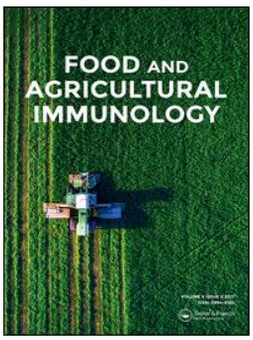

Food and Agricultural Immunology

ISSN: 0954-0105 (Print) 1465-3443 (Online) Journal homepage: https://www.tandfonline.com/loi/cfai20

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To cite this article: Chengcheng Zhao, Bin Zhou, Jing Miao, Xuejiao Li, Songsong Jing, Ding Zhang, Jessica Yijia Wang, Xia Li, Luqi Huang & Wenyuan Gao (2019) Multicomponent analysis and activities for evaluation of *Dioscorea oppositifolia* and *Dioscorea hamiltonii*, Food and Agricultural Immunology, 30:1, 1148-1161, DOI: 10.1080/09540105.2019.1674786

To link to this article: https://doi.org/10.1080/09540105.2019.1674786

Published online: 20 Oct 2019.

FOOD AND AGRICULTURAL IMMUNOLOGY
2019, VOL. 30, NO. 1, 1148–1161
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Multicomponent analysis and activities for evaluation of *Dioscorea oppositifolia* and *Dioscorea hamiltonii*

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ABSTRACT

Dioscorea oppositifolia (Chinese yam) (DO) and *Dioscorea hamiltonii* (DH) are the plants of the genus *Dioscorea* and they are used as health food and traditional Chinese medicine. In order to development of the available DO and DH source, a comparative study of principal components and activities were carried on. DO and DH was qualitatively and quantitively profiled, and we comparatively evaluated their antioxidant (reducing power assay), anti-inflammatory (xylene-induced ear oedema test) and nonspecific immune regulating (carbon clearance test) activities. The 42 and 38 compounds were detected from DO and DH by the method UPLC-DAD-Q-TOF-MS/MS, respectively, as well as 14 compounds are quantified by HPLC and the methodology was examined. This is the first study of the composition and activities of DH, it also indicates that DO and DH consumption would be beneficial to the health. They may be useful in the production of functional food containing an efficacious dose.

ARTICLE HISTORY
Received 15 February 2019
Accepted 30 August 2019

KEYWORDS
Dioscorea oppositifolia; *Dioscorea hamiltonii*; UPLC; HPLC; biological activity

1. Introduction

There are more than 600 species of *Dioscorea spp.* in the world, 93 of which are found in China. The root of *Dioscorea oppositifolia* (DO), commonly named Chinese yam, which is known as the king of the world green health food has been used for centuries. Many tropical countries view DO as an important crop, and DO has became a consequential part of those local residents (Chen & Lin, 2007). DO is not only a common food, but also used as traditional Chinese medicine for the treatment of diabetes, diarrhoea, and other ailments (Ju, Xue, Huang, Zhai, & Wang, 2014). DO contains many chemical components, such as mannan, allantoin, saponins, dopamine, batatasine, phytic acid, amino acids,

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glucoprotein, choline, ergosterol, campesterol and so on, which were extensively studied in the past decades (McAnuff et al., 2005; Mishra & Gaikar, 2004; Yang, Lu, & Hwang, 2003).

In *Dioscorea*, *D. persimilis* and *D. opposita* both belong to *Sect. Enantiophyuum* Uline, which is widely cultivated in southern China. DO is a synonym of *D. opposita*, and D. *hamiltonii* (DH) is a synonym of D. *persimilis*. DO and DH are the two accepted denominations. But DH used to abuse as DO for commercial and medicinal material at the local level and it as an alternative to DO has been controversial for researchers. The study of our group, the starch content and the nature difference between DO and DH is not that significant (Jiang et al., 2014), it is still remained unclear difference of the chemical compositions and biological activities between DO and DH, which is the principal reason of the dispute.

In order to make development of the available DO and DH, the study of principal components and activities of DO and DH was carried on. We investigated the total phenolic acid, total flavonoids, total saponins contents in DO and DH grew in China, which major compounds were identified by UPLC-DAD-Q-TOF-MS and quantified by HPLC (high-performance liquid chromatography) the content of phenolic acids, flavonoids and saponins, and activities (including antioxidant, anti-inflammatory and nonspecific immune regulating activities) of DO and DH were assessed, respectively.

2. Materials and methods

2.1. Plant materials and chemicals

Dry harvested DH was collected from Yulin City, Guangxi Province and DO was provided by Tianjin Zhongxin Pharmaceutical Group Co., Ltd. (China), which was identified by Professor Wenyuan Gao from Tianjin University, China. Standards were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (China). Xylene was purchased from Guangfu Fine Chemical Research Institute (China). Dexamethasone Acetate Tablets (No. H12020122) were purchased from Lisheng Pharmaceutical Co., Ltd. (China). Acetonitrile and acetic acid were both of HPLC grade. Other reagents were of analytical grade.

2.2. Animals

Male Kunming mice (20 ± 2 g) were purchased from the Tianjin Medical Institute (China, License No. SCXK (Jin) 2009-0004). Animals were dwelled in standard plastic cages with wood chip bedding and they were kept in rooms with provided with rodent chow and water ad libitum, and controlled temperature, relative humidity, and a 12-h light/dark cycle. This study was carried through in accordance with the "Regulation for the Administration of Affairs Concerning Experimental Animals" (State Council of China).

2.3. Preparation of extract

DO and DH were ground into powders (50 g) until pass 30-mesh sieve, and then extracted with methanol (400 mL × 3) by refluxing in a heating mantle, each time 2 h. The extraction was prepared at a 0.5 g/mL of the drying herbs concentration in methanol.

2.4. Determinations of total phenolic acid contents

Total phenolic acid (Tp) contents of extracts from DO and DH were determined using Folin-Ciocalteu's reagent as described in Latocha et al. (Latocha, Wolosiak, Worobiej, & Krupa, 2013) with minor modification. Briefly, 0.5 mL sample solution was mixed with 0.5 mL Folin–Ciocalteu reagent, and 3 min later 0.5 mL 10% sodium carbonate solution was added. After incubating at 25°C for 1 h, the absorbance was determined at 765 nm.

2.5. Determinations of total flavonoids contents

Total flavonoids (Tf) contents were measured by the aluminium chloride colorimetric method, which was described by Alothman et al. (Alothman, Bhat, & Karim, 2009).

2.6. Determination of total saponins

Total saponins (Ts) contents of samples were analysed using the method described with minor modification (Li, Wu, Wang, & Li, 2013). The 0.5 mL sample solution with the evaporation of solvent, 0.2 mL of 5% vanillin-glacial acetic acid solution, and 0.8 mL perchloric acid were mixed. After incubating in 70°C water bath for 20 min, 5.0 mL glacial acetic acid was added. The absorbance was measured at 540 nm and the result was calculated as dioscin equivalents.

2.7. UPLC-DAD-Q-TOF-MS/MS analysis

All analyses were performed on an Agilent 1290 UPLC system and connected with the Agilent 6520 Q-TOF mass spectrometer via an ESI interface. The mobile phase was a linear gradient prepared from water (A, containing 0.5% acetic acid), and acetonitrile (B): 0–30 min, 20% B; 30–35 min, 20–70% B; 35–60 min, 70% B; 60–65 min, 70–90% B; 65–85 min, 90% B. For MS detection, the acquisition parameters were as follows: drying gas temperature, 190 °C; nebulizer gas pressure, 1.6 bar; drying gas (N2) flow rate, 6.0 L/min.

2.8. HPLC analysis of monomeric compounds

Phenolic acids and flavonoids compounds were carried out using an HPLC (SHIMADZU-LC-2030C) system. The mobile phases were 0.5% (v/v) aqueous acetic acid (solvent A) and acetonitrile (solvent B). The binary elution system was 0–5 min with 5% B, 5–25 min with 5–10% B, 25–45 min with 10–15% B, 45–80 min with 15–25%B, 80–85 min with 25–55% B, 85–95 min with 55%B, 95–120 min with 55–100%B. Simultaneous monitoring was performed at 280 nm for the phenolic acid and flavonoid compounds, and the compounds were identified by comparison of retention time (Figure 1A). The four saponins compounds were determined using the same HPLC-PAD, and the detection wavelength was at 203 nm. Solvents A and B were water and acetonitrile, respectively. The gradient programme was 0–5 min with 10% B, 5–10 min with 10–25% B, 10–40 min with 25–30% B, 40–45 min with 30–40% B, 45–50 min with 40% B, 50–75 min with 40–70%B, 75–100 min with 70–100%B. Protogracillin, dioscin, trillin and diosgenin in the HPLC chromatogram were identified (Figure 1B).

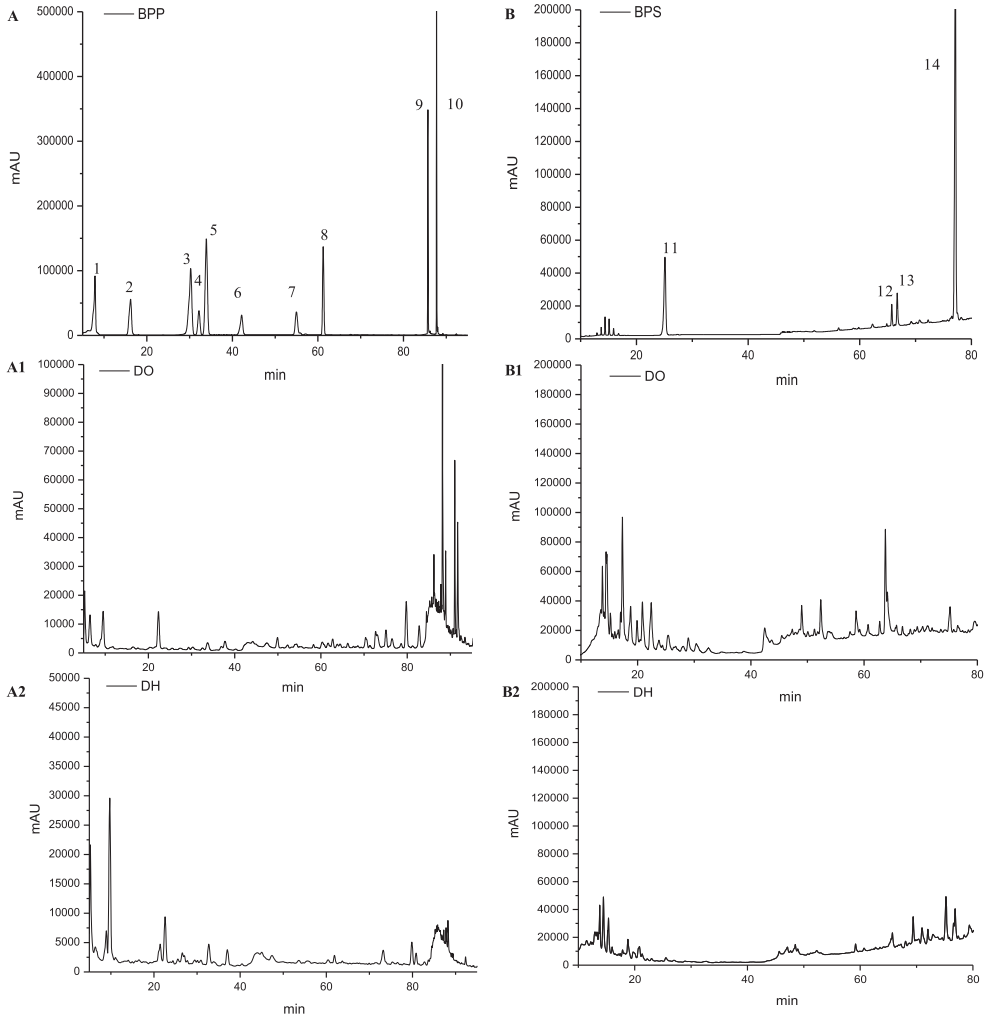

Figure 1. HPLC trace of individual constituents of the standard mixture solution and the samples of DO and DH (A: 10 monomeric polyphenols, A1: sample of DO, A2: sample of DP; B: 4 monomeric saponins, B1: sample of DO, B2: sample of DP). (1) Gallic acid, (2) Protocatechuic acid, (3) Chlorogenic acid, (4) Catechin, (5) Vanillic acid, (6) Syringic acid, (7) p-coumaric acid, (8) Rutin, (9) Quercetol, (10) Kaempferol, (11) Protogracillin, (12) Dioscin, (13) Diosgenin, (14) Trillin.

2.9. In vitro experiments-reducing power

Reducing power was determined as the method of Yildirim A et al. (Yildirim, Mavi, & Karat, 2003) with minor modification. The 1.0 mL sample solution was mixed with 2.5 mL of 0.2 M sodium phosphate buffer (pH = 6.6) and 2.5 mL 1.0% potassium ferricyanide. After incubating at 50 °C for 20 min, added to 2.5 mL 10% trichloroacetic acid and centrifuged. Then 2.5 mL of the supernatant was mixed with 2.5 mL water and 0.5 mL 0.1% ferric chloride. The absorbance at 700 nm was measured.

2.10. In vivo experiments

2.10.1 Xylene-induced ear oedema

The extracts were administered orally for 5 days at the doses of 2 and 6 g/kg, dexamethasone was administered at the dose of 0.01 g/kg (Oluwatoyin, Adewale, & Isaac, 2008). The left ear was considered as control and inflammation action was induced by topical application of xylene (0.1 mL) to the right ear of each mouse. 60 min after oral administration of extracts, oedema was induced in each mouse by using 0.1 mL xylene to the inner and outer surfaces of the right ear. After 90 min, both ears were removed. Swelling degree was detected by the weight increase of each ear, while the inhibition rate was expressed as the weight reduction in comparison with control group.

2.10.2 Carbon clearance test

The samples, dexamethasone, or vehicle was/were administrated orally with the same dose as ear oedema essay for 8 days. 45 min later after the last time administered, 0.1 mL freshly prepared Indian ink (diluted with three times the volume of physiological saline) was injected via the tail intravenous. 20 μL blood sample was taken at intervals of 2 and 10 min from the eye angular vein, mixed with 2 mL 0.1% sodium carbonate solution, and then shook immediately. The absorbance was measured at 600 nm by spectrophotometer. Additionally, body, liver and spleen was weighed. The rate of carbon clearance (K) and phagocytic index (α) were calculated (Jayathirtha & Mishra, 2004).

2.11. Oxidative and inflammatory parameters of DO and DH

Blood samples were collected from the retro-orbital venous plexus, and they were centrifuged at 4000 rpm for 15 min after coagulating in the refrigerator at −4°C. The serums were stored at −80°C. The activity of superoxide dismutase (SOD) and concentration of malondialdehyde (MDA) were measured with biochemical assay kit. Tumour necrosis factor-α (TNF-α) and interleukin-6 (IL-6) concentration were measured with ELISA kits. All the assay kits were purchased from Nanjing Jiancheng Bioengineering Institute (China) and used according to the manufacturer's instructions.

2.12. Statistical analysis

The SPSS version 20.0 statistical software package was used for all statistical analysis. All values were expressed as the mean ± SD (standard deviation). ANOVA were taken and the significant differences were detected by LSD and S–N–K tests.

3. Results and discussion

3.1. Comparison of chemical components of DO and DH

The contents of Tp, Tf and Ts were determined, and average values and standard deviations of the samples from DO and DH are presented in Figure 2, and the chemical components of the DO and DH were determined by UPLC-Q-TOF (Table 1). Further investigation on the main monomeric polyphenols and saponins compounds was

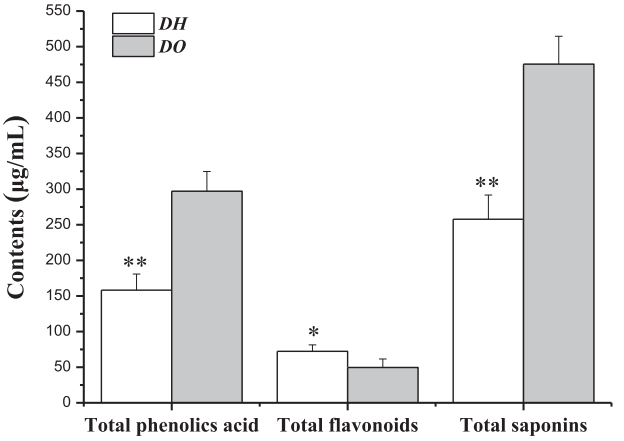

Figure 2. Total phenolic acid, total flavonoids and total saponins contents of DO and DH (μg/mL). Significant correlation at $*p < 0.05$; Significant correlation at $**p < 0.01$.

carried out utilizing HPLC. Ten polyphenols and four saponins were quantified, and the contents of main monomeric compounds of DO and DH are listed in Table 2.

In this work, 42 of compounds were identified from DO, and 38 compounds were detected for the first time in DH. Each of 14 standards has the purity of more than 98.00%. The injection volume was 20 μL. The column temperature was maintained at 30 °C. The standard solution was serially diluted to concentrations to establish calibration curves, and the standard curves were plotted according to the peak areas versus injection content. Precision was calculated by intra-day and inter-day variations. Repeatability was evaluated by assaying six times of the same sample. The stability was conducted for the solution preparation at room temperature. The recovery test was determined to evaluate the accuracy of the method, and the amounts of 14 standard substances were added to known contents of DO samples. Recovery (%) = (amount found – original amount)/amount spiked × 100%. The results were shown in Table 3.

3.2. Antioxidant activities of the extracts from DO and DH

The VC equivalent of the methanol extract of DO was 4.71 mg/g, which was significantly higher than DH (3.30 mg/g). Polyphenols (flavonoids, phenolic acid, proanthocyanidins, diterpenes and so on) have been considered as great antioxidants, which natural or phytochemical antioxidants are secondary metabolites in plants are amongst the antioxidants produced by plants for their sustenance (Adeniran & Sonibare, 2017; Chen et al., 2014; Dai & Mumper, 2010). Therefore, the contents of Tp from DO were 297.03 mg/mL while it from DH contained only 158.21 mg/mL in methanol extracts, and the higher contents of Tp in the extracts could account for the better results found in their reducing power. As natural sources, DO and DH might be introduced for searching sources and may bring new natural products into the food industry with safer and better antioxidants that provide good protection against the oxidative damage, which occurs both in the body and our daily foods. As far as our literature survey could ascertain, little information is available on the in vitro antioxidative activities of DH, and it is the first time that DH was submitted to these studies.

Table 1. Comparative analysis of chemical components in DO and DH.

NO.	t_R (time)	Compound	Molecular formula	Theoretical m/z	Experimental *m/z*	Error	DO	DH
1	2.2	Diosbulbin B	$C_{19}H_{20}O_6$	344.3640	381.0920 $(M+K)^+$	−2.0340	+	+
2	2.4	5-Hydroxy-1,7-bis-(4-hydroxyphenyl)-heptadien-4-en-3-one-hexoside	$C_{25}H_{32}O_9$	476.5240	475.1548 $(M+H)^+$	−2.3692	+	+
3	2.4	Gentisic acid glycoside	$C_{13}H_{16}O_9$	316.2650	317.1050 $(M+H)^+$	−0.1600	+	+
4	2.5	Allantoin	$C_4H_6O_3N_4$	158.1170	182.0871 $(M+Na)^+$	−0.9701	+	+
5	2.6	Cyclo-(L-Phe-L-Tyr)	$C_{18}H_{18}O_3N_2$	310.3520	310.1390 $(M+H)^+$	−1.2113	+	+
6	3.5	Adenine	$C_5H_5N_5$	135.1290	136.0676 $(M+H)^+$	−0.0614	+	+
7	3.6	Gallic acid	$C_7H_6O_5$	170.1220	188.0755 $(M+NH_4)^+$	−0.0470	+	+
8	3.9	Protocatechuic acid	$C_7H_6O_4$	154.1230	155.9790 $(M+H)^+$	0.8560	+	+
9	4.0	β-Sitosterol	$C_{29}H_{50}O$	414.7150	417.1290 $(M+H)^+$	1.4140	+	+
10	4.3	Adenosine	$C_{10}H_{13}N_5O_4$	267.2450	285.2836 $(M+NH_4)^+$	0.0386	+	+
11	5.1	Chlorogenic acid	$C_{16}H_{18}O_9$	354.3140	376.2059 $(M+Na)^+$	−1.1081	+	+
12	5.5	β-daucosterol	$C_{35}H_{60}O_6$	576.8570	578.9245 $(M+H)^+$	1.0675	+	+
13	5.8	Ethyl iso-allocholate	$C_{26}H_{44}O_5$	436.6320	453.3539 $(M+NH_4)^+$	−1.2781	+	+
14	7.3	26-O-β-D-glucopyranosyl-(25R)-furost-5-en-3β,22ξ,26-triol-3-O-β-D-glucopyranoside	$C_{39}H_{65}O_{14}$	757.9370	778.4358 $(M+Na)^+$	−2.5012	+	+
15	7.5	Prosapogenin A of dioscin	$C_{39}H_{62}O_{12}$	722.9140	744.9743 $(M+Na)^+$	−0.9397	+	+
16	9.2	Platyphyllonol pentoside	$C_{24}H_{32}O_8$	448.5130	471.0727 $(M+Na)^+$	−0.4403	+	−
17	34.7	Diosniposide B	$C_{22}H_{26}O_{10}$	450.4420	466.5357 $(M+NH_4)^+$	−1.9063	+	−
18	34.8	6-O-caffeoyl-α-glucose	$C_{15}H_{18}O_9$	342.3030	359.1780 $(M+NH_4)^+$	−1.1250	−	+
19	35.3	Phlorizin	$C_{21}H_{24}O_{10}$	436.4160	479.2044 $(M+K)^+$	3.7784	+	+
20	35.5	Catechin	$C_{15}H_{14}O_6$	290.2720	290.2714 $(M+H)^+$	−1.0006	+	+
21	35.6	3-O-p-Coumaroylquinic acid	$C_{16}H_{18}O_8$	337.0929	359.1651 $(M+Na)^+$	−0.9278	+	+
22	35.8	Vanillic acid	$C_8H_8O_4$	168.1500	185.1124 $(M+NH_4)^+$	−1.0376	+	−
23	36.0	Syringic acid	$C_9H_{10}O_5$	198.1750	242.2896 $(M+K)^+$	5.1146	+	+
24	36.3	Dioscin	$C_{45}H_{72}O_{16}$	869.0570	892.9580 $(M+Na)^+$	0.9010	+	+
25	36.5	Diosgenin	$C_{27}H_{42}O_3$	414.6280	433.2230 $(M+NH_4)^+$	0.5950	+	+
26	36.7	Salicylic acid glycoside	$C_{13}H_{16}O_8$	300.2650	316.2873 $(M+NH_4)^+$	−1.9777	+	+
27	37.0	1,7-Bis-(4-hydroxyphenyl)hepta-4,6-dien-3-one	$C_{19}H_{18}O_3$	294.3500	318.3023 $(M+Na)^+$	0.9523	+	+
28	40.1	Caffeoylshikimic acid	$C_{16}H_{16}O_8$	335.0772	337.2725 $(M+H)^+$	1.1953	+	+
29	40.2	p-coumaric acid	$C_9H_8O_3$	164.1610	184.0737 $(M+NH_4)^+$	1.9127	+	+
30	40.4	β-sitosterolpalmitate	$C_{45}H_{80}O_2$	653.1290	653.3673 $(M+H)^+$	−0.7617	+	−
31	41.7	3-Hydroxy-1,7-bis-(3,4-dihydroxyphenyl)-heptane	$C_{19}H_{24}O_5$	332.3970	353.2696 $(M+Na)^+$	−2.1274	+	+
32	42.0	1-(3,4-Dihydroxyphenyl)-7-(4-hydroxyphenyl)-hept-4-en-3-one	$C_{19}H_{20}O_4$	312.3660	313.2737 $(M+H)^+$	−0.0923	+	+
33	42.2	Hirsutanolol	$C_{19}H_{22}O_6$	346.3800	347.1731 $(M+H)^+$	−0.2069	+	+
34	42.5	Glycerol monopalmitate	$C_{19}H_{38}O_4$	330.5090	331.2829 $(M+H)^+$	−0.2261	+	+
35	42.9	Kaempferol-3-O-rhamnoside	$C_{21}H_{20}O_{10}$	432.3840	454.2906 $(M+Na)^+$	−1.0934	+	+

36	49.1	Rutin	$C_{27}H_{30}O_{16}$	610.5250	635.2526 $(M + Na)^+$	1.7276	+	+
37	49.9	Gracillin	$C_{45}H_{72}O_{17}$	885.0570	889.4246 $(M + H)^+$	3.3676	+	+
38	50.6	Diosniposide A	$C_{21}H_{26}O_8$	406.4320	445.2240 $(M + K)^+$	−0.2080	+	−
39	51.4	Quercetol	$C_{15}H_{10}O_7$	302.2400	301.1400 $(M + H)^+$	−2.1000	+	+
40	55.5	Kaempferol	$C_{15}H_{10}O_6$	286.2400	313.2723 $(M + Na)^+$	4.0323	+	+
41	66.2	Trillin	$C_{33}H_{52}O_8$	576.7710	614.4716 $(M + K)^+$	−1.2994	+	+
42	67.5	Dioscoreside I	$C_{39}H_{60}O_{15}$	768.8960	804.5425 $(M + K)^+$	−3.3535	+	+
43	70.8	6-O-*p*-coumaroyl-α-glucose	$C_{15}H_{18}O_8$	326.3030	327.2260 $(M + H)^+$	1.1331	+	+

Note: "+": representative of the existence of this compound; "−": representative of not exist in this compound.

Table 2. The main chemical compounds contained in DO and DH.

NO.	Samples	DO	DH
1	Gallic acid	3.67 ± 0.10^{b}	6.24 ± 0.07^{bc}
2	Protocatechuic acid	0.69 ± 0.02^{a}	0.65 ± 0.02^{a}
3	Chlorogenic acid	1.20 ± 0.04^{a}	0.93 ± 0.03^{a}
4	Catechin	4.45 ± 0.07^{b}	17.69 ± 0.03^{d}
5	Vanillic acid	2.08 ± 0.05^{a}	ND
6	Syringic acid	37.35 ± 0.49^{h}	26.26 ± 0.42^{f}
7	*p*-coumaric acid	1.65 ± 0.04^{a}	0.96 ± 0.05^{a}
8	Rutin	11.98 ± 0.16^{c}	7.07 ± 0.22^{c}
9	Quercetol	27.76 ± 0.12^{g}	$6.8 \pm 0.17b^{c}$
10	Kaempferol	18.65 ± 0.08^{d}	5.92 ± 0.13^{b}
11	Protogracillin	154.45 ± 2.56^{j}	60.21 ± 1.04^{g}
12	Dioscin	23.64 ± 0.27^{e}	18.21 ± 0.54^{d}
13	Diosgenin	26.02 ± 0.05^{f}	25.00 ± 0.08^{e}
14	Trillin	77.61 ± 0.10^{i}	107.08 ± 1.12^{h}

Notes: The results were given as mean values ± standard deviation ($n = 3$). Units of all values are μg compounds/g dry weight. Different letters (a–j) of each value indicate significant difference at $p < 0.05$.

Table 3. Linearity, precision, repeatability, stability, and recovery of HPLC analysis.

NO.	Linear regression equation[a]	R^2	Intra-day runs RSD[b] (%)	Inter-day runs RSD[b] (%)	Repeatability RSD[b] (%)	Stability RSD[b] (%)	Recovery RSD[b] (%)
1	$y = 2509.187x + 77.786$	0.9962	0.434	0.490	2.593	0.104	0.470
2	$y = 1441.810x + 25.396$	0.9996	1.243	1.129	2.141	0.266	0.945
3	$y = 2394.044x + 98.036$	0.9987	1.042	1.260	2.916	1.138	0.903
4	$y = 648.878x + 21.287$	0.9994	0.642	1.543	1.537	0.786	0.787
5	$y = 5428.473x + 73.067$	0.9994	0.722	1.393	2.254	1.231	1.028
6	$y = 314.942x - 11.984$	0.9970	0.980	1.423	1.304	1.078	0.665
7	$y = 4737.533x + 61.012$	0.9996	1.073	1.403	2.066	0.302	0.728
8	$y = 455.127x + 44.087$	0.9995	1.439	1.549	1.318	0.427	1.070
9	$y = 1091.399x + 14.008$	0.9992	1.628	1.587	0.422	0.842	0.875
10	$y = 1812.314x + 32.705$	0.9999	0.325	1.014	0.400	0.395	0.723
11	$y = 122.319x - 151.619$	0.9978	0.647	1.230	1.660	1.315	1.103
12	$y = 449.374x + 124.423$	0.9998	0.840	0.894	1.132	1.678	0.532
13	$y = 768.041x - 30.957$	0.9998	0.248	0.716	0.204	1.358	1.112
14	$y = 336.269x + 200.055$	0.9998	1.536	0.802	0.133	1.157	0.320

[a] y is peak area and x is injection content (ng).
[b] Relative standard deviation: RSD (%) = 100 × SD/mean.

3.3. Anti-inflammatory effects of the extracts from DO and DH

As shown in Figure 3, extracts of DO and DH have anti-inflammatory activity at the dosages of 2 and 6 g/kg, DO present better inhibitory than DH. As shown in Figure 4, the activity of SOD was significantly decreased in the negative control group, with a significant increase in MDA level compared with treatment of DXM. With the interference of DO and DH groups, the level of SOD in serum was elevated, and the concentration of MDA was declined. As exhibited in Figure 5, IL-6 and TNF-α concentration were also increased respectively in negative control group. These results indicated that the animals were in inflammatory status. The treatment of DO and DH decreased the level of all the inflammatory cytokines. The high dosage of DO showed better efficacy than the groups of DO-Low and DH.

The inflammation is a complex biological process. It is one of main responses of the immune system against infection and irritation (Hartati, Widjanarko, Widyaningsih, & Rifa'i, 2017), which involves a series of events that could be elicited by numerous

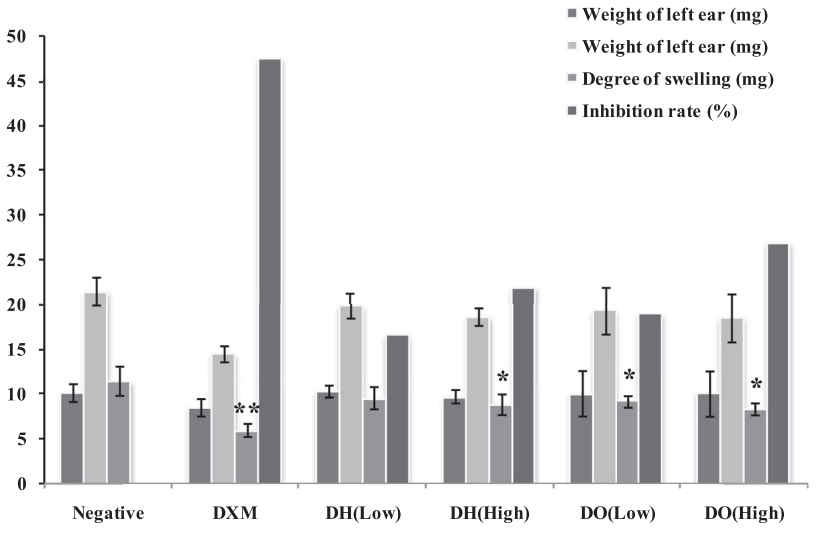

Figure 3. Effects of extraction from DO and DH on xylene-induced ear swelling in mice. Compared with negative control group: *$p < 0.05$, **$p < 0.01$. DXM: dexamethasone.

stimuli such as infectious agents, antigen–antibody interaction and ischaemia (Osadebe & Okoye, 2003). MDA is a biomarker to measure the level of peroxidation. SOD plays a vital role in vivo system to prevent ROS damage. The results suggested the protective

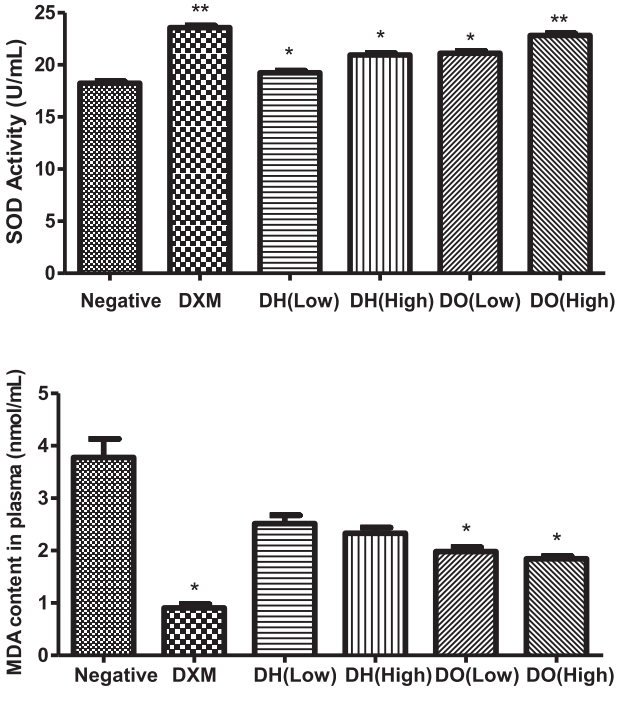

Figure 4. Activities of key antioxidant enzymes SOD and MDA in different groups from DO and DH in mice. Compared with negative control group: *$p < 0.05$, **$p < 0.01$. DXM: dexamethasone.

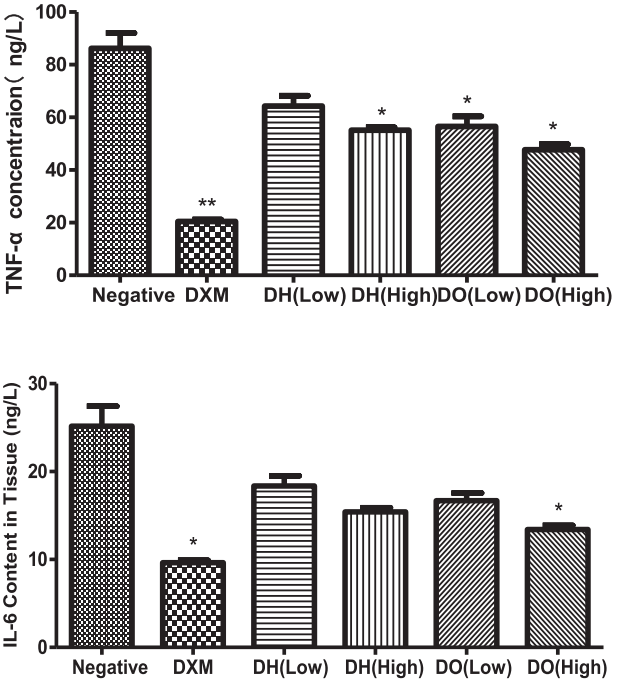

Figure 5. Changes of inflammatory cytokines TNF-α and IL-6 in different groups from DO and DH in mice. Compared with negative control group: *$p < 0.05$, **$p < 0.01$. DXM: dexamethasone.

effect of DO and DH induced oxidative stress. The oxidative stress was prevented by improving SOD activity and decreasing MDA level. Numerous molecules, such as IL-1, IL-6, IL-8, IL-11, TNF-α, PGE2 and nitric oxide, underlie local tissue destruction during the process of inflammation (Hartati et al., 2017; Ikeda, Ueno, Naraba, & Oh-ishi, 2001). The results showed the anti-inflammatory effect of DO and DH that is associated with the reduction of the expression of IL-6 and TNF-α. Diosgenin is a naturally occurring steroidal saponins abundantly present in many plants (Adham, Zaki, & Naim, 2009), which has the biological activity of anti-inflammation. It could stimulate lymphocyte transformation and enhance the phagocytic capability of macrophages in vitro, and remarkably promote the secretion of NO and TNF-α in macrophages. Phenolics compounds are anti-inflammatory, which may be work with the inhibition of COX activity and anti-oxidant activity, and the structure–activity relationship of the phenolics derivatives on the anti-inflammatory action revealed that both C-4 hydroxy and C-3 methoxy radicals of benzyl aldehyde was an important role in anti-inflammatory activities (Lee et al., 2006). The amounts of Ts and Tp of DO were nearly twice that of DH, and DO was better than DH at the same dosage of anti-inflammatory activities; furthermore, the chemical information was available to illustrate the results of vitro anti-inflammatory activities. It showed the anti-inflammatory effect of DO and DH may be mediated through the regulation of anti-oxidative and anti-inflammatory ability, and it is the first time that DH was submitted to these studies.

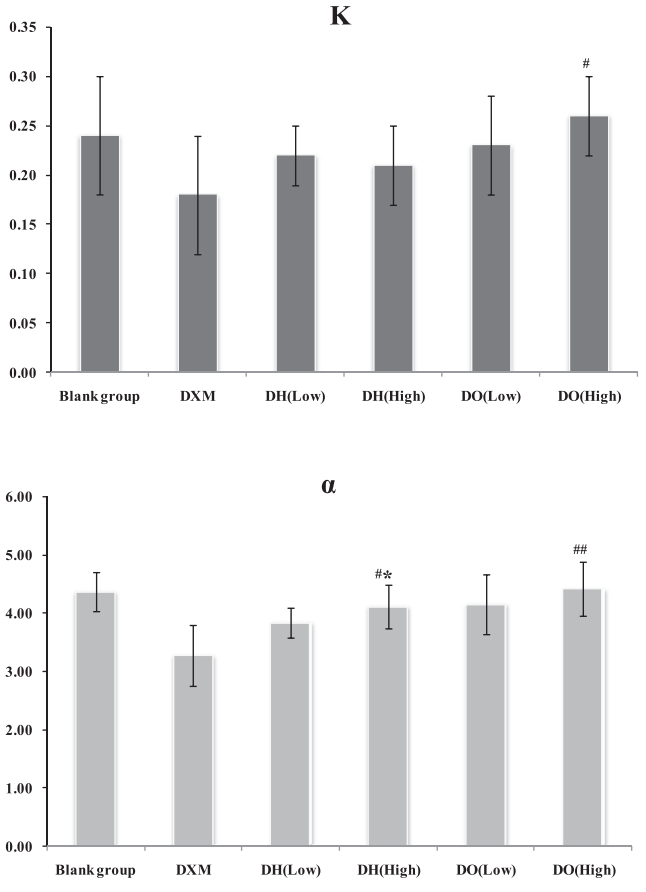

Figure 6. Effects of the extraction from DO and DH on carbon clearance function. Compared with blank group: $^{*}p < 0.05$, $^{**}p < 0.01$. Compared with DXM group, $^{\#}p < 0.05$, $^{\#\#}p < 0.01$. DXM: dexamethasone.

3.4. Effects of the extracts from DO and DH on carbon clearance function

Results are presented in the Figure 6, and the experimental results indicate that DO and DH can enhance the carbon clearance function of mononuclear phagocyte, promote the generation of antibody. DO and DH shows an improved effect on the immune regulation function at high doses, and the phagocytosis was the removal of microorganisms and foreign bodies, dead or injured cells. Modulation of immune response to alleviate disease has been of interest for a long time (Yin, Zhou, Wang, Xu, & Zhang, 2016). Plant saponins and flavonoids play important roles in immune activity, and DO and DH showed Ts contents with 475.5 and 257.8 μg/mL, while having Tf amounts of 49.6 and 72.3 μg/mL, respectively. Diosgenin could improve specific and non-specific cellular immune responses (Driedger & Sporns, 2001). The anti-tumour effects of diosgenin were achieved by immunostimulating properties (He et al., 2012). Only activated cells are susceptible to the modulating effects of flavonoids, which are responding to a stimulus, and stimulated activities of numerous cell types can be influenced by specific flavonoids. DO and DH both stimulate the reticuloendothelial system by significant increase in the phagocytic index, and it is the first time that DH was submitted for this study.

4. Conclusion

The Tp, Tf and Ts contents of methanol extracts from DO and DH were determined and the 42 and 38 compounds were detected from DO and DH by the method UPLC-DAD-Q-TOF-MS/MS, respectively, as well as 14 compounds are quantified by HPLC. The amounts of Ts and Tp of DO were nearly twice that of DH, while the contents of Tf in DH were slightly higher than DO. The activities of DO in equal doses were significantly better than DH. This is the first study of the chemical constituents and activities of DH, and the research results proved that DH as an alternative to DO use as medicine in equal doses is insufficient. In the micromolecule, antioxidant, anti-inflammatory and immune regulation effect of DO and DH indicate that their consumption would be beneficial to health. They may be useful in the production of functional foods containing an efficacious dose.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work was supported by National Key R&D Program of China [grant number 2017YFD0201402]; National Natural Science Foundation of China [grant number 81173487,81673535]; the Innovation Fund for Small and Medium-Sized Technology-Based Firms of Tianjin [grant number 16YDLJSY00090].

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