Chemical Constituents and Coagulation Activity of Amygdalus persica L. Flowers

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Received 12 March 2021; Accepted 9 May 2021; Published 20 May 2021

1.Introduction

Amygdalus persica L., belonging to Rosaceae, is an excellent plant resource that had the concomitant function of both medicine and foodstuff, and with relaxing bowel, diuresis, and reducing swelling effects [1]. A. persica flowers are widely used in traditional Chinese medicine and also used as a healthy food for beauty and constipation treatment, as well as wine and tea drinks [2, 3].

At present, there are few studies on chemical composition and pharmacological activity of A. persica, mainly focusing on the volatile constituents and extraction process of polyphenols. Yuan et al. studied the optimum ultrasonic-assisted extraction technology of polyphenols from A. persica with response surface methodology and found the polyphenols had certain scavenging effects on hydroxyl and DPPH radicals [4]. In addition, Zhang et al. found that the major volatile constituents of A. persica flowers were linolenic alcohol, n-hexadecanoic acid, cyclohexane, and octadecanoic acid [5]. Zeng et al. analyzed the conditions for purification of total flavonoids from A. persica by macroporous resin, with adsorption-desorption ratios as indexes, and optimum resin DM-28 was selected by static adsorption-desorption ratio tests and also found that flavonoids had an obvious antioxidant activity. The IC_{50} values of DPPH and ABTS were 13.47 μg/mL and 32.16 μg/mL, respectively [6].
According to literature research, *A. persica* had the effect of promoting blood circulation and removing blood stasis [7]. In our previous research, we found that the constituents of ethyl acetate extract from *A. persica* flowers showed strong coagulation activity. In the light of previous research, the coagulation activities of *n*-butanol extract of *A. persica* were researched with coagulation parameters in vitro.

2. Methods

2.1. Materials and Reagents. Sodium chloride injection (1603311336) and breviscapine injection (20190813-1) were obtained from Kunming Longjin Pharmaceutical Co., Ltd. (Kunming, Yunnan, China). Yunnan Baiyao (ZJA1708) was obtained from Yunnan Baiyao Group Co., Ltd. (Kunming, Yunnan, China). PT (20190610M), APTT (20200319M), TT (20190521M), and FIB (20191120M) assay kits were purchased from Shenzhen Leidi Life Science Co., Ltd. (Guangdong, China). Sephadex LH-20 was purchased from Pharmacia (Burlington, MA, USA). NMR was recorded on a Bruker Avance AM-400 spectrometer. RAC-030 automatic blood coagulation analyzer was obtained from Shenzhen Leidi Life Science Co., Ltd. (Guangdong, China).

2.2. Plant Material. *Amygdalus persica* flowers were purchased from Anhui Pharmaceutical Zhiyuan Chinese Medicine Co., Ltd., Anhui, China. A voucher specimen (no. 201803027) was identified by Professor Changqin Li of Henan University and deposited in the Institute of Natural Medicine of Huanghe Science and Technology College.

2.3. Animals. Female Sprague Dawley (SD) rats (6–8 weeks, 200–250 g) were obtained from the Experimental Animal Center of Henan Province (Zhengzhou, Henan, China), and the animal certificate number was SCXK 2019-0004. The female SD rats were maintained at 25°C and 45–65% humidity, in a 12 h light/12 h dark cycle, and fed with standard rodent diet and water ad libitum.

2.4. Extraction and Isolation. The extraction work of air-dried *A. persica* flowers (2000 g) with petroleum ether, ethyl acetate, and *n*-butanol have been published in our previous research [8]. And, we get ether extract 28 g, ethyl acetate extract 93 g, and *n*-butanol extract 180 g.

The extracted method of *n*-butanol extract was similar to our previous research [9]. *n*-Butanol extract (180 g) was subjected to a silica gel H medium-pressure liquid chromatography and successively eluted with methylene chloride/methanol (from *v:v* = 100:1–7:3) to obtain eight fractions: Fr.1–Fr.8. Fr.2 (6.81 g) was applied to a silica gel H column chromatography and eluted with petroleum ether/methylene chloride (*v:v* = 25:1–3:1) and then separated with Sephadex LH-20 (petroleum ether/methylene chloride/methanol, *v:v:v* = 9:9:2) to obtain compound 1 (20.0 mg). Fr.3 (5.47 g) was subjected to a silica gel H column chromatography, eluted with methylene chloride/methanol (*v:v* = 35:1–1:1) to three fractions (Fr.3.1–Fr.3.3)

based on TLC analyses. Fr.3.2 was separated on a silica gel H with methylene chloride/ethyl acetate (*v:v* = 10:1) and further separated with Sephadex LH-20 (methylene chloride/methanol, *v:v* = 1:1) to obtain compound 2 (21.0 mg). Fr.3.3 was separated with Sephadex LH-20 (methylene chloride/methanol, *v:v* = 1:1) and Sephadex LH-20 (methanol) to obtain compounds 3 (8.0 mg) and 4 (11.7 mg). Fr.4 (8.20 g) was separated on silica gel H with a stepwise-gradient of methylene chloride/ethyl acetate/methanol (*v:v:v* = 40:1:1–5:1:1) to yield 2 fractions: Fr.4.1–Fr.4.2. Fr.4.2 was separated on silica gel H with methylene chloride/methanol (*v:v* = 15:1) and Sephadex LH-20 (methylene chloride/methanol, *v:v* = 1:1) to give compounds 5 (15.7 mg) and 6 (10.3 mg). Fr.5 (6.40 g) was separated with silica gel column with methylene chloride/methanol (*v:v* = 8:1) and further with Sephadex LH-20 (methylene chloride/methanol = 1:1) to obtain compound 7 (10.5 mg). Fr.6 (11.95 g) was subjected to a silica gel H column chromatography, eluted with methylene chloride/methanol (*v:v* = 20:1–1:1) to three fractions (Fr.6.1–Fr.6.3) based on TLC analyses. Fr.6.1 was purified by silica gel column with methylene chloride/methanol (*v:v* = 10:1–1:1) and further with Sephadex LH-20 (methanol) to yield compounds 8 (11.2 mg) and 9 (7.5 mg). Fr.6.2 was purified by Sephadex LH-20 (methylene chloride/methanol, *v:v* = 1:1) to give compound 10 (5.6 mg). Fr.7 (10.07 g) was subjected to a silica gel H column chromatography, eluted with methylene chloride/ethyl acetate/methanol (*v:v:v* = 35:20:1–2:1:1) to four fractions (Fr.7.1–Fr.7.4) based on TLC analyses. Fr.7.2 was subjected to atmospheric pressure chromatographic column of silica gel H with methylene chloride/methanol (*v:v* = 25:1–2:1) and Sephadex LH-20 (methanol) to obtain compounds 11 (10.7 mg) and 12 (11.8 mg). Compound 13 (20.4 mg) was isolated by the same separation method from Fr.7.4. Fr.8 (7.26 g) was purified by silica gel column with methylene chloride/methanol (*v:v* = 15:1–1:1) and Sephadex LH-20 (methanol) to obtain compound 14 (18.6 mg).

2.5. Coagulation Time Assays In Vitro

2.5.1. Sample Preparation. In this test, blank solvent (ethyl alcohol: 1, 2-propylene glycol: normal saline = 1:1:3) was used as the negative control group, while breviscapine (8 mg) was prepared into 13.33 mg/mL solution with 600 μL blank solvent and Yunnan Baiyao (1 mg) was prepared into 40 mg/mL solution with 25 μL blank solvent and used as positive control groups. The compound (3 mg) was prepared into 3 mg/mL solution with 1000 μL blank solvent. The method was similar to our previous research [10, 11].

2.5.2. Collection of Blood Sample. Rats were anesthetized with chloral hydrate, and the blood was collected from abdominal aorta. Blood was placed in a disposable anticoagulant negative pressure vacuum tube, then mixed gently upside down, and centrifuged at 3,000 rpm at 5°C for 15 min, and the supernatant was taken for use.
2.5.3. APTT Assay. Add 25 μL sample and 50 μL plasma into the test cup, then put it into the coagulation apparatus, automatically add 25 μL APTT reagent, incubate for 5 min at 37°C, and then, add 50 μL CaCl₂. Clotting times were recorded as the value of APTT.

2.5.4. PT Assay. Add 25 μL sample and 50 μL plasma into the test cup, then put it into the coagulation apparatus, and automatically add 100 μL PT reagent. Clotting times were recorded as the value of PT.

2.5.5. TT Assay. Add 25 μL sample and 100 μL plasma into the test cup, then put it into the coagulation apparatus, and automatically add 100 μL TT reagent. Clotting times were recorded as the value of TT.

2.5.6. FIB Assay. Add 25 μL sample and 50 μL plasma into the test cup, then put it into the coagulation apparatus, and automatically add 100 μL FIB reagent. And, the content of FIB was recorded. APTT, PT, TT, and FIB assays were conducted by using an automatic blood coagulation analyzer.

2.6. Statistical Analysis. The results were expressed as mean ± standard deviation (SD). Statistical analysis was performed using SPSS19.0 software, and comparison between any two groups was evaluated using one-way analysis of variance (one-way ANOVA). The difference between groups with P < 0.05 was regarded as statistically significant.

3. Results

3.1. Identification of the Compounds. By correlating melting points, 1H-NMR, 13C-NMR, and MS from the literature values, compounds 1–14 were identified as chlorogenic acid butyl ester (1) [12], rutin (2) [13], protocatechuic acid (3) [14], caffeic acid (4) [15], 5-O-coumaroylquinic acid methyl ester (5) [16], kaempferol-3-O-neohesperidoside (6) [17], quercetin-3-O-β-D-glucoside (7) [18], 3,5-dicaffeoylquinic acid (8) [19], quercetin-3-O-α-L-rhamnoside (9) [20], 5-O-coumaroylquinic acid (10) [16], kaempferol-3-O-α-L-rhamnoside (11) [21], kaempferol-3-O-β-D-galactoside (12) [22], D-glucitol (13) [23], multiflorin A (14) [24]. Flavonoids were the main components [25–27]. All the compounds except compound 2 were identified from A. persica flowers for the first time. The structures of compounds 1–14 are shown in Figure 1.

3.2. Effects on Plasma Coagulation Parameters In Vitro. The APTT, PT, TT, and FIB assays results of A. persica flowers are shown in Table 1 and Figure 2.

In APTT assay, compounds 2, 4, 6, 8, 12, and 14 could remarkably shorten APTT (P < 0.05, P < 0.01, P < 0.001, respectively), while compound 1 could remarkably lengthen APTT (P < 0.01) compared with the blank group. In PT assay, all the compounds except compounds 1 and 10 could remarkably shorten PT (P < 0.05, P < 0.01, and P < 0.001, respectively), and compound 1 could remarkably lengthen PT (P < 0.05) compared with the blank group. Then, the shortening PT effects of compounds 4 and 7 were better than those of Yunnan Baiyao (P < 0.05 and P < 0.01, respectively), and compounds 5, 6, 11, 12, and 13 had no difference with Yunnan Baiyao (P > 0.05). In TT assay, compounds 5, 6, 7, 9, 10, 11, 12, 13, and 14 could remarkably shorten TT (P < 0.05, P < 0.01, and P < 0.001, respectively) compared with the blank group. And, the shortening TT effects of compounds 7, 9, 11, and 12 were better than those of Yunnan Baiyao (P < 0.05, P < 0.01, and P < 0.001, respectively), and compounds 5 and 14 had no difference with Yunnan Baiyao (P > 0.05). In FIB assay, compound 11 could remarkably increase the content of FIB (P < 0.01) compared with the blank group, and the effect of compound 11 was better than that of Yunnan Baiyao (P < 0.05). While compounds 1, 2, 4, 5, 6, 7, 8, 10, 12, and 13 could remarkably reduce the content of FIB (P < 0.05, P < 0.01, and P < 0.001, respectively), and the effects of compounds 1, 2, 4, 5, 10, 12, and 13 were better than those of breviscapine (P < 0.05, P < 0.01, and P < 0.001, respectively), and compounds 6 and 7 had no difference with the breviscapine (P > 0.05).

4. Discussion

Blood coagulation refers to the process in which blood changes from a flowing liquid state to a gel state that cannot flow. Its essence is the process of transforming soluble fibrinogen into insoluble fibrin in plasma. The key to this transformation is the occurrence of a series of complex enzymatic reactions, which require the participation of various coagulation factors [28]. According to the activation pathway of coagulation factor X and the different factors involved in coagulation, blood coagulation was divided into endogenous coagulation pathway, exogenous coagulation pathway, and common coagulation pathway [29].

The measurement of PT, APTT, TT, and FIB is an important index to judge the pathological changes of hemostasis and coagulation system, and it is the most commonly used and basic screening experiment of coagulation system in clinic. APTT mainly reflects the endogenous coagulation pathway and measures the time required for fibrinogen to transform into insoluble fibrin with the participation of Ca²⁺. PT mainly reflects the exogenous coagulation pathway and the overall activity of coagulation factors I, II, V, VII, and X in plasma. TT mainly reflects the time required for fibrinogen to transform into insoluble fibrin eggs in the common pathway of coagulation [30, 31]. FIB reflects the content of fibrinogen, which is mainly synthesized by liver and is the substrate of thrombin. Peptide A and peptide B are hydrolyzed by thrombin, and finally, insoluble fibrin is formed [32].

In coagulation activity assay, compared with the blank group, chlorogenic acid butyl ester (1) could remarkably lengthen APTT and PT and reduce the content of FIB, which indicated the beneficial effect of it on endogenous and exogenous coagulation pathways, and hinder fibrin formation.
Rutin (2), caffeic acid (4), and 3,5-dicaffeoylquinic acid (8) could remarkably shorten APTT and PT and reduce the content of FIB, which indicated the beneficial effect of them on endogenous and exogenous coagulation pathways, and hinder fibrin formation. 5-O-coumaroylquinic acid methyl ester (5), quercetin-3-O-β-D-glucoside (7), and D-glucitol (13) could remarkably shorten PT and TT and reduce the content of FIB, which indicated the beneficial effect of them on exogenous coagulation pathways, and hinder fibrin formation. Kaempferol-3-O-neohesperidoside (6) and kaempferol-3-O-β-D-galactoside (12) could remarkably shorten APTT, PT, and TT and reduce the content of FIB, which indicated the beneficial effect of them on endogenous and exogenous coagulation pathways, and hinder fibrin formation. Quercetin-3-O-α-L-rhamnoside (9) could remarkably shorten PT and TT, which indicated the beneficial
Table 1: Effects of compounds on plasma coagulation parameters in vitro.

| Groups                  | APTT(s)       | PT(s)         | TT(s)         | FIB(g/L)      |
|-------------------------|---------------|---------------|---------------|---------------|
| Blank group             | 15.65 ± 0.35  | 20.80 ± 0.14  | 82.05 ± 1.06  | 112.94 ± 0.72 |
| Yunnan Baiyao           | 10.40 ± 0.85  | 19.10 ± 0.26  | 76.75 ± 0.63  | 115.83 ± 0.62 |
| Breviscapine            | 22.85 ± 0.21  | 22.65 ± 0.49  | 88.35 ± 1.06  | 108.30 ± 0.81 |
| Compound 1              | 16.50 ± 0.14  | 21.05 ± 0.50  | 82.30 ± 4.67  | 106.65 ± 0.47 |
| Compound 2              | 15.00 ± 0.14  | 19.95 ± 0.33  | 80.25 ± 4.45  | 104.81 ± 0.92 |
| Compound 4              | 13.35 ± 0.09  | 17.70 ± 0.71  | 80.40 ± 10.18 | 104.81 ± 1.02 |
| Compound 5              | 15.00 ± 0.85  | 19.20 ± 0.57  | 78.90 ± 1.97  | 106.25 ± 0.90 |
| Compound 6              | 14.90 ± 0.14  | 18.65 ± 0.50  | 79.05 ± 1.34  | 108.49 ± 0.67 |
| Compound 7              | 15.35 ± 0.21  | 18.35 ± 0.07  | 74.95 ± 3.53  | 106.98 ± 0.90 |
| Compound 8              | 14.60 ± 0.71  | 19.60 ± 0.06  | 82.70 ± 0.99  | 110.43 ± 1.09 |
| Compound 9              | 15.56 ± 0.21  | 20.15 ± 0.07  | 71.85 ± 1.20  | 113.27 ± 1.23 |
| Compound 10             | 14.61 ± 0.14  | 20.60 ± 0.14  | 80.40 ± 0.57  | 103.41 ± 0.67 |
| Compound 11             | 15.90 ± 0.57  | 19.20 ± 0.14  | 69.45 ± 4.03  | 118.06 ± 0.93 |
| Compound 12             | 12.30 ± 0.14  | 19.05 ± 0.21  | 73.55 ± 1.34  | 105.16 ± 0.63 |
| Compound 13             | 15.80 ± 0.42  | 19.60 ± 0.70  | 74.00 ± 3.39  | 103.75 ± 1.04 |
| Compound 14             | 14.75 ± 0.49  | 20.00 ± 0.98  | 76.4 ± 0.85   | 112.44 ± 0.93 |

Note. Data represent mean ± SD, n = 4. Compared with blank group, *P < 0.05, **P < 0.01, and ***P < 0.001. Compared with Yunnan Baiyao, ΔP < 0.05, ΔΔP < 0.01, and ΔΔΔP < 0.001. Compared with breviscapine, *P < 0.05, **P < 0.01, and ***P < 0.001.

Figure 2: Effects of compounds from *A. persica* flowers on plasma coagulation parameters in vitro (X ± SD, n = 4). Compared with blank group, *P < 0.05, **P < 0.01, and ***P < 0.001. Compared with Yunnan Baiyao, ΔP < 0.05, ΔΔP < 0.01, and ΔΔΔP < 0.001. Compared with breviscapine, *P < 0.05, **P < 0.01, and ***P < 0.001.

5. Conclusions

In this study, fourteen compounds were isolated and identified from *n*-butanol extract of *A. persica* by various modern chromatographic methods. The coagulation activity assay of the compounds showed that rutin, caffeic acid, kaempferol-3-O-neohesperidoside, and kaempferol-3-O-β-D-galactoside possessed significant procoagulant activity.

effect of it on exogenous coagulation pathways. Kaempferol-3-O-α-L-rhamnoside (11) could remarkably shorten PT and TT and increase the content of FIB, which indicated the beneficial effect of it on exogenous coagulation pathways, and encourage fibrin formation. And, it was for the first time to investigate the coagulation activity of all the compounds except rutin (2), quercetin-3-O-β-D-glucoside (7) and kaempferol-3-O-α-L-rhamnoside (11).
through endogenous and exogenous coagulation pathways, and kaempferol-3-O-α-L-rhamnoside possessed significant procoagulant activity through exogenous coagulation pathways, while chlorogenic acid butyl ester exerted good anticoagulant effects through endogenous and exogenous coagulation pathways in vitro.

Data Availability

The data used to support the findings of this study are included within the article.

Ethical Approval

All the animal procedures were approved by the Ethical Committee in accordance with “Institute Ethical Committee Guidelines” for Animal Experimentation and Care. Animals were housed in standard cages. The experiment was carried out according to the guidelines of the National Institutes of Health for Care and Use of Laboratory Animals and was approved by the Bioethics Committee of Henan University, Kaifeng, Henan, China.

Conflicts of Interest

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

Acknowledgments

This study received financial support from the Key Research Projects of Colleges and Universities in Henan Province (21B360006) and Special Fund Project of Zhengzhou Basic and Applied Basic Research (ZZSZX2020003).

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