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Dynamic Change of Secondary Metabolites and spectrum-effect relationship of *Malus halliana* Koehne flowers during blooming

https://doi.org/10.1515/chem-2018-0043
received December 31, 2017; accepted March 5, 2018.

Abstract: *Malus halliana* Koehne flowers have been used as a Chinese traditional medicine to treat metrorrhagia. In this study, the dynamic changes in its secondary metabolites and spectrum-effect relationship of inhibition on *α*-glucosidase during blooming were investigated. The changes in the contents of three flavonoids (phloretin-4'-*O*-glycosidase, afzeloside, and 3- hydroxyphloridzin) were determined by high performance liquid chromatography (HPLC) and changes in inhibitory effect on *α*-glucosidase were evaluated *in vitro*. Then, spectrum-effect relationship was evaluated by partial least square method. The results indicated that the contents of three flavonoids and inhibition of *α*-glucosidase activity *in vitro* showed a fluctuating downward trend, thereinto, the maximum contents of phloretin-4'-O-glycosidase, afzeloside, and 3-hydroxyphloridzin reached 157.43±0.36, 17.27±0.06 and 22.67±0.35 (mg/g), respectively. In spectrum-effect relationship assay, matched 40 mutual peaks, thereinto, P_2, P_5, P_6-P_{12}, P_{14} (3-hydroxyphloridzin), P_{16}-P_{19}, P_{20} (phloretin-4'-*O*-glycosidase), P_{24}, P_{26}, P_{29}, P_{31}, P_{33}, P_{34}, P_{36}, P_{39} and P_{40} were positively correlated to inhibitory effect on *α*-glucosidase *in vitro*. P_1, P_3, P_4, P_{13}, P_{15}, P_{21}-P_{23}, P_{27}, P_{28}, P_{30} (afzeloside), P_{32}, P_{35}, P_{37} and P_{38} were negatively related to inhibitory effect on α-glucosidase *in vitro*.

Keywords: *Malus halliana* flowers; content change; *α*-glucosidase; spectrum-effect relationship.

1 Introduction

Malus halliana Koehne (*M. halliana*) belongs to the Malus Family [1]. Its flowers have been used as a Chinese traditional medicine to treat metrorrhagia [2], and its edible fruits are used to make health drinks [3]. Previously, our group carried out chemical analysis on this flower and the results showed that *M. halliana* leaves contained flavonoids, terpenoids and andsterols [4]. Our pharmacological research indicated that *M. halliana* leaves possessed the capability of protecting the liver, antioxidant, and inhibition of *α*-glycosidase *in vitro* [5-7]. As part of our continuous investigation, we have isolated seven flavonoids from *M. halliana* flowers and identified them.

At present, the studies on the natural plant are limited, and relatively fewer studies were conducted on the dynamic change. Zhao et al. [8] examined the changes in the content of phloridzin and phloretin in *M. pumila* twigs and leaves. Their results indicated that the contents of phloridzin and phloretin varied greatly in different months and different parts of the plant. Yang et al. [9] analyzed essential oils from *Curcuma longa* L. in Hainan with different harvest times by Gas chromatography–mass spectrometry (GC-MS). Their results showed that volatile components in different harvest periods were basically the same, but their contents were quite different. It is important to study the dynamic changes in the contents of components in natural plant. The purpose of this paper was to clarify the optimum harvest time of *M. halliana* flowers, to clarify the effective components of *M. halliana* flowers, and could potentially impact the quality control of plant based medicine to achieve better pharmacodynamics effects. So, the dynamic changes in the contents of phloretin 4'-*O*-glycosidase, afzeloside and 3-hydroxyphloridzin and the changes in their inhibitory effects on *α*-glucosidase

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of *M. halliana* flowers were investigated, and spectrum-effect relationship was evaluated by partial least square method.

α-Glucosidase is a kind of metabolic enzyme present in the small intestine. Inhibitors of *α*-glucosidase can inhibit the activity of *α*-glucosidase to reduce the absorption of intestinal epithelial cells to glucose and thus, reduce postprandial blood glucose [10,11]. In this paper, inhibitory effects on *α*-glucosidase were evaluated by *α*-glucosidase model *in vitro*.

2 Materials and methods

2.1 General experimental procedures

Multiskan GO (thermo Electron) was used to screen the *α*-glucosidase activity. GRP-9270 incubator was purchased from Shanghai Senxin Experimental Equipment Co. Ltd. KQ-500DB CNC ultrasonic cleaner was purchased from Kunshan Ultrasonic Instrument Co. Ltd (Kunshan, Jiangsu China). Sample screen was purchased from Shangyu 54 Instrument Creen Factory (Zhejiang). A LC-20AT high performance liquid chromatography system (Shimadzu, Kyoto, Japan), equipped with a degasser, a quaternary gradient low pressure pump, the CTO-20A column oven, a SPD-M20A UV-detector and a SIL-20A automatic sampling device. *α*-Glucosidase (pcode1001594504) was purchased from Oriental Yeast Co.,Ltd (Tokyo, JAPAN). 4-Nitrophenyl-*α*-D-glucopyranoside (PNPG, Lot: D00129651) was purchased from Calbiochem Ltd. Acarbose (Lot:100434) was purchased from SERVA Electrophoresis Gmbh Ltd. Methanol for chromatographic grade was purchased from Tianjin Da Mao Chemical Reagent Factory. The ultrapure water was purchased from Hangzhou WahahaBaili Food CO. Ltd. phloretin-4'-*O*-glycosidase, afzeloside, 3-hydroxyphloridzin were isolated and identified from *M. halliana* flowers (HPLC≥98%).

2.2 Plant materials

Malus halliana flowers were collected in Kaifeng City in April 2014 and identified by Professor Chang-qin Li (Institute of Chinese Materia Medica, Henan University). The specimens were deposited in Institute of Traditional Chinese Medicine, Henan University. These samples (2 Kg, dry weight) were extracted with 70% ethanol at room temperature three times to yield the 70% ethanol extract, then they were dissolved with methanol and continuously partitioned with water, 20% methanol-water, 40% methanol - water, 60% methanol - water and methanol on D101 macroporous resin column chromatography. Eluent was concentrated and yield to the water, 20% methanol, 40% methanol, 60% methanol and methanol extract, respectively.

M. halliana flowers were collected in Henan University campus from March 19 (bud just appeared) to March 28 (flower decline), 2016, once a day, each collection for the same plant to obtain 10 samples, and identified by Professor Chang-qin Li (Institute of Chinese Materia Medica, Henan University). The specimens were deposited in Institute of Traditional Chinese Medicine, Henan University. These samples were used to investigate dynamic change and inhibition of *α*-glucosidase spectrum-effect relationship of *M. halliana* flowers during blooming.

2.3 Sample preparation

2.3.1 Sample preparation for HPLC

The ten samples of *M. halliana* flowers (1.0010, 1.0004, 1.0002, 1.0006, 1.0008, 1.0001, 1.0004, 1.0003, 1.0006 and 1.0007 g, respectively) were weighed precisely and extracted with 50 mL of 70% ethanol by CNC ultrasonic instrument for 50 min. The extraction was filtered, and then the filtrate was subsequently filtered through 0.22 μm microporous membrane to obtain sample numbers 1 to 10.

Phloretin-4'-*O*-glycosidase, 3- hydroxyphloridzin and afzeloside were dissolved with 70% ethanol to obtain three standard solutions at the concentrations of 1.68, 0.88 and 0.61 mg/mL. The standard working solutions were prepared by mixing the three stock standard solutions.

2.3.2 Sample preparation for α-glucosidase assay *in vitro*

The extracts of ten samples were prepared according to the above methods and were evaporated under reduced pressure to obtain the corresponding extracts. Then the corresponding extracts were dissolved in moderate dimethyl sulfoxide (DMSO) to obtain the sample solutions (30 mg/mL).

Acarbose, the positive control, was dissolved in moderate DMSO to make a concentration of 30 mg/mL.

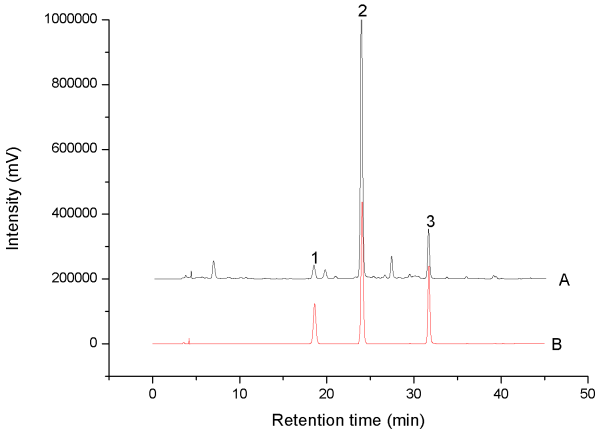

Figure 1: HPLC chromatograms of the sample of *M. halliana* flowers (A) and the standard solution (B). Peak 1: 3- hydroxyphloridzin; Peak 2: phloretin-4'-*O*-glycosidase; and Peak 3: afzeloside.

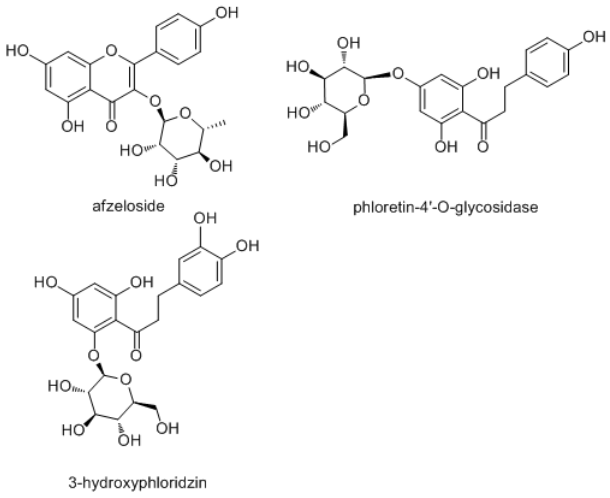

Figure 2: Structure of 3- hydroxyphloridzin, phloretin-4'-*O*-glycosidase and afzeloside

2.4 HPLC analysis condition

Chromatographic separations were performed on a Lichrospher RP-18 column (4.6 mm×250 mm, 5 μm) at 25°C. The flow rate of mobile phase was 0.8 mL·min^{-1} and the UV detection wavelength was 270 nm. Injection volumes were 5 μL. The compositions of mobile phase were methanol (A)-0.2% phosphoric acid aqueous solution (B), the samples were eluted according to the following procedure: 0-10min, 35%A-40%A; and 10-45min, 40%A-80%A.The HPLC chromatograms of the standard working solution and the samples of *M. halliana* flowers were shown in Figure **1**. Structure of the three metabolites were shown in Figure **2**.

2.5 α-Glucosidase assay *in vitro*

The inhibitory activities of the extracts of *M. halliana* flowers on α-Glucosidase were determined by spectrophotometry in a 96-well microtiter plate using PNPG (4-Nitrophenyl-α-*D*-glucopyranoside) as substrate according to the method described by Feng et al. [10] with the following modifications. In short, 8 μL of sample, 112 μL of potassium phosphate buffer (PBS, pH 6.8), and 20 μL of α-glucosidase solution were added to 96-well microtiter plate and incubated at 37°C for 15 min. After pre-incubation, 20 μL of 4-nitrophenyl-β-D-glucopyranoside (PNPG) was added. The mixture solution was vibrated and further incubated at 37°C for 15 min. Subsequently 80 μL of Na_2CO_3 was added to terminate the reaction. The OD value was measured at 405 nm, and each sample was set three parallels. The percent inhibition of α-glucosidase activity was calculated as follows.

$$\text{Inhibition (I\%)} = [1-(OD_{sample}-OD_{blank})/(OD_{control}-OD_{blank})]\times 100\%$$

OD_{sample} stands for the absorbance of samples; OD_{blank} stands for the absorbance of 96-well microtiter plate, when no sample is added; $OD_{control}$ stands for the absorbance of control group.

Ethical approval: The conducted research is not related to either human or animals use.

3 Results

3.1 Methodology validation

3.1.1 Linear relation, limit of detection (LOD) and limit of quantitation (LOQ)

The standard working solution was injected into the chromatographic instrument. The injection volumes of samples were 1, 2, 4, 8, 10, 16, 20 and 25 μL, respectively, according to the chromatographic conditions described above, and the peak areas were recorded. The amount of sample *X* (μg) was taken as the abscissa and the chromatographic peak area *Y* was taken as the ordinate and the standard curves were drawn to obtain the linear regression equations for phloretin-4'-*O*-glycosidase, afzeloside and 3-hydroxyphloridzin, respectively. The LOD and LOQ were determined by the method of signal to noise ratio (SNR), when the SNR was 3, the amount of the measured sample was the LOD, when SNR was 10; the quantity of the sample was the LOQ. Results were shown in Table **1**.

Table 1: The linear regression equations of three compounds.

Compound	Regression equation	r	Linear range (μg)	LOD	LOQ
3-hydroxyphloridzin	Y=1267620X-724	1	0.264~6.600	0.48 ng	1.58 ng
Phloretin-4'-*O*-glycosidase	Y=1566726X-12929	1	0.504~12.600	0.39 ng	1.22 ng
Afzeloside	Y=2620899X-6834	1	0.183~4.583	0.13 ng	0.40 ng

3.1.2 Precision assay

10 μL of the standard working solution was mixed with 3-hydroxyphloridzin, phloretin-4'-*O*-glycosidase, afzeloside and then injected into the chromatographic instrument. The sample was continuously injected 6 times. The peak area value of each compound was recorded. The relative standard deviations (RSDs) of the peak area values for the three compounds were 0.32, 0.07 and 0.07 (%), respectively. The results indicated good precision of instrument.

3.1.3 Stability assay

5 μL of the same sample solution was injected into the chromatographic instrument at 0, 4, 8, 12, 16 and 24 h, respectively. The peak area value of each compound was recorded. The RSDs of the peak area values for 3-hydroxyphloridzin, phloretin-4'-*O*-glycosidase, and afzeloside were 0.21, 0.84 and 1.30 (%), respectively. These results indicated that samples were stable within 24 h.

3.1.4 Repeatability assay

5 μL of the same sample solution was successively injected into the chromatographic instrument. The peak area value of each compound was recorded. The RSDs of the peak area value for 3- hydroxyphloridzin, phloretin-4'-*O*-glycosidase, and afzeloside were 0.18, 0.92 and 1.10 (%), respectively. The results indicated good repeatability of the method to determine 3- hydroxyphloridzin, phloretin-4'-*O*-glycosidase, and afzeloside.

3.1.5 Sample recovery assay

Six shares of known samples were obtained by adding a certain amount of standard substance to determine the content and to calculate the recovery and RSD values. The recoveries of 3-hydroxyphloridzin, phloretin-4'-*O*-glycosidase and afzeloside were 99.7, 99.6 and 100.1 (%), respectively, and the RSDs were 0.76, 0.96 and 1.39 (%). The results showed that the methods were feasible.

3.2 Content determination

5 μL of the tested samples was injected into the chromatographic instrument to determine the contents of 3-hydroxyphloridzin, phloretin-4'-*O*-glycosidase, and afzeloside. The results are shown in Table 2. The contents of 3-hydroxyphloridzin and phloretin-4'-*O*-glycosidase reached the maximum values (22.67±0.35 and 157.43±0.36 mg/g, respectively) when the flower was bud. However, the contents of afzeloside reached the maximum value (17.27±0.06 mg/g) on the second day (bud period). The contents of three flavonoids showed fluctuating trends during the blooming process, but the overall trend was declining. Details were presented in Figure **3**.

3.3 The inhibitory effects of *M. halliana* flowers on *α*-Glucosidase

Initially, we studied the inhibitory effects of different extract from *M. halliana*flowers collected in 2014 on *α*-glucosidase activity. The results showed that different extracts from *M. halliana* flowers all possessed potent inhibition on *α*-glucosidase activity *in vitro*, and the effects of 40% methanol fraction and 60% methanol fraction were better than that of acarbose ($P \leq 0.001$). Detailed results are shown in Table **3**. In this study, we investigated dynamic changes in the inhibitory effects of *M. halliana* flowers collected in 2016 on *α*-glucosidase activity during the blooming process. Results are shown in Table **2.** Inhibition of *α*-glucosidase activity *in vitro* showed a fluctuating trend in the process of bud blooming, but the overall trend was declining. The inhibition effect of *M. halliana* flowers on *α*-glucosidase activity was the strongest when the flowers were collected in bud period. The change trend was shown in Figure **4**.

Table 2: Content determination and inhibitory effect of *M. hallianaflowers* on α- glucosidase (n=3).

Collection time (days)	3- hydroxyphloridzin (mg/g)	Phloretin-4'-O-glycosidase (mg/g)	Afzeloside (mg/g)	α- glucosidase inhibition (%)
1	22.67±0.35	157.43±0.36	15.35±0.10	57.29±0.74
2	18.39±0.34**	117.01±0.11***	17.27±0.06**	27.57±2.36***
3	10.46±0.13***	136.91±0.65***	11.89±0.07***	47.29±3.41***
4	9.79±0.49***	123.53±0.08***	10.47±0.10***	31.95±1.25***
5	9.28±0.17***	106.73±0.10***	12.70±0.08***	24.38±2.33***
6	10.57±0.09**	128.04±0.07***	12.34±0.11***	27.32±2.12***
7	8.26±0.06**	115.35±0.11***	8.79±0.10***	32.45±2.02***
8	7.24±0.06**	125.32±0.21***	9.00±0.05***	27.03±1.26***
9	6.96±0.05**	98.67±0.23***	9.25±0.06***	29.76±2.48***
10	6.91±0.04**	77.68±0.62***	9.72±0.18***	28.82±0.69***

***$P \leq 0.001$, 0.001<**$P \leq 0.01$, as compared with that of sample 1

Table 3: Effects of different extract and compounds from *M. halliana* flowers in 2014 on *α*-glucosidase activity (n=3).

Samples	Inhibition rate (%)
70% ethanol extract of *M. hallianaflowers*	56.71±0.10***
20% methanol fraction	76.89±0.15***
40% methanol fraction	87.49±0.19***
60% methanol fraction	97.66±0.26***
methanol fraction	62.16±0.16***
3- hydroxyphloridzin	11.28±0.13***
Phloretin-4'-O-glycosidase	20.41±0.16***
Afzeloside	27.77±0.10***
Acarbose(positive control)	79.65±0.14

***$P \leq 0.001$, compared with that of acarbose

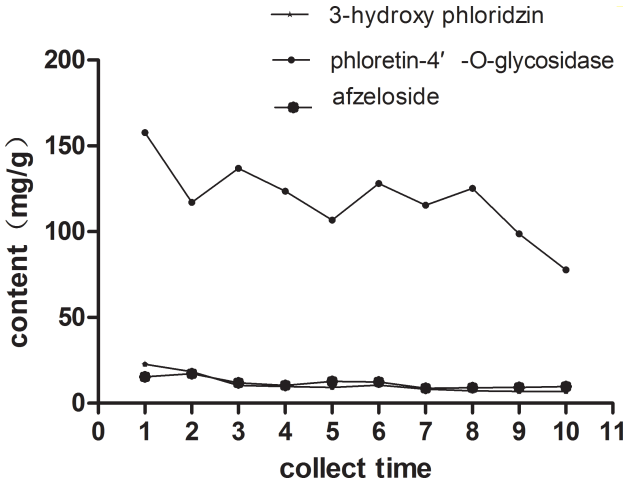

Figure 3: Temporal changes in the contents of 3-hydroxyphloridzin, phloretin-4'-*O*-glycosidase and afzeloside of *M. halliana* flowers during blooming.

3.4 Spectrum-effect analysis

3.4.1 Determination of chromatographic peaks

Chromatographic peaks were analyzed by < chromatographic fingerprint similarity evaluation system 2004 1.0 A version >, the good common chromatogram peaks were selected to carry out multi point correction. Ten chromatograms were matched to get 40 common peaks. The details are described in Figure 5.

3.4.2 Partial least squares regression analysis

Inhibition of *α*-glucosidase activity data of *M. halliana* flowers collected at different times were taken as the dependent variable, and sample quantitative fingerprint chromatographic information was taken as the independent variables. Z score on the data standardization was made by DPS 7.05 software, then the partial least

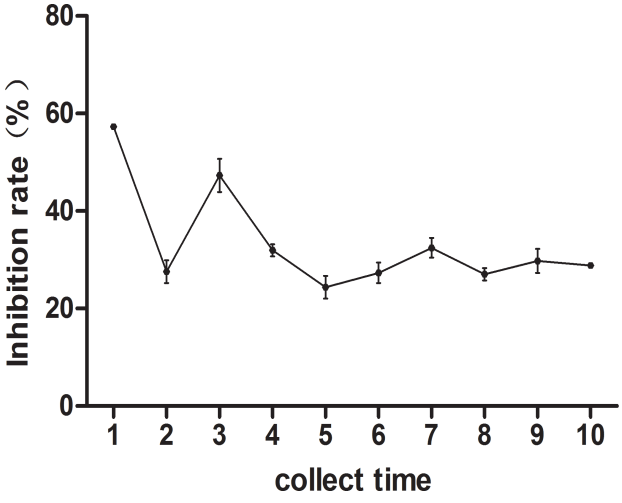

Figure 4: Temporal changes in the inhibition effect of *M. halliana* flowers during blooming on *α*- glucosidase.

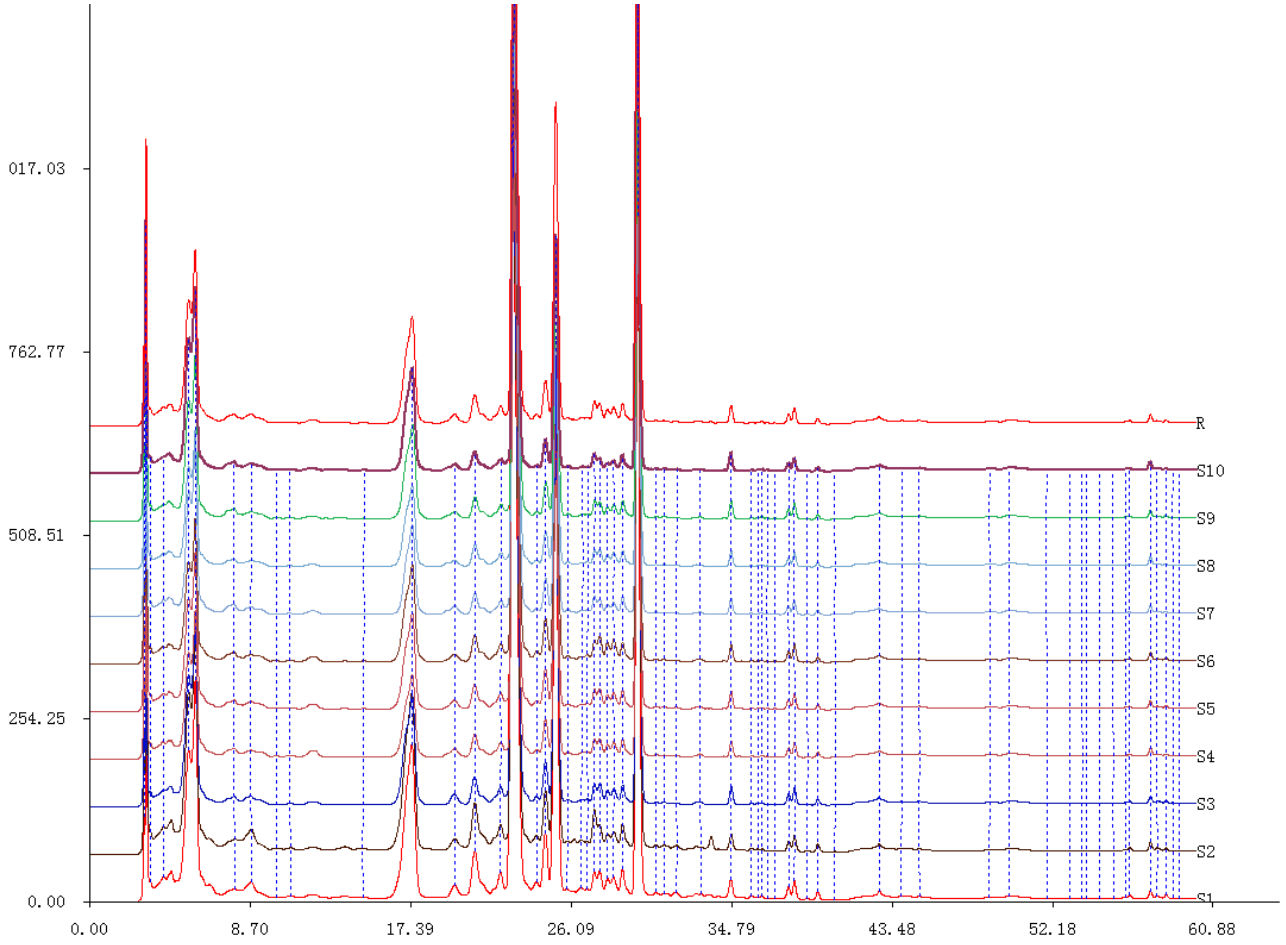

Figure 5: Similarity evaluation chromatogram of *M. halliana*flowers.

squares regression was analyzed and regression equation was obtained as follows:

$$Y_1=0.000000-0.215465X_1+0.146537X_2-0.173677X_3-0.105408X_4+0.030912X_5+0.169936X_6+0.138471X_7+0.023735X_8+0.094997X_9+0.060767X_{10}+0.064152X_{11}+0.142443X_{12}-0.094049X_{13}+0.042021X_{14}-0.086451X_{15}+0.163835X_{16}+0.035735X_{17}+0.017126X_{18}+0.016577X_{19}+0.089480X_{20}-0.021741X_{21}-0.045091X_{22}-0.070270X_{23}+0.039067X_{24}-0.004468X_{25}+0.041819X_{26}-0.042776X_{27}-0.104066X_{28}+0.112307X_{29}-0.130738X_{30}+0.080602X_{31}-0.017265X_{32}+0.018970X_{33}+0.037201X_{34}-0.023101X_{35}+0.087110X_{36}-0.069372X_{37}-0.043112X_{38}+0.237044X_{39}+0.116683X_{40}$$

Where Y_1 is the mean inhibition rate of *α*-glucosidase, and X_i indicates the peak area of the corresponding chromatographic peak.

The graphs of standardized regression coefficients of *α*-glucosidase activity *in vitro* were drawn by using the regression equation coefficients. In 40 common peaks, P_2, P_5, P_6~P_{12}, P_{14} (3- hydroxyphloridzin), P_{16}~P_{19},P_{20} (phloretin-4'-*O*-glycosidase), and P_{24}, P_{26}, P_{29}, P_{31}, P_{33}, P_{34}, P_{36}, P_{39} and P_{40} were positively correlated to inhibition effect of *M. halliana* flowers on α-glucosidase *in vitro* while P_1, P_3, P_4, P_{13}, P_{15}, P_{21}~P_{23}, P_{27}, P_{28},P_{30} (afzeloside), and P_{32}, P_{35}, P_{37} and P_{38} were negatively corerelated to inhibition effect of *M. halliana* flowers on *α*-glucosidase *in vitro*. The details are shown in Figure **6**.

4 Discussion

In this study, we determined the dynamic changes in the contents of three flavonoids in *M. halliana* flowers. The mobile phase was selected according to the method described by Zhao et al. [12] with an appropriate adjustment. The detection wavelength was selected according to maximum absorption wavelength of three flavonoids and set at 270 nm. Extraction solvent was 70% ethanol, because the three flavonoids were isolated

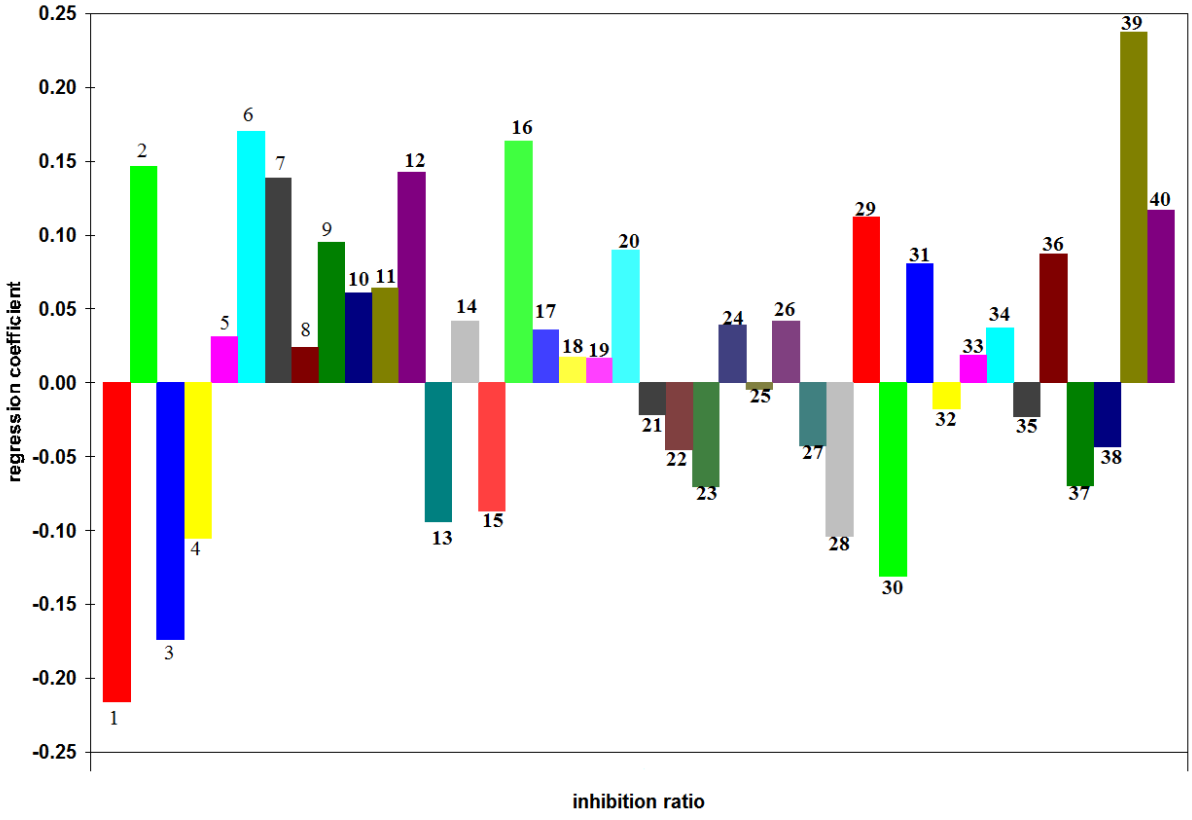

Figure 6: The standardized regression coefficients of inhibition of *M. halliana* flowers on α-glucosidase activity *in vitro*.

from 70% ethanol extract of *M. halliana* flowers. We also investigated ultrasonic times and the ratios of material to liquid by single factor variable method, with the prolonging of time, the content of 3-hydroxyphloridzin and phloretin-4'-*O*-glycosidase firstly increased and then decreased. However, the content of afzeloside firstly decreased and then increased, which may be due to the reason that the longer, the ultrasound time is, more components of medicinal materials are dissolved. There is a mutual interference between the compositions. 50mins was chosen as the ultrasound extraction time. With the increase in the solid-liquid ratio, the contents of three kinds of flavonoids were increased firstly and then became basically stable. When the content reached 1:50, the contents were basically stable. Thus, in this experiment, the ratio of material to liquid was set at 1:50 (g/mL). The method used to monitor the changes in the contents of 3-hydroxyphloridzin, phloretin-4'-*O*-glycosidase, and afzeloside was reliable, simple in operation and highly sensitive.

Previously, we studied inhibitory effects of different extracts from *M. halliana* flowers collected in 2014 on *α*-glucosidase activity and the results showed that different extracts from *M. halliana* flowers all possessed strong inhibition on *α*-glucosidase activity *in vitro*. Hence, we investigated dynamic changes in the contents of components and the inhibitory effects on *α*-glucosidase of *M. halliana* flowers collected in 2016 during the blooming process. The results showed that during flower blooming, the contents of three kinds of flavonoids were exhibiting the downward trends, and their inhibitory effects on *α*-glucosidase activity *in vitro* showed the decreasing trends, indicating that these flavonoids may be major components responsible for the inhibition of *M. halliana* flowers on *α*-glucosidase activity. The results showed that the content of phloretin-4'-*O*-glycosidase was significantly higher than those of 3-hydroxyphloridzin and afzeloside. Huang et al. [13] studied dynamic changes in the content, chemical composition and antibacterial activities of the essential oil from *Cinnamomum japonicum* leaves. Their results showed that the content of essential oil in December was highest and its antibacterial activity was the strongest. In addition, it has been reported that 3-hydroxyphloridzin and afzeloside have many biological activities. However, limited research on phloretin-4'-*O*-glycosidase was reported [14-16].

In order to clarify and define the active ingredients of *M. halliana* flowers, the inhibitory effects of three flavonoids on α-glucosidase activity were evaluated. Their inhibitory effects on α-glucosidase activity were not stronger than that of *M. halliana* flowers extract. This may be due to the reason that the compositions of plant are complex and there is interference among various components in plant.

At present, the study of spectrum-effect relationship is a relatively hot topic, which provides a new way for the quality control of traditional Chinese medicine. Spectrum-effect relationship can be used to reflect the pharmacodynamic material basis of traditional Chinese medicine and is widely used in the field of traditional Chinese medicine [17-21]. Shi et al. [22] studied the antibacterial spectrum-effect relationship of *Lonicerae japonicae* Flos and Lonicerae Flos base on Ultra-performance liquid chromatography (UPLC) and microcalorimetry. The results of canonical correlation analysis showed that chlorogenic acid and 3,4-dicaffeoylquinic acid were the major antibacterial components, and 3,4-dicaffeoylquinic acid could be used as a new markering redient for quality control of *L. japonicae* Flos and Lonicerae Flos. In our research, partial least squares regression was used to examine the relationship between chemical composition and the inhibition of *M. halliana* flowers on α-glucosidase activity. The results showed that 3- hydroxyphloridzin and phloretin-4'-*O*-glycosidase were positively correlated to the inhibitory effect of of *M. halliana* flowers on α- glucosidase *in vitro*, However afzeloside was negatively related to the inhibitory effect of *M. halliana* flowers on α- glucosidase *in vitro*. This result showed that 3-hydroxyphloridzin and phloretin-4'-*O*-glycosidase could be the active ingredients of *M. halliana* flowers responsible for inhibiting α-glucosidase.

5 Conclusion

Based on the above experimental results, the following conclusions can be drawn. If we want to develop and utilize 3-hydroxyphloridzin, phloretin-4'-*O*-glycosidase and afzeloside in *M. halliana* flowers reasonably, it is better to choose the bud stage in the harvest period. Different extracts from *M. halliana* flowers all possessed strong inhibition on α-glucosidase activity *in vitro*. During flower blooming, the contents of three kinds of flavonoids were exhibiting the downward trends, and their inhibitory effects on α-glucosidase activity *in vitro* showed the decreasing trends. The results of spectrum-effect relationship showed that 3- hydroxyphloridzin and phloretin-4'-*O*-glycosidase were positively correlated to inhibitory effect of of *M. halliana* flowers on α- glucosidase *in vitro*. However afzeloside was negatively related to inhibitory effect of *M. halliana* flowers on α- glucosidase *in vitro*. Continuously, the inhibitory effects of three flavonoids on α-glucosidase activity were evaluated. Their inhibitory effects on α-glucosidase activity were not stronger than that of *M. halliana* flowers extract. This may be due to the reason that the compositions of plant are complex and there are synergistic effects among various components in plants.

Conflicts of interest: The authors declare that there are no conflicts of interest regarding the publication of this paper.

Acknowledgements: This work was supported by Henan Province University Science and Technology Innovation Team (16IRTSTHN019), Key Project in Science and Technology of Henan Province (182102410083).

Author's contributions: WYK designed the outline of the article and reviewed the manuscript. LLC and NH conducted the experiments, collected the plant specimens, analyzed and interpreted the data as well as prepared the first draft. WYK and SML critically read and revised the paper. All the authors read and approved the paper before its final submission.

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