bioactivity-based analysis and chemical characterization of hypoglycemic and antioxidant components from *Artemisia argyi*

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ABSTRACT

Diabetes is one of the metabolic disorders in the world. It is the prime reason of mortality and morbidity owing to hyperglycemia which is link with numerus obstacles. *Artemisia argyi* is commonly used as an ingredient in healthy foods as well as an herbal medicine in Asian countries. The present research aims to evaluate the hypoglycemic effects of *A. argyi* and reveal its the potentially active constituents. The chemical composition was identified by HPLC-DAD-Q-TOF-MS, and fractionation was performed by extraction. The fractions were assessed by the blood glucose level, oral glucose tolerance and small intestinal α-glucosidase inhibitory tests, and an analysis of the total phenolic content (TPC), antioxidant and α-glucosidase inhibitory activities. In our efforts to characterize the compounds responsible for hypoglycemic effect, bioactivity-guided fraction of the MeOH extract and chemical investigation of its active EtOAc fraction led to the successful identification of caffeoylquinic acids, which were elucidated by molecular docking, using the crystal structure of *S. cerevisiae* isomaltase (PD code: 3AXI). In summary, this bio-guided search revealed that caffeoylquinic acids from *A. argyi* as potential active constituents displayed with hypoglycemic activity, which provided a basis for further study of pharmacological activity.

1. Introduction

Diabetes mellitus (DM) is the most common chronic disease and is characterized by elevated blood glucose [1]. Reducing postprandial hyperglycemia is one therapeutic proposals for the treatment of Type 2 diabetes [2]. One of the therapeutic approaches for decreasing postprandial hyperglycemia is to inhibit key carbohydrate-hydrolyzing enzymes (α-amylase and α-glucosidase) [3]. Oxidative stress is a pathological consequence of the imbalance between the increased generation of free radicals (oxidants) and the diminished radical scavenging systems (antioxidants) [4]. Hyperglycemia may also be due to the cause-effect relationship of increased oxidative stress [5]. Several lines of evidences suggest that hyperglycemia induced oxidative stress, via the over production of highly reactive free radical, plays a central role in diabetes; owing to decreased efficiency of antioxidant defense system, hyperglycemia induced oxidative stress ultimately leads to the oxidative damage of cellular components [6,7]. Therefore, monitoring only blood glucose levels solely is not sufficient in retarding diabetic complications. As a consequence, an effective drug that has both antioxidant and hyperglycemic properties might be beneficial in modulating diabetes [8]. Although many medicines have been introduced, most are inapplicable to long-term use or a permanent cure. Some researchers hope to develop drugs derived from medicinal plants because they may produce fewer toxic effects. Therefore, according to traditional Chinese theories, the alternative methods of relieving these symptoms are regulating spleen and tonifying the kidney, and traditional Chinese medicine offers dietary supplements to maintain the blood glucose levels in a more secure and healthy way.

Artemisia argyi belongs to the Arteraceae family, which is mainly distributed in China, Japan and several other Asian countries [9]. *A. argyi* exhibits hemostatic, analgesic, and antipruritic effects for the treatment of various ailments, such as uterine hemorrhage,

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dysmenorrhea, abdominal pain and inflammation. *A. argyi* is also regarded as a food ingredient due to its delicious flavor and characteristic smell [10–12]. In China, the *Artemisia* species are also medicinally used to regulate the liver and spleen, and to tonify the kidney [13,14]. Several Studies have reported that *A. argyi* also contains many active compounds such as volatile oil, flavonoids, phenols, terpenoids and glycosides [15,16–19]. Previous investigations have reported that *A. argyi* extracts possess various biological activities including anti-inflammatory, antioxidant, antibacterial, antiviral, anti-hypertensive effects, immunoregulatory, anti-asthmatic, hemostatic, oncogene inhibitory and antimicrobial activities [12,20–22]. However, the hypoglycemic effect of *A. argyi* was lack of study and the investigation of hypoglycemic compounds from *A. argyi* was not sufficient.

Therefore, the search for active compounds of plants capable of being used as hypoglycemic compounds remains a focus of research. In this study, the MeOH extract of *A. argyi* was assessed by the blood glucose level, oral glucose tolerance and small intestinal α-glucosidase inhibitory tests, antioxidant and α-glucosidase inhibitory activities. We found that the MeOH extract of *A. argyi* exerted a hypoglycemic effect and was investigated by HPLC-Q-TOF-MS method and developed for qualitative analysis of phenolic content in *A. argyi*. Based on bioactivity-guided fraction of the MeOH extract, we obtained the active EtOAc fraction and an analysis of the total phenolic content (TPC) was also measured. Antioxidant and α-glucosidase inhibitory activities by the six characteristic compounds isolated from *A. argyi* were also measured. The caffeoylquinic acids, which contribute to the hypoglycemic effect of *A. argyi*, were elucidated by molecular docking.

2. Results and discussion

2.1. Hypoglycemic, antioxidant and α-glucosidase inhibitory activities of MeOH extract of A. argyi

Oral glucose tolerance test (OGTT), which is based on experimental animals, is widely used in the functional evaluation of hypoglycemic drugs and is an important experimental method for evaluating the functions of drugs (such as α-glucosidase inhibitors) [23]. As shown in Fig. 1, a reduction in the mice's blood glucose level was observed for 2 h in all doses used in the study. The blood glucose reached the highest level at 30 min after the intragastric administration of the glucose, and hyperglycemia was maintained until 120 min. The High-Dose and Acarbose groups significantly ($p < 0.01$) prevented an increased of the blood glucose levels at 30 and 60 min, and the Low-Dose group decreased the blood glucose by 12.03% and 23.35% with statistical significance ($p < 0.05$).

The ability of the *A. argyi* MeOH extract to inhibit α-glucosidase activity in the mice's small intestinal mucosa was assessed. We examined the effect of orally administered *A. argyi* MeOH extract on the α-glucosidase activity in the small intestinal mucosa of mice. As shown in Fig. 2, compared to 0, 30, 60, 90 and 120 min after drug administration, the α-glucosidase activity was decreased in a time-dependent manner. The α-glucosidase activity at 60 min had a significant reduction ($p < 0.05$) of 49.07%. Furthermore, the α-glucosidase activity at 90 and 120 min had a significant reduction ($p < 0.01$) of 32.83% and 49.73%, respectively.

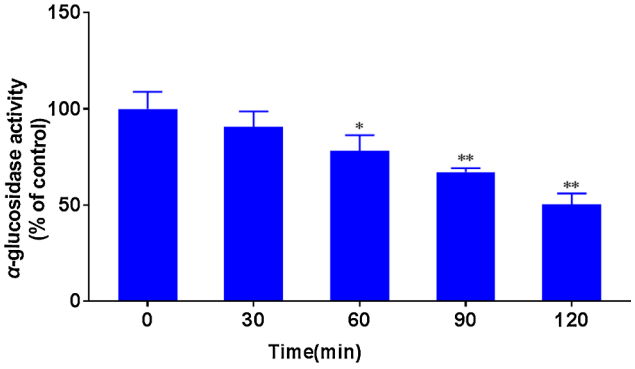


Fig. 2. Effect of MeOH extract oral administration on small intestinal α-glucosidase activity in normal mice. *$p < 0.05$, **$p < 0.01$ significantly different ANOVA followed by Dunnett's *t*-test for comparison with respect to the Control.

As shown in Table 1, the antioxidant activities of the *A. argyi* MeOH extract was measured via three assay methods. The MeOH extract exhibited antioxidant activity: the DPPH scavenging activity (IC_{50}), ABTS and FRAP values were 14.91 μg/mL, 726.29 mmol TEs/g and 583.27 mmol TEs/g, respectively.

The enzymes α-amylase and α-glucosidase are key players in the control of glycemic levels. It is noteworthy that many natural compounds are more effective inhibitors of yeast α-glucosidase activity than mammalian enzymes [24]; therefore, an *in vitro* experimental design was necessary. The MeOH extract showed α-glucosidase inhibitory activity with IC_{50} values was 47.58 μg/mL, which were stronger than the reference acarbose (IC_{50} 125.91 μg/mL) (Table 1).

2.2. Bioassay guided fraction of MeOH extract

The MeOH extract was sequentially solvent-partitioned with PE, EtOAc, *n*-BuOH and H_2O to obtain four main fractions. The four fractions were assessed by the blood glucose level, oral glucose tolerance and small intestinal α-glucosidase inhibitory tests, and an analysis of the total phenolic content (TPC), antioxidant and α-glucosidase inhibitory activities. The EtOAc fraction caused significant hypoglycemic

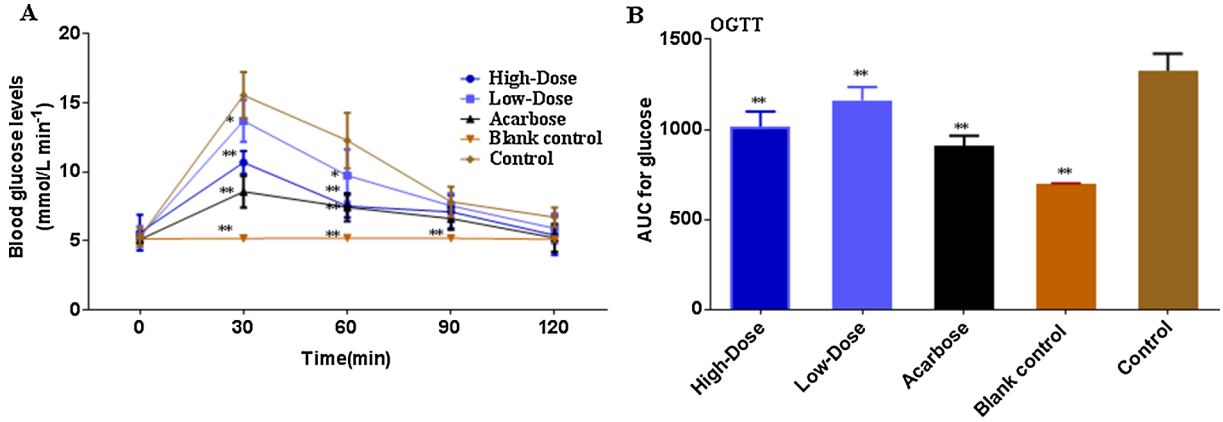


Fig. 1. (A) Blood glucose level at 0, 30, 60, 90 and 120 min after 10% glucose solution injection for MeOH extract. (B) Area under the curve (AUC) for blood glucose over 2 h in glucose tolerance test for MeOH extract. *$p < 0.05$, **$p < 0.01$ significantly different ANOVA followed by Dunnett's *t*-test for comparison with respect to the Control. High-Dose and Low-Dose mice treated with MeOH extract at 400 and 200 mg/kg b.w., respectively.

Table 1
Total phenolic contents, antioxidant capacity (DPPH, ABTS and FRAP) and α-glucosidase inhibitory activity of the MeOH extract and different polarity fractions of *Artemisia argyi*. Mean value ± standard deviation (n = 3).

Extract or fraction	TPC (mg GAE/g DW)	Antioxidant activity			α-glucosidase IC_{50} (μg/mL)[a]
		DPPH IC_{50} (μg/mL)[a]	ABTS (μmol TEs/g)[b]	FRAP (μmol TEs/g)[c]	
MeOH extract	108.56 ± 22.32	14.91 ± 0.14	726.29 ± 63.22	583.27 ± 28.28	47.58 ± 2.02
PE fraction	52.87 ± 1.54	123.54 ± 1.01	44.07 ± 4.23	170.37 ± 5.36	213.22 ± 2.11
EtOAc fracion	899.66 ± 35.17	7.26 ± 0.47	1000.74 ± 0.03	1090.22 ± 27.33	5.48 ± 0.53
n-BuOH fraction	738.77 ± 42.91	12.16 ± 2.00	810.41 ± 4.17	760.92 ± 32.03	30.24 ± 1.14
H_2O fracion	88.31 ± 13.89	101.43 ± 0.14	76.80 ± 5.32	239.75 ± 0.29	158.12 ± 3.58
Trolox[d]		6.41 ± 0.30	3995.04 ± 39.42		
Acarbose[d]					125.91 ± 20.35

Each value is expressed as the mean ± standard deviation (n = 3).
[a] IC_{50} value: the concentration at which the antioxidant activity inhibition ratio reached 50%; 1,1-diphenyl-2-picrylhydrazyl (DPPH) was scavenged by 50%.
[b] The ratio of trolox to samples corresponding to the same free radical scavenging rate (TEAC).
[c] The ratio of $FeSO_4$ to samples corresponding to the same free radical scavenging rate(TEAC).
[d] Reference compounds.

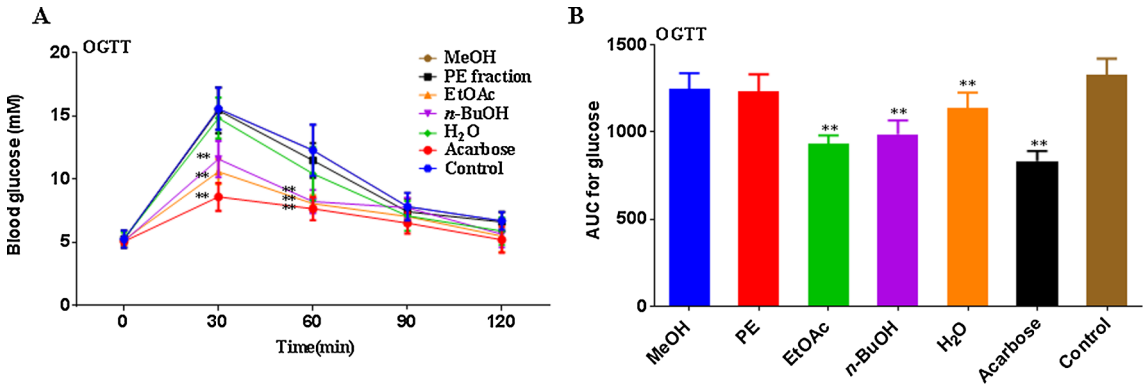


Fig. 3. (A) Blood glucose level at 0, 30, 60, 90 and 120 min after 10% glucose solution injection for different fractions. (B) Area under the curve (AUC) for blood glucose over 2 h in glucose tolerance test for different fractions. *p < 0.05, **p < 0.01 significantly different ANOVA followed by Dunnett's *t*-test for comparison with respect to the Control.

effects. A blood glucose reduction of 49.07% at 60 min was observed. The EtOAc and *n*-BuOH fractions also significantly prevented the increased of the blood glucose levels at 30 and 60 min, while PE and H_2O fractions decreased the blood glucose level without statistical significance at 30 and 60 min (Fig. 3). The results of OGTT showed an improvement in glucose tolerance among the EtOAc group relative to the Blank Control and Control groups. This result indicates an important hypoglycemic effect of the EtOAc fraction. The AUC of EtOAc group showed a significant decrease, indicating that the dynamic of the disappearance of blood glucose was accelerated by the treatment.

According to the results of the OGTT test, the EtOAc extract of *A. argyi* may contains compounds that affect the glucose uptake, utilization and/or insulin secretion or action. This test is usually performed to evaluate if the hypoglycemic action of a drug is involved in the inhibition of intestinal α-glucosidase.

As shown in Table 1, the EtOAc fraction exhibited the highest antioxidant activity: the DPPH scavenging activity (IC_{50}), ABTS and FRAP values were 7.26 μg/mL, 1000.74 mmol TEs/g and 1090.22 mmol TEs/g, respectively. The EtOAc highest showed α-glucosidase inhibitory activity with IC_{50} values being 5.48 μg/mL, which was stronger than the reference acarbose (IC_{50} 125.91 μg/mL). The presence of one or more compounds in the plant extract might be involved in the decrease of blood glucose suggesting that the natural constituents may have act separately or synergistically to induce the hypoglycemic effects [25].

2.3. Total phenolic content

The total phenolic content of the extract and all the fractions were reported as gallic acid equivalents per gram of dried sample. The TPC of the MeOH extract and the other fractions are presented in Table 1. The TPC of the MeOH extract was 108.56 mg GAE/g, whereas the EtOAc fraction had the highest TPC (899.66 mg GAE/g) among the fractions. The results from the current study are in good agreement with other research studies that have reported phenolic rich extracts that exhibit high antioxidant and α-glucosidase inhibitory effects [26]. Furthermore, these results revealed a direct correlation between the antioxidant activity and the α-glucosidase inhibitory activity. The higher phenolic content and antioxidant activity significantly influenced the α-glucosidase inhibitory activity of the samples (p < 0.05).

2.4. Chemical identification of hypoglycemic constituents by HPLC-DAD-Q-TOF-MS analysis

A. argyi MeOH extract was analyzed by HPLC-DAD-Q-TOF-MS in the negative and positive ion mode (Fig. 5). A total of twenty-three compounds, including thirteen flavonoids and ten caffeoylquinic acids, were identified (Table 2, Fig. 6), among which, six compounds, including fraxin (**3**), isoschaftoside (**6**), apigenin-*O*-rutinoside (**11**), 3-caffeoyl-4-feruoyl-quinic acid (**14**), 5,7,4-trihydroxy-6,3′,5′-trimethoxy-flavone (**18**) and 5,7-dihydroxy-8,2′-dimethoxy-flavone (**21**), were characterized for the first time in *A. argyi*. The identities of twelve compounds (**1**–**7**, **11**, **14**, **21**, **22** and **23**) were tentatively inferred based on their fragmentation pathways and previous reports, while the others were unambiguously identified by comparison with their reference standards via retention time.

The main components (**8**, **9**, **13**, **15**, **16**, **17**, **18**, **20** and **22**) were isolated from EtOAc fraction and purified by repeated silica gel and ODS column chromatography. The isolation, structural elucidation and

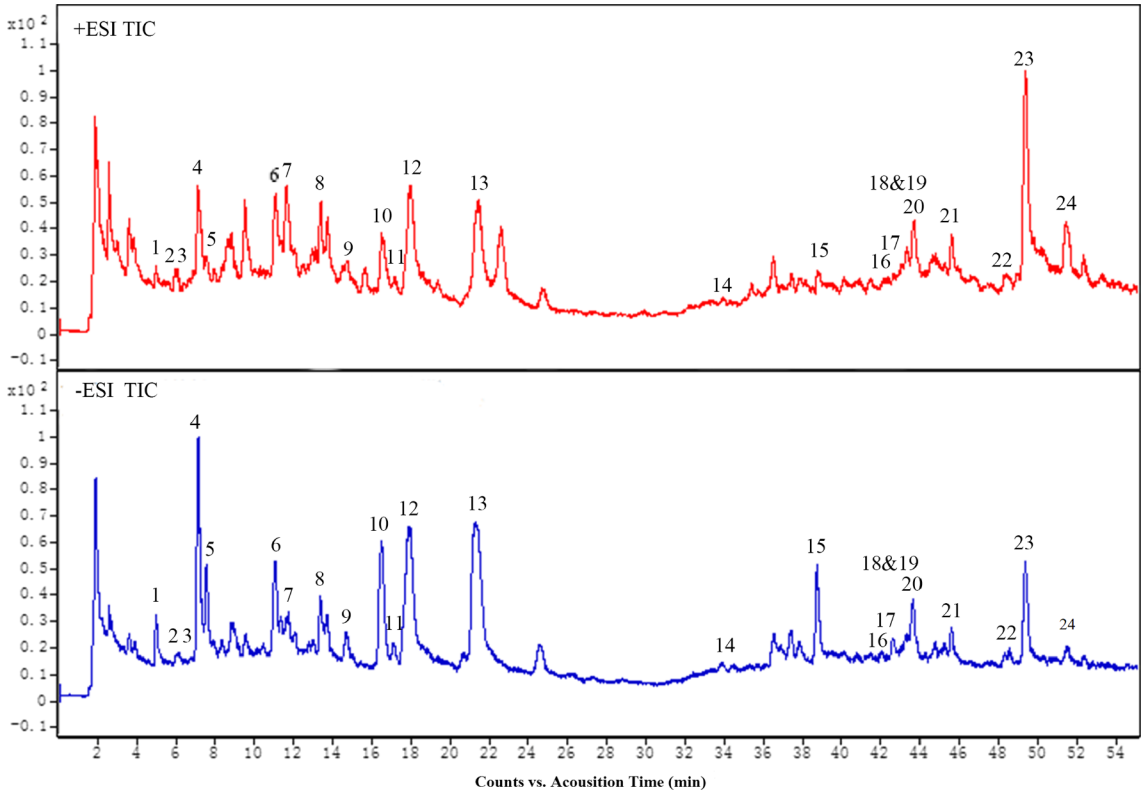
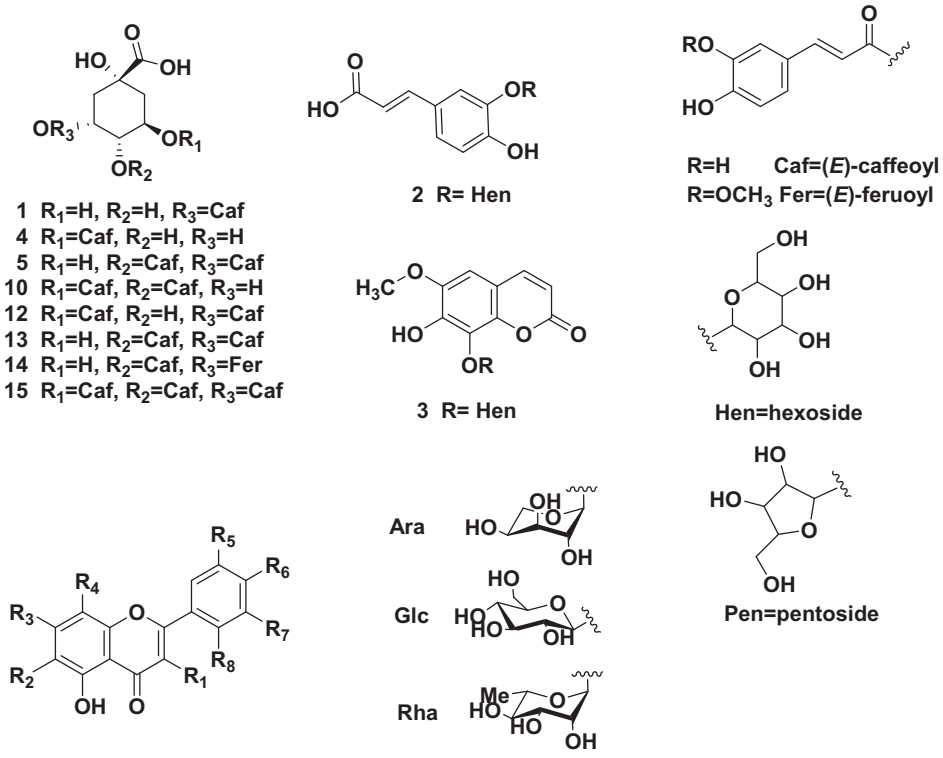


Fig. 5. The typical total ion chromatograms of *A. argyi* acquired by HPLC-DAD-Q-TOF-MS in positive ion mode (A), negative ion mode (B).

Table 2
Identification of 23 compounds from *Artemisia* argyi by developed HPLC-DAD-Q-TOF-MS.

NO.	Retention time, t_R(min)	Formula	$[M-H]^-$ (*m/z*)	Error (ppm)	MS/MS fragments (*m/z*)	Identification	Reference
1[a]	4.997	$C_{16}H_{18}O_9$	353.0879	−0.30	191.0522, 85.0282	Neochlorogenic acid	[27]
2[a]	6.048	$C_{15}H_{18}O_9$	341.0888	−2.82	179.0304, 135.0475	Caffeic acid-hexoside	[27]
3[a,c]	6.159	$C_{16}H_{18}O_{10}$	369.0831	−0.95	207.0492	Fraxin	[28]
4[a]	7.120	$C_{16}H_{18}O_9$	353.0885	−1.83	191.0544, 127.0404, 93.0344, 85.0294	Chlorogenic acid	[27]
5[a]	7.535	$C_{16}H_{18}O_9$	353.0883	−1.43	191.0558, 173.0448, 127.0404, 93.0346, 85.0291	Cryptochlorogenic acid	[27]
6[a,c]	11.092	$C_{26}H_{28}O_{14}$	563.1411	−0.86	503.1185, 473.1065, 443.0998, 383.0788, 353.0678	Isoschaftoside	[29]
7[a]	11.724	$C_{26}H_{28}O_{14}$	563.1412	−1.09	503.1056, 473.1039, 443.0976, 383.0757, 353.0645	Apigenin-C-hexoside-pentoside	[29]
8[b]	13.574	$C_{27}H_{30}O_{16}$	609.1472	−1.75	301.0334, 300.0269, 178.9973	Rutin[b]	[29]
9[b]	14.711	$C_{21}H_{20}O_{12}$	463.1886	−0.92	301.0267, 151.0009	Isoquercitrin/hyperoside	[30]
10[b]	16.466	$C_{25}H_{24}O_{12}$	515.1191	−0.40	173.0447	Isochlorogenic acid B	[31]
11[a,c]	17.115	$C_{27}H_{30}O_{14}$	577.1654	−0.13	269.0452	Apigenin-O-rutinoside	[30]
12[b]	17.925	$C_{25}H_{24}O_{12}$	515.1195	−0.08	135.0447, 191.0555	Isochlorogenic acid A	[31]
13[b]	21.284	$C_{25}H_{24}O_{12}$	515.1193	0.37	191.0548, 179.0348, 173.0450	Isochlorogenic acid C	[31]
14[a,c]	33.881	$C_{26}H_{26}O_{12}$	529.1346	−0.03	367.0950, 161.0232, 135.0440	3-Caffeoyl-4-feruoyl-quinic acid	[32]
15[b]	38.762	$C_{34}H_{30}O_{15}$	677.1504	1.03	515.1186, 353.0869, 335.0753, 179.0339, 173.0451	3,4,5-Tricaffeoylquinic acid	[32]
16[b]	42.093	$C_{15}H_{10}O_5$	269.0455	0.03	138.0381, 151.0025, 117.0324	Apigenin	[33]
17[b]	42.627	$C_{16}H_{12}O_6$	299.0572	−3.59	284.0299, 277.0337, 136.9881, 117.0361, 108.0220	Hispidulin	[33]
18[b,c]	43.259	$C_{18}H_{16}O_8$	359.0782	−2.53	329.0311, 301.0371, 270.0137, 242.0202, 214.0267, 136.9897	5,7,4-Trihydroxy-6,3′,5′-trimethoxy-flavone	
19[c]	43.653	$C_{17}H_{14}O_7$	329.067	−0.54	299.0179, 271.0238, 227.0382, 199.0395	5,7,4′-Trihydroxy-6,3′-dimethoxy-flavone	
20[b]	45.602	$C_{18}H_{16}O_8$	359.0773	−0.10	343.0491, 329.0280, 298.0117	Centaureidin	[41]
21[b,c]	48.542	$C_{17}H_{14}O_6$	313.0721	−0.96	283.0217, 255.0218	5,7-Dihydroxy-8,2′-dimethoxy-flavone	[41]
22[b]	49.369	$C_{18}H_{16}O_7$	343.0826	−0.25	298.0115, 163.0445	Eupatilin	[42]
23[a]	51.468	$C_{19}H_{17}O_8$	373.1934	−1.36	343.0480, 328.0212, 312.9998, 315.0592, 300.0261, 299.0108, 297.0727, 287.0555, 285.0048, 284.0124, 272.0322, 257.0055, 241.0232, 213.0143	5,7,3′-Dihydroxy-6,4′,5′-tetramethoxyflavone	[39]

[a] Compounds identified by comparing retention times and MS data with those of reference compounds.
[b] Identified with the reference compounds.
[c] Reported for the first time in *Artemisia* argyi.



6 R_1=H, R_2=Ara, R_3=OH, R_4=Glc, R_5=H, R_6=OH, R_7=H, R_8=H
7 R_1=H, R_2=Pen, R_3=OH, R_4=Hen, R_5=H, R_6=OH, R_7=H, R_8=H
8 R_1=OGlc-(6-1)-Rha-, R_2=H, R_3=OH, R_4=H, R_5=OH, R_6=OH, R_7=H, R_8=H
9 R_1=OGlc, R_2=H, R_3=OH, R_4=H, R_5=OH, R_6=OH, R_7=H, R_8=H
11 R_1=H, R_2=H, R_3=OGlc-(6-1)-Rha-, R_4=H, R_5=H, R_6=OH, R_7=H, R_8=H
16 R_1=H, R_2=H, R_3=OH, R_4=H, R_5=H, R_6=OH, R_7=H, R_8=H
17 R_1=H, R_2=OCH_3, R_3=OH, R_4=H, R_5=H, R_6=OH, R_7=H, R_8=H
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19 R_1=H, R_2=OCH_3, R_3=OH, R_4=H, R_5=OCH_3, R_6=OH, R_7=H, R_8=H
20 R_1=H, R_2=OH, R_3=OH, R_4=H, R_5=H, R_6=OH, R_7=H, R_8=H
21 R_1=H, R_2=H, R_3=OH, R_4=OCH_3, R_5=H, R_6=H, R_7=H, R_8=OCH_3
22 R_1=OH, R_2=H, R_3=OH, R_4=H, R_5=OCH_3, R_6=OH, R_7=H, R_8=H
23 R_1=OH, R_2=OCH_3, R_3=OH, R_4=H, R_5=OH, R_6=OCH_3, R_7=OCH_3, R_8=H

Fig. 6. Structures of compounds 1–23. Numbering of the peaks refers to Table 2.

nuclear magnetic resonance (NMR) spectroscopic data are shown in the Supplementary material.

Peaks **1**, **4**, **5** displayed a $[M-H]^-$ at *m/z* 353 and were characterized as neochlorogenic acid, chlorogenic acid, and cryptochlorogenic acid. The three isomers yielded the same fragmentation at *m/z* 191, corresponding to a deprotonated quinic acid. A higher peak signal at *m/z* 173 could distinguished **5** from the other isomers [27].

Based on previously reported data [27,30–32], the [caffeic acid–H]$^-$ ion at *m/z* 179 and a series of characteristic ions at *m/z* 161, 135 (indicative of losing a H_2O and a CO_2) were common features of the caffeoyl groups. These MS/MS fragment profiles (*m/z* 179, 161, 135) were observed in compounds **2**, **10**, **12**, **13**, **14** and **15**, indicating they all possessed the caffeoyl moiety.

Flavones are featured in *Artemisia* species, and thirteen flavonoids were identified in *A. argyi*. The regular fragmentation ions of flavones often can be elucidated by a *retro*-Diels–Alder (RDA) cleavage that occurs in the C-ring and aids in the identification [29–35].

Flavonoids and caffeoylquinic acids are a complex group of chemicals that are widely distributed throughout the plant kingdom and form an integral part of the human diet [36]. Therefore, the inhibition of the α-glucosidase found in our experiments may be due to the presence of flavonoids and caffeoylquinic acids [37].

2.5. Method validation for quantitative analysis

The mobile phase consisted of solvent A (MeCN) and solvent B (H_2O with 0.5% HCOOH). The gradient program was as followes: 0–10 min, 10–20% A; 10–27 min, isocratic 20% A; 27–65 min, 20–65% A; and 65–70 min, 65–100% A. The flow rate was maintained at 1.0 mL/min. The column temperature was maintained at 25 °C, the injection volume was 10 μL and the UV detector was set at 330 nm. The LODs and LOQs of five compounds, including chlorogenic acid (**4**), isochlorogenic acid B (**10**), isochlorogenic acid A (**12**), isochlorogenic acid C (**13**), and 3,4,5-tricaffeoylquinic acid (**15**), were in the range of 0.22–1.00 and 0.65–3.30 μg/mL (Table 3). The calibration curves of the compounds demonstrated good linearity ($r^2 \geq 0.9995$) over the designed ranges. The average recoveries obtained showed high recovery rates (92.10–113.00%). The repeatability test (RSD ≤ 2.00%) and the precision test (RSD ≤ 2.00%) indicated the method was reproducible and precise.

The total concentration of the compounds ranged from 0.105 to 1.183 mg/g. Compound **13** was the predominant component, and compound **12** was the second most abundant; compounds **4**, **10**, **17** and **22** were found at lower abundance. The results were consistent with the study reported by Tian F et al [38], in which the content of chlorogenic acid in aerial part of *A. argyi* was 0.18%.

Table 3
Method validation for quantification and the contents of five characteristic compounds in *Artemisia argyi*.

Compounds	Regression equation[a]	R^2	Linear range (μg/mL)	LOD (μg/mL)	LOQ (μg/mL)	Content (mg/g)	Precision (RSD, %)		Repeatability (RSD, %, n = 6)	Stability (RSD, %, n = 6)	Recovery[b] (RSD, %, n = 6)	
							Intra-day (n = 6)	Inter-day (n = 10)			Recovery (%)	RSD (%)
Chlorogic acid (**4**)	y = 29574x + 54149	1.0000	2.90–200.00	0.44	1.45	0.188 ± 0.0061	0.44	1.21	1.32	1.14	107.00	0.21
Isochlorogenic acid B (**10**)	y = 47659x − 175,973	0.9999	4.56–63.50	0.68	2.28	0.975 ± 0.001	0.31	1.02	1.41	1.61	113.00	0.64
Isochlorogenic acid A (**12**)	y = 39790x − 181,097	0.9997	3.30–130.00	1.00	3.30	1.180 ± 0.003	0.74	1.45	1.89	1.84	92.10	0.77
Isochlorogenic acid C (**13**)	y = 23295x + 156788	0.9999	5.64–62.50	0.56	1.88	1.180 ± 0.005	0.51	1.11	1.24	1.31	99.40	0.33
3,4,5-Tricaffeoylquinic acid (**15**)	y = 14233x − 83,648	0.9999	3.91–125.00	0.22	0.65	0.413 ± 0.004	0.62	1.49	2.52	1.81	96.20	1.41

[a] The regressive equations are presented as y = ax + b. y is the peak area, x is the concentration of compound.
[b] Recovery(%) = 100 × (detected-original)/spike.

2.6. Antioxidant and α-glucosidase inhibitory activities of characteristic compounds

Due to the good biological activity of the MeOH extract, five compounds (**4**, **10**, **12**, **13** and **15**) were prepared for further evaluation of their antioxidant and α-glucosidase inhibitory activities. Current studies have reported that chlorogenic acid derivatives showed remarkable inhibitory activity against α-glucosidase [39]. Among the compounds tested (Table 4), four caffeoylquinic acids (**10**, **12**, **13** and **15**) showed strong antioxidant activity (DPPH IC_{50} ranging from 8.74 to 19.41 μmol/mL; ABTS ranging from 14.64 to 21.75 μmol TEs/μmol; and FRAP ranging from 5.48 to 12.32 μmol TEs/μmol) and α-glucosidase inhibitory activities (IC_{50} ranging from 10.79 to 42.09 μmol/L) compared with the references trolox and acarbose. The results revealed that the compounds (**10**, **12**, **13** and **15**) with high contents are mainly responsible for the antioxidant and α-glucosidase inhibitory activities in the *A. argyi* MeOH extract.

2.7. Molecular modeling study

A molecular modeling study was conducted to predict the binding site between α-glucosidase and the four caffeoylquinic acids (**10**, **12**, **13** and **15**) and to confirm the results of the α-glucosidase inhibitory experiments described above (Fig. 7). The best docking pose obtained for compound **10** docked to α-glucosidase was stabilized by four hydrogen bonds to Lys568, Asn489, Lys373 and Glu497, while compound **12** docked to α-glucosidase was stabilized by three hydrogen bonds to Glu497 and Lys568. Compound **13**-α-glucosidase interactions were stabilized by three hydrogen bonds to Lys373 and Lys569. Compound **15** docked to α-glucosidase and was stabilized by six hydrogen bonds to Phe543, Pro320 and Thr358. The calculated binding energy of α-glucosidase with compound **10** (−6.23 kcal/mol), compound **12** (−6.50 kcal/mol), compound **13** (−5.95 kcal/mol) and **15** (−6.50 kcal/mol) supported the α-glucosidase inhibitory potential observed. The molecular docking results of the four caffeoylquinic acids were consistent with α-glucosidase inhibitory activity, and the interactions were dependent on the structure of the compounds. These results indicated the interaction between caffeoylquinic acids and the residues adjacent to the active site of α-glucosidase. Interestingly, it has been reported previously that experimental data to establish these caffeoylquinic acid compounds bind to the active site on α-glucosidase tend to possess cholinesterase activity [40–41].

3. Conclusion

In the current study, we found that the MeOH extract of *A. argyi* showed hypoglycemic effects. In our efforts, characterize the compounds responsible for these hypoglycemic effects, bioactivity-based analysis and chemical investigation of the MeOH extract led to the successful identification of the anti-hyperglycemic constituents, caffeoylquinic acids, which significantly inhibited α-glucosidase with an IC_{50} value ranging from ranging from 10.79 to 42.09 μmol/mL. These fouding provide experimental evidence that *A. argyi* was a foodstuff with the potential for development as a functional food for the treatment of diabetes and the alleviation of diabetes complications. Future studies will focus on the exact mechanism of action responsible for its beneficial effects mediating the hypoglycemic effect.

4. Experimental

4.1. Chemicals and reagents

The reagents used for high-performance liquid chromatography with quadrupole time of flight mass spectrometry (HPLC-Q-TOF-MS) analysis were: water was purified by means of a Millipore Milli Q-Plus system (Millipore, Bedford). All other reagents and solvents used were

Table 4
Antioxidant and α-glucosidase inhibitory activity of five marker of *Artemisia argyi*. Mean value ± standard deviation (n = 3).

Compouinds	Antioxidant activity			α-glucosidase IC_{50} (μmol/L)[a]
	DPPH IC_{50} (μmol/mL)[a]	ABTS (μmol Trolox/μmol)[b]	FRAP (μmol Trolox/μmol)[c]	
Chlorogic acid (**4**)	25.82 ± 1.48	11.31 ± 0.41	4.87 ± 0.03	> 200
Isochlorogenic acid B (**10**)	19.41 ± 1.31	18.21 ± 1.47	9.00 ± 0.21	42.09 ± 3.14
Isochlorogenic acid A (**12**)	18.22 ± 1.29	21.75 ± 0.19	12.32 ± 0.27	28.58 ± 0.24
Isochlorogenic acid C (**13**)	10.04 ± 1.63	15.91 ± 1.06	6.80 ± 0.39	82.76 ± 7.24
3,4,5-Tricaffeoylquinic acid (**15**)	8.74 ± 0.56	14.64 ± 0.01	5.48 ± 0.01	10.79 ± 1.64
Trolox[d]	27.41 ± 0.11			
Acarbose[d]				185.42 ± 1.17

Each value is expressed as the mean ± standard deviation (n = 3). ND, not determined.
[a] IC_{50} value: the concentration at which the antioxidant activity inhibition ratio reached 50%; 1,1-diphenyl-2-picrylhydrazyl (DPPH) was scavenged by 50%.
[b] The ratio of trolox to samples corresponding to the same free radical scavenging rate (TEAC).
[c] The ratio of $FeSO_4$ to samples corresponding to the same free radical scavenging rate.(TEAC).
[d] Reference compounds.

analytical grade, and purchased from Nanjing Chemical Reagent Corp. (Nanjing, China). The reagents used to measure the antioxidant capacity: 1,1-di-phenyl-2-picrylhydrazul (DPPH), 2,4,6-tripyridyls-triazine (TPTZ), 2,2′-azinobis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS), 6-hydroxy-2,5,7,8-tetr-methylchroman-2-carboxylicacid (Trolox), Folin-Ciocalteu reagent and ferric chloride ($FeCl_3$) were purchased from Yuanye Bio Technology (Shanghai, China). The reagents used to measure the *α*-glucosidase inhibitory capacity: BCA kit, *α*-glucosidase powder and p-nitrophenyl-*α*-D-glucopyranoside (pNPG) were purchased from Yuanye Bio Technology (Shanghai, China). Acarbose were obtained from Aladdin (Shanghai, China). Reference compounds, including chlorogenic acid (**4**), isochlorogenic acid B (**10**) and isochlorogenic acid A (**12**), were purchased from Shanghai Yuanye Bio-Technology Co. (Shanghai, China). Reference compounds, including rutin (**8**), isoquercitrin (**9**), isochlorogenic acid C (**13**), 3,4,5-tricaffeoylquinic acid (**15**), apigenin (**16**), hispidulin (**17**), 5,7,4-trihydroxy-6,3′,5′-trimethoxy-flavone (**18**), centaureidin (**20**) and eupatilin (**22**), were isolated from *A. argyi* in our previous study [42].

4.2. Animals

ICR mice (Male, 20 ± 2 g) were obtained from the Experimental Animal Center of Qinglongshan. All experiment procedures were approved by the Institutional Ethics Committee of China Pharmaceutical University.

4.3. Plant samples and preparation of sample solutions

The *A. argyi* herbal materials were provided by Qichun Hubei Pharmaceutical Co. Ltd (Hubei, China) in December 2015. The plant was identified by one of the authors, JZ. A voucher specimen (NO.20140901) was deposited in School of Traditional Chinese Pharmacy, China Pharmaceutical University, China.

The dried leaves (800 g) were extracted 3 times with 500 mL MeOH (60 °C, 3 h × 3). The extracts were combined and evaporated under reduced pressure at 60 °C using a rotary vacuum evaporator to afford the extract, yielding 45.6 g of MeOH extract. The MeOH extract (45.6 g, 6%) was redissolved in water/methanol (1:1, v/v) while a liquid-liquid partition was carried out with petroleum ether (PE), EtOAc and *n*-BuOH for three times each (3 × 500 mL) to eventually obtain the PE, EtOAc, *n*-BuOH and H_2O fractions, respectively. The yield from the extraction were PE (2.7 g, 6%,) EtOAc (11.4 g, 25%), *n*-BuOH (9.1 g, 20%) and H_2O (13.7 g, 30%).

Each accurately weighed reference compounds (**4**, **10**, **12**, **13** and **15**) was dissolved and diluted with 100% MeOH to obtain a series of stock standard solutions, each terminal concentration was 200 μg/mL. A mixed stock standard solution containing six standards (ranging from 0.2 μg/mL to 200 μg/mL) was prepared by adding and diluting each stock standard solution with 100% MeOH to a series of working standard solutions. All solutions were stored at 4 °C and brought to room temperature before use.

4.4. α-Glucosidase inhibitory activity

The method for *α*-glucosidase inhibition assay was adapted from Boue S M et al [43]. In a 96-well plate, 20 μL of sample, 50 μL of 0.1 M buffer control (pH 6.9), or a positive control was added to 10 μL of a 1 U/mL *α*-glucosidase solution and incubated at 37 °C for 20 min. A 20 μL of 5 mM pNPG was added to each well and incubated for 30 min at 37 °C. After incubation, the absorbance was read at 405 nm. Results were expressed as IC_{50} values estimated by a nonlinear regression algorithm. Eq. (1) was used for *α*-glucosidase inhibition activity percentage.

$$\alpha - \text{glucosidase inhibition activity}(\%) = \left(1 - \frac{Abs_{sample} - Abs_{blank}}{Abs_{control} - Abs_{blank}}\right) \times 100\% \quad (1)$$

4.5. Antioxidant activity

The antioxidant activities were measured using three assays including DPPH, ABTS and FRAP assays. The scavenging capacity of DPPH free radical was determined using a previously method [44]. The ABTS assay was followed according to the procedure described [45]. The FRAP assay was carried out according to the method [44].

4.6. Treatment with A. argyi MeOH extract and fractions

The ICR mice were divided into ten experimental groups (n = 10/group): which comprised the following: High-Dose and Low-Dose mice treated with MeOH extract at 400 and 200 mg/kg b.w., respectively.; Acarbose – mice treated with Acarbose at 50 mg/kg b.w.; Blank Control – mice treated with 0.5% CMC-Na; Control – mice treated with nothing. MeOH, PE, EtOAc, *n*-BuOH and H_2O – mice treated with 200 mg/kg b.w. different fractions (MeOH extract, PE, EtOAc, *n*-BuOH and H_2O fractions, respectively.).

4.7. Oral glucose tolerance test (OGTT)

All the groups of mice were subjected to fasting for 12 h. Their glycaemia level was measured prior to the beginning of the fasting (time zero). Afterwards, the animals received an oral load of glucose (2.0 g/kg b.w.). Their blood glucose was measured at 30, 60, 90 and 120 min after glucose administration. Blood glucose concentration (mM) was determined by the blood glucose meter (OneTouch UltraEasy

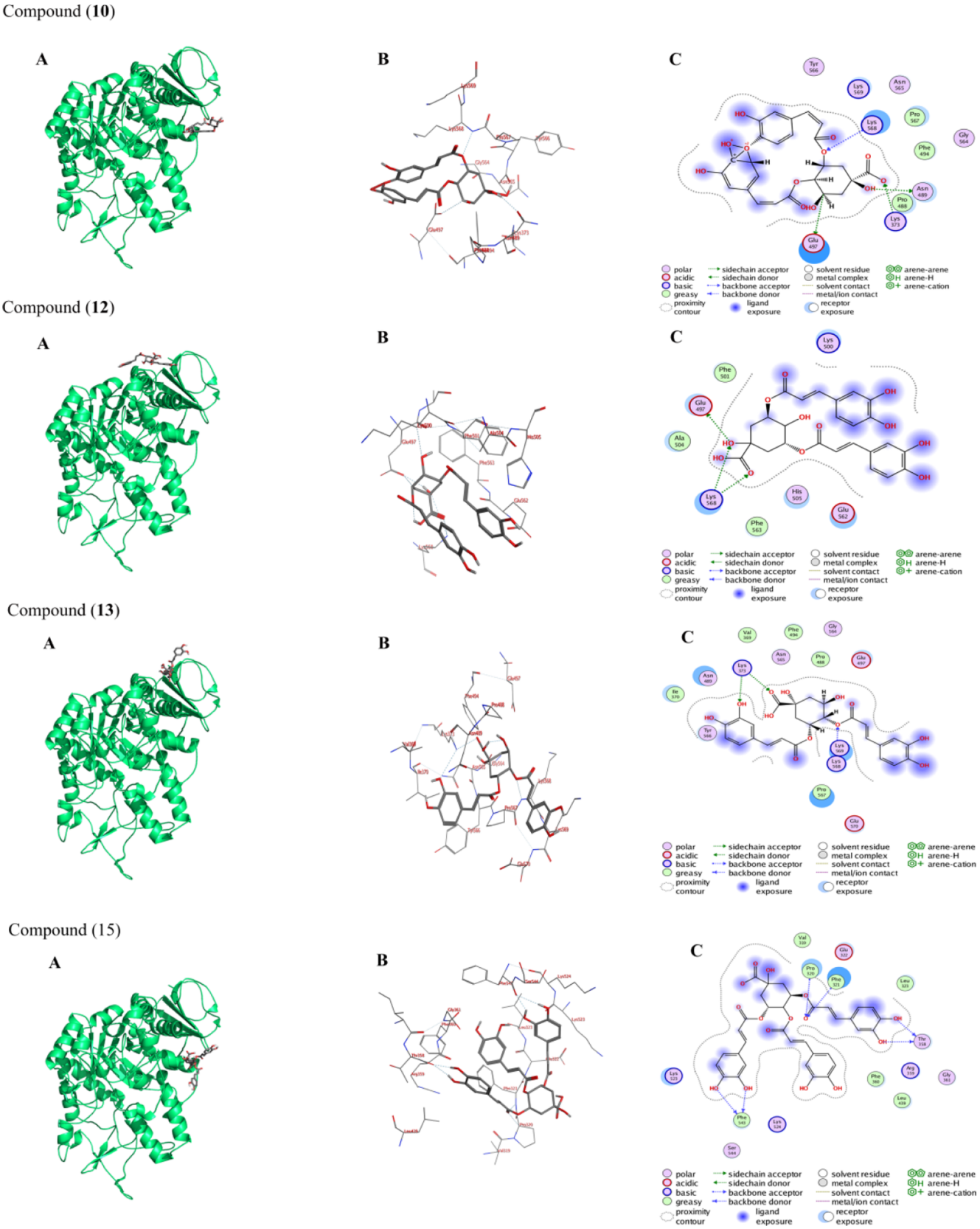

Fig. 7. Best docking pose found between α-glucosidase enzyme (3AXI) (A); 2D binding interactions of the best pose of *α*-glucosidase in complex with **10**, **12**, **13** and 15 (B); 3D binding interactions of the best pose of *α*-glucosidase in complex with **10**, **12**, **13** and **15** (C).

TM test strips and Accu-Chek Performa blood glucose meter, ROCHE). Blood samples were obtained from the tip of tail at the defined time patterns. Eq. (2) was used for the area under the curve of blood glucose level (AUC).

$$AUC=0.5 \times (FBC_0+2 \times FBG_{30}+2 \times FBG_{60}+2 \times FBG_{90}+2 \times FBG_{120}) \times 30 \quad (2)$$

where FBG_0, FBG_{30}, FBG_{60}, FBG_{90} and FBG_{120} are the FBG levels at 0, 30, 60, 90 and 120 min, respectively.

4.8. Small intestinal α-glucosidase inhibitory activity in mice

The Small intestinal α-glucosidase inhibitory activity was performed according to the method [46]. Normoglycemic mice fasted for 12 h were orally administered 400 mg/kg MeOH extract. The mice were sacrificed and the small intestines were resected at 0, 30, 60, 90 and 120 min. The mucosa was homogenized in 0.1 M PBS buffer (1:10 dilution). After centrifugation at 1000 rpm for 15 min, the supernatant was retained and used as an enzyme solutions. Measurement of the intestinal enzyme activity was performed via the aforementioned in *vitro* assays. The supernatant protein was determined with a Bio Rad Protein Assay Kit.

4.9. Apparatus and conditions of HPLC-DAD-Q-TOF-MS analysis

Characterization of chemical constituents was performed on an Agilent 6520 Q-TOF mass spectrometer equipped with a diode array detector (DAD) and electrospray interface (ESI) (Agilent Technologies, Santa Clara, CA). Major operating parameters were as follows: nebulizer pressure of 40 psi, scan spectra from *m/z* 50 to 1000, drying gas temperature of 325 °C, drying gas (N_2) flow rate 8.0 L/min, skimmer of 65 V, frag-mentor voltage of 100 V, capillary voltage of 3500 V and collision energy 25 eV. Data were processed by Agilent Mass Hunter Workstation Data Acquisition Software Version B (Agilent Technologies, Santa Clara, CA).

Quantifications of six characteristic compounds was accomplished on a L-2000 HPLC instrument (Hitachi, Tokyo, Japan) equipped with a ultraviolet (UV) detector, a quaternary pump, loop injection system, and a column oven. Chromatographic separation was performed on a reversed-phase column (PEGASIL ODS C18, 4.6 × 150 mm, 5 μm).

4.10. Quantitative analysis

The established analytical method in Sections 4.2 and 4.9 were subsequently applied to analyze *A. argyi*. Quantitation of five compounds was performed in triplicate and the results were expressed as means.

4.11. Molecular docking study

Molecular modeling studies were carried out in order to confirm the role of **10**, **12**, **13** and **15** in the inhibition of the tested enzymes, exemplarily. Homology modeling was performed by using the crystal structure of *S. cerevisiae* isomaltase (PD code: 3AXI) as the the template [47], which was download from the Protein Data Bank (PDB) website (http://www.rcsb.org).

4.12. Statistical analysis

Results of *in vivo* studies are presented as mean ± standard deviation (SD). Statistical differences between the treatments and the control were tested by one-way analysis of variance (ANOVA) with *Dunnett's t-test* using SPSS version 19.0. The *p*-values are represented as follows: statistically different from control group by *($p < 0.05$) and **($p < 0.01$). In *vitro* experiments, all analyses were carried out in triplicate and the results were averaged. All values were expressed as the mean ± SD; linear regression analyses and IC_{50} calculations were done by using SPSS version 19.0. The molecular docking method was performed using the Auto Dock (4.2) tools. Results were expressed in terms of free energy (kcal/mol; docking score) of ligand–protein binding.

Declaration of Competing Interest

The authors declare no conflicts of interest

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Appendix A. Supplementary material

The isolation and structural elucidation and nuclear magnetic resonance (NMR) spectroscopic data of rutin (**8**), isoquercitrin (**9**), isochlorogenic acid C (**13**), 3,4,5-tricaffeoylquinic acid (**15**), apigenin (**16**), hispidulin (**17**), 5,7,4-trihydroxy-6,3′,5′-trimethoxy-flavone (**18**), centaureidin (**20**) and eupatilin (**22**). Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioorg.2019.103268.

References

[1] S. Venkatesh, G.D. Reddy, B.M. Reddy, M. Ramesh, A.A. Rao, Antihyperglycemic activity of *Caralluma attenuata*, Fitoterapia 74 (2003) 274–279.
[2] A. Mitra, Some salient points in dietary and life-style survey of rural bengal particularly tribal populace in relation to rural diabetes prevalence, J. Ethnobiol. Ethnomed. 2 (2008) 51–56.
[3] A.K. Tiwari, J.M. Rao, Diabetes mellitus and multiple therapeutic approaches of phytochemicals: present status and future prospects, Curr. Sci. 83 (2002) 30–38.
[4] Y. Choi, Y. Jung, S.N. Kim, Identification of eupatilin from *Artemisia argyi* as a selective PPARα agonist using affinity selection ultrafiltration LC-MS, Molecules 20 (2015) 13753–13763.
[5] I.C. West, Radicals and oxidative stress in diabetes, Diabetic Med. 17 (2010) 171–180.
[6] F.A. Matough, S.B. Budin, Z.A. Hamid, N. Alwahaibi, J. Mohamed, The role of oxidative stress and antioxidants in diabetic complications, Sultan. Qaboos. Univ. Med. J. 12 (2012) 5–18.
[7] P.S. Sellamuthu, P. Arulselvan, B.P. Muniappan, S. Fakurazi, M. Kandasamy, Mangiferin from *Salacia* chinensis prevents oxidative stress and protects pancreatic β-cells in streptozotocin-induced diabetic rats, J. Med. Food. 16 (2013) 719–727.
[8] K.V. Pugalendi, B. Ramesh, Impact of umbelliferone (7-hydroxycoumarin) on hepatic marker enzymes in streptozotocin diabetic rats, Indian J. Pharmacol. 38 (2006) 380–383.
[9] T. Akihisa, K. Kawashima, M. Orido, H. Akazawa, M. Matsumoto, A. Yamamoto, E. Ogihara, M. Fukatsu, H. Tokuda, J. Fuji, Antioxidative and melanogenesis-inhibitory activities of caffeoylquinic acids and other compounds from moxa, Chem. Biodiversity. 10 (2013) 313–327.
[10] N. Techen, I. Parveen, Z. Pan, I.A. Khan, DNA barcoding of medicinal plant material for identification, Curr. Opin. Biotechnol. 25 (2014) 103–110.
[11] X. He, Study on *Argy Wormwood* food production and processing, J. Anhui Agric. Sci. 32 (2004) 995–996.
[12] M.J. Abad, L.M. Bedoya, L. Apaza, P. Bermejo, The *artemisia* L. Genus: a review of bioactive essential oils, Molecules 17 (2012) 2542–2566.
[13] G. Zengin, A. Molica, A. Aktumsek, C.M.N. Picot, M.F. Mahomoodally, In vitro and in silico insights of *Cupressus* sempervirens, *Artemisia* absinthium and *Lippia* triphylla: bridging traditional knowledge and scientific validation, Eur. J. Integr. Med. 12 (2017) 135–214.
[14] T.G. Liu, J.X. Chen, G.K. Lv, X.F. Li, J.H. Zhen, X.F. Li, Y.U. He, X.H. Gu, Professor KONG Guang-yi's experience on menstrual diseases studied by using complex system entropy method, J. Beijing. Univ. Tradit. Chin. Med. (2013).
[15] N. Li, Y. Mao, C.H. Deng, X.M. Zhang, Separation and identification of volatile constituents in *Artemisia argyi* flowers by GC-MS with SPME and steam distillation, J. Chromatogr. Sci. 46 (2008) 401–405.
[16] B. Han, Z. Xin, S. Ma, W. Liu, B. Zhang, L. Ran, D. Ren, Comprehensive characterization and identification of antioxidants in *Folium Artemisiae Argyi* using high-resolution tandem mass spectrometry, J. Chromatogr. B 1063 (2017) 84–92.
[17] M. Yoshikawa, H. Shimada, H. Matsuda, J. Yamahara, N. Murakami, Bioactive constituents of Chinese natural medicines. 1. New sesquiterpene ketones with

vasorelaxant effect from Chinese moxa, the processed leaves of *Artemisia argyi* Levl et Vant: Moxartenone and moxartenolide, Chem. Pharm. Bull. 44 (1996) 1656–1662.
[18] L.B. Zhang, J.L. Lv, H.L. Chen, X.Q. Yan, J.A. Duan, Chemical constituents from *Artemisia argyi* and their chemotaxonomic significance, Biochem. Syst. Ecol. 55 (2013) 455–458.
[19] R.X. Tan, Z.J. Jia, Eudesmanolides and other constituents from *Artemisia argyi*, Planta Med. 58 (1992) 370–372.
[20] S. Wang, J. Sun, K.W. Zeng, X.G. Chen, W.Q. Zhou, C. Zhang, H.W. Jin, Y. Jiang, P.F. Tu, Sesquiterpenes from *Artemisia argyi*: absolute configurations and biological activities, Eur. J. Org. Chem. 2104 (2014) 973–983.
[21] S. Toda, Antioxidative effects of polyphenols from leaves of *Artemisia princeps* Pamp. On lipid peroxidation *in vitro*, J. Food Biochem. 29 (2005) 305–312.
[22] H.G. Lee, K.A. Yu, W.K. Oh, T.W. Baeg, H.C. Oh, J.S. Ahn, W.C. Jang, J.W. Kim, J.S. Lim, Y.K. Choe, D. Yoon, Inhibitory effect of jaceosidin isolated from *Artemisia argyi* on the function of E6 and E7 oncoproteins of HPV 16, J. Ethnopharmacol. 98 (2015) 339–343.
[23] K. Yamazaki, T. Inoue, N. Yasuda, Y. Sato, T. Nagakura, O. Takenaka, R. Ckark, T. Saeki, I. Tanaka, Comparison of efficacies of a dipeptidyl peptidase IV inhibitor and α-glucosidase inhibitors in oral carbohydrate and meal tolerance tests and the effects of their combination in mice, J. Pharmacol. Sci. 4 (2007) 29–38.
[24] T. Oki, T. Matsui, M. Osajima, Inhibitory effect of alpha-glucosidase inhibitors varies according to its origin, J. Agric. Food. Chem. 47 (1992) 550–553.
[25] R.J. Marles, N.R. Farnsworth, Antidiabetic plants and their active constituents, Phytomedicine 2 (1995) 137–189.
[26] C. Rodriguezcerdeira, E. Sanchezblanco, Glycolic acid 15% plus salicylic acid 2%: a new therapeutic pearl for facial flat warts, J. Clinic. Aesthetic Dermatol. 4 (2011) 62–64.
[27] E.M. Sánchezsalcedo, M. Tassotti, D.D. Rio, F. Hernándeza, F.H. Martíneza, J.J. Martíneza, P. Menab, (Poly) phenolic fingerprint and chemometric analysis of white (*Morus alba* L.) and black (*Morus nigra* L.) mulberry leaves by using a non-targeted UHPLC-MS approach, Food. Chem. 212 (2016) 250–255.
[28] L. Zhou, J. Kang, L. Fan, X.C. Ma, H.H. Zhao, J. Han, B.R. Wang, D.A. Guo, Simultaneous analysis of coumarins and secoiridoids in *Cortex Fraxini* by high-performance liquid chromatography-diode array detection-electrospray ionization tandem mass spectrometry, J. Pharmaceut. Biomed. 47 (2008) 39–46.
[29] P. Quiferrada, G. Chivablanch, R. Estruch, A comprehensive characterisation of beer polyphenols by high resolution mass spectrometry (LC-ESI-LTQ-Orbitrap-MS), Food Chem. 169 (2015) 336–343.
[30] Z. Fu, Y. Ling, Z. Li, M. Chen, C. Huang, HPLC-Q-TOF-MS/MS for analysis of major chemical constituents of Yinchen-Zhizi herb pair extract, Biomed. Chromatogr. 28 (2014) 475–485.
[31] M.N. Clifford, K.L. Johnston, S. Knight, N. Kuhnert, Hierarchical scheme for LC-MSn identification of chlorogenic acids, J. Agr. Food. Chem. 51 (2003) 2900–2911.
[32] J. Han, M. Ye, X. Qiao, B.R. Wang, D.A. Guo, Characterization of phenolic compounds in the Chinese herbal drug *Artemisia annua* by liquid chromatography coupled to electrospray ionization mass spectrometry, J. Pharmaceut. Biomed. 47 (2008) 516–525.
[33] L. He, Z. Zhang, L. Lu, J. Wang, Z. Song, Z. Yan, J. Miao, Rapid identification and quantitative analysis of the chemical constituents in *Scutellaria indica*, L. by UHPLC-QTOF-MS and UHPLC-MS/MS, J. Pharmaceut. Biomed. Anal. 117 (2016) 125–139.
[34] S. Glasl, P. Mucaji, I. Werner, A. Presser, J. Jurenitsch, Sesquiterpenes and flavonoid aglycones from a Hungarian taxon of the *Achillea millefolium* group, Z. Naturforsch. C. 57 (2002) 976–982.
[35] E.M. Suleimenov, F.M. Smagulova, O.V. Morozova, V.A. Raldugin, I.Y. Bagryanskaya, Y.V. Gatilov, V.I. Yamovoi, S.M. Adekenov, Sesquiterpene Lactones and Flavonoids from *Artemisia albida*, Chem. Nat. Compd. 41 (2005) 689–691.
[36] J.P. Lai, Y.H. Lim, J. Su, H.M. Shen, C.N. Ong, Identification and characterization of major flavonoids and caffeoylquinic acids in three *Compositae* plants by LC/DAD-APCI/MS, J. Chromatogr. B 848 (2007) 215–225.
[37] M.N. Islam, J.C. Ran, H.A. Jung, H.O. Sang, J.S. Choli, Promising anti-diabetic potential of capillin and capillinol isolated from *Artemisia* capillaris, Arch. Pharm. Res. 39 (2016) 340–349.
[38] F. Tian, L. Liu, D.Q. Dou, T.G. Kang, S. David, Chemical comparison of *Artemisia* borealis and three Chinese *Artemisia* plants, J. Pharm. Sci. 16 (2017) 27–31.
[39] C.M. Ma, M. Hattori, M. Daneshtalab, L.L. Wang, Chlorogenic acid derivatives with alkyl chains of different lengths and orientations: potent alpha-glucosidase inhibitors, J. Med. Chem. 51 (2008) 6188–6194.
[40] S. Uysal, A. Ugurlu, G. Zengin, M.C. Baloglu, Y.C. Altunoglu, A. Mollica, L. Custodio, N.R. Neng, J.M. Nogueira, M.F. Mahomoodally, Novel, in vitro, and in silico, insights of the multi-biological activities and chemical composition of Bidens tripartita L, Food Chem. Toxicol. 111 (2018) 525–536.
[41] A. Uysa, O.Y. Ozer, G. Zengin, A. Stefanucci, A. Mollica, C.M.N. Picot-Allain, M.F. Mahomoodally, Multifunctional approaches to provide potential pharmacophores for the pharmacy shelf: Heracleum sphondylium L. subsp. ternatum (Velen.) Brummitt, Comput. Biol. Chem. 78 (2019) 64–73.
[42] T. Akihisa, K. Kawashima, M. Orido, H. Akazawa, M. Matsumoto, A. Yamamoto, E. Ogihara, M. Fukatsu, H. Tokuda, J. Fuji, Antioxidative and melanogenesis-inhibitory activities of caffeoylquinic acids and other compounds from Moxa, Chem. Biodivers. 10 (2013) 313–327.
[43] P. Hu, D.H. Li, C.C. Jia, Q. Liu, X.F. Wang, Z.L. Li, H. Hua, Bioactive constituents from *Vitex negundo*, var. heterophylla, and their antioxidant and α-glucosidase inhibitory activities, J. Funct. Food. 35 (2017) 236–244.
[44] S. Ydjedd, S. Bouriche, R. Lopez-Nicolas, T. Sanchez-Moya, C. Frontela-Saseta, G. Ros-Berruezo, H. Louaileche, D.E. Kati, Effect of *in Vitro* gastrointestinal digestion on encapsulated and nonencapsulated phenolic compounds of carob (*Ceratonia siliqua* L.) pulp extracts and their antioxidant capacity, J. Agric. Food. Chem. 65 (2017) 827–835.
[45] H. Jemai, M. Bouaziz, S. Sayadi, Phenolic composition, sugar contents and antioxidant activity of Tunisian sweet olive cultivar with regard to fruit ripening, J. Agric. Food. Chem. 57 (2009) 2961–2968.
[46] T. Satoh, M. Igarashi, S. Yamada, N. Takahashi, K. Watanabe, Inhibitory effect of black tea and its combination with acarbose on small intestinal α-glucosidase activity, J. Ethnopharmacol. 161 (2015) 147–155.
[47] A. Mocan, G. Zengin, M. Simirgiotis, M. Schafberg, A. Mollica, D.C. Vodnar, G. Crişan, S. Rohn, Functional constituents of wild and cultivated Goji (L. barbarum L.) leaves: phytochemical characterization, biological profile, and computational studies, J. Enzym. Inhib. Med. Ch. 32 (2017) 153–168.