

SUPPLEMENTARY MATERIAL

Chemical composition and biological activities of an essential oil from the aerial parts of *Artemisia gmelinii* Weber ex Stechm

Qian Xu^{1,2}, Luyun Zhang^{2,3}, Shuai Yu², Guangqing Xia², Junyi Zhu^{2,*} and Hao Zang^{2,3,*}

1. College of Medicine, Yanbian University, Yanji 133000, China

2. School of Pharmacy and Medicine, Green Medicinal Chemistry Laboratory, Tonghua Normal University, Tonghua 134002, China;

3. Jilin Ginseng Academy, Changchun University of Chinese Medicine, Changchun 130117, China

Abstract: The aerial parts of *Artemisia gmelinii* Weber ex Stechm were collected from the northeast of China. The essential oil was obtained by hydrodistillation and analysed by GC-MS. A set of 66 compounds were identified representing 99.1 % of the oil composition. The major compounds in the oil were cyclobutaneethanol, endo-borneol, germacrene D, eucalyptol, selin-6-en-4 α -ol, bisabolone oxide A, caryophyllene and terpinen-4-ol. Moreover, the essential oil was evaluated for its antioxidant, antidiabetic, and anticholinesterase activities *in vitro*. Additionally, the antioxidant potential of the oil was evaluated using DPPH and ABTS assays. The oil showed good antidiabetic activity with an IC₅₀ of 63.2 μ g/mL, which was similar to that of the positive control acarbose, and weak anticholinesterase activities. These findings demonstrated that the essential oil of *Artemisia gmelinii* may be a good natural antidiabetic.

Keywords: *Artemisia gmelinii* Weber ex Stechm; GC-MS; antioxidant activity; antidiabetic activity; cholinesterase inhibitory activity; docking studies

Experimental section

Plant material

The plant material (aerial parts of *A. gmelinii*) was collected (Number of Voucher specimen: 17-06-05-001) in June 2017, in Tonghua, Jilin Province and botanically identified as *A. gmelinii* based on similarity to herbarium specimen of Tonghua Normal University.

Extraction of essential oil

EO was hydrodistilled for 5 hours in a Clevenger-type apparatus. In the hydrodistillation, fresh material was chopped by a knife, the relationship between material mass and water volume was 1/5, with the bath temperature of 120 °C. The EO layer was separated, dried with Na₂SO₄ and stored in an amber glass bottle at 4 °C until used.

Chemical composition analysis of essential oil

The chemical composition analysis of essential oils of *A. gmelinii* was carried out at the Medicinal Chemistry Laboratory in Tonghua Normal University. Sample of EO was analyzed on an Thermo Fisher Trace 1300-ISQ GC/MS (Thermo Fisher Scientific), equipped with a SE-54 quartz capillary column (Thermo Fisher Scientific; 30 by 0.25 mm inner diameter, 0.25 µm). Initial GC oven temperature was set at 50 °C, following a temperature increase of 5 °C/min until 200 °C, following a temperature increase of 1 °C/min until 220 °C. The carrier gas was helium at a constant pressure of 500 kPa. For each analysis, 1.0 µL of essential oil solution (Volatile oil diluted 10 times using ethyl ether) was injected in split mode (1:10) at 250 °C. MS transfer line temperature was set at 280 °C, ion source temperature was set at 230 °C, and the mass spectrum of the compounds was obtained in 70 eV (EI mode), 0.2 scan/s, m/z 50-500. Compounds were identified based on the mass spectra (as compared to the Mainlib library), and the concentrations were presented as relative percentages of the area of each peak over the total area. The analysis results are shown in Figure S1.

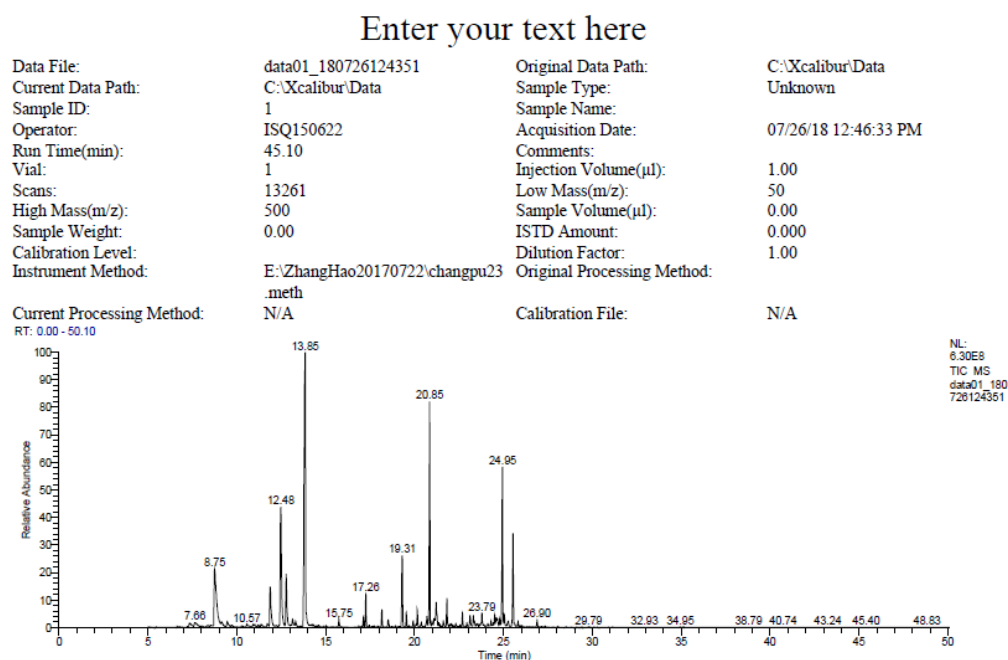


Figure S1 GC-MS analysis results of essential oil

Biological activities

DPPH Radical Scavenging Assay

Each sample (100 μL) in methanol at different concentrations (from 0.15625 to 10 mg/mL) was added to 100 μL of DPPH methanol solution (50 μM). The solution was vortexed in 96-well plates for 10 s and then left at room temperature for 20 min in the dark. The absorbance of the resulting solution was measured at 492 nm on a microplate spectrophotometer (BioTek). *L*-Ascorbic acid and trolox were used as positive references. IC₅₀ values (the concentrations required to scavenge 50% DPPH radicals present in the test solution) were calculated and expressed as means ± SD in μg/mL.

ABTS Radical Cation Scavenging Assay

1 mL 2.6 mM of potassium persulfate was added to 1 mL 7 mM of ABTS•⁺, and the mixture was allowed to stand in the dark at room temperature for 12-16 h before using. The ABTS•⁺ solution was diluted with methanol to provide an absorbance of 0.70±0.02 at 734 nm. The diluted ABTS•⁺ solution (190 μL) was added to sample

fractions (10 μ L) in DMSO at different concentrations (from 0.00625 to 10 mg/mL). A standard curve was constructed by measuring the reduction in absorbance of the ABTS \bullet^+ solution at different concentrations of trolox (0-1 mg/mL). Each treatment was conducted in triplicate. The plates were incubated at room temperature for 20 min in the dark, the absorbance in each well was read at 734 nm on a microplate spectrophotometer (BioTek). *L*-ascorbic acid and trolox were used as positive references. The inhibition rates of ABTS \bullet^+ were calculated according to the following formula: ABTS scavenging rate (%)=[1-(absorbance of compound-absorbance of blank)/absorbance of control] \times 100%. IC₅₀ values were calculated and expressed as means \pm SD in μ g/mL.

Acetylcholinesterase Inhibition Assay

Each sample in 10% DMSO solution(20 μ L) (from 0.078125 to 10 mg/mL) was added to 120 μ L phosphate buffer (pH 8.0, 0.1 M) and 20 μ L of acetylcholinesterase solution (pH 8.0, 0.8 U/mL, in 0.1 M phosphate buffer). The reaction mixtures were incubated at 25 °C for 15 min. Then, 20 μ L acetylthiocholine iodide (ATCI) solution (pH 8.0, 1.78 mM, in 0.1 M phosphate buffer) and 20 μ L 5,5'-Dithiobis-(2-nitrobenzoic acid) (DTNB) solution (pH 8.0, 1.25 mM, in 0.1 M phosphate buffer) were added to each well, the reaction mixtures were incubated at 25 °C for 5 min. Before incubating as well as after that, the absorbance was recorded at 405 nm on a microplate spectrophotometer (BioTek). Donepezil was used as a positive reference. The acetylcholinesterase inhibition activity was expressed as % inhibition and was calculated as follows:

$$\%inhibition = \left(1 - \frac{\Delta A_{sample}}{\Delta A_{control}}\right) \times 100\%$$

Butyrylcholinesterase Inhibition Assay

Each sample in 10 % DMSO solution(20 μ L) (from 0.15625 to 10 mg/mL) was added to 120 μ L phosphate buffer (pH 8.0, 0.1 M) and 20 μ L of butyrylcholinesterase solution(pH 8.0, 0.8 U/mL, in 0.1 M phosphate buffer). The reaction mixtures were incubated at 25 °C for 15 min. Then, 20 μ L butyrylthiocholine chloride solution (pH 8.0, 0.4 mM, in 0.1 M phosphate buffer) and 20 μ L 5,5'-Dithiobis-(2-nitrobenzoic acid) (DTNB) solution (pH 8.0, 1.25 mM, in 0.1 M phosphate buffer) were added to each well, the reaction mixtures were incubated at 25 °C for 5 min. Before incubating as well as after that, the absorbance was recorded at 405 nm on a microplate spectrophotometer (BioTek). Donepezil was used as a positive reference. The butyrylcholinesterase inhibition activity was expressed as % inhibition and was calculated as follows:

$$\%inhibition = \left(1 - \frac{\Delta A_{sample}}{\Delta A_{control}}\right) \times 100\%$$

α -Glucosidase Inhibition Assay

Each sample in DMSO solution(20 μ L) (from 0.53125 to 10 mg/mL) was added to 100 μ L of α -Glucosidase solution (pH 6.9, 0.1 U/mL, in 0.1 M phosphate buffer). The reaction mixtures were incubated at 25 °C for 10 min. Then, 50 μ L p-nitrophenyl- α -D-glucopyranoside (pNPG) solution (pH 6.9, 5 mM, in 0.1 M phosphate buffer) was added to each well, the reaction mixtures were incubated at 25 °C for 5 min. Before incubating as well as after that, the absorbance was recorded at 405 nm on a microplate spectrophotometer (BioTek). Acarbose was used as a positive

reference. The α -Glucosidase inhibition activity was expressed as % inhibition and was calculated as follows:

$$\% inhibition = \left(1 - \frac{\Delta A_{sample}}{\Delta A_{control}} \right) \times 100\%$$

Statistical Analysis

All the experiments were carried out in triplicate and the data are analyzed using SPSS software (Version 22.0) and Origin software (Version 8.0).

Molecular docking studies with α -glucosidase

In contemplation of revealing the binding modes of EO of *A. gmelinii*, docking simulation was performed targeting the crystal structure of α -glucosidase. Since crystal structure for α -glucosidase of *Saccharomyces cerevisiae* is still not available, docking study was conducted using a homology model for α -glucosidase. Preliminary results on sequence analysis of α -glucosidase from *S. cerevisiae* showed that the most suitable template for homology modeling is isomaltase (EC 3.2.1.10, oligo-1,6-glucosidase, MALX3) (PDB ID: 3A4A) from baker's yeast which shares 71% identity and 84% similarity with the target enzyme, α -glucosidase of *S. cerevisiae*. The crystal structure was retrieved from the protein data bank (PDB), the α -glycosidase was pre-docked using the Autodock. The 3D structures of the acarbose and the major components of EO were built by Chemdraw and converted to pdbqt coordinate using Auto dock Tools. In AUTOGRID for each atom type in the ligand, maps were calculated with 0.375 Å spacing between grid points and the center of the grid box was placed at x = 12.583, y = -7.896, z = 12.519. The dimensions of the active site box were set at 40 × 40 × 40 Å.

Flexible ligand dockings were accomplished for the selected compounds. Each docked system was carried out by 50 runs of the Autodock search by the Lamarckian genetic algorithm (LGA). The best poses of the selected compounds were selected for analyzing the interactions between enzyme and the inhibitors. The results were visualized using Discovery Studio Client 2017. Figure S2-S19 showed the binding mode of acarbose and major components of EO.

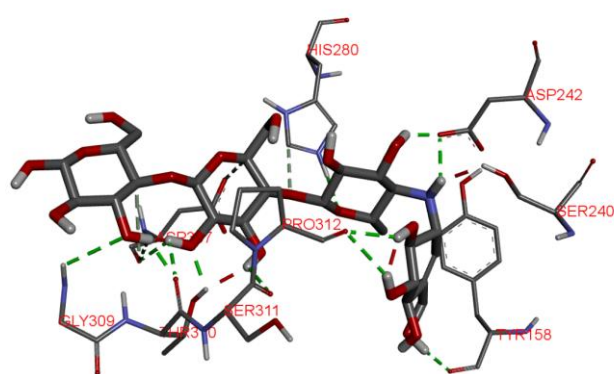


Figure S2 Binding mode of acarbose in the binding pocket of a developed homology model of α -glucosidase.

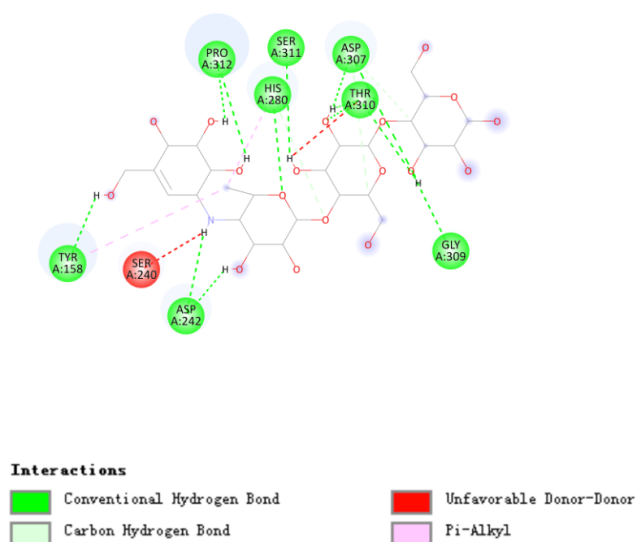


Figure S3 The predicted binding mode of acarbose in the active site pocket.

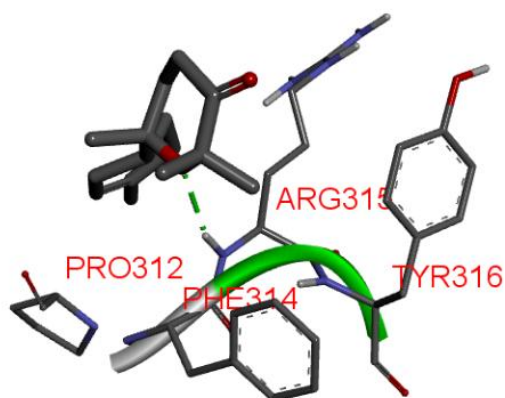


Figure S4 Binding mode of Bisabolone oxide A in the binding pocket of a developed homology model of α -glucosidase.

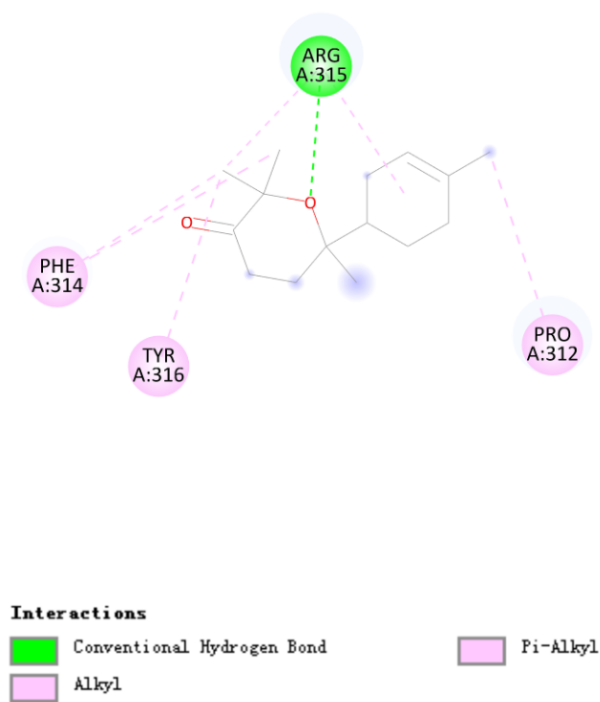


Figure S5 The predicted binding mode of Bisabolone oxide A in the active site pocket.

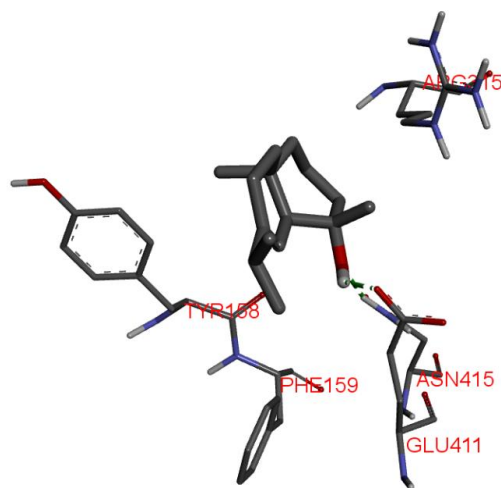


Figure S6 Binding mode of Selin-6-en-4 α -ol in the binding pocket of a developed homology model of α -glucosidase.

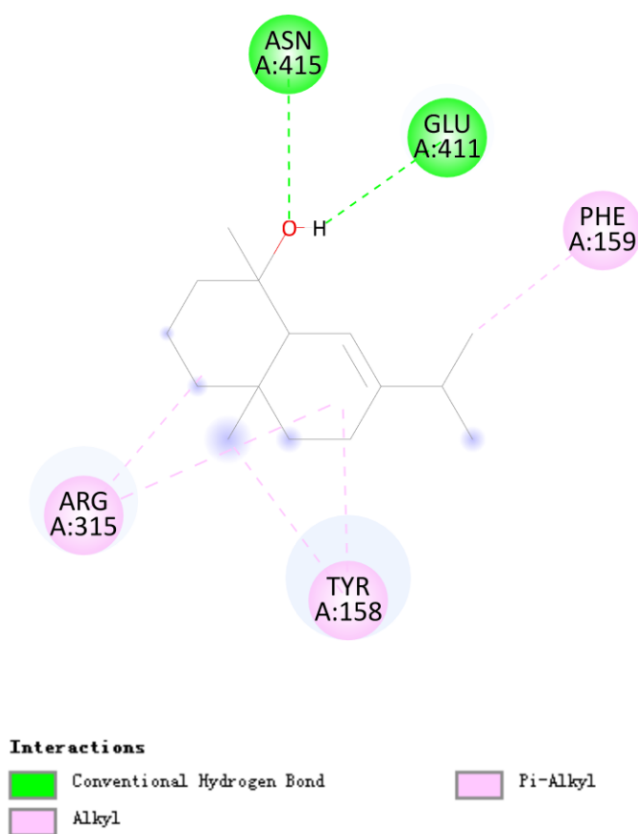


Figure S7 The predicted binding mode of Selin-6-en-4 α -ol in the active site pocket.

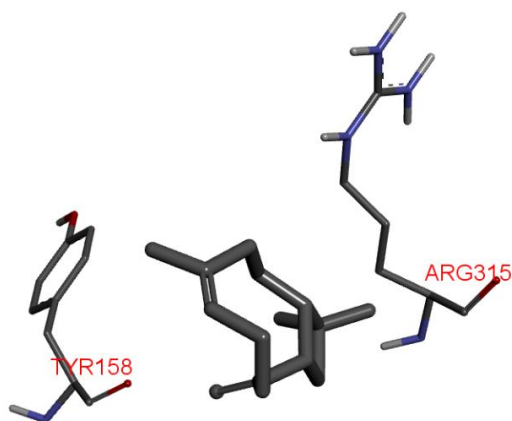


Figure S8 Binding mode of Caryophyllene in the binding pocket of a developed homology model of α -glucosidase.

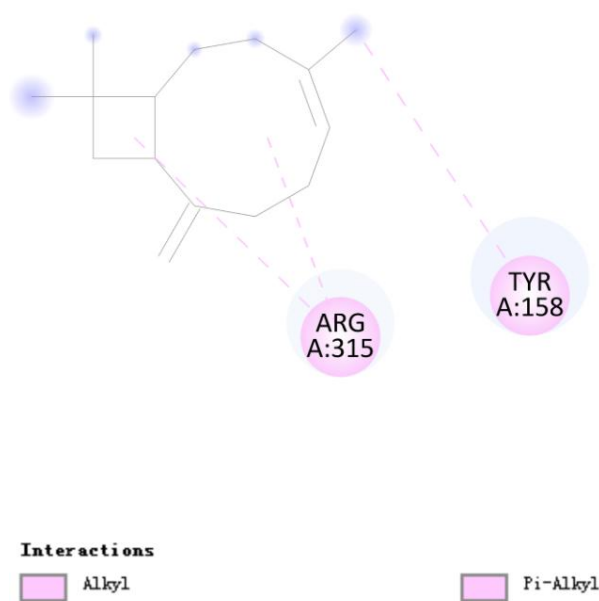


Figure S9 The predicted binding mode of Caryophyllene in the active site pocket.

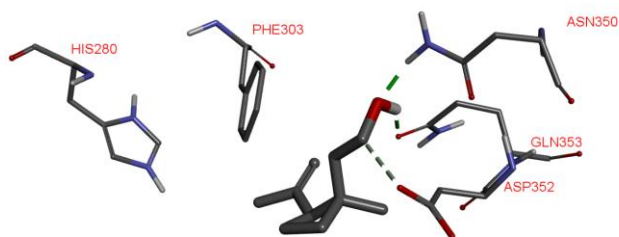


Figure S10 Binding mode of Cyclobutaneethanol in the binding pocket of a developed homology model of α -glucosidase.

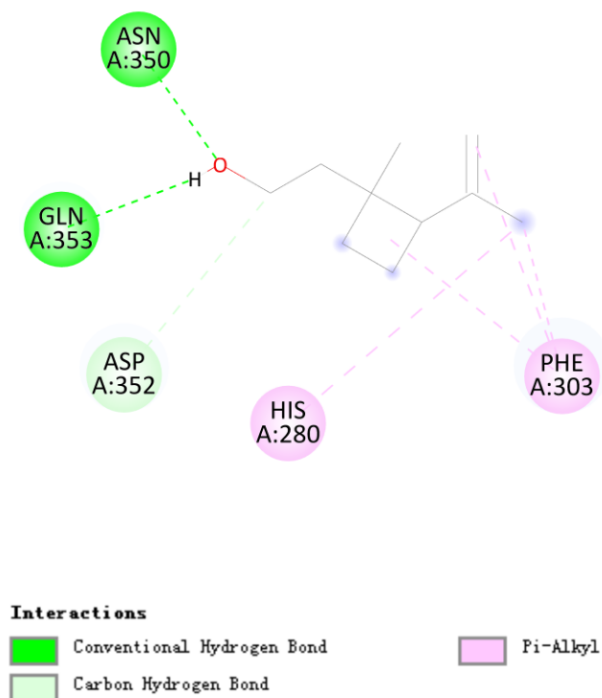


Figure S11 The predicted binding mode of Cyclobutaneethanol in the active site pocket.

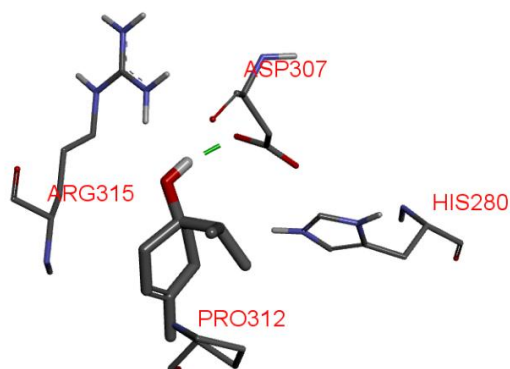


Figure S12 Binding mode of Terpinen-4-ol in the binding pocket of a developed homology model of α -glucosidase.

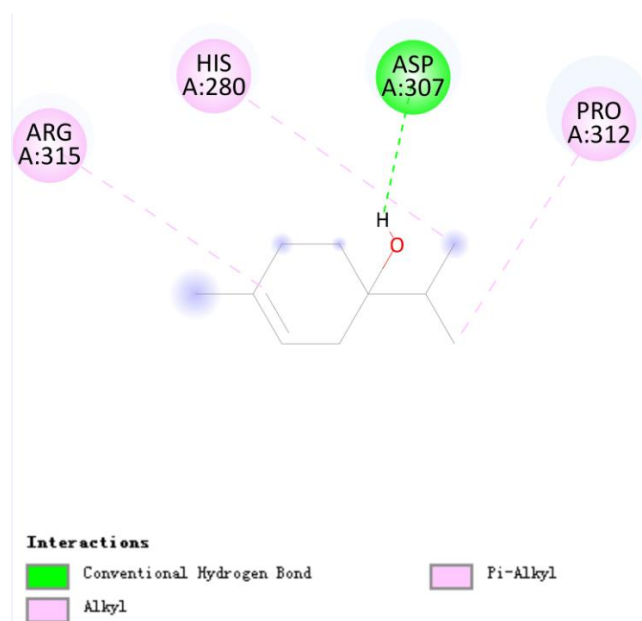


Figure S13 The predicted binding mode of Terpinen-4-ol in the active site pocket.

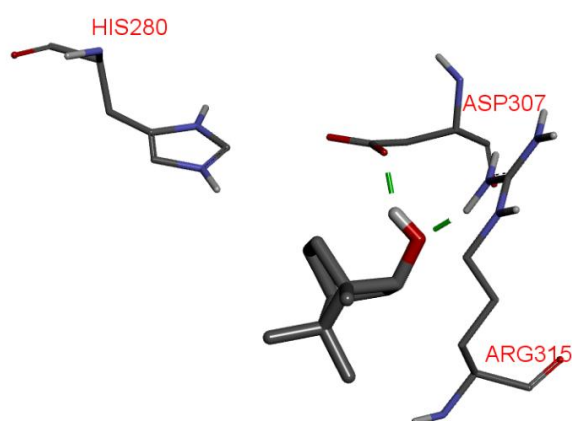


Figure S14 Binding mode of endo-Borneol in the binding pocket of a developed homology model of α -glucosidase.

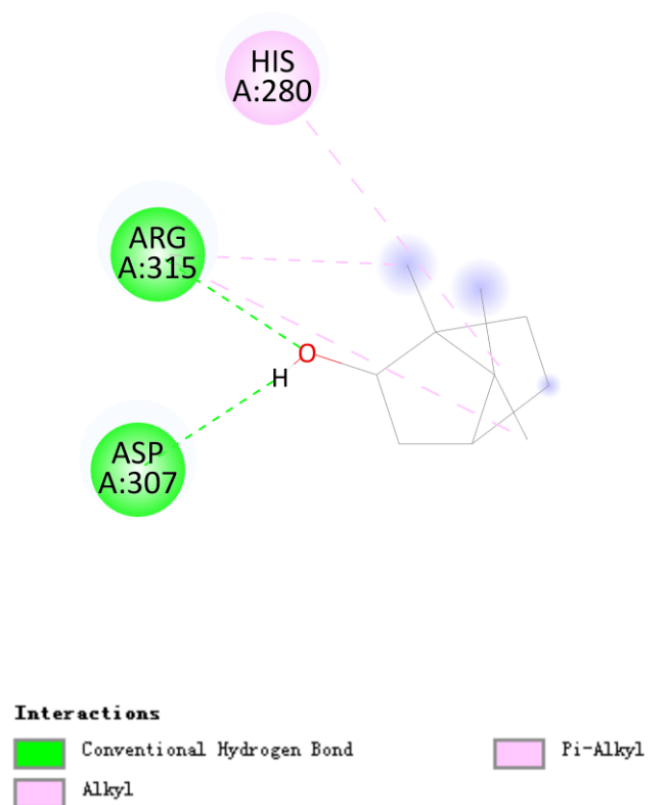


Figure S15 The predicted binding mode of endo-Borneol in the active site pocket.

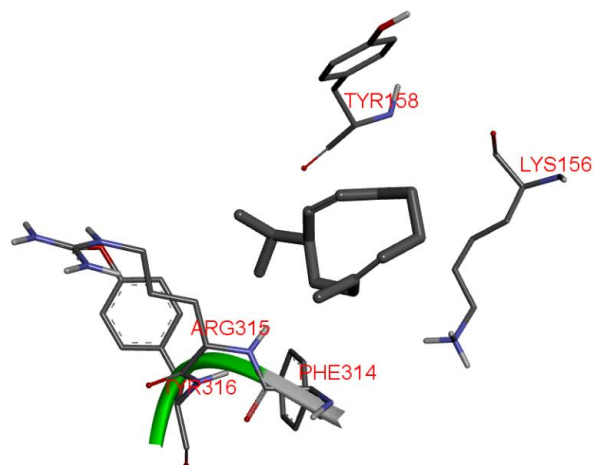


Figure S16 Binding mode of Germacrene D in the binding pocket of a developed homology model of α -glucosidase.

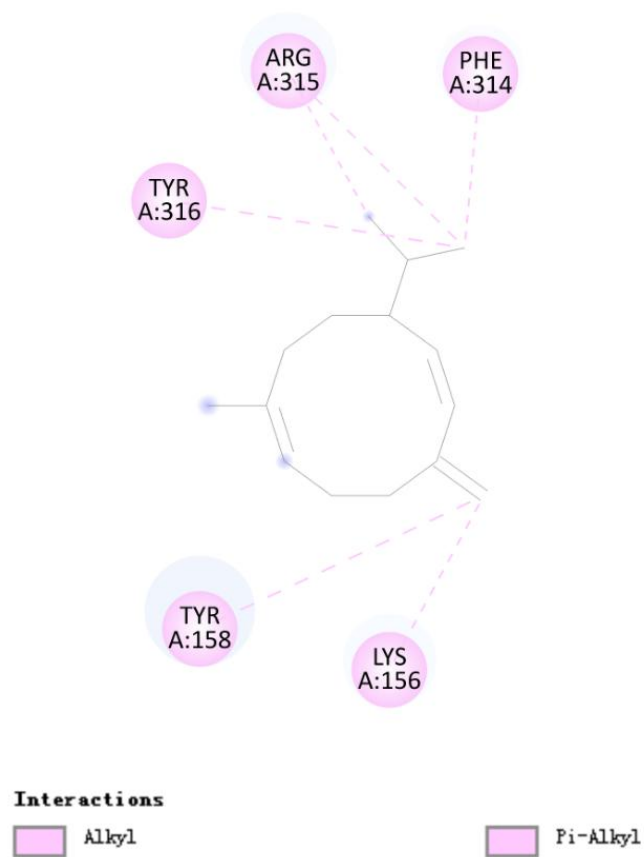


Figure S17 The predicted binding mode of Germacrene D in the active site pocket.

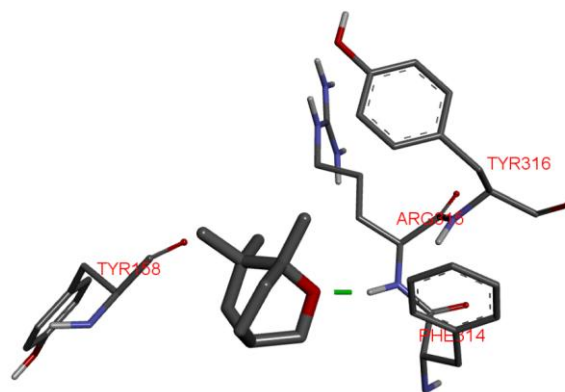


Figure S18 Binding mode of Eucalyptol in the binding pocket of a developed homology model of α -glucosidase.

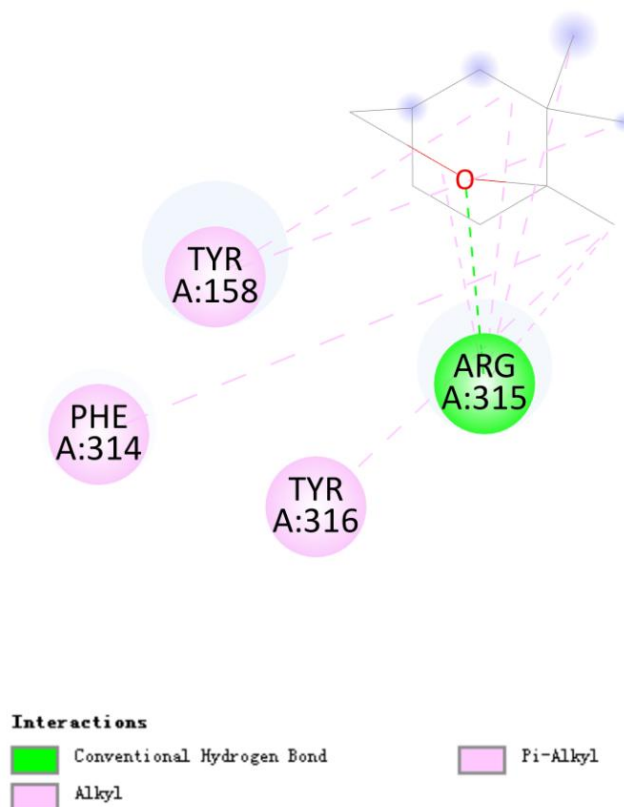


Figure S19 The predicted binding mode of Eucalyptol in the active site pocket.

Table S1 Chemical composition of essential oil of *Artemisia gmelinii*

Compound	RI ^a	%	Compound	RI ^a	%
1-Octen-3-ol	978	0.6	cis-Muurolo-4(15),5-diene	1461	0.2
β -Pinene	980	0.8	(Z,E)- α -Farnesene	1462	0.2
Terpinolene	1088	0.1	γ -Muurolene	1476	0.6
o-Cymene	1022	0.2	γ -Amorphene	1478	0.4
Eucalyptol	1032	8.8	Germacrene D	1480	9.7
γ -Terpinene	1062	0.5	β -Selinene	1492	0.4
cis-4-Thujanol	1066	0.1	Guaia-1(10),11-diene	1498	0.1
trans-4-Thujanol	1068	0.2	β -Cyclogermacrane	1500	1.9
4-Thujanol	1094	0.6	cis- α -Bisabolene	1504	0.8
(Z)-2-Ochtoden-1-al	1103	0.2	Cadina-1(10),4-diene	1508	1.3
Tetrahydrofuran, 2-isobutenyl-4-vinyl	1112	0.1	(-)-Cubebol	1513	0.2
(-)-trans-Pinocarveol	1139	0.2	Diepicedrene-1-oxide	1551	0.1
2-Cyclohexen-1-ol, 1-methyl-4-(1-methylethyl)-, trans	1140	0.1	Nerolidol	1564	0.6
trans-Alloocimene	1142	0.2	Fragranyl 2-methylbutyrate	1575	0.2
(-)-Camphor	1146	3.5	Longipinocarvone	1578	0.6
endo-Borneol	1165	9.8	Caryophyllene oxide	1581	0.5

Terpinen-4-ol	1177	3.7	cis- β -Elemenone	1594	0.1
5-Caranol, trans,trans-(+)-	1185	0.1	Isoaromadendrene epoxide	1612	0.4
Terpineol	1196	0.6	Cadinenol	1614	0.3
(-)-Myrtenol	1212	0.4	Copaborneol	1618	0.8
Cyclobutaneethanol	1216	24.0	Aromadendrene oxide-(2)	1621	0.1
Isobornyl formate	1232	0.1	Junenol	1629	0.2
cis-p-Mentha-1(7),8-dien-2-ol	1234	0.2	Selin-6-en-4 α -ol	1636	8.0
Carvacrol methyl ether	1239	0.1	T-cadinol	1640	0.6
Levo-bornyl acetate	1283	0.4	10,10-Dimethyl-2,6-dimethylenebi cyclo[7.2.0]undecan-5-ol	1644	0.5
Elemene isomer	1344	0.4	11,11-Dimethyl-4,8-dimethylenebi cyclo[7.2.0]undecan-3-ol	1646	0.2
	1346		7-Isopropyl-4a,8a-dimethyl-4a,5,6,	1657	
Fragranyl acetate		1.4	7,8,8a-hexahydro-2(1H)-naphthale none		0.4
Ylangene	1367	0.7	Ylangenol	1666	0.4
(+)-Longicyclene	1372	0.7	Bisabolone oxide A	1679	4.4
α -Copaene	1376	0.3	4(15),5,10(14)-Germacatrien-1-ol	1695	0.2
(-)- β -Cubebene	1389	0.4	5-Isopropyl-6-methyl-3-heptyne-2, 5-diol	1710	0.2
Caryophyllene	1418	3.8	Bisabolol oxide A	1744	0.3
cis- β -Copaene	1428	0.6	Total		99.1
(S,1Z,6Z)-8-Isopropyl-1-methyl-5- methylenecyclodeca-1,6-diene	1439	0.3			

(RI: retention index; MS: mass spectroscopy).

Table S2 Antioxidant activity of essential oil of *Artemisia gmelinii*

Samples	Antioxidant activity	
	DPPH IC ₅₀ (μ g/ml)	ABTS IC ₅₀ (μ g/ml)
Essential oil	2400.0 \pm 8.6	282.5 \pm 1.5
L-Ascorbic acid	1.7 \pm 0.0	4.8 \pm 0.0
Trolox	0.6 \pm 0.0	4.2 \pm 0.0

Table S3 Enzyme inhibitory activities of essential oil of *Artemisia gmelinii*

Samples	Antidiabetic activity	Cholinesterase inhibitory activity	
	IC ₅₀ (μ g/ml)	IC ₅₀ (μ g/ml)	
	α -Glucosidase	AchE	BchE
Essential oil	63.2 \pm 0.3	341.7 \pm 5.7	166.3 \pm 2.9
Acarbose	39.3 \pm 0.2	N.D.	N.D.
Donepezil	N.D.	0.1 \pm 0.0	1.5 \pm 0.0

N.D. Not determined