Anti-hyperglycemic effect of *Potentilla discolor* decoction on obese-diabetic (Ob-db) mice and its chemical composition

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*Potentilla discolor* is used as an ethnomedicine in treatments of diabetes mellitus in China for years. In the present study, the anti-hyperglycemic effects of a clinical active extract (decoction) from *P. discolor* were investigated in Ob-db mice. Four week's treatment of *P. discolor* decoction ameliorated the development of hyperlipidemia, lipid peroxidation and hyperglycemia associated with hyperphagia and polydypsia in Ob-db mice. *P. discolor* significantly attenuated the increase of blood glucose and cholesterol levels in Ob-db mice. These findings clearly provided evidences regarding the anti-hyperglycemic potentials of *P. discolor* decoction. High-resolution liquid chromatography-mass spectrometry/mass spectrometry (HR-LC-MS/MS) was used to analyze the phytochemicals in *P. discolor*. In an comprehensive analysis of phytochemicals in *P. discolor*, thirty-five components were identified or characterized in *P. discolor* decoction and only sixteen of them have been reported in *P. discolor* previously. There are five major components identified in *P. discolor* decoction. One of the major components is a flavonoid sulfate, and this is the first evidence for the presence of sulfated flavonoid in *P. discolor*. Sulfated flavonoids have been reported to improve the complications of diabetes mellitus by inhibition of the aldose reductase in both experimental animals and clinical trials. Therefore, the sulfated flavonoid in *P. discolor* decoction may in part contribute to the anti-hyperglycemic effect of *P. discolor*.

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1. Introduction

Diabetes mellitus has been recognized as the most common metabolic disorders induced by many etiologies, and characterized by hyperglycemia and an abnormal elevation in the blood glucose level. Between 2010 and 2030, there will be a 69% increase in numbers of adults with diabetes in developing countries and a 20% increase in developed countries [1]. Ob-db is characterized by a combination of resistance to insulin action and an inadequate compensatory insulin-secretory response, and is closely associated with obesity [2,3]. Dietary restrictions, exercise and administration of oral glucose-lowering agents are applied widely to control concentrations of blood glucose [4]. Moreover, herbal supplements and other alternative medicines have gradually increased to use for treatment of diabetic disorders. The genus *Potentilla* of Rosaceae family comprises more than 200 species, and is widely distributed in the Northern temperate zone [5]. Of the herbal medications used in diabetic disorders, *P. discolor* is mentioned as one of the important crude materials in oriental medicine for therapy of hyperglycemia and hyperlipidemia. The principal phytochemicals in *P. discolor* have been reported to be polyphenols,
flavonoids and triterpenoids [6,7]. In the previous studies, most investigations of chemical composition of *P. discolor* have been focused on the methanol or ethanol extract. However, most Traditional Chinese Medicine (TCM) remedies are prepared in the form of decoctions and are administered orally. The methanol or ethanol extract of TCM commonly used for pharmacology research could not reflect the real characteristic of TCM. Therefore, limited studies are available on the effects of *P. discolor* decoction on diabetes animal model and its chemical composition.

In the present study, the ameliorative effects of *P. discolor* decoction against diabetic condition were examined in Ob-db mice. The findings could provide evidences to support the use of *P. discolor* decoction as an anti-hyperglycemic TCM. Furthermore, a series of HR-LC-MS/MS analyses was carried out to identify the structures of the components present in *P. discolor* decoction. A comprehensive phytochemical profile of *P. discolor* decoction could be used to correctly explain its anti-hyperglycemic effects.

2. Materials and methods

2.1. Drugs and chemicals

The plant of *Potentilla discolor* Bunge was grown in Bozhou area of Anhui Province, China. Air-dried entire plant was purchased from Xiangning Kang Jin Chinese Herbal Pieces Co., Ltd. (Hubei, China). The herb was identified and authenticated by the taxonomist of Key Laboratory of Chinese Medicine Resource and Compound Prescription (Hubei University of Chinese Medicine), Ministry of Education, China. A voucher specimen (No. 022) was deposited in the Key Laboratory.

Alloxan was purchased from Sigma Ltd. (USA). Metformin was obtained from Zhong Hui pharmaceutical Ltd. (Beijing, China). Cholesterol, triglyceride, blood glucose, superoxide dismutase, malondialdehyde and non-esterified fatty acid assay kits were purchased from Shanghai Mind Bioengineering Co. Ltd. (Shanghai, China).

2.2. Preparation of *P. discolor* decoction

Two kilograms of *P. discolor* were decocted in 20 L of water for 3 h, and the extraction process was repeated for 3 times. The slurry was filtered through a Büchner funnel with a no. 4 Whatman filter paper. The three extracts were combined and concentrated on a rotary evaporator under reduced pressure at room temperature to 3 L. The concentrate was lyophilized to obtain the dry decoction. The dry decoction represented 13.2% of the original *P. discolor* (w/w).

2.3. Animals and treatments

Male mice weighing approximately between 20 and 25 g were acclimatized under standard conditions of 12 h light/dark cycle at 18–23 °C with a humidity of 55–60% for three days prior to the experiments. They were maintained under standard conditions for experiment. Ob-db mice were established by a high-fat diet and alloxan [3,8,9]. A high-fat diet contained basic diet (78.8%), egg yolk (10%), lard oil (10%), cholesterol (1%) and cholate (0.2%). Briefly, mouse was fed a high-fat diet for 4 weeks to prepare the hyperlipidemia mouse. The hyperlipidemia mouse was treated with a twice low-dose intraperitoneal injection of fresh alloxan (60 mg·kg⁻¹·2 of body weight) for establishment of Ob-db mouse. In addition to polyuria and other diabetic features, Ob-db mouse was defined as a blood glucose level of 11 mmol·L⁻¹ or greater in blood obtained by a tail-vein puncture after the last injection of alloxan following overnight fasting. All animals were divided into 6 groups with 8 mice in each group. Mice in control (C) group and Ob-db (O) group were intragastrically administered with 0.5 mL of distilled water one time per day. Positive group (P) was treated with 50 mg·kg⁻¹·d⁻¹ Metformine. *P. discolor* decoction low (PD₉), medium (PD₈) and high (PD₇) dose groups were treated with 0.4 g, 0.8 g and 2.0 g·kg⁻¹·d⁻¹ dry decoction of *P. discolor*, respectively. Metformine and dry decoction were homogenized in 0.5 mL of distilled water for one time intragastric administration. The 0.4 g, 0.8 g and 2.0 g of *P. discolor* decoction powder were equivalent to 3 g, 6 g and 15 g of TCM materials. Food and water intake were monitored every 4 days. Animal use protocols were approved by the Institutional Animal Care and Use Committee of Hubei University of Chinese Medicine.

Blood samples were collected by a tail-vein puncture every 7 days. Oral glucose tolerance test was performed to determine the short-term effect of *P. discolor* decoction on diabetic mice at the end of the study. Tail vein blood samples were withdrawn without anesthesia before (0 min) and 0.5, 1 and 2 h after the administration of 2 g·kg⁻¹ glucose solution. Serum was separated immediately from blood samples by centrifugation and stored at −80 °C until the analysis. After the mice were anesthetized with pentobarbital injection, liver, kidney, epididymal white adipose tissues and other organs were immediately weighted, then frozen and stored at −80 °C. The serum samples were analyzed for cholesterol, triglyceride, blood glucose, superoxide dismutase, malondialdehyde and non-esterified fatty acid.

2.4. Biochemical analysis

Blood glucose levels were measured using a glucose oxidase method with a commercial kit. Serum triglyceride and cholesterol concentrations were determined with assay kits by a glyceral-3-phosphate oxidase method and a cholesterol oxidase method, respectively. The levels of serum superoxide dismutase, malondialdehyde and non-esterified fatty acid were determined by commercial kits according to manufacturer's protocols.

2.5. HR-LC-MS/MS analysis

The lyophilized powder of *P. discolor* decoction was dissolved in 50% aqueous methanol to prepared the dilute solution (0.25 mg·mL⁻¹), and directly analyzed by LC-MS/MS with a 20-μL injection. The components in *P. discolor* were separated using a Alltima reversed-phase C18 column (5 μm, 250 mm×4.6 mm i.d.) with gradient elution of (A) water-formic acid (99.9:0.1, v/v) and (B) acetonitrile-formic acid (99.9:0.1, v/v). The following binary gradient with linear interpolation was used: 0 min, 10% B; 35 min, 40% B; 70 min, 100% B; 75 min, 10% B, at a flow rate of 0.5 mL·min⁻¹. HR-MS/MS analyses were carried out using a MicrOTOF-Q II Focus mass spectrometer (Bruker Daltonics) fitted with an ESI source operating in Auto-MSn mode to obtain fragment ion m/z. The
MS conditions were as follows: capillary, 4500 V; end plate offset, −500 V; collision cell RF, 150 Vpp; dry heater temperature, 180 °C; dry gas flow rate, 5.0 L·min⁻¹; nebulizer pressure, 3.0 bar. The outflows were divided into three shares, and only one share entered the MS detector.

2.6. Statistics

Results are presented as the means±SD. The data were analyzed by one-way analysis of variance (LSD test) to compare treatments vs the diabetic control (O group) using SPSS 19.0 software. P values<0.05 were considered statistically significant.

3. Results and discussion

3.1. General parameters

Established Ob-db mouse in the present study demonstrated typical characteristics of diabetes mellitus, such as increases in food and water intake and polyuria. Food and water intake of the experimental mice are depicted in Fig. 1. The water intake of PDH group was significantly lower than that of O group after an 8-day treatment and continues to decrease until end of experiment. Ob-db mice in PD₅ group shows a moderate decrease of water intake, and the significant changes were observed after a 16-day treatment (Fig. 1A). The food intake in PD₅ group was lower than that in O group, and the differences were statistically significant except for the 0–8th days of treatment (Fig. 1B). As shown in Table 1, Ob-db mice in O, P and PD_L, PD_M and PD_H groups showed a significantly higher body weight compared to that of normal mice in control group. There was no significant changes in the body weight of Ob-db mice after 4 weeks of intragastric administration of P. discolor decoction. In addition, 4 weeks treatment with P. discolor decoction failed to alter the weight of the livers, kidneys and epididymal white adipose tissues of Ob-db mice. However, the weight of the livers and epididymal white adipose tissues of Ob-db mice groups with or w/o treatments of P. discolor decoction were significantly higher than that of normal mice in control group.

There are at least three methods to establish diabetic animal models, namely, diet induced, chemically induced, and spontaneous or genetically modified mice [10]. In this study, a combination of diet and chemical induced diabetes was used for the establishment of Ob-db mouse. Ob-db mouse established by this method mimics human syndromes. As a result,
the high-fat diet significantly increased the body weight of mice compared to that of mice in normal diet group (Table 1). Alloxan injection has been reported to cause a loss of body weight, hyperphagia and polydypsia of mouse [11]. The loss of body weight could be due to dehydration and catabolism of fats and proteins. In this study, injection of alloxan failed to change the body weight of mice fed a high-fat diet, and 4 weeks’ P. discolor decoction consumption did not alter body weight of Ob-db mice in three dose groups (PDl, PDm and PDh). However, P. discolor decoction consumption induced a reduction of glucose levels as well as hyperphagia and polydypsia (Fig. 1). Therefore, the present study gives evidence for the ameliorative effect of P. discolor decoction in diabetes-related symptoms.

3.2. Effect of treatment on the blood glucose levels

The hypoglycemic potency of P. discolor decoction on Ob-db mice was shown in Fig. 2A. Treatment of Ob-db mice with 0.8 and 2.0 g dry decoction·kg\(^{-1}\)·d\(^{-1}\) for 4 weeks results in a significant decrease of the blood glucose values compared with group O. Thirty minutes after glucose administration, the blood glucose concentration was elevated both in control and P. discolor decoction-treated mice (Fig. 2B). However, the increase of blood glucose produced by the glucose administration was significantly improved in the mice treated with 2.0 g dry decoction·kg\(^{-1}\)·d\(^{-1}\) in comparison of the mice in O group. This result showed that P. discolor decoction can enhance glucose utilization in vivo.

Glucose homeostasis largely results from a balance of oxidative stress or the potentiation of the effects of insulin [12].

3.3. Serum lipid measurements

Table 1 shows the effect of P. discolor decoction on the serum lipid profiles. Marked increases in the levels of serum cholesterol, triglyceride and non-esterified fatty acid were observed in O group as compared with C group. Meanwhile, these major markers of hypercholesterolaemia decreased significantly in PDh group compared to that of O group. Moreover, dose-dependent decreases were observed in serum cholesterol, triglyceride and non-esterified fatty acid levels, although there was no significant alteration on serum cholesterol of Ob-db mice in PDl group. Also, significant differences on triglyceride and non-esterified fatty acid levels were not observed in another two decoction-treated groups (PDl and PDm) in responses to 28 consecutive days of treatments.

Diabetes mellitus increases the risk of coronary heart disease associated with profound alterations in the plasma lipid and lipoprotein profile [13]. In this study, the alteration in lipid metabolism was partially alleviated as evidenced by decreased serum cholesterol, triglyceride and non-esterified fatty acid levels after treatment with P. discolor decoction. In addition, 2.0 g dry decoction·kg\(^{-1}\)·d\(^{-1}\) treatment greatly affected serum lipid profiles in PDh group. The lipid-lowering effect of P. discolor decoction in Ob-db mice may be possibly attributed to the hydrolysis of certain lipoproteins and their selective uptake and metabolism by different tissues.

3.4. Anti-oxidant parameters in serum

As shown in Table 1, superoxide dismutase levels in serum significantly decreased in O group as compared with C group. The administration of P. discolor decoction inhibited this decrease, especially in Ob-db mice of PDh group. Furthermore, Ob-db mice induced by HFD and alloxan caused a marked rise in serum malondialdehyde. The levels of malondialdehyde were decreased during 4 weeks’ treatments. Furthermore, the levels of this marker in PDh group tended to be the lowest in all of the P. discolor decoction-treated groups, and had a significant difference compared to that of O group. Increased oxidative stress and oxidative modification products of proteins is commonly found in Ob-db mice, and these alterations may cause tissues to be more susceptible to oxidative damage [12]. An increase in protein glycosylation induced by hyperglycemia under the diabetic condition is a
significant source of free radicals and inducers of oxidative stress [14]. Superoxide dismutase is a metalloenzyme which is involved in the dismutation of the superoxide anion to molecular oxygen and hydrogen peroxide [10]. As a by-product of lipid peroxidation, malondialdehyde concentration reflects the degree of oxidation in Ob-db mice [6]. In the present study, the observed increases in lipid peroxidation under diabetes was partly suppressed by *P. discolor* decoction intake, and the elevated superoxide dismutase and the decrease of malondialdehyde indicated that *P. discolor* decoction might attenuate the pathological condition of diabetes by the control of blood glucose and protein glycosylation.

3.5. Identification of constituents in *P. discolor* decoction

3.5.1. Extraction and analysis strategy

The extraction methods using organic solvents are commonly used for pharmacology research. However, the extraction methods could not reflect the real characteristic of TCM because most TCM remedies are prepared in the form of decoctions. Boiling water extraction and organic solvent extractions would result in different phytochemical profiles in the extracts. The present study used the traditional extraction process (decoction) to investigate the real active components that exist in the clinical active extract (decoction) from *P. discolor*. Constituents in *P. discolor* decoction were monitored by a diode-array detector and HR-MS/MS. HPLC profiles of *P. discolor* decoction were essentially similar to those previously reported [15] (Fig. 3). However, more phytochemicals were found in *P. discolor* decoction by ESI-MS. Five major peaks, 6, 20, 24, 30 and 34 were detected in TIC MSn negative mass spectrum. The compounds 5–17, 24, 25, 27–29 and 34 were newly found in *P. discolor*. The structures of the compounds identified in *P. discolor* decoction were shown in Fig. 4.

3.5.2. Major components

As flavonoid glycosides, the glycosidic bond was easily cleaved to form fragment ions of [M-H-176]− or [M-H-162]− by the facile neutral loss of a glucuronide or a glucoside residue from the corresponding deprotonated molecule ions in the ESI MS/MS. These two diagnostic ions of compound 6, 20, 24 and 30 suggested that they were glucuronide or a glucoside. HR-MS/MS of compound 6 exhibited a deprotonated molecular ion [M-H]− at m/z 653.1070 corresponding to C27H25O19 (calculated for C27H25O19 [M-H]−, 653.0990). The structure of compound 6 was elucidated as quercetin-O-diglucuronide based on product ions from collision-induced dissociation (CID) of [M-H-glcu]− at m/z 477, [M-H-glcu-glcu]− at m/z 301.
Fig. 3. TIC MSn negative mass spectrum (A) and LC-DAD chromatogram spectrum (190–950 nm) (B) of P. discolor decoction.

Fig. 4. The structures of the compounds identified in P. discolor decoction. glucoside; rha, rhamnoside; glu, glucuronide; xyl, xyloside; ara, arabinoside. *Since NMR data and the corresponding standards of these compounds were not available, conjugation position of the glycosides or acetyl could not be determined by LC-MS/MS in this study.
[M-H-glucu-glucu-CO]− at m/z 257 and [M-H-glucu-glucu-C16H17O9]− at m/z 151, and data reported previously [16,17]. Compound 20 exhibited an [M-H]− ion at m/z 461.0768 corresponding to C21H17O12 (calculated for C21H17O12, 461.0720). The ion at m/z 285 was formed by elimination of a glucuronic group from deprotonated molecular ions. Compound 30 exhibited an [M-H]− ion at m/z 593.1578 corresponding to C27H29O15 (calculated for C27H29O15, 593.1506). The ion [M-H-coumaroyl-glu-C8H6O2]− at m/z 257 and [M-H-glu-glu-C8H6O3]− were identified as kaempferol-3-O-glucuronide and potengriffioside A, respectively, as reported previously in P. discolor [15]. For the compound 24, the [M-H]− at m/z 445.0702 (calculated for C27H29O15, 445.0771) with its product ions [M-H-80]− at m/z 269 and [M-H-C16H17O8]− at m/z 151 suggested that compound 24 was apigenin-7-O-glucuronide, as reported previously [18]. In addition, the ions [M-H]− at m/z 923 and 891 in MS/MS of compounds 20 and 24 were identified as a dimeric adduct ion of Kaempferol-3-O-glucuronide and apigenin-7-O-glucuronide, respectively. For compound 34, the [M-H]− at m/z 423.0304 (calculated for C18H17O7SO3, 423.0386) with its product ions [M-H-80]− at m/z 343 indicated a loss of SO3 [19]. The product ions at m/z 328, 313, 298, 285 and 270 from CID was identical to that of 5,2′-dihydroxy-6,7,8-trimethoxy flavone (Tenaxin I) or 5,2′-dihydroxy-7,8,6′-trimethoxy flavone (Skullcapflavon I) (Fig. 5) [20]. The very high intensity of product ion at m/z 298 (69.8%) and the relatively weak intensity of ion at m/z 285 (21.34%) indicated that the flavonoid skeleton of this compound was Tenaxin I [20]. Since the conjugation position of the sulfate substitute of this compound could not be determined by LC-MS/MS in this study, compound 34 was characterized as a flavonoid sulfate, and this is the first report on sulfated flavonoid in P. discolor. The ability to synthesize flavonoid sulfates is not a universal feature for the Potentilla species, and it is the first time to detect the flavonoid sulfate in Potentilla species in the present study.

3.5.3. Caffeoylquinic acids and coumaroylquinic acids

Compounds 5 (HR-MS/MS: m/z 353.0865 [M-H]−), 9 (HR-MS/MS: m/z 353.1023 [M-H]−) and 10 (HR-MS/MS: m/z 353.0947 [M-H]−) had the same [M-H]− ion in accordance with a C16H17O9 formula of chlorogenic acid (calculated for C16H17O9, 353.0872). Their molecular ions [M-H]− yielded four peaks at m/z 191, 179, 173, and 135 in MS/MS (Table 2), which suggested that these three compounds were chlorogenic acids isomers. By comparison of retention time (15.2, 19.3 and 19.9 min) of these three chlorogenic acids isomers on the C18 HPLC column, compounds 5, 9 and 10 were identified here as neochlorogenic acid, chlorogenic acid and cryptochlorogenic acid, respectively. These three chlorogenic acids isomers have been identified by LC-MS/MS as reported previously [21]. Compounds 8 (HR-MS/MS: m/z 337.0973 [M-H]−), 15 (HR-MS/MS: m/z 337.0553 [M-H]−) and 16 (HR-MS/MS: m/z 337.0736 [M-H]−) had the same [M-H]− ion in accordance with a C16H17O9 formula of coumaroylquinic acid (calculated for C16H17O9, 337.0923). Their product ions at m/z 191, 173 and 163 from CID indicated that these three compounds were coumaroylquinic acids isomers, as reported previously [22]. The stereoisomers could be resulted from cis/tran-coumaric acids or different conformational forms of coumaric acids.

![Fig. 5](image-url) HR-TIC-MS2 negative mass spectrum of compound 34 (A) and the structures of Tenaxin I and Skullcapflavon I (B).
<table>
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<tr>
<th>No. in Fig. 3</th>
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<tr>
<td>22</td>
<td>30.2</td>
<td>Quercetin-3-o-glucoside</td>
<td>negative</td>
<td>463 (0.2) 301 (100) 273 (18.9), 151 (20.4)</td>
<td>15</td>
</tr>
<tr>
<td>23</td>
<td>31.9</td>
<td>Kaempferol-3-o-glucoside</td>
<td>negative</td>
<td>465 (0.1) 303 (100) 153 (15.7)</td>
<td>15</td>
</tr>
<tr>
<td>24</td>
<td>32.3</td>
<td>Apigenin-7-o-glucuronide</td>
<td>negative</td>
<td>449 (0.1) 287 (100) 153 (14.6)</td>
<td>15</td>
</tr>
<tr>
<td>25</td>
<td>34.1</td>
<td>Acetyl-kaempferol-o-glucuronide</td>
<td>negative</td>
<td>445 (0.1) 269 (100) 151 (3.4), 891***</td>
<td>18,**</td>
</tr>
<tr>
<td>26</td>
<td>35.1</td>
<td>Apigenin-7-o-glucoside</td>
<td>negative</td>
<td>447 (0.1) 271 (100) 153 (3.9)</td>
<td>15</td>
</tr>
<tr>
<td>27</td>
<td>36.2</td>
<td>Acetyl-apigenin-o-glucuronide</td>
<td>negative</td>
<td>487 (0.1) 269 (100) 445 (2.4), 151 (1.5)</td>
<td>18,**</td>
</tr>
<tr>
<td>28</td>
<td>36.8</td>
<td>isomer of compound 25</td>
<td>negative</td>
<td>489 (0.3) 271 (100) 153 (2.7)</td>
<td>15</td>
</tr>
<tr>
<td>29</td>
<td>38.2</td>
<td>isomer of compound 27</td>
<td>negative</td>
<td>487 (0.1) 269 (100) 151 (3.4)</td>
<td>15</td>
</tr>
<tr>
<td>30</td>
<td>39.5</td>
<td>Potengriffioside A</td>
<td>negative</td>
<td>489 (0.1) 271 (100) 153 (15.3)</td>
<td>15</td>
</tr>
<tr>
<td>31</td>
<td>40.7</td>
<td>Kaempferol-o-glucoside</td>
<td>negative</td>
<td>593 (0.1) 285 (100) 257 (72.9), 151 (6.7)</td>
<td>15</td>
</tr>
</tbody>
</table>

(continued on next page)
3.5.4. Flavonoids

Compounds 18, 19, 21–23, 26, 31–33 and 35 have been reported in *P. discolor* [15], which were also identified in *P. discolor* decoction using HR-molecular ion and its product ions in the present study. The CID ions at m/z 285, 301, and 269 from [M-H]− were due to the loss of a glucuronide or a glucoside from flavonoids glycoside (Table 2). For compounds 25 and 28, the MS/MS spectrum at m/z 285 and 151 presented a nearly identical product ions to that obtained from a major compound 20, except an additional ion [M-H-42]−. And the molecular ions were 42 Da larger than that molecular ion of compound 20 in both negative and positive ionization, which suggested an acetate moiety esterified to compound 20 (kaempferol-O-glucuronide). Therefore, compounds 25 and 28 were characterized as acetyl-kaempferol-O-glucuronide or its isomers. Compounds 27 and 29 show the same product ions at m/z 269 and 151 as major compound 24 (apigenin-O-glucuronide). Taken together with the additional ion [M-H-42]−, these two compounds were characterized as acetyl-apigenin-O-glucuronide or its isomers.

In negative ion mode, the glycosidic bond of C-glycosides flavonoids was not easily cleaved to produce daughter ions of [M-60-120]− and [M-H-90]−. The same ions in accordance with a C26H27O14 formula (calculated for 593.1402 corresponding to C27H29O15 (calculated for 593.1506). The product ions at m/z [M-H-90]−, [M-H-120]−, [M-H-60-90]−, [M-H-90-120]− and [M-H-18-90-120]− were also identified as C-glycosides based on their C-glycosides fragmentation pattern. Compounds 12, 13, 14 and 17 were identified here as vicenin 1, schaftoside, isoschaftoside and vicenin 3, which have been reported in *Desmodium styracifolium* previously [23].

3.5.5. Simple phenolic acids

Compound 1–4 and 7 were characterized as simple phenolic acids and their glucosides based on the product ions at m/z [M-H-CO2]− and [M-H-glu]−. The common fragment (base peak) for all simple phenolic acids in MS/MS spectra with negative mode was [M-H-CO2]−, which was formed by elimination of a carboxy group from deprotonated molecular ions. Compounds 1–4 and 7 were characterized as gallic acid, gallic acid-glucoside, protocatechuic acid, protocatechuic acid-glucoside and vanillic acid-glucoside by the common fragmentation pathway, which involves cleavage of intact sugar and aglycon fragments, and produced an aglycon ion as base peak. These compounds have been identified in dried plums previously [22].

By HR-LC-MS/MS analysis, the phytochemical profiles of *P. discolor* decoction was rather different to that of previous report [15]. Nineteen components were newly found in *P. discolor* decoction, and one of the major components is a flavonoid sulfate. However, myricanthic acid, arjunolic acid, euscaphic acid and some other triterpenoid acids were not detected in *P. discolor* decoction [15]. These compounds may be decomposed during a long-time hot water treatment. Also, the different variants and growing conditions may be another possible factor which lead to a different chemical composition of *P. discolor*.

The efficacy of TCM is a characteristic of a complex mixture of chemical compounds which lead to complexity of mechanisms of pharmacological activity. In the present study, *P. discolor* decoction are rich in flavonoids as the predominant compounds. The similar compounds have been studied separately and as part of a complex food matrix and have shown both, hypolipidemic effect and anti-hyperglycemic properties [27–31]. In addition, sulfated flavonoids have been reported to possess aldose reductase inhibitory activity [32]. Aldose reductase, a key enzyme of the polyol pathway, has been demonstrated to play an important role in the etiology of the complications of diabetes such as neuropathy, cataract formation, nephropathy and retinopathy [33]. The complications of diabetes mellitus can be improved by the inhibitors of
aldose reductase in both experimental animals and clinical trials [34,35]. Therefore, the sulfated flavonoid in *P. discolor* decoction may in part contribute to the anti-hyperglycemic effect of *P. discolor*.

4. Conclusions

Four week’s treatment of *P. discolor* decoction ameliorated the development of hyperglycemia, hyperlipidemia and lipid peroxidation in Ob-db mice. The phytochemical profiles of the clinical active extract (decoction) from *P. discolor* was rather different from that of the extract from extraction using organic solvents. Nineteen components were newly found in *P. discolor*. Also, there are five major components identified in *P. discolor* decoction. One of the major components in *P. discolor* decoction is a flavonoid sulfate, and this is the first evidence for the presence of sulfated flavonoid in *Potentilla* species. Therefore, the sulfated flavonoid in *P. discolor* decoction may in part contribute to the anti-hyperglycemic effect of *P. discolor*. Further work is clearly needed to identify the pure active components and to elucidate their anti-hyperglycemic potency.

Conflict of interest statement

The authors declare no conflicts of interest.

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References

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