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Chemical constituents and coagulation activity of *Syringa oblata* Lindl flowers

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Abstract

The leaves and bark of *Syringa oblata* Lindl are used as folk medicine which has heat-clearing, detoxifying, dampness-removing and jaundice-relieving effects. There are many studies about leaves of *S. oblata* because of its abundant resource, however, less reports about the components of *S. oblata* flowers. The previous studies on *S. oblate* flowers were mainly focused on the volatile components and its traditional pharmacological activity. Thus, this study aimed to investigate the nonvolatile chemical constituents and the coagulation activity of *S. oblate* flowers. The chemical constituents of *S. oblate* flowers were isolated with various column chromatographies and coagulation activity of the major constituents was investigated by assaying the activated partial thromboplastin time (**APTT**), prothrombin time (**PT**), thrombin time (**TT**) and fibrinogen (**FIB**) on plasma of rabbit in vitro. Fifteen known compounds (namely compound **1**-**15**) were isolated from *S. oblata* flowers. Compound **6**, **10**, **11** and **14** were isolated from *Syringa* genus for the first time. Compound **1**, **2**, **4**, **5**, **8** and **9** were isolated from the plant for the first time. The results of coagulation activity showed that water part of *S. oblate* flowers, lauric acid and kaempferol-rutinose significantly shorten PT ($P < 0.001$), TT ($P < 0.001$) and APTT ($P < 0.001$) compared with blank group, thus revealed that water extract of *S. oblate* flowers, lauric acid and kaempferol-rutinose possessed the procoagulant activity, but the effects were not better than that of Yunnan Baiyao as positive control.

Keywords: *Syringa oblata* Lindl flowers, Chemical constituents, Coagulation activity

Introduction

Syringa oblata Lindl, a medicinal plant which has the characteristics of trees or shrubs of the Oleaceae family, is native to north China. *S. oblate* tastes bitter, and has quality of cold. Chinese Materia Medica records that the leaves and bark of *S. oblata* have been used as folk medicine, which have heat-clearing, detoxifying, dampness-removing and jaundice-relieving effect [1].

Many studies were reported on the chemical constituents of *S. oblate* in China. Zhang et al. [2–6] isolated more than 50 compounds from the twigs, bark, leaves, alabastrum, seeds, and seed crust of *S. oblate*. These compounds were identified as oleanolic acid, lupinic acid, lupeol, 4-hydroxyphenethyl alcohol, 3,4-dihydroxyphenylethanol, *p*-hydroxyphenylethanol acetate, 2-(3,4-dihydroxy) phenyl ethyl acetate, *p*-hydroxyphenylethyl propyl ester, (8E)-ligstroside, oleuropein, syringopicroside, lariciresinol and esculetin, respectively. Tian et al. [7] isolated 9 compounds, including (+) pinoresinol-4″-*O*-*β*-D-glucopyranoside, (+) lariciresinol-4-*O*-*β*-D-glucoside, and epipinoresinol-4-*O*-*β*-D-glucopyranoside, from the leaves of *S. oblate*. Zhou [8] reported 2-furancarboxylic, mannitol, cyclohexane-1,2,3,4,5,6- hexaol, succinic acid, *p*-hydroxyphenylethyl alcohol and formononetin isolated from the leaves of *S. oblate*. Yang et al. [9] analyzed the compositions in the essential oil from fruits and leaves of *S. oblate*. Jiao et al. [10] found that the main component of the dried flowers of *S. oblate* were the same as those in the fruits and leaves.

These references indicate that triterpenes, phenethyl alcohol, phenylpropanoid and iridoid compounds are the main components in *S. oblate*. However, the flavonoids,

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organic acids and other constituents have been less reported.

In view of the fact that the *S. oblate* has a wide range of biological activities, including antibacterial, anti-inflammatory, antiviral, anti-tussive and expectorant effect, liver protection and cholagogue etc. [11]. The previous studies on *S. oblate* flowers were mainly focused on the volatile components and its traditional pharmacological activity. Thus, this study aimed to investigate the nonvolatile chemical constituents and the coagulation activity of *S. oblate* flowers.

Methods

Chemicals and material

The chemicals and material were similar to our previous research [12].

Plant material

Syringa oblata flowers were collected in April 2015 from the Kaifeng region of Henan Province, China and identified by Professor Changqin Li. A voucher specimen was deposited in National R & D Center for Edible Fungus Processing Technology, Henan University.

Animal

The male rabbit (approximately 20 months old, weight from 2.0 to 2.5 kg) was provided by Kaifeng Key Laboratory of Functional Components in Health Food (2016-02) to evaluate anticoagulant effect in vitro.

Ethics information

The study obtained ethical clearance from the Ethics Committee of College of Medical, Henan University (NO: 2016-36). The rabbits were treated as per the guidelines on the care and use of animals for scientific purposes.

Extraction and isolation

The extracted method was similar to our previous research [12]. The air-dried flowers of *S. oblata* (1.4 kg) were extracted with 70% ethanol to yield the crude extract (So. TE 378 g). The extract (378 g) was dissolved in MeOH-H_2O (v:v = 3:1, 500 mL), and then mixed with D101 macroporous adsorbent resin. TE was separated by macroporous resin column chromatography, eluted with 20%, 40%, 60%, and 90% ethanol. After evaporation of the solvent, 235 g of water part, 27 g of 20% ethanol part, 61 g of 40% ethanol part, 20 g of 60% ethanol part and 35 g of 90% ethanol part were obtained.

The 60% ethanol part was separated on a silica gel H column by medium pressure liquid chromatography (MPLC), eluted with dichloromethane-methanol (v:v = 1:0–2:1) to obtain 2 fractions (F_1–F_2) based on TLC analyses. F_1 was separated on a silica gel H column by MPLC, eluted with dichloromethane-acetone (v:v = 1:0–0:1) and then separated by Sephadex LH-20 (methanol) to obtain compound **1** (3 mg). F_2 was separated with Sephadex LH-20 (methanol) and Sephadex LH-20 (methanol/water, 3:1, v/v) to obtain compound **2** (18 mg).

40% ethanol part was separated on a silica gel H column by MPLC, eluted with dichloromethane-methanol (v:v = 50:1–1:1) to obtain 6 fractions (P_1–P_7) based on TLC analyses. P_1 was separated with Sephadex LH-20 (dichloromethane/methanol, 1:1, v/v) and Sephadex LH-20 (methanol) to obtain $P_{1\text{-a}}$ and $P_{1\text{-b}}$. $F_{1\text{-a}}$ was subjected to atmospheric pressure chromatographic column of silica gel H with $CHCl_2$-acetone (v:v = 1:0–0:1) to obtain compound **3** (20 mg). Compound **4** (16 mg) was obtained by the same separation method from $F_{1\text{-b}}$. P_2 was subjected to ordinary pressure chromatographic columns of silica gel H with dichloromethane-methanol (v:v = 80:1–15:1), and then separated with Sephadex LH-20 (methanol) to obtain compound **5** (4 mg). P_3 was separated with Sephadex LH-20 (dichloromethane/methanol, 1:1, v/v) and Sephadex LH-20 (methanol), and then subjected to atmospheric pressure chromatographic column of silica gel H with dichloromethane $_2$-acetone- methanol (v:v:v = 50:25:1) to obtain compound **6** (30 mg). P_4 was separated on a silica gel H column by MPLC, eluted with dichloromethane-methanol (v:v = 100:1–5:1), and then separated with Sephadex LH-20 (methanol) to obtain compound **7** (11 mg). P_5 was separated on a silica gel H column by MPLC, eluted with dichloromethane-methanol (v:v = 20:1–1:1), and then separated with Sephadex LH-20 (methanol) to obtain compound **8** (5 mg). P_6 was separated with Sephadex LH-20 (dichloromethane/methanol, 1:1, v/v) and Sephadex LH-20 (methanol) to obtain compound **2** (74 mg). P_7 was subjected to atmospheric pressure chromatographic column of silica gel H with dichloromethane-acetone-MeOH (v:v:v = 1:1:0.1–1:1:0.2) and then separated with Sephadex LH-20 (MeOH) to obtain compound **9** (39 mg).

The 90% ethanol part was separated by MPLC that was filled with silica gel H, eluted with petroleum ether-ethyl acetate (100:1–2:1, v/v) and dichloromethane- methanol (50:1–5:1, v/v) to obtain S_1–S_5. S_1 was subjected to atmospheric pressure chromatographic column of silica gel H with petroleum ether-ethyl acetate-acetone (v:v:v = 100:1:1–2:1:1) and petroleum ether-ethyl acetate (v:v = 100:1–5:1) to obtain compound **10** (86 mg). S_2 was subjected to decompressed chromatographic column of silica gel H with petroleum ether-ethyl acetate (v:v = 50:1–5:1), and then subjected to atmospheric pressure chromatographic column of silica gel H with petroleum ether- dichloromethane (v:v = 1:1–0:1) and petroleum ether-ethyl acetate (v:v = 20:1) to obtain compound **11** (25 mg). S_3 was subjected to atmospheric

pressure chromatographic column of silica gel H with petroleum ether- dichloromethane (v:v = 2:1–0:1) and dichloromethane-methanol (v:v = 20:3–10:1) to merge the same components based on TLC analysis. This part was then subjected to atmospheric pressure chromatographic column of silica gel H with petroleum ether-ethyl acetate (v:v = 20:3) to obtain compound **12** (45 mg). S_4 was recrystallized to obtain white un-dissolved substance and yellow dissolved substance. The white un-dissolved substance was subjected to atmospheric pressure chromatographic column of silica gel H with dichloromethane-methanol (v:v = 50:1–3:1) to obtain compound **13** (39 mg). The yellow substance was separated with Sephadex LH-20 (dichloromethane/methanol, 1:1, v/v) to afford compound **14** (16 mg). S_5 was recrystallized to obtain compound **15** (13 mg).

The coagulation activity of *Syringa oblata* Lindl flowers in vitro

Blood samples were drawn from Rabbit's Auricular vein without anaesthesia. The method was similar to our previous research [12]. APTT, PT,TT and FIB were determined.

For all the tests mentioned above, blank solvent (dimethyl sulphoxide: Tween 80: normal saline = 2:1:17) was used as negative control, while the drugs of breviscapine (13.3 mg/mL) and Yunnan baiyao (5 mg/mL) used in the clinics were used as positive control. All the samples were dissolved in blank solvent. The concentrations of compounds were 5 mg/mL and all the extract samples were 15 mg/mL. PT, APTT, TT and FIB tests were conducted with Semi-Automated Coagulation Analyzer (CPC Diagnostics Pvt. Ltd, India).

Statistical analysis

The results of coagulation activity were expressed as mean ± standard deviation. The data analysis was performed by SPSS19.0 software with single factor analysis of variance (ANOVA One-Way) to determine the significant difference. The difference between groups with $P < 0.05$ and $P < 0.001$ were regarded as significant and highly significant, respectively. Results were shown in Table 1.

Results

Chemical constituents in *S. oblate* flowers

Fifteen known compounds (**1**–**15**) were isolated and identified from *S. oblata* flowers. The structures of compounds were shown in Fig. 1.

Compound 1 Yellow power. The molecular formula was $C_{15}H_{10}O_7$. EI-MS *m/z*: 302[M]$^+$. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 12.49 (1H, s, 5-OH), 7.67 (1H, s, H-2′), 7.55 (1H, d, J = 8.0 Hz, H-6′), 6.89 (1H, d, J = 8.0 Hz, H-5′), 6.40 (1H, s, H-8), 6.18 (1H, s, H-6); ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 146.74 (C-2), 135.66 (C-3), 175.77 (C-4), 156.09 (C-5), 98.15 (C-6), 163.95 (C-7), 93.29 (C-8), 160.65 (C-9), 102.90 (C-10), 121.90 (C-1′), 115,55 (C-2′), 145.01 (C-3′), 147.66 (C-4′), 115.01 (C-5′), 119.90 (C-6′). The above spectral data were basically consistent with those reported previously [13] and thus, compound **1** was identified as quercetin.

Table 1 The effects of *S. oblata* extract and compounds on APTT, PT, TT, and FIB in vitro ($\bar{x} \pm s$)

Groups	PT(s)	APTT(s)	TT(s)	FIB(g/L)
Blank	11.58 ± 0.26	20.03 ± 0.24	17.47 ± 0.36	3.16 ± 0.035
Breviscapine	14.90 ± 0.23***	23.68 ± 0.38***	20.05 ± 0.19***	2.97 ± 0.044**
Yunnan Baiyao	10.65 ± 0.38***	11.35 ± 0.94***	12.68 ± 0.13***	5.00 ± 0.14***
Water part	10.58 ± 0.22***	15.43 ± 0.22***&&&	16.43 ± 0.51***&&&	3.68 ± 0.087***&&&
20% ethanol part	11.83 ± 0.35	8.8 ± 0.32***&&&	16.43 ± 0.26***&&&	3.50 ± 0.11***&&&
40% ethanol part	12.35 ± 0.37***###	14.53 ± 0.66***&&&	16.45 ± 0.10***&&&	4.51 ± 0.077***&&&
60% ethanol part	NT	NT	NT	NT
90% ethanol part	12.03 ± 0.28**###	18.85 ± 0.26**&&&	16.75 ± 0.30**&&&	4.00 ± 0.062***&&&
So.TE	12.63 ± 0.30***###	14.58 ± 0.19***&&&	15.95 ± 0.21***&&&	3.78 ± 0.10***&&&
Lauric acid	10.85 ± 0.26***	18.35 ± 0.26***&&&	15.57 ± 0.34***&&&	3.95 ± 0.033***&&&
Dictamnoside A	11.50 ± 0.26	19.82 ± 0.59	13.93 ± 0.78***&&&	3.12 ± 0.050
Kaempferol-rutinose	10.55 ± 0.21***	17.45 ± 0.25***&&&	16.47 ± 0.29***&&&	3.22 ± 0.24

Results were expressed as mean ± SD, $n = 4$, *NT*: not detected

Compared with blank: *** $P < 0.001$; $0.001 <$ ** $P < 0.01$

Compared with breviscapine: ### $P < 0.001$

Compared with Yunnan Baiyao: &&& $P < 0.001$

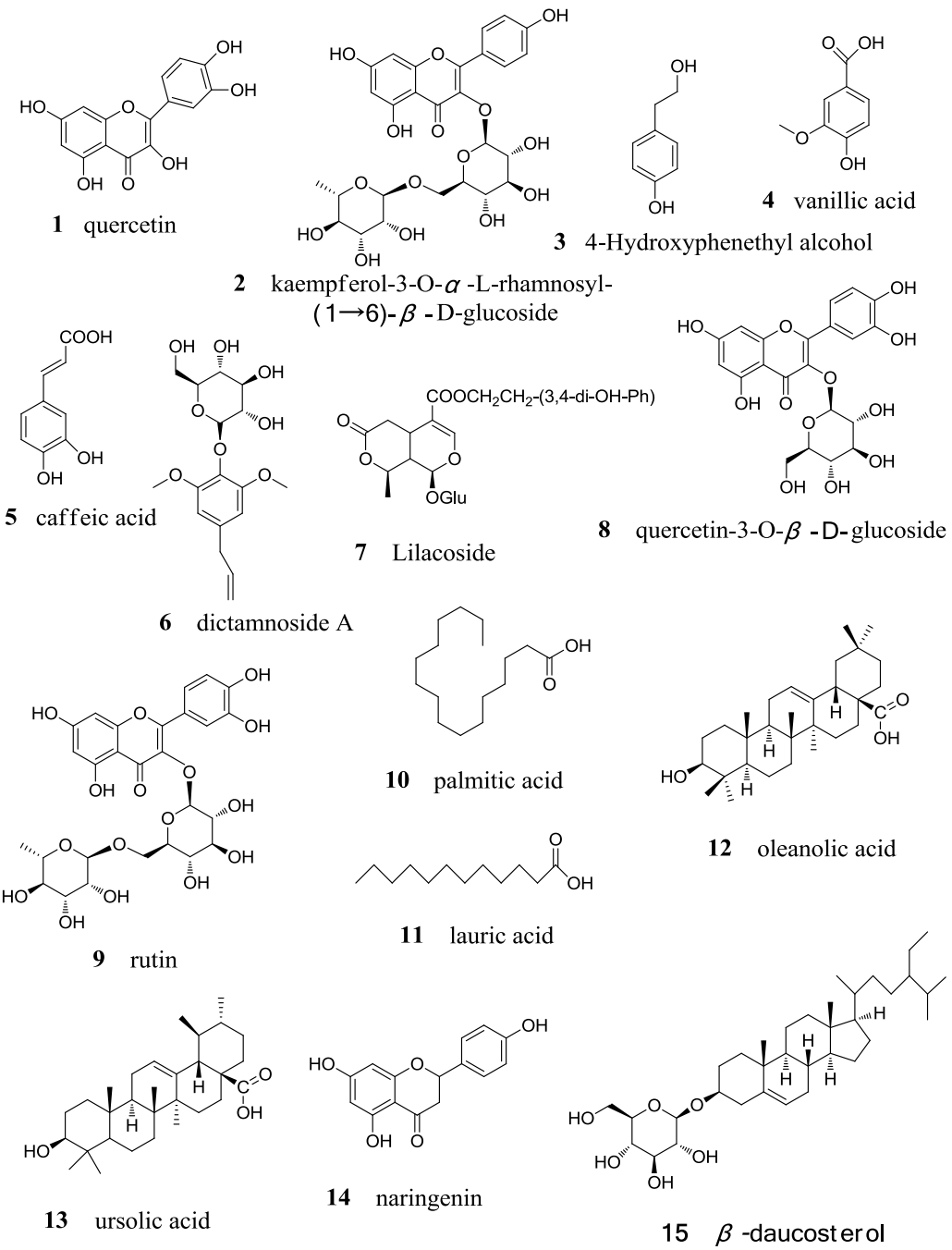

Fig. 1 Structures of compound **1**–**15**

Compound 2 A yellow, needle-shaped crystal. Its molecular formula was $C_{27}H_{30}O_{15}$. EI-MS *m/z*: 594 $[M]^+$. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 12.57 (1H, s, 5-OH), 10.86 (1H, s, 7-OH), 10.14 (1H, s, 4′-OH), 8.00 (2H, d, J = 12.0 Hz, H-2′, 6′), 6.89 (2H, d, J = 8.0 Hz, H-3′, 5′), 6.42 (1H, d, J = 4.0 Hz, H-8), 6.21 (1H, d, J = 4.0 Hz, H-6), 5.32 (1H, d, J = 8.0 Hz, H-1″), 4.44 (1H, brs, H-1‴); ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 156.63 (C-2), 133.26 (C-3), 177.43 (C-4), 161.24 (C-5), 98.76 (C-6), 164.14 (C-7), 93.79 (C-8), 156.90 (C-9), 104.04 (C-10), 120.93 (C-1′), 130.93 (C-2′, 6′), 159.93 (C-4′), 115.14 (C-3′, 5′), 101.36 (C-1″), 74.21 (C-2″), 76.39 (C-3″), 69.96 (C-4″), 75.78 (C-5″), 66.92 (C-6″), 100.81 (C-1‴), 70.39 (C-2‴), 70.63 (C-3‴), 71.85 (C-4‴), 68.29 (C-5‴), 17.77 (C-6‴).

The above data were basically consistent with those reported in the Ref. [14]. Thus, compound **2** was identified as kaempferol-3-*O*-*α*-L-rhamnosyl-(1 → 6)-*β*-D-glucoside (kaempferol- rutinose).

Compound 3 A white powder. The molecular formula was $C_8H_{10}O_2$. EI-MS *m/z*: 138 $[M]^+$. ^{1}H-NMR (C_5D_5N, 400 MHz) *δ*: 7.33 (2H, d, *J* = 8.0 Hz, H-2, 6), 7.18 (2H, d, *J* = 8.0 Hz, H-3, 5), 4.11 (2H, d, *J* = 12.0 Hz, H-8), 3.05 (2H, t, *J* = 7.0 Hz, H-7); ^{13}C-NMR (C_5D_5N, 100 MHz) *δ*: 130.59 (C-1), 130.54 (C-2, 6), 116.07 (C-3, 5), 157.17 (C-4), 39.58 (C-7), 63.86 (C-8). The above data were basically consistent with those reported in the Ref. [15]. Thus, compound **3** was identified as 4-Hydroxyphenethyl alcohol.

Compound 4 A white powder. The molecular formula was $C_8H_8O_4$. EI-MS *m/z*: 168 $[M]^+$. ^{1}H-NMR (C_5D_5N, 400 MHz) *δ*: 8.19 (1H, dd, *J* = 8.0 Hz, 4.0 Hz, H-6), 8.09 (1H, d, *J* = 4.0 Hz, H-2), 7.32 (1H, d, *J* = 8.0 Hz, H-5), 3.74 (3H, s, 3-OCH_3); ^{13}C-NMR (C_5D_5N, 100 MHz) *δ*: 123.34 (C-1), 116.03 (C-2), 148.15 (C-3), 152.58 (C-4), 113.61 (C-5), 124.75 (C-6), 168.98 (C-7), 5.58 (C-8). The above data were basically consistent with those reported in the Ref. [16]. Thus, compound **4** was identified as vanillic acid.

Compound 5 This compound was a yellow-brown, needle-shaped crystal. The molecular formula was determined to be $C_9H_8O_4$. EI-MS *m/z*: 180 $[M]^+$. ^{1}H-NMR (DMSO-d_6, 400 MHz) *δ*: 7.42 (1H, d, *J* = 16.0 Hz, H-7), 7.01 (1H, s, H-2), 6.96 (1H, dd, *J* = 4.0, 8.0 Hz H-6), 6.76 (1H, d, *J* = 8.0 Hz, H-5), 6.18 (1H, d, *J* = 16.0 Hz, H-8); ^{13}C-NMR (DMSO-d_6, 100 MHz) *δ*: 125.72 (C-1), 114.60 (C-2), 144.34 (C-3), 148.09 (C-4), 115.36 (C-5), 115.74 (C-6), 145.55 (C-7), 121.04 (C-8), 168.00 (9-COOH). The above data were basically consistent with those reported in the Ref. [17]. Thus, the compound **5** was identified as caffeic acid.

Compound 6 A white powder. The molecular formula was $C_{17}H_{24}O_8$. EI-MS *m/z*: 566 $[M]^+$. ^{1}H-NMR (400 MHz, C_5D_5N) *δ*: 6.60 (2H, s, H-3, 5), 6.03 (1H, m, H-*β*), 5.11 (2H, m, H-*γ*), 3.74 (6H, s, 2 × OCH_3), 3.92–4.38 (6H, m, H-2′–6′), 3.34 (2H, d, *J* = 8.0 Hz, H-*α*); ^{13}C-NMR (100 MHz, C_5D_5N) *δ*: 153.67 (C-2, 6), 137.82 (C-*β*), 136.26 (C-1), 134.45 (C-4), 115.87 (C-*γ*), 107.13 (C-3, 5), 105.02 (C-1′), 75.98 (C-2′), 78.58 (C-3′), 71.51 (C-4′), 78.28 (C-5′), 62.53 (C-6′), 56.46 (2 × OCH_3), 40.44 (C-*α*). The above data were basically consistent with those reported in the Ref. [18]. Thus, the compound **6** was identified as dictamnoside A.

Compound 7 A yellow powder. The molecular formula was $C_{24}H_{30}O_{13}$. EI-MS *m/z*: 526 $[M]^+$. ^{1}H-NMR (400 MHz, C_5D_5N) *δ*: 7.52 (1H, s, H-3), 6.65 (1H, s, H-5″), 6.63 (1H, d, *J* = 1.5 Hz, H-2″), 6.49 (1H, dd, *J* = 1.5 Hz, 8.5 Hz, H-6″), 4.18 (2H, m, 2 × H-α), 3.02 (1H, m, H-5), 2.72 (2H, t, *J* = 6.5 Hz 2 × H-β), 2.07 (1H, q, H-9), 4.56 (1H, d, *J* = 8.0 Hz, H-1′), 1.41 (3H, d, *J* = 4.0 Hz, 10-Me); ^{13}C-NMR (100 MHz, C_5D_5N) *δ*: 94.84 (C-1), 152.92 (C-3), 107.77 (C-4), 26.82 (C-5), 33.53 (C-6), 171.67 (C-7), 73.49 (C-8), 21.26 (C-10), 166.63 (C-11), 99.23 (C-1′), 73.20 (C-2′), 77.34 (C-3′), 70.16 (C-4′), 76.57 (C-5′), 61.38(C-6′), 128.75 (C-1″), 116.21 (C-2″), 145.12 (C-3″), 143.76 (C-4″), 115.55 (C-5″), 119.62 (C-6″), 64.93 (C-*α*), 33.88 (C-*β*). The above data were basically consistent with those reported in the Ref. [19]. Thus, the compound **7** was identified as Lilacoside.

Compound 8 A yellow powder. The molecular formula was $C_{21}H_{20}O_{12}$. EI-MS *m/z*: 465 $[M]^+$. ^{1}H-NMR (400 MHz, DMSO-d_6) *δ*: 12.65 (1H, s,5-OH), 7.59 (1H, d, *J* = 4.0 Hz, H-6′), 7.57 (1H, d, *J* = 4.0 Hz, H-2′), 6.85 (1H, d, *J* = 8.0 Hz, H-5′), 6.39 (1H, s, H-8), 6.20 (1H, d, *J* = 4.0 Hz, H-6), 5.46 (1H, d, *J* = 8.0 Hz, Glc-H-1″), 3.17–3.24 (5H, m, Rha-H-2″–6″); ^{13}C-NMR (100 MHz, DMSO-d_6) *δ*: 156.11 (C-2), 133.30 (C-3), 177.36 (C-4), 161.18 (C-5), 98.63(C-6), 164.21(C-7), 93.45(C-8), 156.28 (C-9), 103.78 (C-10), 121.52 (C-1′), 115.15 (C-2′), 144.75(C-3′), 148.41(C-4′), 116.16(C-5′), 121.11(C-6′), Glc: 100.92(C-1″), 74.06(C-2″), 77.46(C-3″), 69.92(C-4″), 76.48 (C-5″), 60.95 (C-6″). The above data were basically consistent with those reported in the Ref. [20]. Thus, the compound **8** was identified as quercetin-3-*O*-*β*-D-glucoside.

Compound 9 Was a yellow powder. The molecular formula was $C_{27}H_{30}O_{16}$. EI-MS *m/z*: 610 $[M]^+$. ^{1}H-NMR (400 MHz, DMSO-d_6) *δ*: 12.60 (1H, s, 5-OH), 10.84 (1H, s, 7-OH), 9.68 (1H, s, 4′-OH), 9.19 (1H, s, 3′-OH), 7.55 (2H, d, *J* = 8.0 Hz, H-2′, 6′), 6.85 (1H, d, *J* = 8.0 Hz, H-5′), 6.39 (1H, d, *J* = 4.0 Hz, H-8), 6.20 (1H, d, *J* = 4.0 Hz, H-6), 5.35 (1H, d, *J* = 4.0 Hz, H-1″), 4.38 (1H, brs, H-1‴); ^{13}C-NMR (100 MHz, DMSO-d_6) *δ*: 156.42 (C-2), 133.30 (C-3), 177.37 (C-4), 161.23 (C-5), 98.68 (C-6), 164.07 (C-7), 93.59 (C-8), 156.61 (C-9), 103.98 (C-10), 121.18 (C-1′), 115.23 (C-2′), 144.76 (C-3′), 148.42 (C-4′), 116.26 (C-5′), 121.59 (C-6′), 101.17 (C-1″), 74.08 (C-2″), 76.44 (C-3″), 70.00 (C-4″), 75.91 (C-5″), 67.01 (C-6″), 100.76 (C-1‴), 70.38 (C-2‴), 70.56 (C-3‴), 71.84 (C-4‴), 68.26 (C-5‴), 17.77 (C-6‴). The above data were basically consistent with those reported in the Ref. [21]. Thus, the compound **9** was identified as rutin.

Compound 10 A white solid. The molecular formula was $C_{16}H_{32}O_2$. EI-MS *m/z*: 256 $[M]^+$. ^{1}H-NMR ($CDCl_3$, 400 MHz) *δ*: 0.88 (3H, t, *J* = 9.0 Hz, H-16), 1.23–1.29 (24H, m, H-4–15), 1.61 (2H, m, H-3), 2.34 (2H, t, *J* = 7.5 Hz, H-2); ^{13}C-NMR($CDCl_3$, 100 MHz) *δ*: 179.3 (–COOH), 34.23 (C-2), 24.83 (C-3), 29.21–29.85 (C-4–13), 32.08 (C-14), 22.85 (C-15), 14.27 (C-16). The above data were basically consistent with those reported in the Ref. [22]. Thus, the compound **10** was identified as palmitic acid.

Compound 11 A white solid. The molecular formula was $C_{12}H_{24}O_2$. EI-MS *m/z*: 200 $[M]^+$. ^{1}H-NMR ($CDCl_3$, 400 MHz) *δ*: 0.87 (3H, t, *J* = 7.0 Hz, H-12), 1.26 (16H, m, H-4–11), 1.80 (2H, m, H-3), 2.52 (2H, t, *J* = 8.0 Hz, H-2); ^{13}C-NMR ($CDCl_3$, 100 MHz) *δ*: 175.9(-COOH), 34.89 (C-2), 25.67 (C-3), 29.63–29.99 (C-4–9), 32.14 (C-10), 22.96 (C-11), 14.29 (C-12). The above data were basically consistent with those reported in the Ref. [23]. Thus, the compound **11** was identified as lauric acid.

Compound 12 A white powder. The molecular formula was $C_{30}H_{48}O_3$. EI-MS *m/z*: 456 $[M]^+$. ^{1}H-NMR (C_5D_5N, 400 MHz) *δ*: 5.50 (1H, brs, H-12), 3.45 (1H, dd, *J* = 8.0 Hz, 4.0 Hz, H-3), 3.32 (1H, dd, *J* = 4.0 Hz, 4.0 Hz, H-18), 1.28 (3H, s, H-27), 1.24 (3H, s, H-25), 1.02 (3H, s, H-30), 1.01 (3H, s, H-29), 0.95 (3H, s, H-23), 0.89 (3H, s, H-26); ^{13}C-NMR (C_5D_5N, 100 MHz) *δ*: 38.93 (C-1), 28.09 (C-2), 78.06 (C-3), 39.38 (C-4), 55.80 (C-5), 18.79 (C-6), 39.74 (C-8), 48.11 (C-9), 37.37 (C-10), 23.82 (C-11), 122.54 (C-12), 144.81 (C-13), 42.16 (C-14), 28.31 (C-15), 23.69 (C-16), 46.47 (C-17), 42.00 (C-18), 346.66 (C-19), 30.96 (C-20), 4.21 (C-21), 33.18 (C-22), 28.78 (C-23), 16.55 (C-24), 15.55 (C-25), 17.43 (C-26), 26.17 (C-27), 180.16 (C-28), 233.27 (C-7, 29), 3.76 (C-30). The above data were basically consistent with those reported in the Ref. [16]. Thus, the compound **12** was identified as oleanolic acid.

Compound 13 Was a white powder. The molecular formula was $C_{30}H_{48}O_3$. EI-MS *m/z*: 456 $[M]^+$. ^{1}H-NMR (C_5D_5N, 400 MHz) *δ*: 5.49 (1H, s, H-12), 3.46 (1H, dd, *J* = 8.0 Hz, 8.0 Hz, H-3), 2.65 (1H, d, *J* = 8.0 Hz, H-18), 1.25 (3H, s, H-27), 1.23 (3H, s, H-26), 1.05 (3H, s, H-23), 0.96 (3H, d, *J* = 8.0 Hz, H-29), 0.88 (3H, s, H-24), 1.01 (3H, d, *J* = 8.0 Hz, H-30), 1.02 (3H, s, H-25); ^{13}C-NMR (C_5D_5N, 100 MHz) *δ*: 37.43 (C-1), 28.11 (C-2), 78.09 (C-3), 39.06 (C-4), 55.80 (C-5), 18.77 (C-6), 33.56 (C-7), 39.94 (C-8), 48.02 (C-9), 39.47 (C-10), 23.90 (C-11), 125.63 (C-12), 139.24 (C-13), 42.48 (C-14), 28.80 (C-15), 24.89 (C-16), 48.02 (C-17), 53.52 (C-18), 39.39 (C-19), 39.37 (C-20), 31.06 (C-21), 37.26 (C-22), 28.67 (C-23), 15.67 (C-24), 16.58 (C-25), 17.52 (C-26), 23.61 (C-27), 179.88 (C-28), 17.43 (C-29), 21.42 (C-30). The above data were basically consistent with those reported in the Ref. [16]. Thus, the compound **13** was identified as ursolic acid.

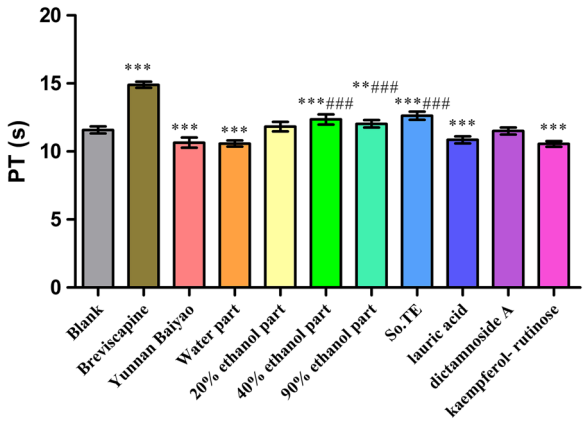

Fig. 2 The effects of *S. oblata* extract and compounds on PT in vitro

Compound 14 A yellow powder. The molecular formula was $C_{15}H_{12}O_5$ EI-MS *m/z*: 272 $[M]^+$. ^{1}H-NMR (C_5D_5N, 400 MHz) *δ*: 12.83 (1H, s, 5-OH), 7.55 (2H, d, *J* = 8.0 Hz, H-2′, 6′), 7.22 (2H, d, *J* = 8.0 Hz H-3′, 5′), 6.49 (1H, d, *J* = 4.0 Hz, H-8), 6.18 (1H, s,H-6), 5.51 (1H, dd, *J* = 4.0 Hz, 4.0 Hz, H-2), 5.32 (2H, s, H-6, 8), 3.33 (1H, dd, *J* = 12.0 Hz, 12.0 Hz, H-3a), 2.90 (1H, dd, *J* = 0 Hz, 4.0 Hz, H-3b); ^{13}C-NMR (C_5D_5N, 100 MHz) *δ*: 79.65 (C-2), 43.29 (C-3), 196.53 (C-4), 165.16 (C-5), 97.22 (C-6), 168.56 (C-7), 96.11 (C-8), 164.03 (C-9), 102.87 (C-10), 129.76 (C-1′), 128.86 (C-2′, 6′), 116.42 (C-3′, 5′), 159.53 (C-4′). The above data were basically consistent with those reported in the Ref. [24]. Thus, the compound **14** was identified as naringenin.

Compound 15 A white powder. The molecular formula was $C_{35}H_{60}O_6$. EI-MS *m/z*: 578$[M]^+$. It was compared with reference substance of *β*-daucosterol, no difference was seen between them in term of the TLC detection. Thus compound **15** was identified as *β*-daucosterol.

Coagulation time test in vitro

In Fig. 2, water part, lauric acid and kaempferol-rutinose could significantly shorten PT ($P < 0.001$) compared with the blank group. The 40% ethanol part, 90% ethanol part and So.TE had significant anticoagulant activity ($P < 0.001$ and $0.001 < P < 0.01$) compared with the blank group. The effects of water part, lauric acid and kaempferol-rutinose were not different with that of Yunnan Baiyao.

In the Fig. 3, all the samples except 60% ethanol part and dictamnoside A could significantly shorten TT

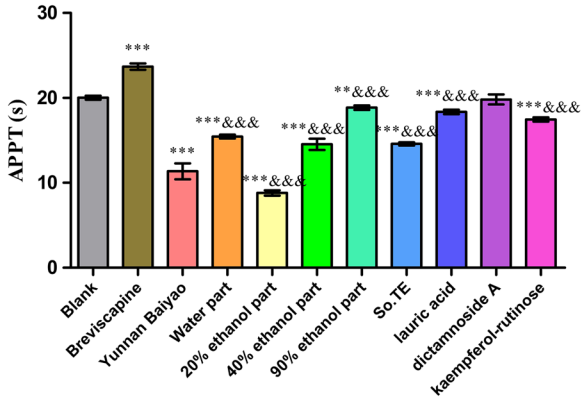

Fig. 3 The effects of *S. oblata* extract and compounds on APTT in vitro

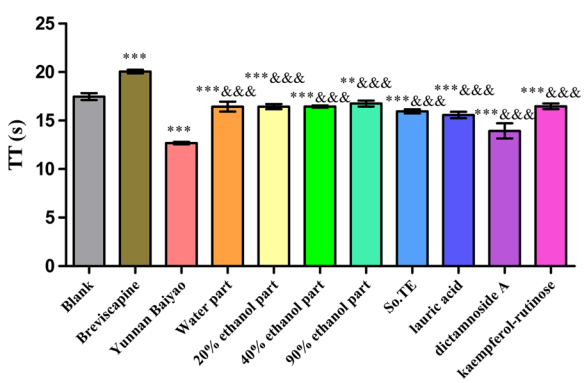

Fig. 4 The effects of *S. oblata* extract and compounds on TT in vitro

($P < 0.001$ and $0.001 < P < 0.01$) compared with the blank group. The procoagulant activity of 20% ethanol part was the best one ($P < 0.001$) compared with the Yunnan Baiyao.

In Fig. 4, water part, 20% ethanol part, 40% ethanol part, 90% ethanol part, So, TE, lauric acid and kaempferol-rutinose could significantly shorten APTT ($P < 0.001$) compared with the blank group. Water part, 20% ethanol part, 40% ethanol part, 90% ethanol part, So.TE, lauric acid and kaempferol-rutinose had procoagulant activity compared with the Yunnan Baiyao, and 20% ethanol part had a higher activity than that of Yunnan Baiyao, while the others were not better than that of Yunnan Baiyao.

In Fig. 5, water part, 20% ethanol part, 40% ethanol part, 90% ethanol part, So.TE and lauric acid all could significantly increase the FIB content ($P < 0.001$) compared with the blank group. The procoagulant activity of the positive control was the best one ($P < 0.001$) compared with the Yunnan Baiyao.

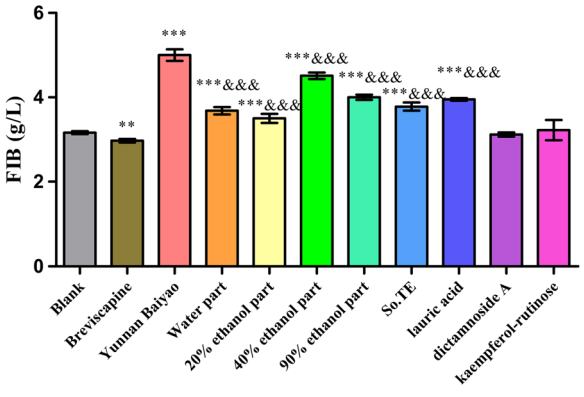

Fig. 5 The effects of *S. oblata* extract and compounds on FIB in vitro

Discussion

Sun et al. [25] found that the volatile compounds in fresh flowers of *S. oblate* during different flowering periods were different. Dong et al. [6] isolated 8 compounds from the alabastrum of *S. oblate*, and they were identified as syringopicrogenin-B, oleandic acid, ursolic acid, lupanic acid, luprol, *p*-hydroxy phenylpropanol, *p*-hydroxy phenylethanol and *β*-sitosterol. Triterpenic acids were the main components. In this study, fifteen known compounds were isolated from *S. oblata* flowers. They were identified as quercetin (**1**), kaempferol-3-*O*-*α*-L-rhamnosyl-(1 → 6)-*β*-D-glucoside (**2**, kaempferol-rutinose), 4-Hydroxyphenethyl alcohol (**3**), vanillic acid (**4**), caffeic acid (**5**), dictamnoside A (**6**), Lilacoside (**7**), quercetin-3-O-*β*-D-glucoside (**8**), rutin (**9**), palmitic acid (**10**), lauric acid (**11**), oleanolic acid (**12**), ursolic acid (**13**), naringenin (**14**), and *β*-daucosterol (**15**). Flavonoids, organic acids and Triterpenic acids were the main components.

The previous studies on *S. oblate* flowers were mainly focused on the volatile components and its traditional pharmacological activity. In the present study we found that the *S. oblate* flowers had a significant procoagulant activity for the first time. Our researches showed that water part, lauric acid and kaempferol-rutinose all displayed a significant procoagulant activity, and that the procoagulant activity of water part, lauric acid, and kaempferol-rutinose were not better than that of Yunnan Baiyao, which was used as the positive control.

Conclusions

In the present study, fifteen compounds were isolated and identified from *S. oblate* flowers, including triterpenic acids, fatty acids and flavonone glycosides etc. Water extract of *S. oblate* flowers, lauric acid and kaempferol-rutinose possessed the procoagulant activity.

Abbreviations
APTT: activated partial thromboplastin time; PT: prothrombin time; TT: thrombin time; FIB: fibrinogen; So.TE: total extract of *S. oblata* flowers; Water part: water extract of *S. oblata* flowers; 20% ethanol part: 20% ethanol extract of *S. oblata* flowers; 40% ethanol part: 40% ethanol extract of *S. oblata* flowers; 60% ethanol part: 60% ethanol extract of *S. oblata* flowers; 90% ethanol part: 90% ethanol extract of *S. oblata* flowers.

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Authors' contributions
WYK and ZHL conceived the research idea. LLC, MYH and PRC conducted the experiment, collected the plant specimens, analyzed and interpreted the data as well as prepared the first draft. WYK, YN and CQL critically read and revised the paper. All the authors read and approved the final manuscript.

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Availability of data and materials
The datasets supporting the conclusions of this article are presented in this main paper. Plant materials used in this study have been identified by Professor Changqin Li. A voucher specimen was deposited in National Center for Research and Development of Edible Fungus Processing Technology.

Competing interests
The authors declare that they have no competing interests.

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