Hindawi
Journal of Food Quality
Volume 2017, Article ID 7950137, 6 pages
https://doi.org/10.1155/2017/7950137

Research Article

Analysis of Chemical Constituents Changing in Physical Process and Nutritional Components of *Malus halliana* Koehne Tea

Zhenhua Yin,[1,2] Yong Zhang,[1,2] Juanjuan Zhang,[1,2] and Wenyi Kang[1,2]

[1] *Huanghe Science and Technology College, Zhengzhou 450063, China*
[2] *Zhengzhou Key Laboratory of Medicinal Resources Research, Zhengzhou 450063, China*

Correspondence should be addressed to Wenyi Kang; kangweny@hotmail.com

Received 24 June 2017; Accepted 23 August 2017; Published 11 October 2017

Academic Editor: Daming Fan

This study aimed to establish a HPLC method for simultaneous determination of the changing of quercitrin, 3-hydroxyphloridzin, and phloridzin in physical process of *M. halliana* tea. Meanwhile, the nutritional compositions were determined, using anthrone-sulfuric acid colorimetry and direct titration determination of total sugar and reducing sugar, respectively, in order to provide theoretical basis for quality control and tea production. The results showed that the regression equations for quercitrin, 3-hydroxyphloridzin, and phloridzin were linear in the range of 0.0972–12.15 μg (r = 0.999 8), 0.0932~11.65 μg (r = 0.999 1), and 0.9~112.5 μg (r = 0.999 6), respectively. The average recoveries ranged from 98.19% to 99.35%. The contents of crude protein and the crude fat were measured by spectrophotometric detection and soxhlet extraction detection, respectively. The contents of total sugar, reducing sugar, the fat, and protein were 6.8 g/100 g, 8.5 mg/100 g, 2.399 g/100 g, and 4.362 g/100 g, respectively, in *M. halliana* tea.

1. Introduction

Malus halliana (M. halliana) Koehne, belonging to the family Rosaceae, is abundant and widely distributed in Jiangsu, Zhejiang, Anhui, Shanxi, Sichuan, and Yunnan provinces in China, which grows in the jungle of slopes or stream sides, commonly cultivated for ornamental industry [1]. *Chinese Materia Medica* records that its taste is light, bitter, and flat, and it can regulate the menstrual function and blood and treat metrorrhagia [2]. In folk medicine, it has been used as traditional Chinese herbal medicine in the treatment of traumatic injury, fractures, and hemorrhage [3]. In recent years, more studies have focused on gardening cuttings, cultivation, breeding, and so on [4, 5]. The *M. halliana*-derived healthy drink has been developed [6].

Drinking tea not only can add some of the necessary trace elements in the human body, but also has a pharmacological function and health effects on the human body. Modern medicine researches have shown that it not only can prevent thrombosis, reduce blood viscosity, increase high-density lipoprotein, and reduce the body's capillary permeability and brittleness, but also possesses certain preventive effects on cardiovascular and cerebrovascular diseases and antiaging and increases immunity [7]. Drinking tea can make people excited, has diuretic impact, results in sterilization, has anti-inflammatory, cardiac, and other effects, enhances memory, and prevents diabetes [8, 9].

Our group has conducted a series of related researches in early stage, including chemical composition [3, 10, 11], pharmacological activity [12–15], and content determination [15]. Flavonoids are characteristic constituents in *M. halliana*, and pharmacological investigations indicate that it possesses antioxidant [12], hepatoprotective [13], α-glucosidase inhibitory [14], tyrosinase-activating [15], and antimicrobial [16] activities.

To date, only *M. hupehensis* is used as drinking tea known as Ning Qing tea, which is widely used as summer cool and refreshing drink in Three Gorges region of China [17]. The main component was phloridzin [18], and there has been a patent to disclose a preparation method and application as antidiabetic, hypolipidemic, antioxidant, and antitumor medicine of *M. hupehensis* tea extract (ZL 200710053716.8).

Our previous research has found that phloridzin is also the main ingredient of *M. halliana* leaves [11]. While the natural resource of *M. halliana* was rich, no studies on the quality control and the analysis of the nutritional compositions of *M. halliana* tea are available in the literatures. Our study was undertaken to adopt the traditional tea processing technology to develop the *M. halliana* tea, and the contents of quercitrin, 3-hydroxyphloridzin, and phloridzin in *M. halliana* tea were measured using high-performance liquid chromatography (HPLC). In the separation and purification of the *M. halliana* tea, we found that quercitrin, 3-hydroxyphloridzin, and phloridzin contained in the tea were more than other flavonoids, for example, juglanin, avicularin, afzelin, and 5,7-dihydroxychromone. In addition, the literature reviews found that the activities of these three compounds in *Malus* genus were more reported. In comprehensive analysis, we used these three compounds as the object of study. The contrast analysis of the changes in the same compositions of *M. halliana* tea before and after processing was also carried out. Meanwhile, the main nutritive components of *M. halliana* tea were studied. Further studies on quality control standards and nutritive components of *M. halliana* tea are essential for providing theoretical basis for quality control and tea production.

2. Materials and Methods

2.1. Material and Reagents. Quercitrin, 3-hydroxyphloridzin, and phloridzin with purity greater than 98% were made by our laboratory. Acetonitrile (HPLC grade) was purchased from Avantor Performance Materials, Inc. (USA). Methanol (HPLC grade) was purchased from Tianjin Shield Fine Chemicals Co., Ltd. The water was Wahaha pure water.

The series of glucose standard solutions (10~100 μg/mL): the glucose (Xiangshui Tianyi Huagong Co., Ltd., Jiangsu) was weighed via drying at constant temperature using a constant temperature incubator (LZJS, Shenzhen in the South China Sea Masson Technology Industrial Co., Ltd.) at 105°C. Metered volume was filled with water to 1000 mL and then shaken well and 1, 2, 4, 6, 8, and 10 mL were sucked from it, respectively. Then they were transferred into six volumetric flasks (100 mL), diluted with water to volume, and shaken well, respectively. They were stored at 0–4°C refrigerator for two weeks.

2.2. Plant. Air-dried leaves were collected from Jinming district, Henan University (Kaifeng, Henan province of China). Among them, the first batch of samples was collected on March 11 in 2016, the second batch was collected on March 16 in 2016, the third batch was collected on March 21 in 2016. The plant was identified as *M. halliana* of family Rosaceae by Professor Changqin Li (Henan University, Kaifeng, China). The voucher specimens were deposited in the Institute of Natural Products, Henan University (number 20160312).

2.3. Preparation of M. halliana Tea. March *M. halliana* leaves were used. The *M. halliana* tea was made by de-enzyming, rolling, drying, and the same processes. Specific tea was processed as described in the patent [19].

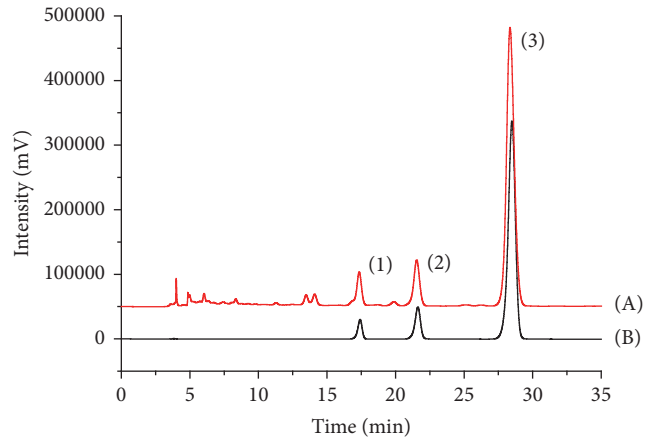

Figure 1: HPLC chromatograms of sample solution (A) and standard solution (B); quercitrin (1); 3-hydroxyphloridzin (2); and phloridzin (3).

2.4. Analysis of Chemical Components

2.4.1. Preparation of Standard Solution and Test Sample Solution. The three standard solutions of quercitrin, 3-hydroxyphloridzin, and phloridzin were prepared in methanol at the concentrations of 486, 466, and 4500 μg·mL^{-1} and stored at 4°C, respectively.

M. halliana leaves or tea (0.02 g, 50 mesh) were mixed with 1 mL of methanol solution in centrifuge tube. The mixture of solid and liquid was centrifuged at 5000 r·min^{-1} for 5 min after being extracted by ultrasonic (50 min, 500 W). Then, the extract was filtrated through a 0.22 μm microporous membrane. The subsequent filtrate was taken as the test sample solution.

2.4.2. Development of the HPLC. LC-20AT high-performance liquid chromatography (Shimadzu) was used and the system was equipped with a degasser, a quaternary gradient low pressure pump, the CTO-20AC column oven, a SPD-M20A UV-detector, and a SIL-20AC autosampler. The data were acquired and processed using LC-solution chromatography data processing system.

Two chromatographic separation steps were all performed on a Agilent TC-C_{18} column (4.6 mm × 250 mm, 5 μm) at a column temperature of 25°C. The flow rate of mobile phase was 0.8 mL·min^{-1}. The UV detection wavelength was set at 270 nm. All the injection volumes were 10 μL. The mobile phase consisted of acetonitrile-1% phosphoric acid (20 : 80, v/v). The HPLC chromatograms of the standard solution and the extract of sample were shown in Figure 1.

2.5. Analysis of Nutritional Components

2.5.1. Determination of Crude Protein Content. 0.5010 g (40 mesh) of *M. halliana* tea powder was accurately weighed. The extraction method of crude protein was determined according to the method as described in Chinese National Standard (GB 5009.5-2016) and ultraviolet spectrophotometric method was used to measure the protein content. At the

same time, blank experiment was carried out with the same method to calculate the total nitrogen content. The protein content formula is as follows: protein content = total nitrogen × 6.25.

2.5.2. Determination of Crude Fat Content. 5.001 g of *M. halliana* tea powder was accurately weighed. Crude fat was measured by the soxhlet extraction method as described in Chinese National Standard (GB 5009.6-2016) previously. The fat content formula is as follows: fat content = $(m_1 - m_0)/m_2 \times 100$, where m_1 refers to quality of absorption bottle and crude fat (g); m_0 represents the quality of absorption bottle (g); m_2 is the quality of sample (g).

2.5.3. The Content Determination of Reducing Sugar. M. halliana tea powder was accurately weighed as 4.0036 g. The reducing sugar extraction method was determined according to the method described in Chinese National Standard (GB 5009.7-2016) and we used direct titration method for calibration. The reducing sugar formula is as follows: the reducing sugar content (%) = $[m_1/(m \times V/250 \times 1000)] \times 100$.

m_1 is quality of the reducing sugar (mg); V is consumption volume of sample solution in calibration (mL); m is quality of sample (g).

2.5.4. The Content Determination of Total Soluble Sugar. 0.1 g of *M. halliana* tea powder was accurately weighed and extracted. The total soluble sugar content was determined according to the method described previously [20]. Meanwhile, the method for determining the total soluble sugar was the same as that used for determining the reducing sugar content. The total soluble sugar formula is as follows: the mass percentage of total sugar = $(c \times f \times V_{\text{Sample total}}/10^6 \times m) \times 10$ (c is sugar concentration of standard curve (μg/mL); f is dilution ratio; $V_{\text{Sample total}}$ is total volume of sample (mL); m is quality of sample (g)).

3. Results and Discussion

3.1. Analysis of Chemical Components

3.1.1. Linearity. The stock standard solutions (0.2, 1, 5, 10, 15, 20, and 25 μL) were accurately injected to chromatographic instrument for the construction of calibration curves, respectively, and the corresponding sample sizes of the injected quercitrin were 0.0972, 0.486, 2.43, 4.86, 7.29, 9.72, and 12.15 μg, respectively; the corresponding sample sizes of the injected 3-hydroxyphloridzin were 0.0932, 0.466, 2.33, 4.66, 6.99, 9.32, and 11.65 μg, respectively; the corresponding sample sizes of the injected phloridzin were 0.9, 4.5, 22.5, 45.00, 67.5, 90.00, and 112.5 μg, respectively. The calibration curves were constructed by plotting the peak areas versus the injected sample quality (μg) of each compound. The results were presented in Table 1. The r values were in the range from 0.9991 to 0.9998, which indicated that the methods displayed good linearity.

3.1.2. Precision. 10 μL of each the stock standard solution was accurately injected to chromatographic instrument. The stock solutions were analyzed in six replicates within one day for determining the precision of the developed assay. The relative standard deviations (RSDs) of peak areas for the three compounds were 0.35%, 0.35%, and 0.79%, respectively. The results indicated that the methods were precise for quantitative analysis of phloridzin, 3-hydroxyphloridzin, and quercitrin.

3.1.3. Stability. The sample solutions were prepared under the optimum extraction conditions and placed at room temperature, and then 10 μL of sample solution was injected into chromatographic instrument at 0, 3, 6, 9, 12, and 24 h, respectively. The RSDs of peak areas for the three compounds were 0.25%, 0.45%, and 0.41%, respectively. The results indicated that the sample solution was basically stable at room temperature within 24 h.

3.1.4. Repeatability. Six test sample solutions were processed under the optimum extraction conditions, and 10 μL of each solution was then injected to chromatographic instrument for analyzing. The RSDs of peak areas for the three compounds were 0.33%, 2.38%, and 1.24%, respectively, indicating that the analytical methods have the acceptable level of repeatability.

3.1.5. Recovery. Nine batches of leaves and flowers samples of *M. halliana* were prepared and divided into three groups, respectively. Then three standard substances at three different amounts were added to the leaves and flowers samples. The spiked samples were prepared according to the optimum extraction conditions. All the calculated recovery values of the analytes ranged from 98.19 to 99.35% and the RSDs were 1.6%, 1.4%, and 1.9%, respectively. The results demonstrated that the methods were reasonable and feasible.

3.1.6. Sample Analysis. The mass fractions of phloridzin, quercitrin, and 3-hydroxyphloridzin in original leaves and tea were presented in Table 2 ($n = 3$), respectively.

3.2. Analysis of Nutritional Components

3.2.1. The Content Determination of Crude Protein. According to the above experimental method, the standard curve of crude protein was drawn. We took extract of *M. halliana* tea and measured its crude protein content. The field experiments were carried out with six replications and then the data were expressed as mean.

The absorbance of *M. halliana* tea was 0.0136, when it was substituted into the standard curve and the crude protein content of *M. halliana* tea was 4.362 g/100 g. Protein played an important role in the body, which provided power and energy for the protein metabolism and maintained the normal operation of all kinds of tissues. It also can strengthen the body resistance, hypoxia, and fatigue resistance and have high nutritional value, which is the important nutritional protein for old body [21]. The crude protein content of *M. halliana* tea was 4.362 g/100 g which explained that it had more amino acids, which could promote the formation of aroma in tea. And the stated amino acids colloid in tea soup, which played an important role in keeping clear of the

Table 1: Regression equations and linear ranges for three compounds.

Compound	Regressive equation	r	Linear range/μg
Phloridzin	$Y = 1165813.0631X + 976247.0924$	0.9996	0.9~112.5
Quercitrin	$Y = 1306866.7475X + 58713.4808$	0.9998	0.0972~12.15
3-Hydroxyphloridzin	$Y = 654528.6618X + 41738.1201$	0.9991	0.0932~11.65

Here Y is the peak area; X refers to injection amount (μg).

Table 2: The contents of three flavonoids in *M. halliana* tea and original leaves for different periods (mg/g).

Compound	Period					
	I		II		III	
	Tea	Original leaves	Tea	Original leaves	Tea	Original leaves
Phloridzin	448.118	382.687	299.486	301.121	504.680	251.456
Quercitrin	9.919	4.237	14.877	7.077	7.412	7.182
3-Hydroxyphloridzin	143.630	112.306	110.055	91.911	137.408	84.023

beverage and the stability of the colloidal solution. It also had offset function of the nervous system excitement caused by caffeine. So *M. halliana* tea can enhance the body resistance, hypoxia, and fatigue resistance and have high nutritional value.

3.2.2. The Content Determination of Crude Fat. According to the method and formula, the crude fat of *M. halliana* tea was 2.399 g/100 g. The lipid composition in tea played a positive role in the formation of the tea aroma, which could accelerate the absorption of fat-soluble vitamins and prevent vitamin deficiency disease. The fat content in tea was less confirmed by medical research, but it had function of improving human immunity, preventing cancer, and so on. Therefore, the special population who had liver disease, hypertension, hyperlipidemia, and pancreatic function not congruent also easily drinks this in addition to the ordinary people.

3.2.3. Determination of Reducing Sugar Content. The cupric tartrate solution consumption of *M. halliana* tea was 9.98. According to the formula, the reducing sugar of *M. halliana* tea was 8.5 mg/100 g.

3.2.4. Determination of Total Soluble Sugar Content. According to the above method, the standard glucose was diluted into six concentrations with gradient dilutions; then the series of glucose standard solution concentrations were taken as the abscissa and absorbance was taken as the ordinate to make a standard curve and calculate the regression equation; the equation was $y = 0.003 \times X + 0.005$ ($R^2 = 0.999$).

The absorbance of total soluble sugar was taken into the equation to draw standard curve for the total sugar contents of sample, and all the field experiment results were carried out with six replications and then the data were expressed as mean. According to the method and formula, the total soluble sugar of *M. halliana* tea was 6.8 g/100 g.

The contents of total sugar and reducing sugar, which are taken as the criteria for evaluating the plant nutrition, are the important components in food flavor and nutrition. Tea polysaccharide had antidiabetic, hypolipidemic, antioxidant, and immunoregulation function [22]. From the results of total soluble sugar and reducing sugar, *M. halliana* tea can be developed as a health care food in the prevention and treatment of those diseases.

4. Conclusions

The contents of glycosides, being made up of sugar and nonsaccharide components, are higher than those in other kinds of medicinal materials such as root, peel, flower, and fruit. Glycosides as the active ingredients coexisted with a variety of enzymes in many traditional Chinese medicines. Thus, they can be hydrolyzed by those enzymes. Therefore, Chinese traditional medicine containing glycosides can inhibit enzyme activity and protect glycosides by frying, cooking, and other methods, which can effectively control the glycosidase solution [23].

Figure 2 showed that the three flavonoids contents of all the whole *M. halliana* tea were higher than that of the original leaves, the phloridzin contents of which were the highest. The possible reason was that glycosides digestion ability is reduced and glycosides component content of *M. halliana* tea after stir-frying increased, or there might be some components by dissolution and decomposition and they might turn into new compositions. Different *M. halliana* teas came from different original leaf source, and the contents of 3-hydroxyphloridzin and phloridzin presented a decreasing and then increasing trend, and the content of quercitrin displayed an increasing and then reducing trend. Compared with our group previously related research [15], we found that the significant difference in the contents of three flavonoids existed in different harvest periods.

Phloridzin as the main ingredient in *M. halliana* [11], which had antidiabetics, improving memory, antioxidant, anticancer, estrogenic, and antiestrogenic activities [24–26], was a competitive inhibitor of glucose transport [27]. Phloridzin has extensive application and helps in development in food, new drugs, and natural health food because of the low toxicity characteristic. *M. halliana* is one of China's unique

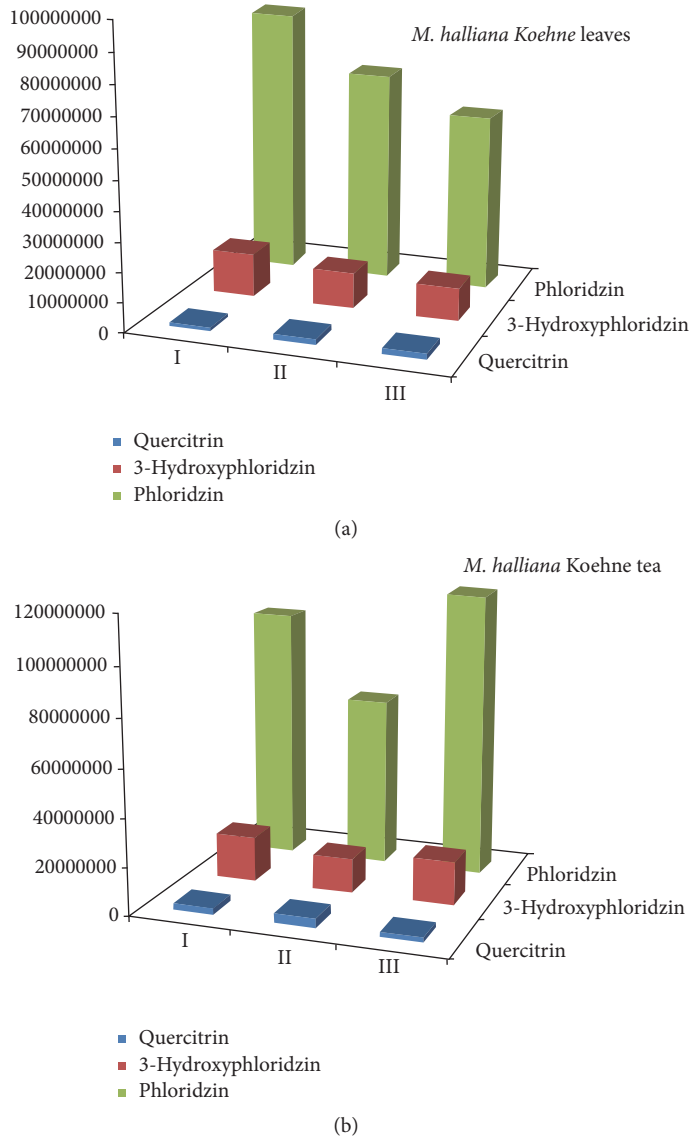

Figure 2: The contents of three flavonoids in *M. halliana* tea and original leaves for different periods.

plants and cultivated in various regions, but mainly as ornamental plant. Because of its rich resources, wide distribution, and easiness of breeding, cutting, and grafting, it brings favorable conditions for mass production. The processing tea of *M. halliana* was similar to green tea production process and it could be harvested in summer and autumn. Our study developed *M. halliana* tea as a health care drinking and established a HPLC method for simultaneous determination of quercitrin, 3-hydroxyphloridzin, and phloridzin; meanwhile, the analysis of the same compositions' change of *M. halliana* tea before and after processing was contrasted.

Through the analysis of the main nutrients of *M. halliana* tea, we found that diversity of nutrients, containing the total sugar, reducing sugar, crude protein, crude fat, and other nutrients, is the tea nutrition characteristic. Therefore, the nutrient contents of *M. halliana* tea are quite high, and some nutrients are quite rich, which is helpful to comprehensive understanding of the benefits of drinking tea. This study showed that *M. halliana* tea had certain development in food and nutrition health care value. We believed that it will become very popular after comprehensive analysis of the main nutrition compositions and efficacy components of *M. halliana* tea.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

Authors' Contributions

Zhenhua Yin and Yong Zhang contributed equally to this work.

Acknowledgments

This work was supported by Henan Province University Science and Technology Innovation Team (16IRTSTHN019), Key Project in Science and Technology Agency of Henan Province (152102310171; 17B360005), Key Project in Science and Technology Agency of Zhengzhou City (20150341), and Project in Science and Technology Agency of Kaifeng City (1603111).

References

[1] D. J. Yu, "*Malus halliana Koehne*," in *Flora Reipublicae Popularis Sinicae*, vol. 36, p. 380, Science Press, Beijing, China, 1974.

[2] L. R. Song, "*Malus halliana Koehne*," in *Chinese Materia Medica*, vol. 10, p. 158, Shanghai Scientific Technology Press, 1999.

[3] P. F. Yuan, Z. Q. Ji, and W. Y. Kang, "Volatile constituents of the buds and flowers of *Malus halliana Koehne*," *Natural Product Research and Development*, vol. 22, pp. 1036–1039, 2010.

[4] A. X. Chu, Y. J. Yang, G. G. Tang, and L. L. Tong, "Studies on numerical taxonomy of the *Malus halliana* Koehne cultivars in Henan," *Acta Horticulturae Sinica*, vol. 36, no. 3, pp. 377–384, 2009.

[5] A. X Chu and G. G. Tang, "Cultivar investigation and classification of *Malus halliana Koehne* in Henan province," *Acta Agriculturae Universitatis Jiangxiensis*, vol. 30, no. 6, pp. 1090–1096, 2008.

[6] M. Yu, Y. L. Yin, and Y. J. Min, "Study on the processing technology for *Malus halliana Koehne* healthy drink," *Journal of Anqing Teachers College (Natural Science Edition)*, vol. 9, no. 9, pp. 50-51, 2003.

[7] X. M. Wang, H. J. Wang, and S. L. Li, *Discussion on the Health of Tea Words*, Chines pharmaceutical economics, S2, 2015.

[8] T. Shiono, K. Yamamoto, Y. Yotsumoto et al., "Selective decaffeination of tea extracts by montmorillonite," *Journal of Food Engineering*, vol. 200, pp. 13–21, 2017.

[9] W. Hao, M. Wang, and M. Lv, "The Inhibitory Effects of Yixing Black Tea Extracts on A-Glucosidase," *Journal of Food Biochemistry*, vol. 41, no. 1, Article ID e12269, 2017.

[10] F. J. Feng, Z. H. Yin, W. Zhang, Q. J. Zhang, and W. Y. Kang, "Analysis of fat-soluble components in leaves of *Malus halliana koehne* by GC-MS," *Chinese Journal of Experimental Traditional Medical Formulae*, vol. 20, no. 20, pp. 100–103, 2014.

[11] F. J. Feng, *Studies on the bioactive constituents of Malus halliana Koehne*, Guizhou University, 2015.

[12] X. M. Kong, W. Zhang, C. Q. Li, J. F. Wei, and W. Y. Kang, "Study on antioxidant activity of *Malus halliana Koehne*," *Natural Product Research and Development*, vol. 25, no. 12, pp. 1748–1751, 2013.

[13] *Study on antioxidant activity and hepatoprotective effects of Malus halliana Koehne and Chaenomeles Speciosa*, 2014, Henan University.

[14] W. Zhang, M. F. Chang, J. Y. Jin, Q. Shen, J. J. Wang, and W. Y. Kang, "α-Glucosidase inhibitory activity of *Malus halliana Koehne*," *Chinese Journal of Experimental Traditional Medical Formulae*, vol. 20, no. 4, pp. 84–86, 2014.

[15] C. C. Lu, L. Zhao, Y. X. Huang, X. Shao, and W. Y. Kang, "Simultaneous determination of quercitrin, phloridzin and 3-hydroxvl phloridzin in leaves of *Malms halliana* by ultrasound-assisted ionic liquid-reversed phase liquid chromatography," *China Journal of Chinese Materia Medica*, vol. 41, no. 11, pp. 2131–2136, 2016.

[16] W. Gu, "Bioactive metabolites from alternaria brassicicola ML-P08, an endophytic fungus residing in *malus halliana*," *World Journal of Microbiology and Biotechnology*, vol. 25, no. 9, pp. 1677–1683, 2009.

[17] D. Y. Guo, J. Li, K. Dong, and Y. Z. Wang, "Optimization of preparation technology for *Malus hupehensis* effervescent tablets by multi-index comprehensive evaluation method," *Chinese Traditional Patent Medicine*, vol. 33, no. 9, pp. 1617–1620, 2011.

[18] R. Fang, Q. Yang, L. Li, J. T. Xiang, and Y. Z. Wang, "Determination of phloridzin in *Malus hupehensis*," *Food Science and Technology*, vol. 6, pp. 195-196, 2008.

[19] W. Y. Kang, X. M. Kong, and Z. H. Yin, *Application of* Malus halliana Koehne *tea and its water extracts*, ZL10382888.5, China, 2013.

[20] X. Wong, G. Xin, and Y. X. Li, "Study on the determination of total sugar in potato starch by anthrone colorimetry," *Food research and development*, vol. 34, no. 17, pp. 86–88, 2013.

[21] L. Shen, X. Wang, Z. Wang, Y. Wu, and J. Chen, "Studies on tea protein extraction using alkaline and enzyme methods," *Food Chemistry*, vol. 107, no. 2, pp. 929–938, 2008.

[22] L.-L. Du, Q. Y. Fu, L. P. Xiang et al., "Tea polysaccharides and their bioactivities," *Molecules*, vol. 21, no. 11, article no. 1449, 2016.

[23] R. Zhou, Y. G. Gao, P. Zng, C. L. Jiang, and L. X. Zhang, "Effect of processing on active components and efficacy of traditional chinese medicine," *Chinese Journal of Experimental Traditional Medical Formulae*, vol. 21, pp. 209–212, 2015.

[24] Z. H. Liang, X. H. Liu, and S. M. Gong, "Evaluation of anti-hyperglycemic activities of phloridzin in diabetic mice," *African Journal of Traditional, Complementary and Alternative Medicines*, vol. 13, no. 3, pp. 209–218, 2016.

[25] J. Wang, M. H. Chung, B. Xue, H. Ma, C. Ma, and M. Hattori, "Estrogenic and antiestrogenic activities of phloridzin," *Biological and Pharmaceutical Bulletin*, vol. 33, no. 4, pp. 592–597, 2010.

[26] J. L. Hall, R. T. Reilly, K. L. Cottrill, W. S. Stone, and P. E. Gold, "Phlorizin enhancement of memory in rats and mice," *Pharmacology, Biochemistry and Behavior*, vol. 41, no. 2, pp. 295–299, 1992.

[27] M. M. Boccia, S. R. Kopf, and C. M. Baratti, "Phlorizin, a competitive inhibitor of glucose transport, facilitates memory storage in mice," *Neurobiology of Learning and Memory*, vol. 71, no. 1, pp. 104–112, 1999.